Functional and speciality beverage technology

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Preface

A change has been taking place in the beverages sector in recent years. Consumers no longer consider beverages simply as thirst-quenchers, but rather as health products that have a content of specific ingredients which form part of their lifestyle. This development in functional beverages addresses different needs and lifestyles – to boost energy, fight ageing, fatigue and stress, and target specific diseases – and the sector is still expanding. According to ACNielsen's marketing information, the carbonated soft-drink category fell 5% across all areas in 2007, whilst at the same time the functional beverages category expanded, with 30% growth in the energy beverages category. Projected global sales for 2010 in the functional beverages category are 34 billion. These new developments will be more tailor-made, addressing the different concerns of consumers. The opportunity will no longer be about new beverages development, but beverages development for the right health concerns.

Studies show that consumers are not choosing low- or no-calorie beverages to manage their weight, but instead prefer nutrient-dense, no-calorie beverages. These preferences are setting new challenges in the development of beverages and the utilisation of drinks as functional foods. We have progressed research of functional beverages, to incorporate consumer awareness of health and the demand for this new development in beverages. This is increasing the challenge for research and development teams to obtain more scientific data about healthy molecules and to develop functional drinks with good taste, texture and flavour.

This is one of the first books specifically on functional beverages and it covers the different aspects involved in their development in a sector that is still evolving. The book examines the different sources for beverage development and the various aspects of research and development, ranging from ingredients for stability, texture and flavour to ingredients with health-specific properties.

The book is divided into four main parts. The first section focuses on the ingredients and technologies which are a key element in the development of new functional beverages. Although consumers are demanding healthy beverages, they still want products with good texture, flavour and taste. The different chapters of this first section cover stability texture, new types of sweeteners, probiotics, fortification and technology for extended shelf life.

The second part of the book highlights new developments in dairy-based beverages. Dairy has long been recognised as a healthy food and research has been undertaken to examine the healthy properties of the various compounds found in milk. The different chapters cover the increasing healthy properties of milk, from feeding cows, to milk- and whey-based healthy drinks and the number of constituents in milk with healthy properties (omega 3 fatty acids, probiotics, bioactive proteins and peptides). Finally, new results are presented on the small molecules of the fat globule membrane, which possess healthy properties.

The third part describes fruit- and plant-based beverages. Although the properties of soya beverages have long been known, these chapters present new developments in their healthy properties. This section also examines new developments in functional fruit drinks with bioactive components and a number of developments realised with different polyphenols (antioxidant properties). New functional fruit beverages with specific molecules are now available. Other chapters in this section discuss tea and coffee and the healthy properties of the different compounds.

The final section of the book is devoted to consumer habits and gives some guidelines about healthy beverages in the context of major development. The first chapter describes the importance of consumers' perceptions in the development of new functional drinks, highlighting the importance of testing consumer attitudes towards beverage development. The second chapter sets out important guidelines in the context of this new development of functional beverages, based on the need for healthy beverages for consumers.

The present volume has been compiled with the assistance of world-renowned scientists who are experts in their fields, and I would like to thank them all for their contribution to this important publication. I hope that both academia and the food and beverages industry will find this a useful work of reference on the various challenges in the development of functional beverages.

Dr Paul Paquin

Part I

Beverage ingredients and technology

1

Ingredient selection for stabilisation and texture optimisation of functional beverages and the inclusion of dietary fibre

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Abstract: A functional beverage is usually formulated by adding functional ingredients and/or by reducing levels of ingredients considered less healthy such as sugar and fat. The composition of the beverage has a direct impact on the ingredient selection needed to achieve the targeted texture and the stability of the beverage. To overcome these challenges the use of ingredients such as hydrocolloids and emulsifiers is key to monitoring the stability and texture of the final product. Tools for understanding the key parameters to consider when selecting the ingredients to texture and stabilise a defined beverage are given. The addition of fibre as a functional ingredient within the beverage matrix can have a direct effect on the mouthfeel, texture and flavour release in a chosen beverage system. The type of fibre ingredient must be chosen and evaluated carefully to ensure desirable textures and flavour profile.

Key words: beverage, dietary fibre, hydrocolloids, stability, texture.

1.1 Introduction

The challenges faced when developing functional beverages are numerous. A functional beverage is usually formulated by adding functional ingredients and/or by reducing levels of ingredients considered as less healthy, such as sugar and fat. The objective is to secure health claims, yet develop stable beverages with an appealing flavour profile and mouthfeel throughout their shelf-life.

Incorporating the necessary amount of a functional ingredient into a beverage to allow for a health claim or to reach the targeted functional property targeted is often a challenge as these 'functional' ingredients may

have a direct impact on the texture (e.g. fibres) and or on the stability (e.g. minerals, proteins). Reducing the fat or sugar level to aim for 'lower/no/reduced' type of claim has a direct impact on the texture of the beverage and in some cases on the stability.

These challenges in product development are increased by the nature of the finished product: a very low viscosity matrix. On a general basis, beverages are less robust to formulation and process variations than more viscous products.

The term beverage covers a very wide variety of types of food matrices and compositions. Classification can be made according to the formulation

- dairy-based beverages
  - acid
  - neutral
- plant-based products
  - containing protein beverages
  - without protein beverages
    - acid
    - neutral

The composition has a direct impact on the selection of the ingredients needed to achieve the targeted texture and stability of the beverage. Process parameters such as homogenisation, heat treatment (time, temperature) and filling temperature also play a key role. The use of ingredients such as hydrocolloids and emulsifiers are key to ensuring the stability and texture of the final product.

Tools to determine which key parameters to consider when selecting the ingredients to texture and stabilise a defined beverage will be given. The approach is relatively general as details for each beverage category are addressed in the following chapters.

1.2 Challenges faced when formulating functional beverages with a good stability, texture and mouthfeel

Prior to formulating it is important to identify and define the final functionality we wish the ingredients to fulfil.

1.2.1 Definitions of texture and stabilisation in beverages

In order to quantify or measure texture, a viscosity measurement is often referred to. Viscosity, however, describes only one attribute of the product as it is measured at a given shear rate. Texture is, on the other hand, a very wide term which covers a number of rheological properties and sensory descriptors. Texture covers the whole appearance and sensation when drinking the beverage:

- appearance when pouring;
- glazing on the bottle;
- first mouth sensation;
- coating of the mouth;
- residual perception.

The importance of the rheological behaviour, and more specifically, of flow properties of gums (hydrocolloids) is that the behaviour can be related to the mouthfeel or textural properties of the gum (Glicksman, 1982).

Stabilisation is a very wide and general term which relates to many different, complex biophysicochemical mechanisms. Each stabilisation parameter needs to be properly described and categorised in order to be addressed. Generally, a stabilised beverage refers to a homogeneous and smooth beverage. Depending on the beverage formulation and final functionalities targeted, stabilisation can be further defined as follows:

- *Particle stabilisation*: The particles such as pulps, cacao particles and minerals are evenly suspended throughout the beverage.
- *Emulsion stabilisation*: There is no oil or fat ring on the top of the beverage container or bottle.
- *Protein stabilisation*:
  - the proteins contained in the beverage have not flocculated, nor become sediment;
  - the beverage exhibits a smooth and non-sandy mouthfeel.
- *Stabilisation of the texture*, often including the functionalities mentioned above. The viscosity and appearance is homogeneous throughout the beverage, meaning
  - no gel points, or lumps;
  - no viscosity gradient;
  - no layer formation;
  - no phase separation;
  - no clarification;
  - no flocculation.

Each parameter has a different ingredient solution and depends on the formulation and the process of the beverage.

1.3 Mechanism involved in texture and stabilisation of beverages

1.3.1 Thickening and gelling agents

Hydrocolloids can roughly be divided into two categories on the basis of their functionality in beverages: thickening agents and gelling agents. Thickening agents provide texture to the beverage, but are not capable of suspending particulates. They slow the settling of particles or the rising

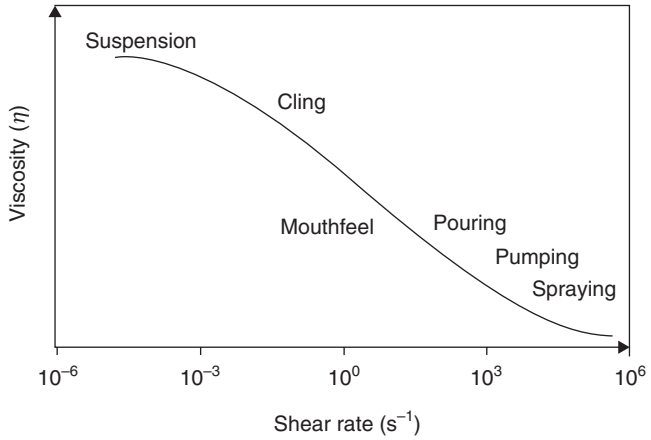

Fig. 1.1 Idealised flow curve of a solution of a thickening agent and typical shear rates associated with different functionality requirements. Inspired from Sworn (2007).

of oil droplets, but they cannot stop separation from occurring (Hoefler, 2004). Gelling agents form links between their molecules, building a three-dimensional network. The result is that the particles or oil droplets become permanently suspended in the matrix and do not separate out, provided that the density of the particulates is lower than the yield value created by the network (Hoefler, 2004).

In the particular case of protein stabilisation in acid beverages, casein and more generally food proteins will tend to agglomerate and form a sediment. In these cases the proteins are also very sensitive to dehydration and can easily become sandy after heat treatment. In order to avoid protein agglomeration, chalky texture and sedimentation, formulation with specific hydrocolloids that have a direct interaction with the proteins through electrostatic interactions is essential.

Most food proteins (isoelectric point around 5) can form complex coacervates with anionic polysaccharides such as high ester pectins, carboxymethylcellulose (CMC) or propylene glycol alginate (isoelectric point around 3.5) in the intermediate region where the two macromolecules carry opposite net charges: pH above the isoelectric point of the polysaccharide below the isoelectric point of the protein.

The strength of the polysaccharide – protein interaction will depend on several factors, such as the distribution of the carboxyl groups on the backbone, the three-dimensional protein structure and the distribution of the ionisable groups on its surface. The whole interaction will also depend on factors such as pH, ionic strength, and presence of sugars or fat (Futo, 1993).

The ideal way of characterising a beverage is to measure its flow curve. Figure 1.1 shows the idealised flow curve of a thickener when measured

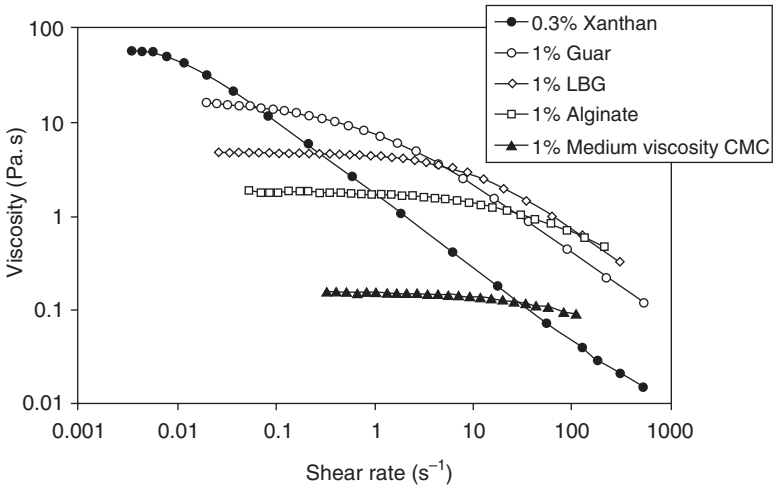

Fig. 1.2 Flow curves for various hydrocolloid-thickening agents in 1% NaCl (Sworn, 2007).

over a broad range of shear rate, and superimposed on this are some typical processes and characteristics. The choice of the thickener will depend on the beverage formulation, process, equipment to disperse and cost in use. Figure 1.2 shows the flow curves of a number of hydrocolloids at various concentrations and serves to illustrate that through the careful choice of the hydrocolloid and concentration the flow behaviour can be tailored to suit specific needs in terms of processing and sensory qualities (Sworn, 2007).

Various studies on the stimuli associated with the oral evaluation of viscosity show that the shear rate range involved is between 1 and 100 s^{-1}. Other studies have shown a more narrow range of 20–50 s^{-1} (Glicksmann, 1982). Thus when developing a beverage it is recommended to measure the flow curves of beverages and/or assess the beverage using a sensory evaluation. Rheology measurements of viscosity versus shear rate (flow curves) therefore make it possible to:

- qualitatively measure and characterise existing beverages and use them as targets for duplication;
- characterise various beverages containing different gums so that the best one is selected for the required mouthfeel (Glicksmann, 1982).

It is, however, very important to note that this could be very time consuming and requires specific rheometers and methods. Indeed beverages have an extra difficulty in that the viscosities are very low and small particles are often present. Sensory evaluation is the most commonly used method.

In beverage matrices the concentration of the polymer is usually in the area of or slightly above the critical concentration (C^*) as the viscosities of

beverages are very low compared to other food matrices. This adds a challenge to the formulation of beverages: to suspend particles in a beverage a three-dimensional network is needed (minimum interaction of polymers together), yet the viscosity of the beverage should remain low.

1.3.2 Emulsifier agents

Emulsifiers are substances that reduce the interfacial tension of two normally immiscible phases, allowing them to mix and form a stable emulsion and thus avoid flocculation, creaming and coalescence of the different phases during storage that may lead to the emulsion breakdown. The emulsification of oil in water (o/w) emulsions nearly always occurs under turbulent conditions (Becher, 1983), and the relative contribution of interfacial tension to oil droplet disruption is negligible compared with the homogenisation energy applied.

Emulsifier agents are used in beverages to stabilise o/w types of emulsion such as high-fat milk beverages, and juice beverages containing flavours based on essential oils, and addition of omega-3 oil for example, to assure stability throughout shelf-life.

1.4 Selecting the ingredients' properties and functionalities

This section gives only a very general and short overview of the main functionalities of each ingredient in beverages. For more information please refer to the bibliography at the end of this chapter. Ingredients are listed in alphabetical order.

Hydrocolloids are water-soluble polysaccharides. They are mainly of vegetal origin (ground, cultivated or grown through bio-fermentation).

1.4.1 Alginate

Sodium alginate is extracted from brown seaweeds (Phaeophyceae). The alginic acid, the free acid form of alginate, is extracted from the seaweed in alkaline conditions, then precipitated and ion-exchanged (e.g. with potassium). The most commonly used alginate in food is sodium alginate (E 401) (Onsoyen, 1999).

Alginate is a cold-soluble polymer. It does not require any heating to hydrate. It has very high affinity with calcium to which it bonds to form thermo-irreversible gels. During the hydration step it is therefore critical to avoid all contact with free calcium.

To meet the target viscosity for a defined application, a choice is made from many different viscosity grades of alginate. In a non-ionic environment such as water, alginate is a pure thickener. In order to increase the viscosity at low alginate concentrations in non-dairy systems a small amount of a

slightly soluble calcium salt (such as calcium sulphate, calcium citrate) may be added after hydration of the alginate. Calcium ions will react immediately with the alginates to create a three-dimensional network. Depending on the level of calcium and the nature of the alginate (high gel strength or low gel strength) this will either only increase the viscosity or create a sufficient network to suspend particles in a water system (acid or neutral). It is important to note that this system will be highly sensitive to any change in pH and in calcium content (e.g. coming from fruit), and alginate precipitates below its pK_a value (pH: 3.5) (Onsoyen, 1999).

In neutral milk alginate provides a nice, smooth, rich and fat-like mouthfeel provided it is either in an integrated form with emulsifiers, calcium sequestrants (e.g. tetra sodium pyrophosphate is added) or it is added alone above 75 °C (under agitation and preferably mixed with other dry ingredients). If lumps are formed at this stage with the free calcium they will be irreversible.

1.4.2 Propylene glycol

Propylene glycol alginate (PGA) is the propylene glycol-ester of alginic acid. The main difference between PGA and sodium alginate is that the latter, being a salt, is ionic whereas PGA, being an ester of a polymeric acid, is essentially non-ionic in character. Therefore PGA is not easily precipitated by interaction with acids or divalent metal salts. This difference therefore enables PGA to be used in acidic or calcium-rich products which would not tolerate sodium alginate. In addition the introduction of an ester group into the molecule confers a degree of lipophilic character and consequent surface activity, making PGA suitable in some applications where emulsification is necessary. PGA is therefore utilised in the stabilisation of milk proteins under acid conditions as well as for pulp and oil ring stabilisation in juice drinks. PGA is completely soluble in both cold and hot water.

The viscosity of PGA is a function of the degree of polymerisation of the alginate chain (indirectly the molecular weight). The degree of esterification is another feature of PGA to take into consideration. Indeed, it indicates the percentage of carboxylic groups in the alginate chain that have reacted with propylene oxide. As described in Section 1.3.1, the remaining unesterified acid groups retain some negative charge even as low as pH 2.75 (in the case of PGA) and will participate in a weak but significant cross-linking with calcium and proteins which, at this low pH, carry a net positive charge (Onsoyen, 1999).

1.4.3 Carrageenan

Carrageenan is a polysaccharide with a high molecular weight. It is extracted from a red seaweed of the class Rhodophyceae. It consists of repeating galactose and 3.6 anhydrogalactose units, both sulphates and non-sulphated,

joined by alternating alpha 1–3 and beta 1–4 glucosidic linkages. The number and position of the ester groups on the repeating galactose units differ from the three main types of carrageenan: kappa, iota and lambda. Depending on the species of the red seaweed and the extraction process, carrageenan is more or less dominated by one of these three types. Each type has its own characteristic functionality which has to be taken into consideration when choosing the right one for a given application. Carrageenan is an ingredient of choice for all neutral dairy applications due to its high reactivity with proteins and extremely low dosages.

In neutral milk applications, the carrageenan interacts with the dairy proteins to form a stabilising network which is able to suspend particles such as cocoa in chocolate milk. The network prevents protein–protein interaction and aggregations during storage. To ensure full functionality of this network, it is essential to keep the beverage below 20 °C.

In neutral milks, kappa carrageenan exhibits strong gelling properties and high milk reactivity, which can cause partial gelation and/or whey separation due to a strong contraction of the network. Iota carrageenan has a tendency to cause latent thickening during storage due to its shear reversibility. Lambda carrageenans are normally known as thickeners owing to their rather weak gelling properties. Proper selection, controlled processing and standardised functionality result in the carrageen that provides the best performance in flavoured milk.

Carrageenan is generally unsuitable for acid dairy products; the low pH increases the electrostatic interactions between protein and carrageenan, producing unstable aggregates, which flocculate and separate (Imeson, 2000).

1.4.4 Cellulose gum and microcrystalline cellulose

Cellulose gum or carboxymethylcellulose (CMC)

The manufacture of cellulose gum involves treating cellulose (from cotton or wood pulp) in alkali solution followed by an esterification reaction with monochloacetic acid (Murray, 2000). Cellulose gum is a linear molecule composed of two repeating anhydro-glucose units joined through a 1,4-glucosidic linkage. Each anhydro-glucose unit contains three hydroxyl groups, which in theory can be substituted. The average number of hydroxyl groups substituted per anhydro-galactose unit is known as the degree of substitution (DS) (Murray, 2000). There are two main factors influencing the properties of cellulose gum: (1) the degree of polymerisation ((DP) in direct correlation with the viscosity) and (2) the DS (playing a key role in protein protection in acidified milk drinks, as described in Section 1.3.1). Cellulose is cold soluble and exhibits crystal clear solutions.

Thanks to its numerous grades generating a wide choice of viscosities and DS, cellulose gum is widely used in beverages, in all types of juice-based

drinks, where it contributes to mouthfeel, and in acidified milk beverages, where it plays a key role to maintain a smooth and homogeneous texture throughout shelf-life.

Colloidal microcrystalline cellulose
The raw material for microcrystalline cellulose (MCC) is wood pulp. The primary MCC particles composing the colloidal type of MCC (Avicel® RC and Ceolus®SC) are aggregated MCC particles (75 to 91% w/w) whose surface has been coated with water soluble polymers (Iijima and Takeo, 2000), most frequently (9–25%) cellulose gum (Imeson and Humphreys, 1999).

Colloidal MCC requires very specific conditions to be fully functional: (1) the use of high shear equipment and a homogeniser is mandatory for milk applications and (2) it must be dispersed in water before any other ingredients. If possible soft and hot water should be used (Imeson and Humphreys, 1999; Iijima and Takeo, 2000).

Colloidal MCC dispersions form a three-dimensional network which exhibits a yield stress and thixotropic behaviour and is very heat stable (Imeson and Humphreys, 1999). This has direct and unique application in beverages, especially in neutral milk applications, to prevent whey separation and oiling off (as carrageenan) but more particularly to efficiently suspend calcium in calcium-enriched milks. It is not sensitive to calcium variations. Colloidal MCC also imparts a very creamy mouthfeel to milk beverages and is therefore well suited to low-fat neutral milks.

1.4.5 Emulsifiers

See Section 1.3.2 for introduction. Emulsifiers are defined as substances that reduce the interfacial tension between two immiscible phases (in the case of beverages between oil and water), enhance emulsification and increase emulsion stability. Food grade emulsifiers are polar lipids manufactured from edible fats and oils or fatty acids, polyvalent alcohols (e.g. glycerol) and organic acids (e.g. lactic acid). Monoglycerides and their organic derivates are the most commonly used emulsifiers in foods worldwide. However, molecules that have emulsifying properties, meaning they are amphiphilic (i.e. possess both hydrophobic and hydrophilic parts), can be of other origins. In beverage applications, molecules such as PGA and gum arabic may also be used as emulsifiers. The two latter are functional in fruit juices containing lipophilic vitamins or essential oils for example.

Emulsifiers such as monoglycerides (DIMODAN® HP at 0.1–0.15%) can also contribute to a creamy mouthfeel in low-fat dairy-based formulation as well as prevent creaming in a full-fat neutral milk or soy drink.

The main factors which determine the ‘quality’ of the newly formed emulsion are (1) the adsorption of the emulsifier to the droplet surface,

(2) the homogenisation process and (3) stability during formation of the emulsion. The HLB value is the most common tool used to select emulsifiers, and is based on the relative percentage of hydrophilic to lipophilic groups within the molecule. HLB values in range of 8 to 18 are suited to o/w emulsions. The concentration of emulsifier is the function of the oil droplet size – the smaller the droplets, the higher the emulsifier dosage.

1.4.6 Gellan gum

Gellan gum is an extracellular polysaccharide secreted by the microorganism *Sphingomonas elodea*. Gellan gum forms gels at low concentrations. Hot solutions are cooled in the presence of gel promoting cations. It is available in a substituted form (referred to as high acyl (HA)) and the unsubstituted form (referred to as low acyl (LA)). Gel properties depend on the degree of substitution – HA producing soft, elastic gels and LA producing hard, brittle gels.

The use of calcium and sodium sequestrants is strongly recommended to enable the hydration of LA gellan gum, unless the gum is introduced above 80 °C in the solution. In food containing sugar, LA gellan gum should be hydrated prior to the addition of sugar, which should then be added hot. The hydration of HA gellan gum depends much less on the concentration of ions, and generally heating to 85–95 °C is sufficient to fully hydrate the gum both in water and in milk systems.

Gellan is commonly used as a gelling agent. It can, however, be used to prepare structured liquids which are extremely efficient suspending agents. These structured liquids are gelling systems that have been subjected to shear either during or after the gelation process. These pourable systems are often referred to as 'fluid gels'. A formulation example of a fluid gel for pulp suspension is given in Table 1.6 (Sworn, 2007).

These fluid gels can also be used in a wide range of neutral dairy products and soy-based products. HA gellan gum is tolerant of a wide range of UHT conditions, and products can be filled at higher temperatures than with carrageenan (Valli and Morrison, 2002).

1.4.7 Guar and locust bean gum

Guar gum and locust bean gum are galactomannans extracted from the endosperm of the guar plant (*Cyamopsis tetragonoloba*) and the carob tree (*Ceratonia siliqua*) respectively. The endosperm halves are then ground to the desired fineness. Both guar gum and locust bean gum consist of a linear chain of mannose units linked by 1–4 β-D glycosidic bonds which have statistically a 1,6-bound galactose unit forming a side branch on every two mannose unit for guar gum and on every four mannose units for locust bean gum. Owing to this chemical structure, guar gum is cold soluble and locust bean gum is hot soluble.

They are both pure thickeners, and their main functionality is to contribute to viscosity and mouthfeel. They are commonly used in all types of non-dairy beverage (i.e. juice drinks). In near-waters, however, they are not transparent even at typical beverage concentrations (0.05–0.2%). In dairy beverages they are always used in association with another hydrocolloid playing a 'stabilising function' (i.e. pectin for acidified dairy beverages, carrageenan for neutral protein-based beverages) as they contribute 'only' to mouthfeel.

The main differences between guar gum and locust bean gum is their dosage to achieve same viscosity (higher for locust bean gum), their cost in use (higher for locust bean gum), creamy mouthfeel and flavour release (improved for locust bean gum). Alternatives to locust bean gum as a thickener in beverages are depolymerised guar gum grades, which exhibit a more Newtonian flow curve, better defined viscosity at low concentrations and increased stability to acidic conditions (owing to lower molecular weight) as well as superior microbiological quality (an example of a commercial grade is MEYPRODOR™ 100).

1.4.8 Gum arabic (acacia gum)

Gum arabic is the natural gum exuded by various species of acacia, the main source of commercial gum arabic being *Acacia senegal* and *Acacia seyal*. They are very complex, highly branched polysaccharides found as mixes of salts, protein and sugars (mainly galactose) (Anderson *et al*., 1990). Gum arabic dissolves in cold water and exhibits very low viscosity (30% of gum arabic has lower viscosity than 1% of CMC at low shear rates) (Phillips and Williams, 2000). Gum arabic is stable in acid conditions and widely used as an emulsifier in the production of concentrated citrus and cola flavour oils for application in soft drinks. The gum is able to inhibit flocculation and coalescence of the oil droplets over several months. Furthermore the emulsions remain stable for up to a year when diluted up to 500 times in sweetened carbonated water prior to bottling.

A typical formulation may contain 20% gum arabic, 10% flavour oil, 5% weighing agent, while the final beverage may contain 0.1–0.2% of the above described emulsion, 0–12% of sugar and 0.2% citric acid/colouring (Phillips and Williams, 2000).

1.4.9 Pectin

Pectin is extracted from fruit. The major sources are citrus peel and apple pomace. Pectin is essentially a linear molecule with 'smooth' regions comprising galacturonic acids. These acid groups may be free or naturally esterified with methanol and of 'hairy' regions containing mainly rhamnose, galactose, xylose, arabinose and galacturonic acid in a branched structure.

Commercial pectin is defined as high ester (HE) or low ester (LE) depending on the number of galacturonic acid groups that are esterified.

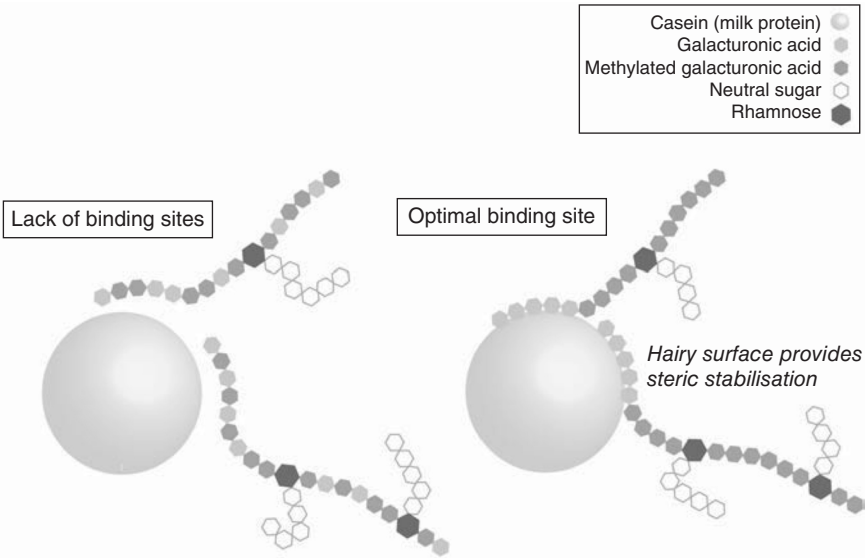

Fig. 1.3 Theoretical representation of different pectin molecular structure types, and their interaction with proteins. Inspired from Futo (1993).

If this number is greater than 50% the pectin is defined as HE pectin, less than 50% denotes LE pectins. Generally, HE pectins are the most suited pectin types for use in non-dairy and acidified dairy beverages.

In water-based beverages such as juice beverages, HE pectins are widely used as mouth-feel improver, especially in no added sugar juice beverages. Low concentration pectin solutions can be considered as Newtonian and show low viscosity. HE pectins impart clean mouthfeel and good flavour release. LE pectins are not recommended for this application as they are highly dependent on the level of calcium present in the media.

In acidified milk drinks, as described in Section 1.3.1, stability problem encountered in this application can be avoided by using HE pectin. As shown in Fig. 1.3, with its controlled block structure of non-methylated galacturonic acid units, HE pectin can bind to the protein surface as the blocks are negatively charged at pH between 3.6 and 4.5 (the typical pH of an acidified milk beverage). Bound to the proteins, the long pectin molecules protect them from re-aggregation through steric stabilisation (Futo, 1993).

1.4.10 Xanthan

Xanthan gum is a heteropolysaccharide produced by fermentation using the bacterium *Xanthomonas campestris*. The primary structure of the molecule is composed of a backbone of 1,4-linked β-D-glucose with side chains

containing two mannose and one glucoronic acids. Half of the terminal mannose carry a pyruvic acid, which confers to the molecule a negative charge independent of the pH (Noble and Urlacher, 1999).

Xanthan gum hydrates immediately in cold water and is extremely stable to pH (from 2.5 to 11), heat and shear. Xanthan gum exhibits very high viscosities at low shear rates (this also at low concentration such as the ones used in beverages; 0.01–0.1%) with a very strong pseudo-plasticity character (instantaneous shear-thinning behaviour) and ability to recover initial viscosity immediately upon removal of shear (Noble and Urlacher, 1999).

These properties are especially well suited for use in all non-dairy beverage (acid and neutral) where xanthan gum is mainly used to enhance mouthfeel. Clear grades exist such as GRINDSTED® Xanthan Clear. In dairy-based beverages xanthan gum is not recommended as it has a tendency to generate flocculation or separation.

Below 0.15% (below typical usage level in non-dairy beverage) xanthan gum alone is not able to suspend particles over time in a beverage. Association with other gums such as locust bean gum and/or guar gum, with which xanthan has a synergy, is needed (a commercial example of such specific blends is GRINDSTED® JU 086).

The main functionalities of the ingredients mentioned in Section 1.4 are summarised in Table 1.1.

1.5 How to use hydrocolloid ingredients in beverages

This section is also applicable to the fibres described in Section 1.7.2. In order to efficiently use a hydrocolloid it is essential that it is fully hydrated at some time during the beverage manufacturing, preferably at the beginning. Hydrocolloids swell very quickly in water and tend to form lumps easily. Therefore the hydrocolloid is less effective and may have a negative impact on the texture or on the stability of the final product.

Gelling agents are obtained in a dried or precipitated state. They must be dissolved before they can be induced into the gelled state. It is important to note that a gelling agent cannot hydrate in its gelling conditions. The gelling agent must be hydrated outside its gelling conditions and then conditions induced, by lowering the temperature (e.g. kappa carrageenan) or by adding calcium (e.g. alginate). Similar rules apply for thickening agents (Hoefler, 2004).

To enable the hydrocolloid to be fully functional two key steps must be respected: the hydrocolloid must first be dispersed and then be hydrated. The key to lump-free hydrocolloid dispersions is to slightly separate the hydrocolloid particles from each other just before they touch the water surface and start hydrating. Particle size plays a key role: the bigger the particle the better the dispersion. It is important to note that potentially all grades of hydrocolloids and fibres can be in granular or agglomerated form.

Table 1.1 Main functionalities of hydrocolloids in beverages

Functionalities in the beverages	Mouthfeel enhancer	Stabilisation of proteins		Suspension of particles	Stabilisation of emulsions
Properties of the ingredient	Thickening	Protein interaction		Ability to form a network (0)	Emulsifying properties
		pH < 4.6	Neutral pH		
Alginates	Yes (1)	No		Yes	No
Kappa, iota, Hybrids Carrageenans	Yes (3)	Precipitates	Yes	Yes	No
Lambda carrageenans	Yes	Precipitates	Yes	No	No
Cellulose gum	Yes	Yes	Whey separation	No	No
Colloidal MCC	Yes	No	No	Yes	No
Gellan gum	Yes	No		Yes	No
Guar gum	Yes	No		No	Yes (4)
Gum arabic	Yes	No		No	Yes
Locust bean gum	Yes	No		No	No
High ester pectin	Yes	Yes	No	Yes	No
Propylene glycol alginate	Yes	Yes	No	No	Yes
Xanthan gum	Yes	No	Precipitates	No (5)	No
Emulsifiers (6)	Yes	No	No	No	Yes

These should be taken as general recommendations and guidelines. The described functionalities depend on the usage levels, the beverage formulation and the process.
(0) In specific condition of concentration, pH, ions, etc.
(1) When no free calcium is available in the beverage.
(2) In presence of calcium, or other divalent ions.
(3) If filling temperature below gelling temperature.
(4) Only some specific grades of depolymerised guars, such as Meyprodor 5 from Danisco.
(5) At typical beverage viscosities xanthan alone is not able to suspend particles; however, specific combinations of Xanthan, LBG, and guar gum are more efficient than xanthan alone in juice beverages.
(6) As defined in section 1.4.5

This physical form of the powder eases the dispersion/hydration step, while contributing to reduce dust when used. (Commercial examples are the grades GRINDSTED® GUAR EASY, GRINDSTED® Xanthan Supra.)

1.5.1 Dissolution conditions

Hydrocolloids may be hot or cold water-soluble as described in Table 1.2. Once dispersed, the cold-water hydrocolloids may fully hydrate after a

Table 1.2 Hydrocolloid properties to be considered when formulating and processing

Ingredient	Requires heat to be functional (1)	Functionality affected by electrolytes	E number
Sodium alginate	No	Yes (2) Ca^{2+}	E 401
Carrageenans	Yes (1)	Yes (3) (2) K^+, Ca^{2+}, Na^+	E 407
Cellulose gum	No	Yes	E 466
Colloidal MCC	No (4)	No	E 460
Gellan gum	Yes	Yes (2) K^+, Na^+, Ca^{2+} Mg^{2+}	E 418
Guar gum	No	No	E 412
Gum arabic	No	Yes	E 414
Locust bean gum	Yes	No	E 410
Pectin high ester (HE)	Yes (5)	Yes (3)	E 440
Propylene glycol alginate	No	No	E 405
Xanthan gum	No	Yes	E 415

(1) Usually above 85 °C
(2) Will form gels in presence of these ions.
(3) Requires heat except lambda types that are cold soluble.
(4) Requires homogenisation to be activated.
(5) Pectin is cold soluble but will take time; hydration at 80 °C for 5–10 min is recommended.

sufficient agitation time at cold temperatures. The hot-soluble hydrocolloids will hydrate only in hot water and require subsequent heat treatment (80–90 °C) for complete hydration. However, these have the advantage of being easily dispersed in cold water. The more water available in the system, the faster and more efficient the hydration of the hydrocolloid. If a pre-solution is not used, it is recommended that the hydrocolloid is added at an early stage in the process, especially before addition of acid and large quantities of solids such as sugar. If a hydrocolloid is sensitive to ions, these ions must not be available to the hydrocolloid in the hydration media (either not present or sequestered).

Various recommended methods for optimum dispersion and hydration applicable in beverage factories are described below.

1.5.2 Making a pre-solution

Aqueous pre-solution (direct addition)

If a high-speed mixer is available, the hydrocolloid can be dispersed and hydrated by direct addition to water, stirring vigorously as the powder is added. The powder should be added slowly into the vortex and once it has been added, the mixing speed should be reduced to prevent air incorporation. Suitable mixers are turbo mixers, Silverson mixers or a high-speed

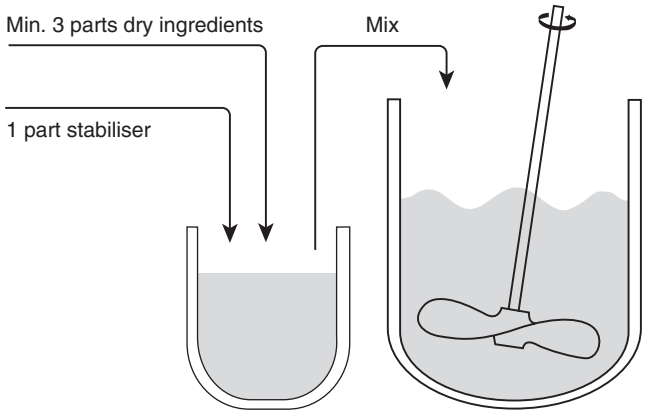

Fig. 1.4 Making a pre-solution: aqueous pre-solution (direct addition).

propeller mixer. For some hydrocolloids it is possible to use cold water to disperse and hydrate but in these cases you may need to extend the mixing time. The pre-solution can then be added directly to the beverage system.

Aqueous pre-solution (premixing with sugar)
In this method (Fig. 1.4) the hydrocolloid is dry-mixed with 5–10 parts of sugar before addition to water at approx. 80 °C. Alternatively the pre-mixed stabiliser can be added to cold water and then heated to approx. 80–90 °C. The mixing intensity required for this method is much lower as the sugar improves the dispersibility of the hydrocolloid, thereby ensuring fast, efficient hydration. The pre-solution can then be added directly to the beverage system.

Pre-solution using a carrier
Hydrocolloids are, as the name implies, mainly hydrophilic. Therefore the hydrocolloid may be conveniently dispersed in non-aqueous liquids (e.g. oils, syrup, alcohol, propylene glycol). This particular property may be useful for incorporating hydrocolloids into systems that do not allow fast mixing. One part of hydrocolloid is dispersed, lump-free, into 5–10 parts oil, syrup, etc., and this dispersion is then added to the final product.

1.5.3 Direct addition (no pre-solution)

If the hydrocolloid is pre-mixed with 5–10 parts of sugar, it can be added directly provided that the total soluble solids do not exceed 20% during hydration. Depending on the solubility of the hydrocolloid this can be in cold or hot (80–90 °C) water. Once hydrated the remaining ingredients can be added. As with the previous method a low to medium stirring intensity is required.

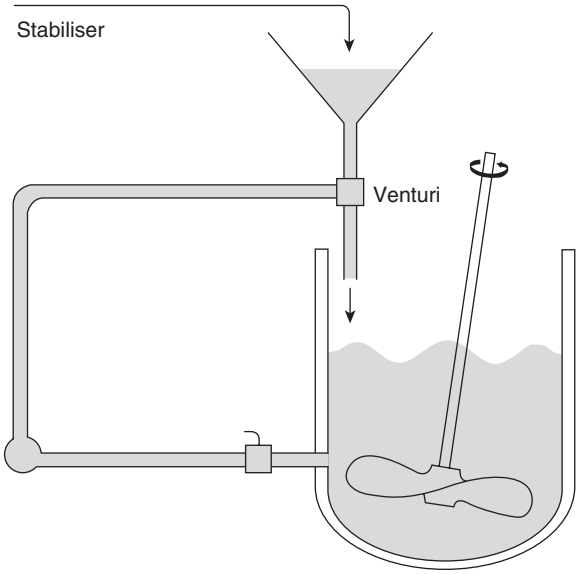

Fig. 1.5 Incorporation with a venturi valve or tri-blender.

1.5.4 Incorporation with a venturi valve or tri-blender

The hydrocolloid is added into the funnel, which is situated on the venturi (Fig. 1.5). At sufficiently high pumping pressure the hydrocolloid is drawn through the opened tap into the liquid.

1.6 Formulation considerations

Figure 1.6 and Table 1.3 give general tools to guide the user through the first selection of ingredients, together with indication on usage levels. In this section more attention has deliberately been given to soy-based beverages.

1.6.1 Soy-based beverages

In addition to off-notes, soy-based beverages often suffer poor mouthfeel typically related to the presence of large particles, giving a sandy, gritty or chalky sensation or a lack of creaminess due to low fat content. The physical instability of the protein dispersion which causes large particles can often be solved by optimising the raw material sources (hydration characteristics) manufacturing process and formulation, including stabilisers. When lack of creaminess is a problem, mouthfeel can be significantly improved by combining optimal textural ingredients and emulsifiers with the right flavouring.

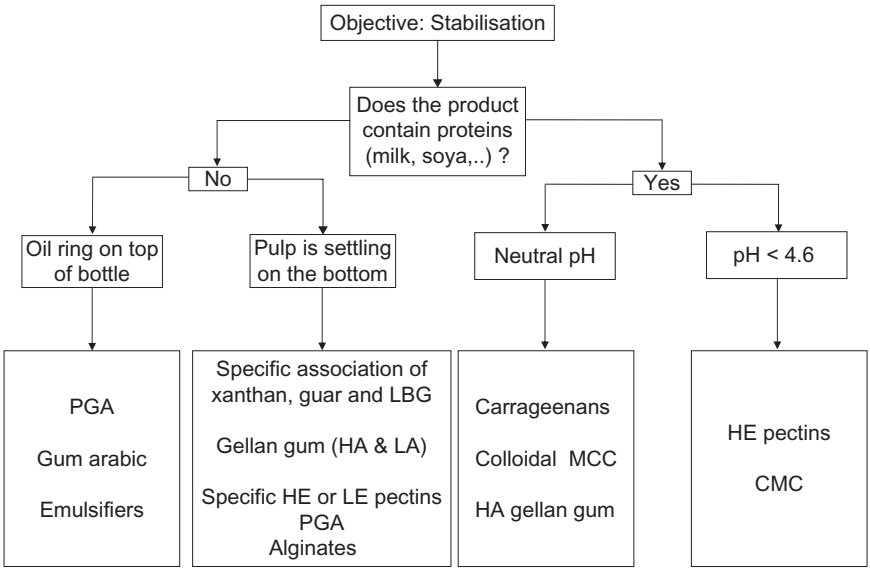

Fig. 1.6 Ingredient selection by type of beverage applications: simplified hydrocolloid selection chart for processed beverages.

Table 1.3 Main applications of hydrocolloids and typical usage levels in beverages

	Typical beverage applications				Typical usage levels (*) (% w/w)
	Protein-based		Non-protein based		
	Neutral (e.g. UHT, pasteurised milk)	Acid (e.g. acidified milk, or milk & juice)	Acid (e.g. juice drinks)	Neutral (e.g. functional waters)	
Alginates	X		X	X	0.1–0.3
Carrageenans	X			X	0.01–0.2
Cellulose gum		X	X	X	0.1–0.4
Colloidal MCC	X		X	X	0.1–1
Gellan gum	X		X	X	0.02–0.05
Guar gum	X	X	X	X	0.05–0.2
Gum arabic			X	X	0.001–0.005
Locust bean gum		X	X	X	0.05–0.2
Pectin high ester (HE)		X	X		0.2–0.5
Propylene glycol alginate		X	X		0.1–0.3
Xanthan gum			X	X	0.01–0.2

(*) Depends on the type of application: protein/non-protein, acid/neutral.

Table 1.4 Example of a neutral soy drink formulation

Composition	Percentage
Coconut fat, 31 °C	1.50%
Soya isolate (90% protein)	2.80%
Sucrose	2.00%
Salt	0.10%
DIMODAN® HP	0.20%
GRINDSTED® Carrageenan CL 110	0.03%
Water	93.37%
Flavouring	++

Emulsion improvement

When the soy-based beverage contains fat, it may be necessary to consider stabilising the fat droplets to prevent fat separation and creaming off. This is achieved by the addition of emulsifiers such as Dimodan®HP. Thereby the fat phase can be dispersed into the water phase. Emulsifiers increase the stability of the fat emulsion, enhance the colour and impart a smooth and creamy texture whereby the overall mouthfeel is improved. In addition emulsifiers may function as defoaming agents during processing, a common problem related to soy-based beverages.

Stability improvement of neutral soy-based beverages

Carrageenan and soy protein molecules react with each other, probably through electrostatic and hydrophobic interactions forming a complex. This reaction seems, however, not to be as strong as in carrageenan–milk systems because for soy products more carrageenan is usually used to stabilise the final product than the carrageenan dosages for milk systems.

Specific association of stabilisers (like RECODAN™) or specific grades of carrageenan (like GRINDSTED® Carrageenan CL) prevent sedimentation and separation of the protein system and avoid the precipitation of cocoa particle by interacting with the soy proteins, giving the beverages the targeted mouthfeel and appearance.

When formulating with calcium, a non-soluble calcium source is preferred. When formulating with vitamins, it is recommended to check and adjust the pH to 7. An example of a neutral soy drink formulation is given in Table 1.4.

Stability improvement of acidic soy-based beverages

An acidic protein system will usually require stabilisation. One of the most efficient ingredients at low pH is high-ester pectin. For this specific application the pectins are usually based on a highly controlled molecular structure for optimal interactions with proteins and possible additional calcium, as illustrated in Fig. 1.3.

Table 1.5 Example of a soy-juice calcium-enriched formulation

Ingredients	Percentage
Soy protein isolate	1.10
Orange juice concentrate 65°Brix	3.30
Sugar	8.00
GRINDSTED® Pectin ASD 540	0.20
Vanilla flavouring	+
Lemon flavouring	+
Calcium lactate	0.92
Water	Up to 100%
pH 3.9	

In directly acidified soy-based beverages, the pectin should be incorporated prior to the addition of acid or juice. For cultured beverage the pectin must be added after fermentation or the protein system will collapse. The optimal dosage of the pectin depends greatly on the formulation (level of protein and ions) and process. Generally a high protein level and a high heat treatment tend to increase the dosage. Table 1.5 gives an example of a soy-juice calcium-enriched formulation.

1.6.2 Acidified milk beverages

Compared with yoghurt or quark, acidified milk beverages contain much less protein, and therefore the challenge is not so much to stabilise the casein gel network but more to maintain the individual casein flocculates in suspension. This is one of the main reasons why acidified milk beverages undergo high homogenisation during processing, in order to break down the casein flocculates and generate particles with a 'stabilisable' density. Typically, HE pectin is best suited for beverages below pH 4.2 and cellulose gum for pH 4.2 (Fox *et al*., 1993). Choice of the dosage of the stabiliser is highly dependent on the formulation, and more specifically on the protein content. Specific grades of HE pectins and cellulose gum with specific molecular structure exist for defined problematics such as high protein content formulations or for calcium enriched formulations.

1.6.3 Neutral milks

When formulating with a functional ingredient in neutral milk such as omega-3, vitamins or an added source of calcium, it is important to ensure that the selected ingredient does not have a major effect on colour or flavour, but also on pH; if this is the case the pH should be readjusted prior to heat treatment, especially if the ingredient induces a decrease in pH, which could cause major instability of the product over shelf life.

When adding a calcium source to the neutral milk, an ingredient solution based on colloidal MCC is preferred, as this does not react with calcium,

Table 1.6 Example of a calcium-enriched UHT fresh milk

Composition	Percentage
Whole milk (or low-fat/skim milk)	99.45%
RECODAN™ CAL X-TRA	0.55%

Blend RECODAN™ CAL X-TRA with cold milk. UHT treat at 140 °C for 3 seconds. Cool to 75 °C and homogenise down-stream at 200 bar. Cool to 25 °C and fill aseptically. The product will have a shelf-life of approximately 6 months.

Table 1.7 Recipe for a pulp suspension beverage using HA gellan gum (Sworn, 2000)

Ingredients	Weight (g)	Percentage
Water	338.10	67.62
Fruit juice	100.0	20.0
Sugar	60.0	12.0
HA gellan gum	0.25	0.05
Tri-sodium citrate dehydrate	0.25	0.05
Citric acid anhydrous	0.9	0.18
Potassium citrate	0.5	0.1

Preparation:
1. Blend the HA gellan gum with the trisodium citrate dehydrate and disperse in the water.
2. Heat the dispersion to 90 °C to hydrate the gum.
3. At 90 °C add the remaining dry ingredients and the fruit juice.
4. Cool to room temperature while mixing to form the fluid gel.

and will ensure stability during storage. An example of a formulation is given in Table 1.6.

1.6.4 Nectars and juice drinks

The stabilisation of particles in juice beverages is challenging; indeed the network needs to be sufficient to suspend the particles, yet the viscosity of the beverage must remain low. The viscosity of such beverages is so low that it can do little to suspend the particle, and this only for a very short period of time given the scale of the shelf-life.

The yield stress value of the beverage is the critical parameter that will secure the suspension of the particles. However, this depends on the particle density, the difference between the density of the particle and the beverage, as well as the amount of particles in the beverage.

Tables 1.7 and 1.8 give two examples of formulation for particle suspension. The formulation in Table 1.8 is very robust to formulation as it is not sensitive to calcium levels, pH variations, fruit types. However, both formu-

Table 1.8 Recipe for a pulp suspension beverage using GRINDSTED® JU 086

Ingredients	Percentage
Water	85.00–78.00
Sucrose	6.50–5.00
Orange juice concentrate	3.00–15.50
GRINDSTED® JU 086 (*)	0.08–0.14
Citric acid powder	0.10–0.20
Potassium sorbate	0.03
Orange flavouring	To suit

GRINDSTED® JU 086 is a specifically formulated system based on guar gum LBG and xanthan.

1. Mix dry ingredients with sugar
2. Add to cold water under vigorous agitation
3. Add juice concentrates
4. Adjust pH with citric acid
5. Add flavours
6. Pasteurise at 90 °C/30 s
7. Cool and fill at 10–20 °C
   Cooling is mandatory to allow the network to form. Formulation is robust to pH, dosage, fruit type, calcium content variations.

lations need to be cooled to a minimum 20 °C to allow a three dimensional network to form in which the particles can be suspended.

Mouthfeel selection in juice drinks

When the degree of mouth coating was evaluated by a panel trained in descriptive sensory analysis, the ratings where highly correlated with viscosity versus rate of shear behaviour. As the degree of mouth coating decreased, the deviation from a Newtonian viscosity characterisation increased. Gums that exhibited a high degree of shear thinning (pseudoplasticity), such as xanthan, were not mouth coating in the mouth (Glicksmann, 1982).

Recent Danisco studies also demonstrate that at the same measured viscosity, therefore different dosages, hydrocolloids generate different mouth coating and viscosity in mouth. For example, in a juice drink (10% fruit, Brix 12, pH 3.5) with the same measured viscosity (30 mPa s, at 10 °C, 10 rpm measured on the ULA Brookfield LV ULA rheometer), GRINDSTED®cellulose gum BEV150 was found to give to the beverage a high viscosity in the mouth and impression of mouth coating, whereas GRINDSTED® xanthan gum Ultra gave a much lighter mouth coating and viscosity in mouth effect (evaluation made by 11 trained panellists). Depending on the viscosity chosen to evaluate the sensory descriptors, the choice of hydrocolloids can determine flavour release; at viscosities below 10 mPa s, flavour release is the same for all tested hydrocolloids, whereas at higher viscosities (i.e. 30 mPa s) some hydrocollods such as GRINDSTED® Pectin RS 461 impart a higher flavour release to the beverage than other hydrocolloids.

1.7 Increasing the fibre content of beverages

1.7.1 Rationale for increasing fibre intake

The main benefits of fibre include decreased transit time, normalised bowel function, normalisation of blood lipids and attenuation of blood glucose. Many disorders are associated with inadequate consumption of dietary fibre. These include constipation, diverticulitis, haemorrhoids, diabetes, cardiovascular disease (CVD), bowel cancer, other cancers and obesity. Individuals who consume fibre-rich diets may reduce the risk of developing these diseases through complex mechanisms that are still being researched.

Some dietary fibre ingredients are also described as prebiotic dietary fibre because of their effects in the colon. The concept of prebiotics was first defined by Gibson and Roberfroid (1995), and the definition of the term 'prebiotic' was further refined by Gibson *et al*. (2004):

> *a prebiotic is a selectively fermented ingredient that allows specific changes, both in composition and/or activity in the gastrointestinal microflora that confers benefits upon host wellbeing and health.*

According to the definition a prebiotic candidate must also demonstrate:

- resistance to gastric acidity, hydrolysis by mammalian enzymes and gastrointestinal absorption;
- fermentation by gastrointestinal microflora;
- selective stimulation of the growth and/or activity of intestinal bacteria associated with health and well-being.

The term prebiotic is used to refer to the colonic fermentation that specifically increases the number of bifidobacteria and lactobacilli as these are thought to be beneficial to the host. In 2007 the Food and Agriculture Organization (FAO), proposed a broader definition that stated 'a prebiotic is a non-viable food component that confers a health benefit on the host associated with modulation of the microbiota' (Anon, 2007).

Recommendations on the amount of dietary fibre that should be consumed daily vary from country to country. These differences are due to variations in the definition of dietary fibre and methods of analysis. However, despite these differences, in developed countries there is consistently a gap between the recommended daily dose and actual consumption levels. In the United Kingdom, the British Nutrition Foundation recommends a fibre intake of 18 g/day based on the non-starch polysaccharide (NSP) method of analysis or 24 g/day based on Association of Analytical Communities (AOAC) methodology. The American Dietetic Association recommends a dietary intake of 20–30 g of dietary fibre a day. For optimal gut health WHO/FAO recommend that 25 g dietary fibre must be consumed daily.

Strategies for increasing fibre consumption in the diet include supplementation by adding fibre to processed foods and drinks and/or increasing the consumption of foods such as wholegrain foods, fruit and vegetables. Functional fibres such as oligosaccharides, inulin, polydextrose and resistant maltodextrins are becoming increasingly popular as fibre forms to be added to processed foods and beverages. These are gaining broad acceptance as convenient forms of dietary fibre, dependent on local legislation. Functional fibres have a role to play in the supplementation of beverages as they can be used in greater amounts than hydrocolloids or other fibres with less textural impact. This ultimately means that products can be formulated with enough dietary fibre present to make a claim.

1.7.2 Aspects of formulating with dietary fibre ingredients

When adding dietary fibre into beverages, there are many aspects to be taken into consideration. The choice of ingredient(s) will depend on the type of beverage to be fortified and the intended characteristics of the product.

Digestive tolerance

Digestive tolerance of dietary fibre ingredients and prebiotics is a complex and important issue. The tolerance is influenced by the chemical nature of the ingredient, the dose, format of food or drink fortified, amount of time taken to consume, consumption occasion (outside meal times, with meals, etc.), consumption frequency and individual factors (Marteau and Flourie, 2001). The point at which digestive discomfort becomes unacceptable is difficult to define as it is very subjective. This subjectivity makes an acceptable dose difficult to evaluate (Roberfroid and Slavin, 2000). Individuals will have differing levels of tolerance to different ingredients and different ideas of what is acceptable. It is important to note that tolerance to new ingredients may improve over time. However, if a beverage produces an undesired reaction, consumers will not repeat their purchase. Therefore it is essential that formulators should be aware of dose levels and forecast consumption patterns to ensure there are no adverse issues.

Physical properties

Physical properties of interest include solubility, dispersibility, particle size, viscosity, density, pH stability, process stability, colour, clarity and flavour. These can affect the manufacturing process and/or the textural properties, final mouthfeel and look of the beverage. For aqueous systems it is preferable to add soluble, easily dispersible ingredients that require no or little additional processing. It is possible to add less soluble, ingredients providing the particles are stabilised properly, preventing floury or grainy textures. Less soluble ingredients are more suitable for opaque beverages such as dairy beverages or fruit-based smoothies. Ingredients that have high

viscosity at low usage levels are somewhat limited in their use as a sole dietary fibre ingredient. Typically ingredients that elicit high viscosities tend to be used to stabilise and/or modify mouthfeel of beverages such as guar gum, pectins, etc. Usually they cannot be added in sufficient quantities alone to provide enough dietary fibre to make a claim. They can, however, be added to 'top up' fibre levels.

Stability
The specific stability requirements for the beverage manufacturer will depend on the process and shelf-life of the intended product. In some cases instability to acidic conditions can result in hydrolysis of the fibre ingredient. This can produce a variety of effects depending on the source of dietary fibre. Examples of hydrolysis in the finished product are: increased level of sweetness, loss of mouthfeel, loss of stabilisation and texture and an increase in coloration. The rise of degradation products inevitably means a reduction in the amount of dietary fibre polymer present in the beverage, which has regulatory implications if a claim is being made. As nearly all dietary fibre ingredients are by nature carbohydrates, some hydrolysis can be expected over a long shelf-life. This loss can be reduced by modifying the manufacturing process, formulation, storage conditions and intended shelf-life.

Nutritional content/composition
Beverages are normally supplemented with dietary fibre in order to establish a nutritional claim. In this context the composition of the fibre ingredient is important. Levels of fibre in the ingredient and residual sugars are important parameters to note. Ingredients containing high levels of fibre will need less to make a claim and potentially have less textural and cost impact on the final beverage. Ingredients containing high levels of residual sugars can prevent a sugar-free claim being made.

Selection of dietary fibre ingredients
There are many different ingredients on the market today with dietary fibre-like properties. The following sections provide a short introduction to some dietary fibre ingredients suitable for fortification of beverages.

1.7.3 Polydextrose

Description
Polydextrose is a low molecular weight randomly bonded polysaccharide of glucose. All possible glycosidic linkages are present, with the 1,6 predominating. It has an average DP of 12 and an average molecular weight of 2000. Polydextrose was originally developed as a bulking agent but it has also gained popularity as a source of dietary fibre in many countries worldwide. This is due to its indigestibility in the small intestine and incomplete

fermentation in the large intestine. Studies have shown that polydextrose increases faecal bulking and softening, decreases colonic pH and positively impacts the colonic microflora (Jie *et al.*, 2000). It has a calorie content of 1 kcal/g (4 kJ/g) and is considered to contain 90% dietary fibre.

Tolerance

Nine clinical studies were carried out with polydextrose to evaluate gastrointestinal toleration. These studies demonstrated that polydextrose is well tolerated because it has a high molecular weight, low osmotic potential and slow fermentation rate. It has been demonstrated that its mean laxative threshold is 90 g/day, and a single dose of 50 g is tolerated (Flood *et al.*, 2004). Studies have demonstrated enhanced bifidobacteria and lactobacillus counts with the consumption of polydextrose at doses as low as 4 or 5 g/day (Jie *et al.*, 2000; Tiihonen *et al.*, 2008). Studies have shown that polydextrose is fermented along the entire length of the colon, resulting in beneficial effects for the entire colon. Colonic fermentation of polydextrose increases the production of short-chain fatty acids (SCFAs), namely acetic acid, propionic acid and butyric acid. Wang and Gibson (1993) reported high generation of propionic and butyric acid compared with other carbohydrates. The molar ratio of acetate, propionate and butyrate was 61:25:14. A high rate of butyrate is desirable as it is thought to be particularly beneficial to colonocyte health.

Polydextrose, originally developed by Pfizer and now marketed by Danisco, has been refined to meet a wide range of application needs under the brand of Litesse®. Litesse® is an improved form of polydextrose. The Litesse® family contains three variants, varying from bland tasting and slightly yellow in colour to being slightly sweet and colourless in solution. Litesse® is available in powder, granulated and in a 70% liquid form. Litesse® polydextrose has many physical and chemical attributes that are favourable for use in beverages. Tate & Lyle also manufactures polydextrose.

Solubility

Polydextrose is very soluble, allowing clear solutions of greater than 80% w/w to be created at 25 °C in water without haze. Polydextrose has good dispersibility properties in all beverage formats. As with many ingredients good mechanical mixing is required to prepare concentrated solutions. Granulated polydextrose offers high dispersibility with minimum dusting.

Stability

Studies indicate that polydextrose is stable throughout typical beverage process and storage conditions at pH 3 and 7 (Beer *et al.*, 1991). Polydextrose is a complex branched molecule and contains a wide range of glycosidic bonds which consequently are resistant to hydrolysis. Studies of model beverage systems containing polydextrose have shown resistance

to hydrolysis over a broad range of pH and temperatures (Beer *et al.*, 1991).

Viscosity
Litesse® is viscous at high levels (70% solution), and it has a higher viscosity than sucrose solutions or high-fructose corn syrup (HFCS) at similar concentrations. However, at practical use levels it has a low viscosity and can help retain mouthfeel in sugar-free or low sugar beverages.

Organoleptic properties
Polydextrose is essentially non-sweet but can be used to provide the bulk and mouthfeel often lost with the removal of sugars. Furthermore, polydextrose can improve the flavour of beverages containing high-potency sweeteners by reducing the bitter, acidic notes and improving the sugar flavour. At addition levels of below 3 g/100 ml it is difficult to detect so it can be used in most beverage applications. Differing grades are suitable for different beverage applications. Litesse® *Two* is suitable for drinks that have a high colour, such as juices, cordials and smoothies, as it imparts a slight yellow colour in solution. Litesse® *Ultra* is especially suitable for clear, low-flavoured products such as flavoured and near-water drinks owing to its minimal flavour and textural impact. Litesse® can be used in a variety of beverages including carbonated and non-carbonated, concentrated and ready-to-drink, hot and cold beverages. Examples include, fruit and/or vegetable juice drinks, smoothies, meal replacements, milk, dairy and soy-based drinks, sport and energy drinks, tea and coffee, creamers and water.

1.7.4 Inulin and fructo-oligosaccharides

Inulin and fructo-oligosaccharides (FOS) are related products based on fructose. Inulin is a linear molecule consisting of 3–60 fructose units linked by β (2-1) bonds (fructosidic). FOS has between three and seven units of fructose and is either produced from controlled hydrolysis of inulin or synthesised from sucrose.

There are many different commercial variants of inulin and FOS from a range of suppliers including Orafti, Sensus, Cosucra and Cargill. These differ in respect to sugar content, form and average DP. Standard inulin has a DP of <10, while high-performance inulin has an average DP of 23. FOS on the other hand has an average DP of 7. Inulin has a caloric value of 1.5 kcal/g (Roberfroid, 2000) and is considered to be 100% dietary fibre in its pure form. Inulin is available in powder and granular format. FOS is available in powder and liquid formats.

Tolerance
The digestive tolerance of inulin and FOS are well documented. Briet *et al.* (1995) demonstrated that doses of FOS under 20 g/day were well tolerated

and resulted in only minor digestive complaints (flatulence). Other studies have also found FOS to be well tolerated at 15 and 17 g/day (Ellegaard *et al*., 1997; van Dokkum *et al*., 1999). However, it was shown that inulin and FOS are completely fermented in the colon. Furthermore, it has been demonstrated that inulin and FOS are fast fermenting dietary fibre and ferment quickly after entering the colon. Inulin and FOS have both been shown to stimulate bifidobacteria and lactobacillus and so conform to the definition of prebiotic. Stimulation of bifidobacteria was shown at doses of between 5 and 15 grams a day. The molar ratio of acetate, propionate and butyrate for inulin was 72:19:8 and for oligofructose was 78:14:8 (Wang and Gibson, 1993; van Dokkum *et al*., 1999).

Solubility

A range of variants with different DP profiles are suitable for different applications. Variants with higher DP values tend to be more insoluble in water than those with lower DPs and tend to be more suitable for beverage applications requiring weak gel structures such as milk-based drinks and yogurt drinks. Shorter chain inulin is more suited to aqueous beverages such as near water drinks. The dispersibility of inulin is good under agitation. FOS is very soluble up to 80% w/w. It can be used in a wide range of beverages.

Stability

Inulin is heat and process stable; however, it is susceptible to acid hydrolysis and may break down at pH lower than 3.7. The degree of hydrolysis will depend on temperature and duration of exposure to the acidic environment. FOS is heat and process stable. Like inulin it is susceptible to hydrolysis in acidic conditions under pH 3.7. Use below pH 3.7 could still be possible in short shelf-life products such as refrigerated juices.

Organoleptic properties

Inulin has very little sweetness and also has a bland taste. Therefore it can be added to beverages at practical use levels with limited change on attributes. It would be necessary to combine inulin with a bulk or high-potency sweetener to add sufficient sweetness to a beverage system. Longer chain inulin can improve body and texture of low-fat dairy drinks and other products. FOS sweetness largely depends on the composition of the commercial product. Sweetness can vary between 0.3 and 0.65 (determined by grade) depending on residual sugars present. The sweetness profile of FOS is similar to sucrose and it has a synergistic effect when combined with high-potency sweeteners. It can round out flavours from high-potency sweeteners and may also enhance fruit flavours.

Inulin and FOS allow for fibre fortification in beverages such as carbonated and non-carbonated drinks, dairy and dairy replacement drinks, dry mixes, near-water drinks, short shelf-life juice drinks and juices, sport and energy drinks, tea and coffee, and water.

1.7.5 Resistant maltodextrins

Resistant maltodextrins are a result of chemical modification of resistant starch to gain advantageous physical and physiological properties. Fibersol® 2 and Nutriose® are examples of commercialised resistant maltodextrins by Matsutani and Roquette respectively. Resistant maltodextrins are also commercialised by Tate and Lyle under the Promitor™ brand. The average molecular weight of Fibersol® is 2000 and is composed of 1–2, 1–3, 1–4 and 1–6 glucosidic bonds. It is highly branched in nature which means that it is only partially hydrolysed by human digestive enzymes. Fibersol is considered to contain 90% dietary fibre. Nutriose® is made by chemical dextrinisation of wheat or corn starch followed by refining, purification and drying. It is an agglomerated soluble dextrin with a high fibre content of 85%. It contains 1–2, 1–4 and 1–6 glucosidic linkages which are all indigestible to human digestive enzymes.

Tolerance

Animal studies and a limited number of human studies have shown Nutriose® to be well tolerated up to 45 g/day. Above 50 g/day some digestive discomfort was reported (Van den Heuval *et al.*, 2004). Its high molecular weight and slow fermentation rates result in good tolerance levels. It is estimated that 90% of Fibersol® 2 reaches the colon and approximately 50% of that is fermented. The remaining is 50% is excreted. According to a review by Ohkuma and Wakabayashi (2001), Fibersol® 2 increases bifidobacteria at doses as low as 3.75 g/day. Nutriose® is partially absorbed in the small intestine (15%), 75% fermented in the colon and the remaining 10% excreted. Studies indicate that there was a significant increase in lactobacilli (human models) following consumption of a high dose of 45 g/day of Nutriose®.

Although Nutriose® and Fibersol® 2 belong to the same classification as resistant maltodextrins, studies suggest that they have differing properties as a consequence of their different molecular structures.

Solubility

Fibersol® is very soluble in solution up to 70% w/w at 2 °C. It is readily dispersible in water and so is compatible for many types of beverages including dry mixes, co-dried and dry-blended mixes. There are grades available which are clear in solution.

Viscosity

The viscosity of Fibersol® is lower than conventional maltodextrins despite the DE value being similar to 10 DE maltodextrin. Nutriose® has a relatively low viscosity at levels required to obtain a fibre claim.

Stability

Fibersol® 2 is resistant to high-temperature processing so it can be sterilised, retorted and pasteurised without any degradation of fibre. It is very

stable to process and acidic conditions and does not show signs of hydrolysis (retrogradation) or haze over long storage times. Fibersol® is also non-hygroscopic and can be used to help protect other more hygroscopic ingredients in a dry blend. Nutriose® is thermally stable to acidic conditions. It is also non-hygroscopic.

Organoleptic properties
Fibersol 2 has a sweetness of less than 10% that of sucrose. Fibersol® 2 modifies and improves the sweetness profile and aftertaste profile of many HIS, allowing flavour and mouthfeel improvements to a variety of low-calorie foods. Likewise Roquette report that Nutriose® also shows mouthfeel improvement. Nutriose® FB is low in sugars so can be used to formulate tooth-friendly or sugar-free products.

1.7.6 Galacto-oligosaccharides

Galacto-oligosaccharides (GOS) are non-digestible carbohydrate polymers made up of galactose with glucose end units. They are produced from lactose by means of enzymatic conversion using β-galactosidase. Commercially available GOS also contain mixtures of lactose, glucose and galactose. The oligosaccharide fraction varies in chain length and type of linkage between the monomer units. Other species include galactans (gal-beta(1,3)-gal; gal-beta(1,6)-gal; gal-alpha(1,6)-gal). The energy value for GOS is 1–2 kcal/g (Salminen *et al*., 1998). GOS is soluble up to 70% w/w and is one-third as sweet as sucrose. It also has resistance to acidity so is suitable for adding to all beverage systems. GOS has demonstrated prebiotic effects *in vivo* and *in vitro*. GOS have been shown to be bifidogenic at 10 g/day (Ito *et al*., 1990; Bouhnik *et al*., 1997). There was also an increase in lactobacilli, while numbers of Bacteroidaceae and *Candida* spp. were reduced (Bouhnik *et al*., 1997).

Stability
Studies have demonstrated GOS to be stable under high-temperature processing and acidic storage conditions. There is no hydrolysis when held for 10 minutes at 160 °C at neutral pH, 120 °C at pH 3 and 100 °C at pH 2 (Sako *et al*., 1999). Other studies have showed stability of GOS: 30 min at 120 °C, 3 h at 100 °C and 3 h at 80 °C at pH ranges between 3 and 7.

Tolerance
Human studies suggest that GOS are well tolerated. Daily consumption of 15–20 g/day is well tolerated with no side effects (van Dokkum *et al*., 1995). GOS are suitable for inclusion in many beverages such as carbonated and non-carbonated, hot and cold drinks, fruit juices, smoothies, near-water, meal replacement, milk and dairy replacement drinks.

1.7.7 Gum arabic (acacia gum)

Acacia gum is described in Section 1.4.8. It has been commercialised by Alfred Wolff, Colloides Naturels International (CNI), Kerry International and others. The fibre content of acacia gum is between 80% and 90% w/w. Acacia gum is a complex polysaccharide that is not digested in the small intestine but is fermented in the large intestine by colonic microflora (Phillips, 1998). It therefore conforms to the definitions of dietary fibre. Acacia gum is highly fermentable but ferments slowly along the length of the colon. This reduces the incidence of gastrointestinal distress as gas production occurs more slowly (Cherbut *et al*., 2003).

Tolerance

The tolerance of acacia gum was assessed by determining the frequency and severity of gastrointestinal symptoms such as flatulence, bloating, borborymi, abdominal cramps and diarrhoea and by estimating the effect on faecal excretion. Cherbut *et al*. (2003) reported that the first feeling of flatulence was perceived by participants at doses higher than 50 g/day. This is due to the high-branched structure, resulting in slow fermentation rates and good toleration. Acacia gum is completely fermented in the colon.

Solubility

Acacia gum is soluble in water up to 43–48% v/v. It is not soluble in ethanol; solutions made with acacia gum do not require any premixing. They are not completely clear, which means that acacia gum is suitable for beverages where clear, water-like colour is not essential.

Viscosity

The highly branched nature of acacia gum means that it has a low viscosity at high concentrations. At 40% w/w, the average viscosity is 1000 mPa·s and shows Newtonian behaviour.

Stability

Acacia gum is stable in acidic environments and has widely been used as an emulsifier in beverages. Stability in acidic beverages has been demonstrated at pH 3.0, 37 °C for 3 months. No hydrolysis of acacia gum was seen.

Organoleptic properties

Gum acacia is bland at fibre usage levels. It improves mouthfeel and oil stability while not masking the flavour release. It is suitable for fibre fortification in a variety of beverage products including near-water, fruit juices and concentrates.

1.7.8 Partially hydrolysed guar gum (PHGG)

A description of guar gum is given in Section 1.4.7. Traditionally guar is used as a viscosity-building and water-binding agent. The high viscosity of

guar at low levels limits its use as an ingredient in beverages as well as a dietary fibre. Typical use levels for beverages are between 0.05% and 0.1%. Partially hydrolysing guar gum increases the potential uses of the ingredient as a nutritional supplement and as a dietary fibre. The nutritional, metabolic and analytical properties of PHGG correspond to native guar gum. Guar gum is considered to be a 100% fermentable dietary fibre. Partially hydrolysed guar gum has a fibre content of 85% w/w. PHGG has been commercialised by Taiyo International under the brand name of Sunfiber®. PHGG is indigestible in the small intestine but is fermented in the colon by colonic microflora (Tomlin *et al*., 1986, 1989). Studies demonstrating the efficacy of PHGG as a prebiotic have shown that consumption of 7 g PHGG three times a day resulted in a significant increase in bifidobacterium and lactobacillus counts (Okubo *et al*., 1994).

Tolerance
Dietary levels of up to 20–40 g/day were well tolerated without adverse effects (Meier *et al*., 1993; Takahashi *et al*., 1993). PHGG is non-digestible in the small intestine by mammalian enzymes. It is, however, readily and completely fermented in colon by intestinal microflora (Balascio *et al*., 1981).

Solubility
The main advantage of PHGG over native guar gum is improved solubility which makes incorporation into beverages at a higher level than 1% possible. PHGG is cold soluble and makes tasteless solutions up to and above 10% w/w. PHGG is stable at low pH and is cold water soluble. The viscosity of 5% PHGG is 10.5 mPa s and it is clear in solution.

Organoleptic properties
PHGG is bland tasting at low levels 5% w/w. PHGG is suitable for fortifying a range of beverages including fruit juices, near waters and concentrates.

1.8 Future trends

Recent trends suggest a move towards formulations with ‘natural’ ingredients (no EU legal definition to date) and clean labelling. Likewise there has been an increased interest in health and wellness in general. This includes the increasing awareness of the benefits of dietary fibre consumption, which has resulted in many beverages being produced with fibre claims. There is a clear trend towards beverage development with prebiotic claims and future developments towards ‘synbiotic’ claims.

1.9 Sources of further information and advice

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Handbook of Hydrocolloids (2000), Edited by G. O. Phillips and P. A. Williams, Woodhead Publishing Limited, Cambridge.

Thickening and Gelling Agents for Food (1999), 2nd edition, edited by Alan Imeson, Aspen Publishers, Inc., Gaithersburg, MD, 1999.

Anonymous, available from Danisco upon request:

Pectin and Health – TM 33-2e

Health benefits of pectin, guar and alginate as dietary fibres and beyond – TP 24-1e

Functional ingredients solutions for soy-based beverages – TM 2079 – 2e

Taste and texture – HO 2029-1e

Stabilisers in ready-to-drink beverages – a users guide – TM 4519-1e

Stabilisers systems for still drinks – TM 4507-2e

Danisco stabilisers for high viscous juice drinks – HO 4505-1e

Danisco stabilisers for low viscous juice drinks and – HO 4506-1e

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2

Developments in sweeteners for functional and speciality beverages

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Abstract: Consumers express real concerns about the calorie content of sugar-sweetened beverages, whether they are traditional beverages or newer products containing added nutritional ingredients. Removal of sugar and its replacement by a high-potency sweetener frequently introduces a number of difficult sensory challenges, including reductions in mouthfeel, alterations in the temporal delivery of sweetness and distortions to the volatility of flavour compounds. Additionally, producers of functional and speciality beverages may be required to offer versions that simultaneously contain little or no unnecessary energy, while also being free from artificial additives. This particular challenge is leading to the development of natural high-potency sweeteners and compounds that function as sweet taste enhancers. The sensory and technical challenges associated with sweetening speciality beverages and other new ingredient developments that assist this process are discussed in this chapter.

Key words: technical challenges in preparing sugar-free beverages, natural high potency sweeteners, sweetness potentiators, improving the taste of beverages containing novel sweeteners.

2.1 Introduction

The food industry has been formulating products that are marketed on some form of 'better for you' platform for many years. However, in what might be seen as something of a paradox, many of these 'better for you' products are formulated to deliver *less* nutrition than their standard equivalents. For example, fat may successfully be removed from many dairy, spread and sauce products, leading to a variety of 'reduced', 'low' and 'free' label declarations, and sugar is often removed from products to create 'diet' and

'sugar-free' versions of beverages, desserts, confectionery and table-top preparations. Today, however, the food industry is now frequently seeking to *add* nutrition to many foods and beverages through addition of ingredients such as soluble and insoluble dietary fibre, vitamins and minerals, essential fatty acids and pre- and probiotics. Consumer demand for many of these newer speciality products is increasing, but not at the expense of what can be considered those traditional nutritionally modified versions (sugar/fat reduced) as markets for these latter products also continue to rise.

Consumers in many developed markets regularly express real concerns about the calorie content of sugar-sweetened beverages, whether they are traditional beverages or those newer products that may contain added nutritionally beneficial ingredients. Essentially, carbohydrate-based sweeteners are viewed as delivering sweetness and energy only. In response to these concerns, beverage companies are able to prepare products with zero calorie high-potency sweeteners, so providing the sweetness of sugar with a non-caloric, alternative ingredient. Thus, these high-potency sweeteners provide no positive nutrition, conferring no intrinsic benefits to foods or beverages. The nutritional rationale for using them in product formulations is straightforward; their use almost always leads directly to reductions in both the caloric density and sugars' content of the products in which they are formulated, as well as conferring benefits to dental health, and collectively it is these features that are behind the increasing use of high-potency sweetener ingredients in today's markets. Thus, although high-potency sweeteners are not intrinsically 'healthy', their use in an appropriate way can be very beneficial to the health of consumers.

Removal of sugar as a sweetener and its replacement by a high-potency sweetener frequently introduces a number of difficult sensory challenges, including reductions in mouthfeel, alterations in the temporal delivery of sweetness and distortions to the volatility of some flavour compounds. Consumers also exhibit a high degree of individual variation in the perception of sweeteners, adding to the complexity of formulating products. In addition, technical challenges are introduced, including the potential for foaming of carbonated beverages, solubility issues with some sweeteners and problems related to sweetener stability. These sensory and technical challenges are discussed in this chapter.

Additionally, there is an interesting dilemma often faced by producers of functional and speciality beverages who may be challenged by their consumers to offer versions that simultaneously contain little or no unnecessary energy, while also being free from artificial additives. This particular challenge is leading to some interesting developments in the sweetener field. These include the development of a number of natural high-potency sweeteners and discovery and development of compounds that function as sweet taste enhancers.

2.2 Sensory challenges of preparing sugar-free beverages

2.2.1 Introduction

The removal of sucrose, high-fructose syrup and/or glucose syrup from beverages and their replacement by high-potency sweeteners introduce a number of technical challenges. These include a need to restore the sensory mouthfeel originally provided by carbohydrate sweeteners since reductions in soluble solids also reduce the perceived mouthfeel of beverages, effects that many dedicated consumers of full calorie products find unacceptable. Another important consequence of using high-potency sweeteners is that their temporal character will almost certainly not be a perfect match for the temporal sweetness profile provided by sucrose, leading potentially to an imbalance between sweetness and acidity or sourness. High-potency sweeteners may deliver non-sweet side/aftertastes, such as bitterness and astringency, and these must also be overcome for acceptable products to be prepared. Finally, reducing the soluble solids content of beverages increases the volatility of some non-polar compounds and reduces the volatility of the polar chemicals within a flavour formulation, thus potentially effecting quite significant changes in flavour delivery.

2.2.2 Restoring sensory mouthfeel

An inevitable consequence of removing soluble solids in the form of sucrose, fructose syrups and/or glucose syrups from beverages is a reduction in the perception of 'mouthfeel'. Consumers frequently describe diet or sugar-free beverages as 'thin' or 'watery' and for many these sensations are perceived as a negative.

Replacing lost body/mouthfeel is not as straightforward as might be imagined, however, as the seemingly simple solution of adding back solids in the form of ingredients such as maltodextrins clearly is counter-productive in that adding maltodextrin also adds back calories. Use of a low-calorie bulk sugar substitute such as polydextrose, although promoted for this functionality by the commercial supplier due to its Newtonian viscosity characteristics (Auerbach *et al*., 2006), adds substantial cost. Polysaccharides such as pectin and xanthan gum have also been proposed for use in low-calorie beverages in the expectation that the perception of mouthfeel is directly related to viscosity. However, this formulation approach has not been adopted widely, probably because mouthfeel may be as much a consequence of sweet taste quality as it is dependent on solution viscosity and because viscosity derived from polysaccharides can have quite profound flavour modification effects (Cook, 2006).

2.2.3 Modifying temporal profiles

Many natural and synthetic high-potency sweeteners deliver sweet tastes that are slower in onset and longer in duration than the sweet taste from

sucrose, and this characteristic introduces undesirable taste imbalances in products so sweetened (Carr *et al*., 1993). This situation applies to varying degrees to sweeteners such as aspartame, sucralose, neotame and most of the naturally occurring high-potency sweeteners, including thaumatin, stevioside and glycyrrhizin. In contrast, sweeteners such as saccharin and acesulfame-K are generally considered to display a very rapid onset and rapid decay of sweetness. Thus, blending of high-potency sweeteners in products has become more or less standard practice as it is well known that so doing produces sweetness with a temporal profile closer to that of sucrose (Bakal, 1991; Walters, 1993). In particular, blends of aspartame or sucralose with acesulfame-K have become widely selected blends for low-calorie foods and beverages. The probable reason that sweetener blends taste better than individual sweeteners is that the concentrations in a blend are each lower than if one sweetener had been used on its own. As the off-tastes develop faster than sweetness as concentration is increased, so a blending approach minimises off-tastes and tailors temporal profiles to specific needs (Walters, 2006).

Temporal sweetness profiles may also be modified beneficially through the use of additives. Burge and Nechutny (1978) reported that sugar acids such as glucuronic acid can improve the sweetness profiles of the protein sweeteners thaumatin and monellin. A number of other organic acids also apparently shorten lingering sweetness. For example, tannic acid was identified by Shamil (1998) as an additive that is able to shorten the lingering sweetness character of sucralose, and hydrophobic organic acids (e.g. cinnamic acid) and hydroxy amino acids (e.g. serine and tyrosine) appear to be able to modify the temporal profile of neotame successfully without delivering any taste of their own at the particular concentrations used (Prakash *et al*., 2001). In this particular study, the authors present data that suggest these acids induce their effects by interacting competitively at the receptor rather than by interacting directly with the sweetener in solution. Malic acid is promoted as a partial alternative to citric acid for use in some beverages on the basis that its temporal acidity profile more closely matches (and hence balances) the time–intensity sweetness profiles of high-potency sweeteners such as sucralose.

2.2.4 Reducing bitterness and other aftertastes

Carbohydrate sweeteners generally deliver pure sweetness with no associated bitter, liquorice or metallic aftertastes. In contrast, high-potency sweeteners almost always elicit some non-sweet tastes in addition to sweetness and so much effort has been expended seeking to reduce or eliminate these non-sweet tastes.

The simplest and most widely practised approach to improving the taste of sweeteners is through the use of blends of high-potency sweeteners rather than single ingredients (e.g. Hanger *et al*., 1996). Blends will, in

addition to offering cost savings through the phenomenon of sweetness synergy, almost inevitably lead to improvements in taste quality through reductions in bitterness and other off-tastes, as has been conclusively demonstrated (for a comprehensive review of the benefits of blending see Walters, 2006).

In addition to blending as a route to improving sweetness profiles, various additives have been claimed to be useful in modulating the bitterness of high-potency sweeteners. During the 1970s, patents were granted to cover the use of additives such as calcium chloride, arabinogalactan, neodeosmin, D-tryptophan and cream of tartar for their individual impact on the bitter/metallic aftertaste of saccharin (Lindley, 1999) and a range of specific flavour ingredients are reported to function as sweetness enhancers and/or taste quality improvers (e.g. Smith *et al*., 1996; Barnett and Yarger, 1989). However, it is important to acknowledge that there are few, if any, independent data that demonstrate and measure the quantitative reductions in bitterness or metallic taste induced by high-potency sweeteners.

2.2.5 Balancing sweetness and flavour release

Replacing sugars with high-potency sweeteners naturally changes the concentration of soluble solids in a beverage. This change in soluble solids concentration can have an impact on the volatility of individual chemical compounds that provide product flavour, as has been demonstrated by Nahon *et al*. (1998). This study was designed to study the release of volatile compounds from model beverages sweetened with sucrose or cyclamate and containing an orange flavour. Mixtures of sucrose and cyclamate were prepared to be iso-sweet and the interactive effects between both sweeteners and a water-soluble orange aroma were studied instrumentally. In this study, statistically significant increases in the volatility of the most volatile flavour compounds were found as sucrose concentrations were increased. These results suggest that replacing sucrose with a high-potency sweetener may alter flavour impact and balance in beverages, thus implying that there may be a need to work closely with flavour suppliers to ensure that these characteristics can be re-adjusted successfully to match those of the sucrose sweetened equivalent.

2.2.6 Consumer variability

There are genetic variations in taste perception. Individuals vary in their perception of and preference for sweetness and consumers also vary in their sensitivity to bitterness that frequently occurs as an aftertaste with high-potency sweeteners (Bartoshuk, 2000). Populations also display individual variation in their perception of sweeteners. For example, the bitter taste of saccharin is stronger to some individuals than others (Bartoshuk, 1979) and there is anecdotal evidence that aspartame tastes like sucrose to some

individuals, whereas to others it has a clear off-note (Beauchamp, 1999). Other factors such as age, environment and culture also play a part in taste preferences (Beauchamp, 1999) which means that consumers with different demographics and/or locations are likely to display different preferences for sweetness. Although there is little that product developers can do to satisfy the specific taste preferences of the complete target consumer population, it remains an important factor to take into account during the formulation process, perhaps even leading to regional variations in formulations, as happens for many established and successful food products.

2.3 Technical challenges in the preparation of sugar-free beverages

Removal of sugar from beverage formulations can, depending on the identity of the alternative sweetener(s) selected, introduce difficult technical challenges that need to be addressed for successful product development. If the beverage is to be carbonated and contains aspartame as sweetener there is potential for excessive foaming to occur. This is particularly apparent in caramel-containing beverages and necessitates a slowing of bottling operations so as to prevent or reduce what is known as 'fobbing', the overflow that occurs as carbonated water is added to the beverage concentrates. The effects of fobbing can be reduced through maintenance of low beverage syrup temperatures and also through use of low concentrations of anti-foaming agents.

Some sweeteners are quite difficult to dissolve in solution and also may have quite low absolute solubilities. Low absolute solubility, as with sweeteners such as aspartame and neohesperidin dihydrochalcone, means that it is difficult to maintain useful stock solutions. There may also be implications for product formulations, particularly for powdered beverage mixes, which use powdered sweetener. Making-up such beverages in the home may result in particles of undissolved sweetener floating in the drink and may thus lead to products of varying sweetness intensity. Careful attention to the selection of sweetener having appropriate granulation can help to minimise this particular problem.

Beverages prepared using sucrose as a sweetener have the advantage that the breakdown products of sucrose hydrolysis (glucose and fructose) are also sweet. The resulting invert sugar produced delivers sweetness of virtually identical intensity (and quality) as the sucrose from which the breakdown products are derived. However, when using alternative, low-calorie sweeteners the situation is invariably different in that a loss of sweetener results in a proportionate loss of sweetness. Since beverages are required to have quite lengthy shelf-lives and may also be subjected to extreme and adverse storage conditions (particularly in hot climates), sweetener stability can become a very pertinent issue. Of the low-calorie

sweeteners in use today, aspartame is probably the most labile, but its successful use in beverages has been greatly assisted by raising beverage pH and by paying careful attention to product distribution channels.

2.4 Developments in natural high-potency sweeteners

2.4.1 Introduction

The challenges inherent in sweetening functional and other speciality beverages are generally similar to those challenges of sweetening more mainstream products. Naturally, taste quality is important, as is the cost of sweetness, but perhaps of equal importance is how consumers view each alternative sweetener and its appropriateness for use in particular functional or speciality beverages. Given that most consumers will normally be selecting functional or speciality beverages for some beneficial health or physical performance attributes, it really would not be appropriate to sweeten such products with a sweetener about which there were some real or imagined health concerns. These considerations have been behind a continuing search for natural high-potency sweeteners since there is no doubt that the 'natural' cachet is viewed as beneficial by many consumers, irrespective of the rationality of such opinions. Thus, attempts to identify and develop natural high-potency sweeteners have been on-going for many years and now seem to be leading to some potentially interesting progress.

There has been recent research and development activity on three main natural high-potency sweeteners; the steviol glycoside rebaudioside A, an extract of the Chinese *lo han guo* fruit containing a sweetener known as mogroside, and monatin from a shrub indigenous to South Africa. The progress of these development activities is reviewed.

2.4.2 Rebaudioside A

Rebaudioside A is a diterpene glycoside occurring in the leaves of *Stevia rebaudiana*, a shrub indigenous to Paraguay in South America. The plant produces a number of steviol glycosides (there are believed to be at least eight different glycosides), all of which are sweet, with stevioside and rebaudioside A having been commercial in some parts of the world for many years. For example, there are established and successful markets for stevioside in Japan and other countries in the region, as well as in Brazil.

Stevioside (Fig. 2.1) elicits a clear bitter/liquorice aftertaste that makes it difficult to use in many foods. The sensory properties of stevioside and rebaudioside A have been compared (Table 2.1) and the data show clearly the sensory benefits that will result from formulating beverages and other products with rebaudioside A as opposed to stevioside. This extensive research has identified rebaudioside A as eliciting a cleaner sweetness,

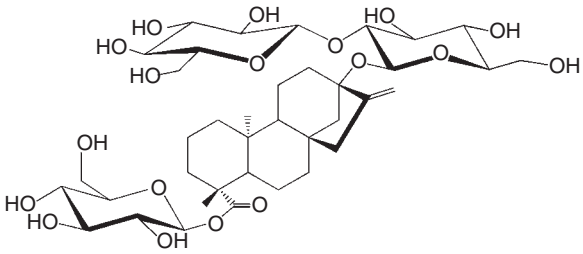

Fig. 2.1 Stevioside.

Table 2.1 Potencies and taste profiles of stevioside and rebaudioside A (DuBois *et al.*, 1991)

Compound	Sweetness potency (× sucrose)	Taste quality (sweet/bitter/other)*
Stevioside	190	62/30/8
Rebaudioside A	170	85/12/3

* Percentage of the total taste sensation.

probably due to the fact that it is a more polar molecule than stevioside, and this has resulted in plant-breeding attempts to select for rebaudioside A. Now, steviol glycoside preparations that are predominantly rebaudioside A are available with at least one commercial product claimed to consist of > 99% rebaudioside A. Purification from the *Stevia* leaves generally involves aqueous extraction, selective extraction into an organic solvent, ion exchange and crystallisation (Kinghorn and Soejarto, 1986).

This ability to produce almost pure rebaudioside A has stimulated much development activity into the optimisation of its sensory delivery, culminating in publication of a large body of patent applications that identify formulation routes to improving the taste of rebaudioside A (e.g. Prakash and DuBois, 2007). Rebaudioside A is characterised as delivering potent sweetness, being approximately 300 times as sweet as sucrose at practical use levels, with associated bitter and liquorice/anise aftertastes. In addition, the sweetener displays an unusual sensory characteristic in that the first taste is very much sweeter than subsequent tastes, leading to potential complications in formulating it into successful beverages. Nonetheless, with pure rebaudioside A now available, it is to be expected that formulation techniques will be identified that overcome many or all of these sensory limitations successfully.

Steviol glycosides including rebaudioside A are not yet approved for use within Europe or North America. There is safety and regulatory activity on-going, as evidenced by a 2004 review of steviol glycosides by Joint FAO/WHO Expert Committee on Food Additives (JECFA) that led to a

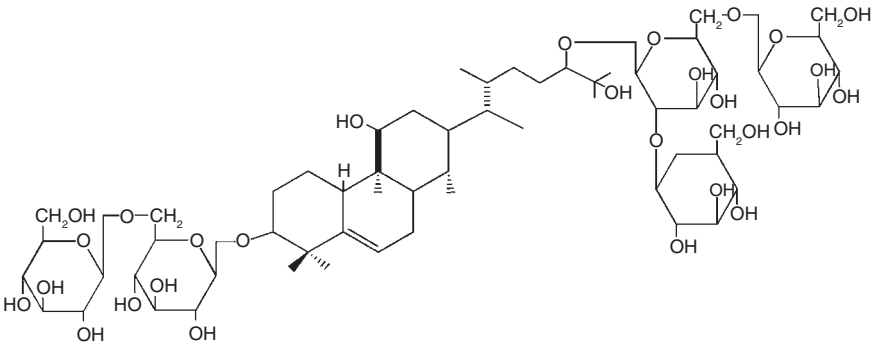

Fig. 2.2 Mogroside V (major sweet principle of *lo han guo*).

temporary acceptable daily intake (ADI) of 2 mg/kg body weight being granted (reported in Lindley, 2006). For this 'temporary' designation to be converted to 'permanent', JECFA required that additional information regarding the pharmacological effects of steviol glycosides be provided for their review. Extracts of *Stevia rebaudiana* containing stevioside are permitted as foods and food additives in Japan, South Korea, Brazil, Argentina and Paraguay. In the United States, refined extracts are available in health food outlets, labelled as 'dietary supplements' and there are table-top sweetener products marketed (Kinghorn *et al*., 2001).

2.4.3 *Lo han guo* (mogroside)

Siraitia grosvenorii is a Chinese plant of the cucumber or melon family, the fruit of which is used indigenously as a food, beverage and traditional medicine. The sweet principles of the plant are known as mogrosides (derived from the original name of the plant as *Momordica grosvenorii*), and are triterpene glycosides. *Lo han guo* is one of the common names of the plant.

The plant produces a number of mogrosides, of which the most common have been designated mogroside IV and mogroside V. Isolation of these two mogrosides has been described and their sensory properties established. Mogroside V (Fig. 2.2) is the most abundant component, occurring at about 1% in the dried fruit, and its sweetness potency is usually described as being approximately 200–250 times that of sucrose. The literature contains no detailed description of the flavour profile of *lo han guo* or mogroside V, but the sweetener is known to exhibit many of the taste characteristics common to natural high-potency sweeteners, including a delay to reaching maximum sweetness and an aftertaste that contains liquorice and cooling elements (Lindley, 2006). Current commercial products are quite crude extracts of the *lo han guo* fruit and so additional non-sweet aftertastes, such as 'grassy' and 'grain-like' notes, are frequently perceived.

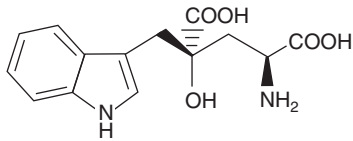

Fig. 2.3 Monatin.

The traditional use of the fruit is to prepare an aqueous extract in hot/boiling water and then to consume this extract as a tea or tonic, although there is not known to be much experience of its use in 'mainstream' food and beverage applications. However, because of its 'natural' designation, it would seem logical that *lo han guo* extracts might be considered as an appropriate sweetener for use in functional and other speciality beverages. *Lo han guo* fruit extracts are considered foods in China. It has self-affirmed generally recognised as safe (GRAS) status in the United States and a GRAS petition is under review there.

2.4.4 Monatin

Monatin is a derivative of the amino acid tryptophan. It is found in the roots of a South African shrub, *Schlerochiton ilicifolius*, but since the shrub is not amenable to cultivation, successful commercialisation of monatin will depend on either identification of a synthetic route suitable for scale-up and/or a biochemical route that may preserve this sweetener's natural status, at least in some markets. Recent studies indicate that monatin can be synthesised according to synthetic schemes that might be commercially viable (Amino and Kurihara, 2003) and that biochemical routes to its synthesis may also be a viable alternative (Hicks and McFarlan, 2005). The sweetener and its derivatives have also been used as model compounds in attempts to model the sweet taste chemoreception mechanism (Bassoli *et al*., 2005). Again, as is the case with the sweetener *lo han guo*, no detailed descriptions of the sweet taste profile of monatin have been published, although its relatively small molecular size (Fig. 2.3) indicates it is likely to lack the licorice/lingering characteristics displayed by most natural high-potency sweeteners.

2.4.5 Other natural sweeteners

Brazzein is a small molecular weight protein detected in the ripe fruit of *Pentadiplanadria brazzeana* (van der Wel *et al*., 1989). Owing to its small molecular size, it has been an interesting molecule for study with the effects of mutations on structure and function being examined (Assadi-Porter *et al*., 2005) and models of multi-site brazzein : receptor interactions developed. These models have been used in two studies to design smaller molecular weight peptides that contain the putatively critical structural features

necessary for receptor interaction, but neither has yielded sweet peptides, thus suggesting a more extensive structure is required for sweetness (Temussi, 2002; Assadi-Porter *et al*., 2005). Although an efficient bacterial production system has been developed for brazzein (Assadi-Porter *et al*., 2000), and it is also possible to express brazzein protein in corn seed embryo, thus opening the interesting possibility of producing pre-sweetened cereals with 'no added sugar' (Lamphear, 2005). The taste characteristics of the sweetener are substantially different from those of sucrose and so its commercial utility is projected to be limited.

Work to identify new natural potent sweeteners continues, as they are perceived to be able to pass more quickly through the regulatory process and enjoy a more consumer-friendly image. There are many inaccessible areas of the world, particularly rainforests, containing undiscovered plant species that may yield new compounds with potent sweet taste. Some companies have entered into commercial bio-divining agreements with local bodies that may yield new flavour compounds while providing a source of revenue for ecological preservation programmes. Potential new natural sweet-tasting compounds can be screened rapidly using genomic techniques (Kemp, 2006).

Genetic modification may provide a route to production of natural potent sweeteners. Easily-grown crop plants may be implanted with the genes necessary to express naturally occurring sweeteners (Fry, 2005). Attempts have been made to modify yeast cells to produce thaumatin (Weickmann *et al*., 1989) and more stable forms of monellin (Kim *et al*., 1991).

The ideal is to find a natural, stable, safe potent sweetener with the taste properties of sucrose, as this would be more acceptable to consumers and potentially easier to gain regulatory approval (Lindley, 1999; Fry, 2005).

2.5 Sweetness potentiators

2.5.1 Introduction

There is a sound rationale for identifying and developing compounds that potentiate sweetness. Sweet taste potentiators are expected to be valuable tools for the food industry because:

- an effective sweetness potentiator might reduce costs by enabling the same level of sweetness to be achieved using less sweetener (assuming the potentiator added was less expensive than the sweeteners eliminated);
- effective potentiation of the sweetness of sucrose by a compound with no intrinsic taste would permit the development of lower-calorie products, particularly beverages, having the taste quality normally associated with sucrose.

Effective potentiation of the sweetness of sucrose would allow development of reduced calorie products, particularly beverages, while not requiring the use of artificial sweeteners.

There is currently significant research effort seeking to identify sweetness potentiators. Belief in their existence is based, at least in part, on the observation that many potent sweeteners contain hydrophilic structural features that are responsible for their sweet taste and lipophilic structural features believed to govern their potency. Thus, identification of a suitable structure that interacts at the binding site 'for potency' and tasting it in combination with a sweetener may enhance the sweetness of that sweetener. While this hypothesis is yet to be proven, it provides enough justification to conduct an appropriate search for such compounds. As with the design of new sweeteners, however, sweet taste potentiator structures may be identified more readily following detailed determination of receptor structure and receptor binding sites. Until then, high-throughput screening and standard structure–activity relationship studies must be employed. That said, some sweet taste potentiator compounds have been described.

2.5.2 Alapyridaine

Alapyridaine is a Maillard reaction product formed when a mixture of glucose and L-alanine is heated. Chemically, alapyridaine is the inner salt of *N*-(1-carboxyethyl)-6-(hydroxymethyl)pyridinium-3-ol. Naturally, it is one of many compounds formed when glucose and alanine are heated, but its isolation and confirmation that it functions as a sweetness enhancer were achieved by applying the comparative taste dilution analysis technique (Ottinger *et al.*, 2003). Ottinger and co-workers demonstrate that alapyridaine reduces the sweetness threshold concentrations of quite diverse structures, including glucose, sucrose, L-alanine and aspartame. They also show that, in common with the structure–activity relationships of sweet compounds, the stereochemistry of alapyridaine is critical; (+)-(*S*)-alapyridaine is effective as a sweet taste enhancer, (−)-(*R*)-alapyridaine is not. Interestingly, there is a strong pH-dependency to the effect with alapyridaine proving to be substantially more effective at pH 7 and pH 9 than at pH 5 or below. This observation suggests it is the de-protonated pyridinium-3-olate that is the physiologically active form of alapyridaine and also has important commercial implications. Since the most likely commercial applications for a sweetness potentiator are soft drinks, the great majority of which are prepared at acidic pH (around pH 3 or pH 4), alapyridaine would therefore not be a functional sweetness potentiator in these applications.

Nonetheless, the discovery of alapyridaine has some important implications. Firstly, it appears to confirm that compounds capable of potentiating sweetness do exist. Secondly, although unlikely to be of commercial utility itself, alapyridaine could be an important lead compound in the develop-

ment of structure–activity relationships in the search for further novel sweetness potentiators.

2.5.3 Substituted benzoic acids

There is a wide range of benzoic acid derivatives that display taste-modifying characteristics. Some are known to enhance sweetness whereas others have been described that inhibit sweet taste perception.

2,4-Dihydroxybenzoic acid is a flavour ingredient generally recognised as safe by the Flavor Extract Manufacturers Association (FEMA) in the United States for use in a broad range of food and drink applications. Although slightly sweet in its own right, it also has the capacity to enhance the sweetness delivery of other sweeteners and, at appropriate use-levels, to improve the quality of taste delivered by high-potency sweeteners (Merkel and Lindley, 2006). The taste effects of this hydroxybenzoic acid are similar to those described (Barnett and Yarger, 1989) for other dihydroxybenzoic acids as well as benzoic acid derivatives substituted with hydroxy and amino groups, e.g. 3-hydroxy-4-aminobenzoic acid.

Barnett and Yarger (1986) also describe the sweetness-enhancing effects of 3-aminobenzoic acid and, interestingly, demonstrate a pH-dependency of the enhancing effect opposite to that of alapyridaine. 3-Aminobenzoic acid enhances sweetness when present in solution at acid pH, but not at neutral pH.

All of these modified benzoic acids deliver some sweetness when tasted alone, thus raising the possibility that they are functioning more as efficient sweetness synergists, rather than as true sweetness potentiators.

2.6 Improving the taste of beverages containing novel sweeteners

One of the main difficulties of formulating good-tasting functional beverages is that the ingredients added that delivery functionality will frequently contribute tastes of their own, thus exacerbating any sensory challenges that may be consequent on the use of high-potency sweeteners. There may, for example, be a requirement to mask tastes associated with certain vitamins and minerals, particularly the B vitamins, dietary fibres and even essential fatty acids. These challenges need to be addressed as they may arise because there are no hard and fast ‘rules’ that can be followed in order for them to be overcome. Generally, the first approach will be to work with flavour suppliers and to use their particular expertise to help address any problems. With respect to any off-tastes that may be a consequence of the sweeteners being used, formulation guidance has already been provided within this chapter.

2.7 Sources of further information and advice

The proceedings of an American Chemical Society symposium on sweetener chemistry have been published (Walters *et al*., 1991) and that volume provides an excellent grounding on these topics. Many excellent and relevant chapters can be found in the books *Optimising Sweet Taste in Foods* (Spillane, 2006) and *Modifying Flavour in Food* (Taylor and Hort, 2007), both published by Woodhead Publishing. The book series *Alternative Sweeteners* provides detailed information on individual sweeteners (O'Brien Nabors and Gelardi, 1986, 1991; O'Brien Nabors, 2001), as does the publication *Sweeteners and Sugar Alternatives in Food Technology* (Mitchell, 2006).

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3

Probiotics as ingredients in functional beverages

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Abstract: Good viability is a prerequisite for optimal probiotic functionality and therefore probiotic products should contain high enough levels of the specific probiotic strain(s) throughout the storage and during consumption. All food applications have some kind of limitations regarding the viability and stability of probiotics. These limitations typically involve factors such as the acidity of the food matrix and storage time and temperature of the product. This chapter focuses on probiotic beverages made of milk, fruits or vegetables. The nature of the probiotic beverage (raw materials, fermented or non-fermented, etc.) sets its own demands for the probiotic strains. Therefore special attention has to be paid to the selection of the suitable probiotic strain for a specific food application. Selecting a strain with good technological properties will be a key factor when novel food applications for probiotics are developed.

Key words: probiotic, beverage, *Lactobacillus*, *Bifidobacterium*, stability.

3.1 Introduction: the range of probiotic beverages and trends in the fortification of beverages with probiotics

Probiotics are currently defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Anon., 2002). Probiotics are available for the consumers in foods, food supplements and pharmaceutical products. Lactobacilli and bifidobacteria have been used as probiotics in food applications in Europe whereas some other microbes (e.g. enterococci) are available as supplements or pharmaceutical products. Traditionally probiotics have been incorporated into dairy products, mainly fermented ones such as yoghurt (also drink), cheese, quark, cultured buttermilk and dairy drink. Other probiotic food products include

milk, ice cream, fruit and berry juices and drinks, recovery drinks, cereal-based drinks and snacks. Europe has traditionally had a strong position on the probiotic food market which is expressed today as a wide range of probiotic food products available for the consumers (Halliwell, 2002; Hilliam, 2004). Daily-dose dairy drinks have been the largest growing probiotic product type on European market (Mellentin, 2006).

However, also other probiotic beverages (e.g. fruit and berry juices) are increasing in popularity. In the studies of Fasoli *et al*. (2003), Temmerman *et al*. (2003) and Gueimonde *et al*. (2004) *Bifidobacterium animalis* subsp. *lactis* was the only *Bifidobacterium* species detected in probiotic foods regardless what was claimed on the food product label. Also in the study of Masco *et al*. (2005) this subspecies was by far the most commonly detected *Bifidobacterium*. The popularity of *B. animalis* subsp. *lactis* is due to its superior technological properties (e.g. tolerance to oxygen and low pH) compared with other *Bifidobacterium* species (Truelstrup Hansen *et al*., 2002; Matsumoto *et al*., 2004; Mättö *et al*., 2004). In Europe *Lactobacillus rhamnosus* GG (LGG® is the trademark owned by Valio Ltd, Finland) is perhaps the most diversely used probiotic in various foods (both dairy and non-dairy). According to Valio's web-page (www.valio.fi) LGG-containing foods are available in at least 15 European countries under various brand names. Some examples of probiotic beverages on European market are listed in Table 3.1.

Table 3.1 Examples of probiotic beverages on European market (information obtained mainly from the World Wide Web)

Product types and trade names	Probiotic microorganisms in the products as stated by the manufacturer (starter microbes are not listed)
Milk and drinkable fermented milks *(including cultured buttermilk, yoghurt drink, dairy drink)*	*Lactobacillus acidophilus* / 'acidophilusbacteria', *L. acidophilus* LA5, *L. casei* (incl. F19, 431, Defensis, Shirota), 'casei', *Lactobacillus* LGG, *L. johnsonii* La1, *L. rhamnosus* (incl. LB21), *L. reuterie*, *Bifidobacterium* / bifidobacteria, *B. lactis, Bifidobacterium* BB12
A-fil, Actimel, Aktifit, AB-piimä, Bella Vita, BioAktiv, Biola, Casilus, Cultura, Emmifit, Fit & Aktiv, Gefilac, Gefilus, Kaiku Actif, Kidius, Lc1go, LGG+, Onaka, Öresundsfil, Philura, Rela, Verum, Vifit, ViktVäktarna, Vitality, Vivi Vivo, Vive+, Yakult, YOMO plus, Yoplait	
Fruit juices and drinks	*Lactobacillus* LGG, *L. reuteri, L. plantarum* 299v, *Lactobacillus casei* F19
Gefilus, Rela, Proviva, Cultura	

* Invalid names are indicated by quotes. Starter microbes are not listed.

3.2 Probiotics and their health-effects

There is a growing demand for developing foods with specific functionalities for increasing the health and well-being of the consumers. Among the health issues that over half of US consumers are concerned about preventing are heart disease, vision problems, lack of energy, overweight, joint disease, high cholesterol and blood pressure, memory concentration problems, diabetes, osteoporosis, frequent cold and flu, blood sugar imbalance, acid reflux and intestinal regularity (Sloan, 2007). Owing to the rapidly ageing populations in the United States and Europe the functional food sector will in the future be even more condition-oriented than today. In the adult population combating lifestyle-related diseases and in the elderly the possibility of averting or delaying age-associated degenerative diseases are important targets for functional foods. Many consumer groups such as children and elderly, and those with impaired immune functions (e.g. due to diabetes) can be prone to food-related diseases and gastrointestinal (GI) disorders. The symptoms of many GI disorders and diseases can potentially be alleviated and prevented by consuming probiotic foods. Microbes tested for their probiotic potential include *Lactobacillus* spp. (e.g. *L. acidophilus, L. reuteri, L. casei, L. johnsonii, L. plantarum, L. rhamnosus*), *Bifidobacterium* spp. (e.g. *B. bifidum, B. infantis, B. animalis, B. longum, B. breve*), some other bacteria (*Enterococcus faecalis, Escherichia coli* and *Bacillus cereus*) and yeasts (*Saccharomyces boulardii, Saccharomyces cerevisiae*) (Alvarez-Olmos and Oberhelman, 2001).

The health-effects attributed to probiotics are diverse. These include alleviation of lactose-intolerance symptoms, treatment of viral and antibiotic-associated diarrhoea, reduction of symptoms of antibiotic treatment of *Helicobacter pylori*, alleviation of atopic dermatitis symptoms in children and prevention the risk of allergy in infancy, alleviation of symptoms of IBD (inflammable bowel disease) and IBS (irritable bowel syndrome), and enhancing the immune response (Reid *et al*., 2003; O'May and Mcfarlane, 2005). Probiotics are postulated to be effective especially in the cases where the condition is at least partially caused by GI tract microbiota imbalance (due to an exogenous pathogen or indigenous GI microbiota population shifts). Many of the health effects are still debated in the literature and recent meta-analysis studies have sometimes given contradicting conclusions of the probiotic efficacy (some of the meta-analysis studies are listed in section 3.6). Thus, more high-quality clinical trials are still needed to support the probiotic health claims.

3.3 Probiotic production technologies

Although the market for probiotic foods has expanded and developed substantially, surveys of probiotic products on the market yet today reveal

quantitative and qualitative deficiencies especially regarding labelling and viability of probiotic strain(s) (Coeuret *et al*., 2004; Masco *et al*., 2005). This indicates that probiotic production technologies are not often optimal for the specific probiotic strains in question. Good viability is generally considered a prerequisite for optimal probiotic functionality (Saarela *et al*., 2000) and therefore probiotic products should contain high enough levels of the specific probiotic strain(s) throughout the storage and during consumption. In the definition of probiotics there are three important issues: viability, dose and health-effects. Of these the first two are directly linked to probiotic production technologies which should enable probiotics to retain their viability during all the production steps and even in the GI tract of the consumer. For the successful production of high-quality probiotic products a solid knowledge on the characteristics of the production strain is necessary. The properties of probiotic strains can vary substantially, sometimes even among the strains representing the same species. Thus the special characteristics of probiotic strains have to be known taken into account during the production and formulation. Viability losses of probiotics occur easily if the microbes encounter stressful situations and conditions. Microbes can become stressed for several reasons: they have unique optimal growth conditions and maintaining these optimal conditions in batch cultures in fermenters is difficult since due to the growth and diminishing nutrient concentrations their environment changes constantly (Rallu *et al*., 1996). Various down-stream processing steps of probiotic production (such as harvesting, freezing, drying and other manipulations) unavoidably cause stress to microbes. During the formulation stage matrix to which the strain is formulated, pH, gas atmosphere, possible accompanying microbes, and storage time and temperature affect probiotic viability and stability (Kailasapathy and Rybka, 1997; Saxelin *et al*., 1999; Saarela *et al*., 2000; Heller, 2001).

3.3.1 Enhancing and maintaining probiotic viability and stability during production

Growth of a probiotic strain in a fermenter is the first production step affecting the viability and stability of the final probiotic product. Probiotic growth is nowadays typically optimised using statistical process parameter evaluation, experimental design and process optimisation (Ha *et al*., 2003; Liew *et al*., 2005; Mättö *et al*., 2006). Another way to affect cell physiology during growth is, instead of performing a free-cell batch fermentation, to immobilise and grow cells in a suitable carrier matrix in a continuous culture. Immobilised cells typically show altered morphology, membrane composition and metabolism, and increased tolerance to antimicrobial compounds compared with free cells (Doleyres and Lacroix, 2005). Additionally, immobilised cell technology allows achieving high stability and volumetric productivity of the cultures (Doleyres *et al*., 2002). Another way to temporarily alter the probiotics' cell physiology is to utilise the natural

stress responses of the cells. Stress responses can be utilised, e.g. to transiently improve the tolerance of the probiotic strain to adverse conditions such as low pH (Lorca and de Valdez, 2001; Maus and Ingham, 2003; Saarela *et al*., 2004), heat (Desmond *et al*., 2002; Ananta and Knorr, 2004) and drying (Desmond *et al*., 2002; Prasad *et al*., 2003). Stress tolerance of probiotic strains has also been permanently improved by overproducing stress response genes in *L. plantarum, Lactobacillus paracasei* and *Lactobacillus salivarius* (Derzelle *et al*., 2003, Corcoran *et al*., 2006, Sheehan *et al*., 2006).

After harvesting the probiotic cells are stabilised by freezing, drying (freeze- or spray-drying) or encapsulation. To enhance the survival of probiotics during these steps, protectants, which help to diminish the treatment-induced injuries, are used. For the cryoprotection of lactic acid bacteria and probiotics a large selection of compounds has been used, the most common of these being skim milk (with or without supplements) and various carbohydrates (ranging from simple sugars to fibres) (Hubalek, 2003; Carvalho *et al*., 2004; Saarela *et al*., 2006b). Some of these compounds can also be used as thermoprotectants during spray-drying. For the probiotic encapsulation carriers or supporting material used include alginate, carrageenan, cellulose acetate phthalate, chitosan, gelatine, gum arabic and starch (Krasaekoopt *et al*., 2003). Basic encapsulation techniques are extrusion and emulsion, but cells can also be encapsulated during freeze- and spray-drying. Encapsulation is often the only option to maintain the viability of very sensitive strains.

Unless probiotics are consumed as capsules or powders they are further formulated into foods. During this formulation step several things need to be considered such as the composition (nutrients, antimicrobials), structure (oxygen permeability, water activity) and pH of the food matrix, and possible interactions with starter microbes in fermented food matrices. Growth of probiotics in non-fermented foods is not desirable (due to possible off-flavour formation), but their growth during the production of fermented foods can lower process costs and increase the adaptation of probiotics leading to enhanced viability. The starter microbes in fermented foods can sometimes inhibit probiotics but they can also enhance their survival by producing beneficial substances or by lowering the oxygen pressure (Kailasapathy and Rybka, 1997; Saxelin *et al*., 1999; Saarela *et al*., 2000; Vinderola *et al*., 2002). In beverages the most important factor affecting probiotic viability is probably the pH. Shelf-stable beverages typically have pH values below 4.4 to ensure their microbial stability (Eckert and Riker, 2007); e.g. fruit juices usually have a pH below 4 or even 3 (Saarela *et al*., 2006b), and this low pH value combined with long storage periods is very demanding for most probiotic strains, especially those representing bifidobacteria.

The final step in probiotic production process is packaging. The packaging material should be a good oxygen barrier (e.g. glass or aluminium foil) to promote the survival of especially anaerobic probiotic bacteria (bifidobacteria) (Saarela e*t al*., 2000). For most probiotic products transpor-

Table 3.2 Factors affecting the viability and stability of probiotics in beverages

- Probiotic strain properties and the way the probiotic has been formulated into the beverage
- Chemical composition of the beverage base (nutrients vs. antinutritives)
- pH and the presence of organic acids (especially if the beverage is fermented or if it is made of fruit)
- Additives (nutrients vs. antinutritives)
- Accompanying microbes (in fermented beverages; inhibition vs. enhancement)
- Packaging (oxygen barrier properties)
- Storage time and temperature

tation and storage at constant refrigerated temperatures are necessary. Storage temperature is an important determinant of the shelf-life; with increasing temperatures viability losses can occur rapidly (Saxelin *et al*., 1999). Factors affecting the viability and stability of probiotics in beverages are listed in Table 3.2.

3.4 Growth and stability of probiotics in dairy beverages and in juices

Beverages supplemented with probiotics can potentially be produced from a multitude of raw materials including milk, fruits and berries, cereals and legumes. Traditional fermented drinks made from cereals are still commonly consumed today in many parts of the world. Although these products can potentially benefit health, like many other fermented products, there is no literature available about specific probiotic strains being used in these traditional beverages. Hence, traditional fermented beverages were not included into this review (for more information see, e.g., Prado *et al*., 2008). The present review thus focuses on dairy and fruit-based beverages.

3.4.1 Probiotics in dairy beverages

Growth in milk and production of fermented dairy beverages

Probiotic *Lactobacillus* and *Bifidobacterium* strains typically grow poorly in milk owing to their low proteolytic activity, inability to utilise lactose, or due to special needs for certain growth factors missing in milk (Kailasapathy and Rybka, 1997; Gomes and Malcata, 1999; Østlie *et al*., 2003; Roy, 2005). In attempts to improve the probiotic growth in milk various nitrogen and carbon sources and other substances have been added into milk to boost the growth of probiotic strains. For probiotic bifidobacteria fructooligosaccharides (FOS) (Shin *et al*., 2000); caseinomacropeptides (CMP) and whey protein concentrate (WPC) (Janer *et al*., 2004); and tryptone (Østlie *et al*., 2003) and yeast extract (Stephenie *et al*., 2007)

have been shown to promote the growth in milk. For probiotic lactobacilli certain amino acids, nucleotide precursors and an iron source (Elli *et al*., 1999); *Lactobacillus delbrueckii* subsp. *bulgaricus* cell extracts (with β-galactosidase and protease activities) (Gaudreau *et al*., 2005); tryptone and fructose (Østlie *et al*., 2003); and inulin, lactulose, raftilose and Hi-maize (Desai *et al*., 2004) have proved useful growth-promoters in milk. Since probiotic strains vary in their ability to utilise lactose and proteins in milk the growth-promoting capability of different substances is largely species- or even strain-specific.

Probiotics can be incorporated into fermented milk products in several ways. Perhaps the most typical is to add the probiotic culture together with starters as DVI (direct vat inoculation) culture. Since the fermentation typically does not occur in conditions optimal for the probiotic strain, the probiotic does not usually grow much during this mixed fermentation. To promote probiotic growth fermentation can be performed in two separate batches: one batch of milk is fermented with the probiotic (in optimal conditions for it) and another batch with starters. After fermentation the two products are combined to generate the final probiotic fermented milk product. A third way of producing probiotic fermented milk products is to use the probiotic culture alone as the fermenting starter.

During the production of probiotic fermented milks several factors have to be considered, including the slow growth of many probiotic strains in non-supplemented milk, the unsuitability of production conditions (e.g. the low fermentation temperature) for the growth of probiotics, and that probiotic growth can result in the formation of off-flavours (e.g. bifidobacteria produce acetic acid, which gives a vinegar-like taste) (Gomes and Malcata, 1999; Saxelin *et al*., 1999; Saarela *et al*., 2000; Østlie *et al*., 2003). In the cases when probiotics and starter microbes are both present during fermentation a special emphasis has to be put on finding suitable probiotic–starter combinations (Saxelin *et al*., 1999). This is important because starter microbes may produce antimicrobials (e.g. hydrogen peroxide, high amounts of lactic acid) that are harmful to the probiotic. On the other hand, starters may also enhance the growth and survival of probiotic cultures by producing growth substrates or by reducing the oxygen pressure (Kailasapathy and Rybka, 1997; Saarela *et al*., 2000; Vinderola *et al*., 2002).

Survival in milk

There is relative little published information on the survival of probiotics in non-fermented milk compared with fermented milk products such as yoghurt. Typically probiotics, especially bifidobacteria, survive better in non-fermented milk than in fermented milk. This is mainly due to the higher pH values of non-fermented products compared to fermented ones, but also the presence of starter bacteria and their other metabolites besides organic acids may negatively affect the stability of probiotics (Kailasapathy and Rybka, 1997). The stability of probiotics in milk is mainly determined by two things:

the strain and formulation properties and the storage time (storage times longer than 2 weeks often cause decrease in viability) (Hughes and Hoover, 1993; Sanders *et al*., 1996; Usman and Hosono, 1999; Saarela *et al*., 2003, 2006a; Martinez-Villaluenga *et al*., 2006). Supplements such as raffinose family oligosaccharides (RFOs), can improve the probiotic storage stability (Martinez-Villaluenga *et al*., 2006), probably in a strain-specific manner.

One of the factors affecting the stability of bifidobacteria in milk is their oxygen sensitivity, which varies between strains (Shah, 2000; Roy, 2005; Bolduc *et al*., 2006). Bolduc *et al*. (2006) showed that electrochemical reduction of milk, as well as deaeration or addition-reducing agents (cysteine) enhanced the survival of oxygen-sensitive bifidobacteria in milk during storage at 7 °C.

There is little data on the survival of probiotics in drinkable fermented milks such as cultured buttermilk. There is, however, a range of probiotic drinkable fermented drinks on the market (Tamime *et al*., 2005). Similarly to for example probiotic stability in yoghurt, factors such as pH (organic acids), flavour compounds and accompanying microbes (starter bacteria) affect the viability and stability of probiotics in drinkable fermented milks (Saarela *et al*., 2000).

Effect of milk on the functional properties of probiotics

Several *in vitro* studies have shown that milk or milk components can protect lactic acid bacteria and bifidobacteria against low pH and also against bile (Conway *et al*., 1987; Charteris *et al*., 1998; Fernández *et al*., 2003; Saarela *et al*., 2006a). The protection against low pH is at least partially explained by the buffering effect of milk, but milk components also have additional protective effects. In the bile test the pH of the test solution is around 7, which indicates that the buffering effect of milk components does not play a role in the protection against bile acids (Saarela *et al*., 2006a). Fermented milk products can also protect bacteria against harmful effects of the upper GI tract. Since cheeses have a markedly higher pH than fermented milks (4.8–5.6 vs. 3.7–4.3) they provide a better protection against low pH conditions. Furthermore, the structure (matrix) and the relatively high fat content of cheeses provide further protection against harmful conditions of the upper GI tract (Gardiner *et al*., 1999; Boylston *et al*., 2004). However, a suitable level of acid stress during food matrix formulation can help bacteria to survive in subsequent harsher acidic conditions (Leverrier *et al*., 2005). The protective effect of milk has also been shown *in vivo*, when the survival of differently formulated probiotics through the human GI tract has been studied (Saxelin *et al*., 2003).

3.4.2 Probiotics in juice

Growth in juice

Probiotic growth in juice has been much less studied than that in milk. Growth studies have been limited to vegetables since in fruit juices micro-

bial fermentation is typically an unwanted phenomenon. Yoon *et al.* (2004, 2005, 2006) showed that probiotic lactobacilli were able to grow and produce acid in non-supplemented tomato, beetroot and cabbage juice. Fermentation of tomato juice by probiotic lactobacilli has also been reported by King *et al.* (2007). Rakin *et al.* (2007) further supplemented beetroot and carrot juices with brewer's yeast autolysate to facilitate the fermentation. In the study of Savard *et al.* (2003) mixed vegetable juice (made of carrots, cabbage and onions; initial pH 6.3) was fermented with nine different probiotic *Lactobacillus* and *Bifidobacterium* strains. All strains grew in the medium but with variable final cell densities.

Since fruit juices typically contain prohibitory levels of organic acids (Vinderola *et al.*, 2002) and have a low pH natural fruit juices are not good growth media for probiotic bacteria. In addition to acids, fruit, especially berries, also contain various phenolic compounds which are inhibitory to bacteria (Puupponen-Pimiä *et al.*, 2005; Howell, 2007).

Survival in juice

Organic acids also impair the survival of probiotics in juices. pH of the juice is an important determinant of the probiotic viability: pH values below 4 are typically detrimental to most probiotic strains (Savard *et al.*, 2003; Ainsley Reid *et al.*, 2007; Sheehan *et al.*, 2007). Probiotic bacteria vary in their tolerance to organic acids and low pH. Lactobacilli (especially *L. acidophilus* and *L. casei* groups) are generally considered to be more resistant to acidic environments than bifidobacteria (Champagne and Gardner, 2005). Bifidobacteria are reported to be sensitive to pH values below 4.6 (Boylston *et al.*, 2004), and thus fruit juices (with typical pH values between 3 and 4) are poor supporters of their viability and stability. However, the acid resistance of bifidobacteria, like lactobacilli, varies and *B. animalis* strains are clearly more acid resistant than the strains of other *Bifidobacterium* species (Mättö *et al.*, 2004). However, in addition to pH the raw materials of the juice and the nature of organic acids they contain play a role here. It has been, for example, shown that at the same pH cranberry juice is more inhibitory to probiotics than pineapple juice, probably due to high levels of benzoic acids it contains (Sheehan *et al.*, 2007). However, depending on the juice, the probiotic strain and the formulation conditions the stability in juice can still be acceptable (at least 10^6–10^7 cfu/ml) after a couple of weeks' cold storage.

In the study of Savard *et al.* (2003) seven *Lactobacillus* strains and two *Bifidobacterium* strains were added into vegetable juice made of carrots, cabbage, beet and onions (pH 3.65 or 6.5) and stored at 4 °C for up to 90 days. Several of the strains were fairly stable at pH 3.65 for about a month, after which time the viability typically started to decline more rapidly. The stability was better in the juice with the higher pH. In the studies of Yoon *et al.* (2004, 2005, 2006) the stabilities of *L. acidophilus*, *L. casei* and *L. plantarum* were studied in fermented beet and tomato juice (and for *L. casei* and *L. plantarum* also in fermented cabbage juice).

Vegetable juices were first fermented with the test strains and then stored at 4 °C for 4 weeks. The final pH values of the juices – being between 3.4 and 5.0 – were determined by the fermentative capability of the test strains. The three strains showed variable stability in different juices. However, both *L. acidophilus* and *L. casei* were fairly stable in tomato juice. Since tomato juice had a low pH (approx. 3.5) acidity was not the only factor affecting probiotic stability in these studies. In a mixed vegetable juice (made of tomatoes, beets, carrots, spinach, celery, lettuce, parsley and watercress) with fairly high pH (4.35), the stability of the probiotic *L. rhamnosus* proved to be excellent over 2 weeks' storage at 4 °C (Ainsley Reid *et al*., 2007).

In the study of Sheehan *et al*. (2007) *L. salivarius*, *B. lactis, L. casei*, *L. rhamnosus* and *Lactobacillus paracasei* were added into orange juice (pH 3.65) and pineapple juice (pH 3.4) and stored at 4 °C for up to 12 weeks. *L. casei* and *L. rhamnosus* were the most robust strains in orange juice, whereas *L. paracasei* was the most robust strain in the pineapple juice. In general, strains survived better in the orange juice than in the pineapple juice. One important factor for the survival in the orange juice was its higher pH compared with pineapple juice. In the study of Saarela *et al*. (2006a) *B. animalis* subsp. *lactis* cells were freeze-dried with either reconstituted skim milk or sucrose as a carrier and freeze-dried cells were then formulated into three fruits' juice (made of orange, grape and passion fruit; pH 3.7) and stored at 4 °C for 6 weeks. The storage stability of the sucrose-formulated cells proved to be better than that of the skim milk formulated cells. When the similarly formulated juices were stored at 20 °C the decline in viability was more rapid. In another study by Saarela *et al*. (2006b) *L. rhamnosus* fresh cells formulated with different carriers (sucrose, oat flour, wheat dextrin and polydextrin) were studied for stability in apple juice (pH 3.5) for 12 weeks at 4 and 20 °C. Oat fibre-formulated cells showed the best stability at both temperatures. Surprisingly both studied *L. rhamnosus* strains formulated with oat flour were more stable at 20 °C than at 4 °C.

Effect of juice on the functional properties of probiotics

There is very little data about the effect of juice formulation on the functional properties of probiotics. According to Saxelin *et al*. (2003) the faecal recovery of *L. rhamnosus* GG consumed in juice was inferior to that of formulated in milk or cheese.

3.5 Future trends

Probiotics are today found in a wide range of food products. All food applications have some kind of limitation regarding the viability and stability of probiotics. These limitations typically involve factors such as the acidity

of the food matrix and storage time and temperature of the product. The nature of the probiotic beverage (raw materials, fermented or non-fermented, etc.) sets its own demands for the probiotic strains. Typically in non-fermented beverages the growth of probiotics is an unwanted phenomenon, whereas in fermented beverages it can be desirable. Therefore special attention has to be paid to the selection of the suitable probiotic strain for a specific food application. Selecting a strain with good technological properties will be a key factor when novel food applications for probiotics are developed. The increasing availability of lactic acid bacterial genome sequences will in the future enable utilising this information also in the studies related to probiotic technology, e.g. following the expression of important genes for probiotic survival during processing (e.g. stress and acid tolerance genes) and identifying novel genes important for the technological functionality of probiotics, thus enabling better control and optimisation of the growth than that currently possible (Klaenhammer *et al*., 2005; Klijn *et al*., 2005). Emerging knowledge of the genes important for the technological functionality and development of the genetic manipulation tools for probiotic bacteria will enable specific tailoring of the technological properties. Whether genetically modified probiotic strains will eventually be incorporated into foods in addition to more potential medical applications remains to be seen. Consumer concerns (Lähteenmäki *et al*., 2002; Grunert *et al*., 2004) and legislative issues will remain a concern here. Another, in a way opposite, approach to the genetic approach is to develop novel probiotic products from traditionally home-made fermented products. Fermentation is an ancient way to store food and improve its safety and nutrition value. Home-made fermented foods are still nowadays commonly produced in the developing countries, especially in the rural areas, although their consumption is declining (Watson *et al*., 1996). Non-commercial fermented products contain vast and largely unknown diversity of lactic acid bacteria (and also other microbes), among which novel probiotic strains could potentially be identified. Preserving this biodiversity is very important in the modern world where the globalisation of markets will eventually lead to the situation where the same foods are available all over the world, and paradoxically, although a wider selection of foods is available for the consumer, the microbial biodiversity of the foods will dramatically diminish owing to the harmonisation of production technologies.

3.6 Sources of further information and advice

More information about the potential probiotic health effects is available, e.g. in the recently published meta-analysis studies:

Deshpande G, Rao S, Patole S (2007), 'Probiotics for prevention of necrotising enterocolitis in preterm neonates with very low birthweight: a systematic review of randomised controlled trials', *Lancet*, **369**(9573), 1614–20.

Elahi B, Nikfar S, Derakhshani S, Vafaie M, Abdollahi M (2008), 'On the benefit of probiotics in the management of pouchitis in patients underwent ileal pouch anal anastomosis: a meta-analysis of controlled clinical trials', *Dig Dis Sci*, **53**(5), 1278–84.

McFarland L V (2007), 'Meta-analysis of probiotics for the prevention of traveler's diarrhea', *Travel Med Infect Dis*, **5**(2), 97–105.

Osborn D A, Sinn J K (2007), 'Probiotics in infants for prevention of allergic disease and food hypersensitivity', *Cochrane Database Syst Rev*, Oct 17;(4):CD006475.

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Tong J L, Ran Z H, Shen J, Zhang C X, Xiao S D (2007), 'Meta-analysis: the effect of supplementation with probiotics on eradication rates and adverse events during *Helicobacter pylori* eradication therapy', *Aliment Pharmacol Ther*, **25**(2), 155–68.

Introduction of a Qualified Presumption of Safety (QPS) approach for assessment of selected microorganisms referred to EFSA – Opinion of the scientific committee can be found here: http://www.efsa.europa.eu/EFSA/efsa_locale-1178620753812_1178667590178.htm

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4

Fortification of beverages with vitamins and minerals

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Abstract: There has been a long history of micronutrient addition to soft drinks and beverages. Recent studies on the fortification of beverages with vitamins and minerals have demonstrated positive health effects, particularly in target populations in developing countries. The addition of micronutrients to liquid products can present a number of technological challenges and a good knowledge of the chemistry of the product is required to prevent ingredient interactions. Vitamins are inherently unstable and their stability is affected by a number of factors such as oxygen in the liquid, heat, ultraviolet light and the presence of other substances. The use of mineral salts to provide the nutritional minerals and trace elements requires considerable care and a good knowledge of their chemical and physical properties and interactions. It is essential that fortified beverages are subjected to realistic stability studies during product development.

Key words: fortified beverages/drinks, vitamins, vitamin stability, nutritional minerals, functional drinks.

4.1 Introduction

There has been a long history of micronutrient addition to soft drinks and beverages, either for restoration or enhancement of vitamins lost or reduced during processing or for fortification. For example, orange juice can often have levels of vitamin C restored, and sometimes enhanced, while specialised convalescent beverages may contain a range of vitamins and minerals.

The addition of micronutrients to liquid products can present a number of technological challenges. The challenges have increased with the desire to develop products intended to contain substances other than vitamins and minerals with a physiological effect, such as the carotenoids and polyphenols.

4.2 Fortification of beverages and health benefits

Beverages can be fortified with micronutrients for a number of reasons, the main one being to replace or enhance the vitamin and mineral content of the drink to improve its nutritional contribution to the diet. A good example of this would be a convalescent drink which has been specially formulated to deliver predetermined levels of nutritionally important vitamins and minerals to aid recovery from illness. Similar products are developed as infant formulae drinks intended for babies in their first year and, at the other end of the age scale, geriatric beverages for the elderly with eating or digestive problems.

Such products would be considered in most legislative environments to be dietetic foods or foods for special nutritional uses and the composition of some, particularly infant formulae, may be controlled by legislation. Fortification is also common in malted bedtime drinks, such as Horlicks, which tend to have a relatively high level of calcium and a wide range of vitamins. As bedtime drinks are not specifically targeted at defined sectors of the population but at the population as a whole, they do not normally come under the definition of a dietetic product.

Although dietetic products are developed with a sound nutritional rationale, there are also a number of products on the market which have been enhanced or fortified with micronutrients primarily to obtain a marketing differentiation for the product, although there is usually some nutritional justification. An example could be the addition of calcium to an orange-based breakfast drink. Such a drink is aimed mainly at women concerned about osteoporosis.

While the restoration of vitamins in fruit juices has been carried out for many years, particularly with orange juices and healthy fruit and vegetable juice blends, there have been trends towards the fortification of soft drinks, both carbonated and still. Vitamin C has been the most common but there are products containing B vitamins, calcium and vitamin D (Coca Cola) and vegetable juices fortified with the carotenoid lutein and promoted for eye health. In some cases the fortificants and the scientific rationale behind their usage has to be questioned.

An important aspect of beverage fortification which appears to have been under-utilised is that of public health in developing countries and under-privileged communities. Studies carried out by researchers at Cornell University, USA, on pregnant women and children in Tanzania showed improvements in nutritional and general health status when provided with a fortified drink.[1] Following on from this work similar studies have been undertaken in the Philippines[2] and Bangladesh.[3]

In the Tanzanian study on children the drink was based on an orange-flavoured powder fortified with 11 vitamins and minerals. The micronutrients selected were the minerals iron, zinc and iodine and vitamins A, E, C, folic acid, niacin, thiamin (B_1), riboflavin (B_2) and pyridoxine (B_6). The

levels of the micronutrients were from 30% to 120% of the US recommended dietary allowances.[4]

Results from the study showed that the fortified drink not only significantly improved observed nutritional deficiencies but also brought almost twice as much weight gain and 25% greater height gain in the children who consumed the drink compared with those who drank a placebo. Most of the children with moderately severe anaemia showed a significant improvement in iron levels while a number of those on the placebo showed a reduction in iron level.

A similar study on 439 pregnant women in Tanzania using the drink showed that the risk of anaemia dropped by 51% in the women who consumed the drink. In addition, the mothers who had been on the fortification showed improvements in the vitamin A level in their breast milk compared with those on the placebo. As up to two-thirds of pregnant women in the developing world suffer from anaemia and do not take iron pills regularly, a palatable fortified drink may prove to be a feasible alternative.[5]

4.3 Micronutrients

Until the end of the 20th century the category of micronutrients was considered to comprise the 13 recognised vitamins and a number of minerals and trace elements. More recently, a large number of substances with a demonstrated physiological effect have been found to have properties with benefits to human health and have been added to functional foods and supplements. These have become more accepted and popular and now cover a wide range of substances. The more common ones are the non-vitamin A contributing carotenoids such as lutein and lycopene and certain groups of plant polyphenols such as the anthocyanidins and procyanidins.

The focus of this chapter is on vitamins and minerals. The vitamins are classified into two groups, the oil-soluble vitamins, of which there are four, and the nine water-soluble vitamins (Table 4.1). In addition, beta-carotene is included as pro-vitamin A as it can be used by the human body as a vitamin A source if there is a deficiency of the animal-sourced retinol. There are 15 minerals and trace elements which are generally recognised as nutrients for humans and also a small number which have been claimed to have nutrient functions, but for which the scientific evidence is not yet convincing.

4.4 Formulating with vitamins

Vitamins can be described as a heterogeneous group of substances brought together by an impure definition. Originally, the vitamins were considered to be a group of organic nutrients required in very small amounts for

Table 4.1 Vitamins and some commonly used synonyms

Vitamin	Synonyms
Fat-soluble	
Vitamin A	Retinol
Vitamin D_2	Ergocalciferol
Vitamin D_3	Cholecalciferol
Vitamin E	α-, β- and γ-Tocopherols and α- tocotrienol
Vitamin K_1	Phylloquinone, phytomenadione
Vitamin K_2	Menaquinone, farnoquinone
Vitamin K_3	Menadione
Water-soluble	
Vitamin B_1	Thiamin
Vitamin B_2	Riboflavin
Vitamin B_6	Pyridoxal, pyridoxine, pyridoxamine
Vitamin B_{12}	Cobalamins, cyanocobalamin, hydroxocobalamin
Niacin	Nicotinic acid (vitamin PP)
Niacinamide	Nicotinamide (vitamin PP)
Pantothenic acid	–
Folic acid/folates	Folacin (vitamin M)
Biotin	Vitamin H
Vitamin C	Ascorbic acid

healthy metabolism and which must be obtained from food as they cannot be synthesised in adequate amounts by the human body. However, more recent knowledge of vitamin D indicates that it no longer fits into the definition as most people obtain their vitamin D from the effects of the sun on the skin.

As previously stated, vitamins are categorised as being either fat soluble or water soluble. This not only has a biological significance but also a technological one, particularly when considering the fortification of liquid products.

The 13 vitamins have totally different and diverse chemical structures which affect their behaviour both *in vivo* and *in vitro*. The diversity of chemical structures means that no two vitamins behave in a similar manner in a product matrix. One of the most important aspects in relation to product development is that none of the vitamins is stable and some are far more unstable than others in certain matrices. This makes the fortification of a liquid a complex challenge.

The forms of vitamins that can be used are limited by commercial availability and in many countries, such as those in the European Union, by legislation. A combination of these two factors in many cases reduces the options open to the formulator. When selecting the vitamins and their chemical forms it is essential to have a good knowledge of the other ingredients and additives intended to be used in the formula.

4.5 Water-soluble vitamins

4.5.1 Vitamin C

Although a number of compounds possess vitamin C activity, the most important is L-ascorbic acid. Vitamin C is widely distributed in nature; it can occur at relatively high levels in some fruits and vegetables and is also found in animal organs such as liver and kidney. Small amounts can be found in milk and other meats.

Ascorbic acid is the enolic form of 3-keto-1-gulofuranolactone. The endiol groups at C-2 and C-3 are sensitive to oxidation and can easily convert into a diketo group. The resultant compound, dehydro-L-ascorbic acid, also has vitamin C activity. The D-isomers do not have vitamin activity.

The L-ascorbic acid in fruits and vegetables is easily oxidised to the dehydro-L-ascorbic acid. In fresh foods the reduced form normally predominates but processing and storage increase the proportions of the dehydro form. Commercially, vitamin C is available as L-ascorbic acid and its calcium, sodium and magnesium salts (the ascorbates). It is also available as ascorbyl palmitate and can be used in this form as an antioxidant in processed foods. Ascorbic acid and the ascorbates are relatively stable in dry air but are unstable in the presence of moisture.

Ascorbic acid is readily oxidised in aqueous solutions, first forming dehydro-L-ascorbic acid which is then further and rapidly oxidised. Conversion to dehydroascorbic acid is reversible but the products of the latter stages of oxidation are irreversible.

Ascorbic acid is widely used in soft drinks and to restore manufacturing losses in fruit juices, particularly citrus juices. Research has shown that its stability in these products varies widely according to the composition and oxygen content of the solution. It is very unstable in apple juice but stability in blackcurrant juice is good, possibly as a result of the protective effects of phenolic substances with antioxidant properties.

The effect of dissolved oxygen is very significant. As 11.2 mg of ascorbic acid are oxidised by 1.0 mg of oxygen, 75–100 mg of ascorbic acid can be destroyed by 1 litre of juice. Vacuum treatment stages are normally added to the process to deaerate the solution to reduce the problem. It is also important to avoid significant head-spaces in containers of liquids with added ascorbic acid, as 3.3 mg of ascorbic acid can be destroyed by the oxygen in 1 cm^3 of air.[6] Different production and filling processes can have a significant effect on the retention of vitamin C in drinks. For example, the ascorbic acid loss in a drink packed in a 0.7 litre glass bottle with a partial deaeration of the water and vacuum deaeration of the drink immediately before filling was 16% of the same product filled without any deaeration.

Traces of heavy metal ions act as catalysts to the degradation of ascorbic acid. Studies on the stability of pharmaceutical solutions of ascorbic acid showed that the order of the effectiveness of the metallic ions was

$Cu^{2+} > Fe^{2+} > Zn^{2+}$. A Cu^{2+}–ascorbate complex has been identified as being intermediate in the oxidation of the ascorbic acid in the presence of Cu^{2+} ions. Other work on model systems has shown that copper ion levels as low as 0.85 ppm were sufficient to catalyse oxidation, and that the reaction rate was approximately proportional to the square root of the copper concentration.

Work with sequestrants has shown that ethylenediamine tetra-acetate (EDTA) has a significant effect on the reduction of ascorbic acid oxidation, with the optimal level of EDTA required to inhibit the oxidation of vitamin C in blackcurrant juice being a mole ratio of EDTA to [Cu + Fe] of approximately 2:3.[7,8] Unfortunately, EDTA is not a permitted sequestrant for fruit juices in many countries. The amino acid cysteine has also been found effectively to inhibit ascorbic acid oxidation.

Cu and Fe ions play such a significant part in metal-catalysed oxidation of ascorbic acid that the selection of process equipment can have a marked effect on the stability of vitamin C in drinks. Contact of product with bronze, brass, cold rolled steel or black iron surfaces or equipment should be avoided and only stainless steel, aluminium or plastic should be used.

The rate of ascorbic acid degradation in aqueous solutions is pH dependent, with the maximum rate at about pH 4. Vitamin C losses can occur during frozen storage and work has shown that oxidation of ascorbic acid is faster in ice than in liquid water. Frozen orange concentrates can lose about 10% of their vitamin C content during 12 months' storage at –23 °C (–10°F).[9]

Light, either in the form of sunlight or white fluorescent light, can have an effect on the stability of vitamin C in milk, with the extent of the losses being dependent on the translucency and permeability of the container and the length and conditions of exposure. Bottled orange drinks exposed to light have been found to lose up to 35% vitamin C in 3 months.[10]

It can be seen from the above description that the stability of vitamin C in drinks is affected by a wide range of factors including product composition, oxygen content, pH and processing. All these have to be taken into consideration to ensure that the vitamin C levels can be maintained throughout the period of the developed shelf-life.

4.5.2 Thiamin (vitamin B_1)

Thiamin is widely distributed in living tissues. In most animal products it occurs in a phosphorylated form, and in plant products it is predominantly in the non-phosphorylated form. Commercially, it is available as either thiamin hydrochloride or thiamin mononitrate. The hydrochloride contains 79% thiamin and the mononitrate 81%. These values are important when calculating the active vitamin levels from the different salts.

A considerable amount of research has been carried out on the heat stability of thiamin and its salts. The destruction of thiamin by heat is more

rapid in alkaline media. Vitamin B_1 losses in milk, which has an average fresh content of 0.04 mg thiamin per 100 g, are normally less than 10% for pasteurised milk, between 5 and 15% for UHT milk and between 30 and 40% for sterilised milk.[11] Between 30 and 50% of the vitamin B_1 activity can be lost during the production of evaporated milk.

Thiamin is very sensitive to sulphites and bisulphites as it is cleaved by sulphite. This reaction is rapid at a high pH and is the cause of large losses of the vitamin in fortified juices, drinks and liquid supplements where sulphites and bisulphites are used as preservatives. Where the pH is low, such as in citrus fruit juices, the bisulphite occurs mainly as the un-ionised acid, and thiamin losses in such systems are not significantly different from those in products not containing bisulphite.[12] It has also been reported that thiamin is cleaved by aromatic aldehydes.

Thiamin is decomposed by both oxidising and reducing agents. If it is allowed to stand in alkaline solution in air it is oxidised to the disulphide and small amounts of thiothiazolone. It is unstable in alkaline solutions and becomes increasingly unstable as the pH increases. The stability of the vitamin in low pH solutions such as fortified fruit drinks is very good.

A range of food ingredients have been shown to have an effect on the stability of thiamin. In general, proteins are protective of the vitamin, particularly food proteins such as egg albumin and casein. When heated with glucose, either as a dry mixture or in solution, a browning analogous to a Maillard reaction can occur. This reaction is similar to the reaction between sugars and amino acids and may be important in the loss of thiamin during heat processing. Work has shown that fructose, invertase, mannitol and inositol can actually retard the rate of destruction of thiamin.[12]

In common with some other vitamins, the stability of thiamin is adversely affected by the presence of copper ions. This effect can be reduced by the addition of metal-chelating compounds such as calcium disodium EDTA. The heavy metals appear to influence thiamin stability only when they are capable of forming complex anions with constituents of the medium.

A problem associated with the addition of thiamin to fortified drinks and beverages is the unpleasant flavour and odour of the thiamin salts. The breakdown of thiamin, particularly during heating or in the presence of sulphites, may give rise to off-flavours and the compounds derived from the degradation of the vitamins are believed to contribute to the cooked or 'meaty' flavours found in a number of foods and drinks.

4.5.3 Riboflavin (vitamin B_2)

Riboflavin is the most widely distributed of all the vitamins and is found in all plant and animal cells, although there are relatively few rich food sources. It is present naturally in foods in two bound forms, riboflavin mononucleotide and flavin adenine dinucleotide. Plants and many bacteria can synthesise riboflavin and it is also found in dietary amounts in dairy products.

Riboflavin is available commercially as a crystalline powder that is only sparingly soluble in water. As a consequence, the sodium salt of riboflavin-5′-phosphate, which is more soluble in water, is used for liquid preparations.

The most important factor influencing the stability of this vitamin is light, with the greatest effect being caused by light in the 420–560 μm range. Fluorescent light is less harmful than direct sunlight, but products in transparent containers can be affected by strip lighting in retail outlets.

Riboflavin and riboflavin phosphate are both stable to heat and atmospheric oxygen, particularly in an acid medium. In this respect, riboflavin is regarded as being one of the more stable vitamins. It is degraded by reducing agents and becomes increasingly unstable with increasing pH. While riboflavin is stable to the heat processing of milk, one of the main causes of loss in milk and milk products is from exposure to light. Liquid milk exposed to light can lose between 20 and 80% of its riboflavin content in 2 hours, with the rate and extent of loss being dependent upon the light intensity, the temperature and the surface area of the container exposed.

4.5.4 Niacin

The term ‘niacin’ is generic for both nicotinic acid and nicotinamide (niacinamide) in foods. Both forms have equal vitamin activity, both are present in a variety of foods and both forms are available as commercial isolates. Niacin occurs naturally in the meat and liver of hoofed animals and also in some plants. In maize and some other cereals it is found in the form of niacytin, which is bound to polysaccharides and peptides in the outer layers of the cereal grains and is unavailable to humans unless treated with a mild alkali. Both forms of niacin are normally very stable in foods and drinks as they are stable to atmospheric oxygen, heat and light in both aqueous and solid systems.

4.5.5 Pantothenic acid

In nature, pantothenic acid is widely distributed in plants and animals, but is rarely found in the free state as it forms part of the coenzyme A molecule. It is found in yeast and egg yolk and in muscle tissue, liver, kidney and heart of animals. It is also found in a number of vegetables, cereals and nuts.

Pantothenic acid is optically active and only its dextrotatory forms have vitamin activity. Losses of pantothenic acid during the heat processing of drinks are normally not very large. Milk generally loses less than 10% during processing. Free pantothenic acid is an unstable and very hygroscopic oil. Commercial preparations are normally provided as calcium or sodium salts. The alcohol form, panthenol, is available as a stable liquid but is not widely used in foods.

The three commercial forms, calcium and sodium D-pantothenate and D-pantothenol, are moderately stable to atmospheric oxygen and light but only when protected from moisture. All three compounds are hygroscopic, with sodium pantothenate being the worst.

Aqueous solutions of both the salts and the alcohol form are thermolabile and will undergo hydrolytic cleavage, particularly at high or low pH. The compounds are unstable in both acid and alkaline solutions and maximum stability is in the pH range of 6 to 7. Aqueous solutions of D-pantothenol are more stable than the salts, particularly in the pH range 3 to 5.

4.5.6 Folic acid/folates

Folic acid (pteroylglutamic acid) does not occur in nature but can be produced commercially. The naturally occurring forms are a number of derivatives collectively known as folates or folacin, which contain one or more linked molecules of glutamic acid. Polyglutamates predominate in fresh food, but on storage these can slowly break down to monoglutamates and oxidise to less biologically available folates. The folic acid synthesised for food fortification has only one glutamic group.

For many years folic acid was the only source of this vitamin for food and drink fortification until an isolated folate, 5-methyltetrahydrofolic acid, became available in 1999. By 2005, following official safety reviews and approvals, it was considered suitable for use in foods and supplements.

Most of the stability studies have been carried out with the commercially available folic acid, which has been found to be moderately stable to heat and atmospheric oxygen. In solution it is stable at around pH 7 but becomes increasingly unstable in acid or alkali media, particularly at pH less than 5. Folic acid is decomposed by oxidising and reducing agents. Sunlight, and particularly ultraviolet radiation, has a serious effect on the stability of folic acid. Cleavage by light is more rapid in the presence of riboflavin. This reaction can be retarded by the addition of the antioxidant butylated hydroxyanisol (BHA) to solutions containing folic acid and riboflavin.[13]

The stability of the folates in drinks during processing and storage is variable. Folic acid loss during the pasteurisation of milk is normally less than 5%. Losses in the region of 20% can occur during the ultra-high temperature (UHT) treatment and about 30% loss is found after sterilisation. UHT milk stored for 3 months can lose over 50% of its folic acid. The extra heat treatment involved in boiling pasteurised milk can decrease the folic acid content by 20%.

Stability studies carried out on 5-methyltetrahydrofolic acid showed that its degradation in all the model systems could be described by first-order reaction kinetics.[14] The thermostability of the folate was enhanced at a pH of 7. The study also investigated the pressure stability of the folate

in fruit and vegetable juices subjected to high pressure processing and at different temperature/pressure combinations. It was found that it was relatively pressure stable at temperatures lower than 40 °C and that both the temperature and pressure stabilities were enhanced in the presence of ascorbic acid.

4.5.7 Pyridoxine (vitamin B_6)

Vitamin B_6 activity is shown by three compounds: pyridoxol, pyridoxal and pyridoxamine. These are often considered together as pyridoxine. Vitamin B_6 is found in red meat, liver, cod roe and liver, milk and green vegetables. The commercial form normally used for food fortification is the salt, pyridoxine hydrochloride.

Pyridoxine is normally stable to atmospheric oxygen and heat. Decomposition is catalysed by metal ions. Pyridoxine is sensitive to light, particularly in neutral and alkaline solutions. One of the main causes of loss of this vitamin in milk is sunlight, with a 21% loss being reported after 8 hours' exposure.[11]

Pyridoxine is stable in milk during pasteurisation but about 20% can be lost during sterilisation. Losses during UHT processing are around 27%, but UHT milk stored for 3 months can lose 35% of this vitamin.

4.5.8 Vitamin B_{12}

The most important compound with vitamin B_{12} activity is cyanocobalamin. This has a complicated chemical structure and occurs only in animal tissue and as a metabolite of certain microorganisms. The other compounds showing this vitamin activity differ only slightly from the cyanocobalamin structure. The central ring structure of the molecule is a 'corrin' ring with a central cobalt atom. In its natural form, vitamin B_{12} is probably bound to peptides or protein. Vitamin B_{12} is commercially available as crystalline cyanocobalamin, which is a dark red powder. As human requirements of vitamin B_{12} are very low (about 1–2 μg a day), it is often supplied as a standardised dilution on a carrier.

Cyanocobalamin is decomposed by both oxidising and reducing agents. In neutral and weakly acid solutions it is relatively stable to both atmospheric oxygen and heat. However, it is not particularly stable in alkaline solutions and strong acids. It is sensitive to light and ultraviolet radiation, and controlled studies on the effect of light on cyanocobalamin in neutral aqueous solutions showed that sunlight at a brightness of 80 000 lux caused a 10% loss for each 30 minutes of exposure, but exposure to levels of brightness below 3000 lux had little effect.[15]

Vitamin B_{12} is normally stable during pasteurisation of milk but up to 20% can be lost during sterilisation. The stability of vitamin B_{12} is significantly influenced by the presence of other vitamins.

4.5.9 Biotin

The chemical structure of biotin is such that eight different isomers are possible and, of these, only the dextro-rotatory or D-Biotin possesses vitamin activity. D-Biotin is widely distributed, but in small concentrations, in animal and plant tissues. It can occur both in the free state (milk, fruit and some vegetables) and in a form bound to protein (animal tissues and yeast). It is commercially available as a white crystalline powder.

Biotin is generally regarded as having a good stability, being fairly stable in air, heat and daylight. It can, however, gradually be decomposed by ultraviolet radiation, particularly if the drink is in a transparent container. Biotin in aqueous solutions is relatively stable if the solutions are either weakly acid or weakly alkaline. In strong acid or alkaline solutions the biological activity can be destroyed by heating.

4.6 Fat-soluble vitamins

As their category name implies, the fat-soluble vitamins can only be 'dissolved' in fats and oils. None is water soluble and this makes the addition to water-based soft drinks and beverages very difficult.

4.6.1 Vitamin A

Nutritionally, the human body can obtain its vitamin A requirements from two sources: from animal sources as forms of retinol, and from plant sources from beta-carotene and related carotenoids. Both sources provide a supply of vitamin A, but by different metabolic pathways. In terms of stability the two sources are different from each other.

Retinol

Vitamin A is one of the more labile vitamins, with retinol being less stable than the retinyl esters. The presence of double bonds in its structure makes it subject to isomerisation, particularly in an aqueous medium at acid pH. The isomer with the highest biological activity is the all-*trans* vitamin A. The predominant *cis* isomer is 13-*cis* or neovitamin A which only has a biological activity of 75% of the all-*trans* isomer; 6-*cis* and 2, 6-di-*cis* isomers, which may also form during isomerisation, have less than 25% of the biological activity of the all-*trans* form of vitamin A. The natural vitamin A sources usually contain about one-third neovitamin A while most synthetic sources generally contain considerably less. For aqueous products where isomerisation is known to occur, mixtures of vitamin A palmitate isomers at the equilibrium ratio have been produced commercially. Vitamin A is relatively stable in alkaline solutions.

Vitamin A is sensitive to atmospheric oxygen with the alcohol form being less stable than the esters. The decomposition is catalysed by the presence of trace minerals. As a consequence of its sensitivity to oxygen, vitamin A

is normally available commercially as a preparation that includes an antioxidant and often a protective coating. While BHA and butylated hydroxytoluene (BHT) are permitted in a number of countries for use as antioxidants in vitamin A preparations, the recent trend has been towards the use of tocopherols (vitamin E).

Both retinol and its esters are inactivated by the ultraviolet component of light, and rapid losses can occur in transparent containers. In general, vitamin A is relatively stable during processing, with the palmitate ester more stable to heat than retinol. It is normally regarded as stable during milk processing, and food composition tables give only small differences between the retinol contents of fresh whole milk, sterilised and UHT-treated milk.[16] However, prolonged holding of milk at high temperatures in the presence of air can be shown to result in a significant decrease in the vitamin A activity.

β-Carotene as provitamin A

There are a small number of carotenoids with provitamin A activity. A provitamin is a compound that can be converted in the body to a vitamin. These compounds are generally found as naturally occurring plant pigments which give the characteristic yellow, orange and red colours to a wide range of fruits and vegetables. Some can also be found in the liver, kidney, spleen and milk. The provitamin A with the greatest nutritional and commercial importance is β-carotene. The stability of the carotenoids is similar to vitamin A in that they are sensitive to oxygen, light and acid media.

It has been reported that treatment with sulphur dioxide reduces carotenoid destruction in vegetables during dehydration and storage. A study with model systems showed that the stability of β-carotene was greatly enhanced by sulphur dioxide added either as a sulphite solution to cellulose powder prior to β-carotene absorption or as a headspace gas in containers of β-carotene. While it was found that the β-carotene stability was improved by increasing the nitrogen levels in the containers, the stability was even greater when the nitrogen was replaced by sulphur dioxide. Comparative values for the induction period were 19 hours for β-carotene samples stored in oxygen only, 120 hours in nitrogen and 252 hours in sulphur dioxide.[17]

There is some evidence of a protective effect from ascorbic acid on β-carotene and other provitamin A carotenoids both in liquid and powder form.[18,19] It would appear that in these circumstances the ascorbic acid is acting as an antioxidant, protecting the carotenoids from rapid oxidation. Products containing β-carotene should be protected from light and headspace air should be kept to the minimum.

4.6.2 Vitamin E

A number of naturally occurring substances exhibit vitamin E activity, including the α-, β-, γ- and δ-tocopherols and α-tocotrienols. Dietary sources

of vitamin E are found in a number of vegetables and cereals, with some vegetable oils such as wheatgerm, sunflower seed, safflower seed and maize oils being particularly good sources. Both synthetic and naturally sourced forms of vitamin E are available commercially. While the natural sources of the tocopherols, which also have the highest biological activity, are in the '*d*' form, the synthetic versions can only be produced in the '*dl*' form. Both the '*d*' and '*dl*' forms are also commercially available as esters.

There is a considerable difference in the stability of the tocopherol forms of vitamin E and the tocopherol esters. Although vitamin E is regarded as being one of the more stable vitamins, the unesterified tocopherol is less stable due to the free phenolic hydroxyl group.

Vitamin E is unusual in that it exhibits *reduced* stability at temperatures below freezing. The explanation given for this is that the peroxides formed during fat oxidation are degraded at higher temperatures but are stable at temperatures below 0 °C and, as a consequence, can react with the vitamin E. It has also been shown that α-tocopherol may function as a pro-oxidant in the presence of metal ions such as iron.

α-Tocopherol is readily oxidised by air. It is stable to heat in the absence of air but is degraded if heated in the presence of air. α-Tocopherol is readily oxidised during processing and storage. One of the most important naturally occurring sources of tocopherols are the vegetable oils, particularly wheat germ and cottonseed oils. *dl*-α-Tocopheryl acetate is relatively stable in air but is hydrolysed by moisture in the presence of alkalis or strong acids to free tocopherols.

4.6.3 Vitamin D

Present in nature in several forms, dietary vitamin D occurs predominantly in animal products with very little being obtained from plant sources. Vitamin D_3, or cholecalciferol, is derived in animals, including humans, from ultraviolet irradiation of 7-dehydrocholesterol found in the skin. Human requirements are obtained both from the endogenous production in the skin and from dietary sources.

Vitamin D_2 (ergocalciferol) is produced by the ultraviolet irradiation of ergosterol, which is widely distributed in plants and fungi. Both vitamins D_2 and D_3 are manufactured for commercial use. Vitamins D_2 and D_3 are sensitive to light and can be destroyed relatively rapidly if exposed to light. They are also adversely affected by acids.

Preparations of vitamin D in edible oils are more stable than the crystalline forms, and the vitamin is normally provided for commercial usage as an oil preparation or stabilised powder containing an antioxidant (usually tocopherol). The preparations are usually provided in lightproof containers with inert gas flushing.

The presence of double bonds in the structure of both forms of vitamin D can make them susceptible to isomerisation under certain conditions.

Studies have shown that the isomerisation rates of ergocalciferol and cholecalciferol are almost equal. The isomerisation of ergocalciferol has been studied in powders prepared with calcium sulphate, calcium phosphate, talc and magnesium trisilicate. It was found that the isomerisation was catalysed by the surface acid of these additives.[20]

Crystalline vitamin D_2 is sensitive to atmospheric oxygen and will show signs of decomposition after a few days' storage in the presence of air at ambient temperatures. Crystalline cholecalciferol (D_3) is also destroyed by atmospheric oxygen but is relatively more stable than D_2, possibly because it has one fewer double bond. The vitamin D_3 naturally occurring in milk appears to be relatively stable to heat processing.

4.6.4 Vitamin K

Vitamin K occurs in a number of forms. Vitamin K_1 (phytomenadione or phylloquinone) is found in green plants and vegetables, potatoes and fruits, while vitamin K_2 (menaquinone) can be found in animal and microbial materials. The presence of double bonds in both vitamins K_1 and K_2 makes them liable to isomerisation. Vitamin K_1 has only one double bond in the side chain in the 3-position whereas in K_2 double bonds recur regularly in the side chain. Vitamin K_1 exists in the form of both *trans* and *cis* isomers. The *trans* isomer is the naturally occurring form and is the one that is biologically active. The *cis* form has no significant biological activity.

The various forms of vitamin K are relatively stable to heat and are retained after most heating processes. The vitamin is destroyed by sunlight and is decomposed by alkalis. Vitamin K_1 is only slowly decomposed by atmospheric oxygen.

Vitamin K is rarely added to food products but is found in supplements and the most common commercially available form is K_1 (phytomenadione), which is insoluble in water. A water-soluble K_3 is available as menadione sodium bisulphite.

4.7 Vitamin–vitamin interactions

One of the least expected and less understood aspects of maintaining the stability of vitamins in foods is the detrimental interaction between vitamins. This can lead to the more rapid degradation of one or more of the vitamins in a beverage. These interactions should be taken into consideration when vitamins are used to restore or fortify products presented in the liquid (aqueous) phase such as soft drinks or fruit juices. Most of the work in the area of vitamin–vitamin interactions has been carried out by the pharmaceutical industry in relation to the development of liquid multivitamin preparations.

Four of the 13 vitamins have been identified as having interactions with each other with deleterious effects. These are ascorbic acid (vitamin C),

Table 4.2 Principal vitamin–vitamin interactions

Activator	Increased instability
Ascorbic acid	Folic acid
Ascorbic acid	Vitamin B_{12}
Thiamin	Folic acid
Thiamin	Vitamin B_{12}
Riboflavin	Thiamin
Riboflavin	Folic acid
Riboflavin	Ascorbic acid

Adapted from Berry Ottaway.[10]

thiamin (vitamin B_1), riboflavin (vitamin B_2) and vitamin B_{12}. The principal interactions are given in Table 4.2. Other interactions have been identified that can be advantageous, particularly in increasing the solubility of the less soluble vitamins in aqueous solutions. For example, niacinamide has been shown to act as a solubiliser for riboflavin and folic acid.

4.8 Vitamin overages

As no two vitamins will degrade at the same rate in a drink at any one set of conditions, the technologist has to determine the rates of deterioration of each vitamin and then increase the amount added to the product during manufacture to ensure that the label claim is met throughout the life of the product.

The difference between the formulated and declared levels is known as the 'overage'. The amount of overage will vary according to the inherent stability of the vitamin, the conditions under which the product is processed and packed, the packaging materials selected and the anticipated shelf-life of the product.

Overages are normally expressed as a percentage of the declared value so that an input of 45 mg of vitamin C and a declared amount of 30 mg would give an overage of 50%. For products where the added vitamins are the only significant source of these nutrients in the food, the overages are usually calculated as a percentage of the amount required in the product at the end of its shelf-life.

When determining the overage for a vitamin in a product, consideration must also be given to the total amount of the vitamin in the product, particularly in the case of vitamins A and D where there may be safety concerns. As vitamins A and D are often the most unstable vitamins in a product, overages tend to be higher than those of the other vitamins. The consequences of large overages must be considered, and at all times the amount of overage added must be the minimum necessary and well within any safety levels for the vitamin.

The shelf-life of a product is often dictated by commercial pressures which must take into account the time taken for the product to reach the consumer and the range of temperatures that it may be subjected to during the time between its manufacture and sale to the consumer. Once this information has been established, the vitamin overages to achieve the required shelf-life have to be assessed. The only realistic estimations of the shelf-life and required overages are those obtained by stability trials on the product carried out in the packaging to be used and at the anticipated storage conditions.

Methodology using the Arrhenius model has been developed which allows predictions to be made for both shelf-life and overages.[21,22] As already stated, this is based on the assumption that the degradation of most of the vitamins follows 'first order' or 'zero order' kinetics. The precision of the technique has been found to be related to the number of storage temperatures that can be used and the number of samples that can be taken from each temperature. Typical storage temperatures used for stability studies are 0, 25, 35, 40 or 45 °C and 50 or 55 °C. Ideally, at least three temperatures should be used and the selection of the higher temperatures depends on the composition of the product under test, as phase changes (e.g. solid to liquid, liquid to gaseous) during storage should be avoided. The tests should normally run for at least 24 weeks with samples from each temperature being removed at predetermined intervals and stored at 0 °C. All samples are stored at 0 °C until the final samples are taken and all are assayed at the same time. The data are analysed using the Arrhenius equations. The data obtained enable estimates to be made of overage amounts for each vitamin to meet a given shelf-life, assist in the comparison of different packaging materials and also help to identify potential stability problems. Although it can be demonstrated that the technique has some limitations, work has shown that, if all the experimental controls are maintained, useful predictions of a product's stability can be obtained.

4.9 Addition of minerals

The addition of nutrient minerals and trace elements to beverages and soft drinks requires a good knowledge of basic chemistry and considerable care. The minerals can cause greater problems in a formulation than the vitamins. Unless the mineral is present in significant quantities in a food source which can be used as an ingredient in the product, the mineral has to be added as an organic or inorganic salt. An example of ingredients providing the required mineral(s) would be the use of dried skimmed milk powder in a bedtime or convalescent drink. In such a case the milk powder could contribute calcium, potassium and phosphorus to the drink.

More usually the mineral/trace element has to be added in the form of one of its organic or inorganic salts. In many countries, and particularly

those in the European Union, the salts are specified by law. While most legislation provides a number of options for each element, the choices can be severely reduced when selecting for use in liquid products.

When considering a mineral salt for use in a beverage it is essential that the various relevant chemical, physical and physiological properties of the salt are available to the formulator. The choice of salts can be critical in the context of solubility, palatability and chemical stability, such as an effect on pH or reactions with other components in the drink. In addition, the salts all contain different proportions of the element. For example, the following sources of calcium have very different amounts of the element:

Salt	% Calcium
Calcium carbonate	40.04
Calcium citrate	24.12
Calcium lactate	18.37
Calcium gluconate	9.31

The importance of these differences is that the selection of a particular source affects the weight of salt needed to achieve the nutritional requirements, and this can affect the chemistry of the liquid. Using the properties given above and an 800 mg per serving of calcium, the amount of calcium carbonate needed would be almost 2 g whereas the amount of calcium gluconate would be almost 8.6 g. To put these figures into the context of a 200 ml/serving beverage, the carbonate would make up 1% of the weight whereas the gluconate would be 4.3%. These differences in the amount of input into a formula can have a significant effect on the quantity and stability of the product. Compromises often have to be made between the marketing desires and the technological realities of a product.

4.9.1 Solubility

Possibly the most critical factor in the selection of a salt is its solubility in water. Although this statement may appear obvious, it is surprising how often this aspect is overlooked in the early stages of the product development of a drink. Data are available on the solubility of salts in water under different conditions, as shown in Table 4.3. It can be seen from this table that the solubilities of the salts for each element vary widely, and for the trace elements shown in the table the choice of salt is limited. Although the values given in Table 4.3 refer to the solubility in water, the solubility can be further decreased by the presence of other substances added to the water.

4.9.2 Elemental contribution from a salt

Also of importance in the development of a formulation is the amount of the element present in the salt. The amount of the element depends upon

Table 4.3 Comparative solubility in water of selected salts of calcium, iron and magnesium

Salt	Solubility g salt/100 ml H_2O
Calcium chloride	74.5
Calcium lactate	5.4
Calcium gluconate	3.3
Calcium phosphate (monobasic)	1.8
Ferrous sulphate	15.7
Ferrous lactate	2.1
Ferrous fumarate	0.14
Ferrous carbonate	0.007
Magnesium sulphate	71.0
Magnesium oxide	0.0006
Magnesium citrate	Slightly soluble

the molecular formula of the salt, with a general rule of thumb being that an organic salt contains much lower levels of the element than most inorganic salts. In liquid products, a high level of input of a salt can have a number of effects, including the shelf stability of the product and particularly on any vitamins in the product.

The elemental content of the salt is calculated from the molecular formula and molecular weight, taking into consideration the water of crystallisation. Thus, for calcium chloride, a generally permitted source of calcium, the molecular formula is $CaCl_2$ and the molecular weight 110.99 (the atomic weight of calcium being 40.08 and chlorine 35.45). The calcium content calculates as 36.11% of the salt. To meet a label claim of 400 mg calcium per serving it would be necessary to add 998 mg calcium chloride.

To obtain the precision necessary to have confidence in the label claim the purity of the approved commercial food sources of calcium chloride should also be taken into consideration. The monograph for calcium chloride given by the Food Chemicals Codex is for an assay of not less than 93.0% $CaCl_2$.[23]

4.9.3 pH

The addition of mineral salts to a liquid product can affect the pH of the solution and can have a deleterious effect on the stability of vitamins and some bioactive plant compounds. The selection of the salt is important and it may require testing to determine its effects on the pH of the formula. The relative pH of some common salts is shown in Table 4.4.

4.9.4 Human absorption

The absorption of the element by the human gut is influenced by a number of factors, including the form (salt) in which it is ingested. The area of

Table 4.4 Comparison of the pH of solutions of selected salts

Salt	pH
Zinc sulphate	4.5
Magnesium sulphate	6 to 7
Potassium chloride	7 (neutral)
Sodium bicarbonate	8.3
Magnesium oxide	10.3

human absorption of micronutrients is complex and is influenced not only by the food in which the nutrient is carried but also by the diet and the presence or absence of specific substances. A commonly used example is the enhanced absorption of iron in the presence of ascorbic acid (vitamin C). However, a number of elements such as zinc, copper and manganese are competitive absorption inhibitors of iron.

It cannot be assumed that the nutrient element (e.g. calcium, iron or zinc) is equally absorbed from each salt. While some data are available on the relative absorption of the nutrient element from a range of salts, technological considerations often dictate the choice of a salt.

4.9.5 Chemical interactions

Unwanted chemical reactions can occur in food products even at very low moisture levels, such as those found in food supplement tablets. In liquid products the reactions are accentuated owing to the presence of water and, in the case of liquids that have not been deaerated, oxygen. It is often the case, when problems occur in products, that the basic principles of chemistry have been forgotten. The precipitates that were formed in the chemistry lessons at school will also be found in a poorly formulated product.

Formulators should also be aware that micronutrient sources can react with food additives used for technological purposes. For example, the combination of vitamin C (ascorbic acid) added as a nutrient source and sodium benzoate as a preservative can result in the formation of benzene in the drink. This has already been the cause of a very expensive product recall of a major brand.

4.10 Future trends

The market trend towards functional foods and drinks brings a number of challenges to the area of fortified beverages, and proposed formulations should be carefully assessed for their technical feasibility. In many ‘functional’ drinks the focus has been on substances other than vitamins and minerals which have been shown to have physiological effects on the human

body. These include certain carotenoids such as lutein and lycopene; polyphenols; a large number of substances extracted or isolated from botanical sources and a smaller number extracted or isolated from animal sources.

In terms of the technological considerations the issues of solubility, stability and interactions equally apply to these substances and detailed stability studies should be carried out on proposed formulations before proceeding to commercial production. This is particularly important if the product also contains vitamins for which label claims are made for their content. With regard to vitamins and minerals research is continuing to develop more effective water-dispersible forms of the oil-soluble vitamins (A, D, E and K) and carotenoids.

4.11 Conclusions

When trying to formulate a new fortified beverage there is a tendency to lean towards the marketing brief that it must contain specified vitamins and minerals at nutritionally significant levels. As can be seen from the preceding sections of this chapter, there are some products that cannot be produced owing to interactions between ingredients. Vitamin C will not survive in the presence of copper ions, and thiamin (vitamin B_1) can be destroyed by the sulphites often present in glucose syrup and fruit juices. From the first example it can be seen that a liquid product cannot contain both vitamin C and copper, and from the second one that all traces of sulphites and bisulphites must be removed from the ingredients if fortification with thiamin is required.

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5

Fortification of beverages with products other than vitamins and minerals

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Abstract: This chapter looks at current trends in the functional beverages industry and at the functional ingredients used to realize and meet consumer demand. The chapter first describes the health benefits of polyphenols, carotenoids, oils, sterols, natural stimulants and botanicals and discusses their value for functional beverages. Future trends for the functional beverages industry and specifically for the ingredients discussed are then analyzed.

Key words: polyphenols, carotenoids, oils, sterol, natural stimulants, botanicals, functional beverages.

5.1 Introduction

The functional beverages industry is increasingly driven by the consumers' recognition of the link between diet and health, and by the affluence of the aging baby-boom generation that is willing to pay for products that will enhance their lives.

In terms of health concerns, general immunity has become the principal focus of both beverage manufacturers and consumers. This is reflected in the marked rise of the sales of drinks based on probiotics, green tea, antioxidant 'superfruits' such as pomegranate, açai, acerola, noni and mangosteen, and other fruits such as blackcurrants, blueberries and cranberries that can be strongly marketed for their immune benefits. Green tea catechins or polyphenols are also widely used for their antioxidant, anti-aging and skin health properties in the cosmeceutical drinks sector.[1] In terms of heart health, plant sterols and omega-3 feature strongly. Another factor that currently shapes the functional beverage market is the consumers' trend towards natural and organic ingredients in their foods and an increasing distrust of artificial ingredients such as sweeteners or colorings.

These trends offer many possibilities for manufacturers to make their products stand out from the competition by adding natural, and specifically botanical, ingredients. Addressing health concerns through fortification of beverages, specifically by combining multiple ingredients, is an approach uniquely suitable to functional beverages. For many substances, science has provided proof for certain effects on health or well-being, making it possible to increase a product's appeal by adding claims, provided the active substances are present in an effective concentration, and of course provided the claims are within the limits stipulated by relevant regulations.

The sectors for functional beverages that lend themselves especially well for botanical additives are sports drinks and wellness drinks. Popular additives in sports drinks are energy-promoting substances such as caffeine or taurine, and extracts such as epigallocatechin galleate (EGCG). In the wellness sector, near-water beverages with added herbal extracts such as ginger, gingko or Melissa are well established on the market.

Adding large molecules such as herbal extracts to functional beverages, however, presents its own challenges to the manufacturer, since there are stability issues to be taken in consideration. Also, botanical substances are rarely tasteless, especially in concentrations needed for effectiveness, and steps need to be taken to mask any undesirable taste or scent. Lipid substances, too, are not soluble in water-based beverages unless they are microencapsulated or emulsified.

Most of all, taste is still king. The most functional drink will fail if the consumer does not like it. Therefore, care needs to be taken with formulation concepts, ingredient selection and processing in order to create a drink that is stable while tasting good. To avoid much of this, manufacturers are offering premixes that have overcome most obstacles, making it possible for the beverage manufacturer to combine premixes in order to arrive at a new formula.

5.2 Polyphenols

Polyphenols are a group of chemical substances found in plants, characterized by the presence of more than one phenol group per molecule. Polyphenols are generally further subdivided into hydrolyzable tannins, which are gallic acid esters of glucose and other sugars; and phenylpropanoids, such as lignins, flavonoids, and condensed tannins.[2]

The main source of polyphenol antioxidants is nutritional, since they are found in a wide array of phytonutrient-bearing foods. For example, most legumes; fruits such as apples, blackberries, blueberries, cantaloupe, cherries, cranberries, grapes, pears, plums, raspberries and strawberries; and vegetables such as broccoli, cabbage, celery, onion and parsley are rich in polyphenol antioxidants. Red wine, chocolate, green tea, olive oil, bee pollen

and many grains are alternative sources. The principal benefit of ingestion of antioxidants seems to stem from the consumption of a wide array of phytonutrients; correspondingly, the role of dietary supplements as a method of realizing these health benefits is the subject of considerable discussion.[3]

In view of the reported synergistic effects, it makes sense to fortify beverages with combinations of polyphenols rather than with single substances. Many manufacturers offer premixes that not only make full use of these synergistic effects afforded by combining polyphenols, but also have been stabilized for the beverage manufacturing process.

Polyphenols of plant origin are currently under investigation for a multitude of health benefits, mostly with the aim to use them as ingredients in dietary supplements. Much research still needs to be done on recommended daily allowances and other safety issues of these isolated phytochemicals. Generally, polyphenol antioxidants are claimed to reduce the risk of atherosclerosis, reduce development of cancers, reduce oxidative damage to the DNA and thus the aging process, and to be anti-inflammatory.

Among the simple polyphenols, hydroxtyrosol is found in olive oil and fruit. Caffeic acid is part of green coffee extract. Oleuropein is extracted from olives. Ferulic and phytic acid are both found in cereal grains and seeds. All these simple polyphenols have antioxidant activity and act as chain breakers or metal chelators.

Special mention should be made of the green tea catechins. Here, we find catechin, epicatechin, epigallocatechin, epicatechin gallate, gallocatechin gallate, and EGCG. The health benefits of EGCG, in particular, are so manifold that it has been called a 'vitamin'. Among others, it has anticancer and anti-arthritis properties, protects from Alzheimer's, and even has weight loss properties because it can raise the resting metabolic rate (RMR).[4]

Recent research suggests that combining tea polyphenols with citrus juice or vitamin C might increase absorption of antioxidants up to 13-fold.[5] This could be excellent news for tea beverage manufacturers. Another study found that green tea polyphenols inhibit growth of pathogenic bacteria in the gut, but not that of commensal bacteria. This finding indicates that these polyphenols may even have prebiotic properties.[6]

Hydrosytyrosol is an active polyphenol extracted from olive fruit. Glanbia Nutritionals offers OlivActiv, an extract claimed to contain 35% of hydroxytyrosol. Since it is extracted from the olive fruit rather than the leaf, the polyphenol molecules in this extract are smaller and more rapidly and completely absorbed, resulting in greater bio-availability.[7]

Powergrape, a grape extract by French active ingredients manufacturer Berkem, has been standardized in terms of polyphenols and flavanols. The ingredient is especially being proposed to the energy market. Studies supporting the energy claim have been conducted, according to the company.[8]

5.3 Carotenoids

The function of carotenoids in functional beverages is providing antioxidant (singlet oxygen quenchers) properties and acting as secondary stabilizers of other antioxidants. Natural sources of carotenoids include carrots, tomatoes, spinach, maize and citrus. Also, carotenoids are sources of natural colors, and are, as such, preferred by the consumer over artificial colorings.

Carotenoids are oil-soluble. Therefore, making stable emulsions is a prior requirement for adding them to water-based beverages. First and foremost, formulators must consider carotenoids' lipophilic nature. Carotenoids are not water soluble and only slightly oil soluble. To overcome the issue, many suppliers are offering water-dispersible beadlets, which are particularly useful in beverage fortification. One trick to avoid beverage ringing is to first make a carotenoid stock solution – a pre-dilution of the beadlets in water – prior to adding it to the beverage production line. Since most carotenoids are colored, there exist special challenges when adding them to near-water products. Also, carotenoids, like many antioxidants, are sensitive to the presence of oxygen during manufacturing and storage.

Juices are one of the most common product categories incorporating carotenoids. Not only are they colored, their taste also effectively masks any issues arising from added carotenoids. When a color emulsion such as one containing carotenoids is destabilized, an oily ring often forms. This, too can be prevented by utilizing special emulsifier systems.

- *Alpha-carotene*: A provitamin A precursor found in foods such as sweet potatoes, pumpkins and yellow and red peppers. It has half the provitamin A potency of beta-carotene, but is more effective than beta-carotene in quenching singlet oxygen.
- *Beta-carotene*: The all-*trans* isomer of beta-carotene is the major source of dietary retinoids. It functions as a chain-breaking antioxidant by trapping free radicals. It is found in many orange foods (carrots, apricots, cantaloupe) and green leafy vegetables (collard greens, spinach, kale).

  Beta-carotene is a popular replacement for vitamin A fortification. Since it is converted to vitamin A in the body, it is safer to use in a product than vitamin A, which has possible issues of overdose, and is more stable to light. It provides multiple benefits, among them boosting the number of cells involved in fighting infection, while possessing potent antioxidant qualities. Its presence in the body lessens the risk of cardiovascular disease.

  There are some indications from a recent study that long-term supplementation with beta-carotene may reduce cognitive decline. No such indications were found in studies where subjects took the supplements, but the time frame was less than 10 years. This result could prove a convincing marketing argument for fortifying beverages with beta-carotene. Natural beta-carotene sources include broccoli, Brussels sprouts, cabbage, turnip and cauliflower.

There are many offers of beta-carotene powders and emulsions on the market. They typically vary in terms of potency and choice of ingredients. Also, there is a trend in the market towards allergen and animal-free products that has to be taken into account. Among others, Cognis Nutrition & Health offers Betatene natural beta-carotene to manufacturers.[9]

- *Lycopene*: This red pigment is found in watermelon, blood oranges and papaya; however, 85% of American dietary intake comes from tomatoes and tomato products. Lycopene is particularly effective at quenching singlet oxygen free radicals.

5.3.1 Xanthophylls

- *Astaxanthin*: A pigment known for giving the pink color to salmon, shrimp and other seafoods (and fed to farmed fish for this purpose), as well as flamingo feathers. It is a potent quencher of singlet oxygen.
- *Cryptoxanthin*: A provitamin A precursor found in oranges, tangerines, peaches and nectarines that is used to color butter. It is capable of quenching singlet oxygen.
- *Lutein*: Abundantly found in green leafy vegetables and corn, lutein is known to support the prevention of macular degeneration. Most commercial extractions use marigold petals. Lutein is found in highest levels in the retina and protects the eyes by filtering blue light, which can cause photodamage, and neutralizing free radicals.
- *Zeaxanthin*: A complementary carotenoid found with lutein in green leafy vegetables and corn. It is found in the highest levels in the macular region of the eye, and absorbs blue-violet light and quenches free radicals.

DSM has developed patented systems for encapsulation for use in beverage fortification. LycoRed is offering BLT (beta-carotene, lutein and tomato lycopene), a multi-carotenoid blend of various suspensions of micronized nutrient crystals. The concept is a 'dispersible, all natural, ready-to-use formulation'.[10]

Overseal has recently developed a unique high-strength emulsion derived from a natural source of mixed carotenes. The new 10% pigment product completes the Em-Seal® mixed carotene range, aimed particularly at the soft drinks market.[11] A product example for lutein is the functional ingredient Xangold lutein ester from Cognis. LycoRed's own store of branded ingredients, which it also offers independently to the industry: Lyc-O-Beta beta-carotene; Lyc-o-Lutein; and natural Red lycopene.

5.4 Oils

'Healthy' oils offer numerous health benefits. They can also act as vehicles for fat-soluble vitamins and, along with other fats, are important for for-

mulation aspects of taste and mouthfeel. Oils require special techniques such as micro-encapsulation or emulsifying if they are to be added to water-based beverages. They are also often prone to oxidation. Oil-soluble antioxidants such as vitamin E, therefore, are often added in order to extend shelf life.

5.4.1 Omega-3

The key members of the omega-3 family of EFAs are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). EPA and DHA themselves are found naturally in oily fish, while ALA is found in flaxseed and various vegetable oils and nuts. ALA can be converted into EPA and DHA. However, this conversion process is thought by some experts to be slow and inefficient.[12]

The health benefits of omega-3 fatty acids are manifold and undisputed. They promise improved cardiovascular health, joint health, brain development and visual acuity in children. They have also been linked to reduced symptoms of depression, cancer prevention, protection against type 2 diabetes, and alleviation of skin disorders, asthma and irritable bowel syndrome. While not all of these health benefits have been researched completely, new and ongoing studies continue to build confidence in the omega-3 fatty acids market. Like vitamins and minerals, omega-3 and omega-6 fatty acids are essential to our well-being, providing a multitude of benefits which are unmatched by very few nutritional ingredients, which makes these essential fatty acids an attractive ingredient for fortified beverages.[13]

As ingredients, omega-3 fatty acids are susceptible to oxidation and subsequent rancidity problems. Also, they are large, fat-soluble molecules. That makes them inherently unstable, and they will rise like cream in water-based beverages.

Advances in processing technologies have improved the stability of omega-3 fatty acids as functional ingredients and increased the success rate of their inclusion in a diverse array of food and beverage products. Various companies provide microencapsulated or deodorized powders. Microencapsulated powders are processed from fish oil that is emulsified within a solution containing antioxidants and coating agents, which is then dried to form a powdered product.[13]

An alternative to using fish-derived omega-3 oils is sourcing it from algae or flax seeds. This removes one of the more disadvantageous sensory aspects of fish oils – the smell. Other exotic omega-3 sources include blackcurrant, borage, chia, cranberry, hemp, perilla, purslane and pumpkin.

Frutarom USA has also recently launched a range of salvia-derived oils for use as a vegetarian source of fatty acids, among them Alina, a patented plant source of omega-3 fatty acids. These oils, according to the company, are particularly suited to use in functional foods because of good stability.[14]

5.4.2 Conjugated linoleic acid (CLA)

Considered one of the 'good fats', CLA is found, among other places, in cow's milk. In animal studies, small amounts of CLA have blocked all three stages of cancer and have slowed the growth of various tumors. A good supply of CLA in the diet also seems to play a part in the reduction of breast cancers, according to a recent survey. A recent meta-analysis also indicates that CLA can aid in weight loss, especially for yo-yo dieters, as it could potentially prevent weight and fat regain following a diet.

Cognis Nutrition and Health offers Tonalin CLA, sourced from safflower oil, as ingredient to manufacturers.[9]

5.5 Sterols

Phytosterols have a chemical structure similar to cholesterol, yet sufficiently different to block low-density lipoprotein (LDL) cholesterol absorption in the intestine, thus leading to its elimination from the body. In fact, the US Food and Drug Administration (FDA) allows health claims indicating that phytosterol-containing products can lower the risk of heart disease. The claim also covers plant stanols, which are chemically related to sterols in both the esterified and unesterified forms.[15]

The most abundant plant sterols are sitosterol, campesterol and stigmasterol. Plant sterols can be added to just about any food, condiment or beverage. And when adding fiber, formulators can elevate the cholesterol-lowering potential of a particular food. For example, combining ADM's (Decatur, IL) Fibersol 2 or Cargill's Barliv with plant sterols can provide powerful cholesterol-lowering benefits in one product.[16]

Plant sterols – recognized for their cholesterol-lowering power when added to margarines, salad dressings and other fats – have also been found to be effective in reducing 'bad' LDL cholesterol levels, when added to orange juice.[17]

In humans, there is a good likelihood that a dose of 0.8–1.0 g of free sterol equivalents per day, properly solubilized, administered in two or three servings with a meal, will reduce LDL cholesterol by 5% or more and that this reduction in LDL cholesterol will correlate with an approximate 6–10% reduction in coronary heart disease risk at age 70.[18]

A recent study found that concerns over possible over-consumption of plant sterol by regular consumers of products enriched with plant sterol or stanol esters were unfounded. The average intake of sterols by subjects regularly consuming such fortified products was well below the recommended daily intake. However, serum sterol levels rose by 22% (sitosterol) and 103% (campesterol) compared with the subjects who did not use fortified products regularly. Whether the increased serum sterol concentrations result in adverse effects needs to be investigated in future post-launch monitoring studies.[19]

Another study, published in the *American Journal of Clinical Nutrition*, found that daily consumption of low fat milk containing 1.6 g phytosterols was effective in reducing LDL levels by 8% after 6 weeks. Most studies up to now have focused on high-fat products as carriers for the sterols. This result is of particular interest to the functional beverage industry, as low-fat dairy products are rising in appeal compared with full-fat products.[20]

Commercially, plant sterols are currently contained in bars (Logicol – Australia, Benecol – UK), vegetable oils (Ekona – Japan; NutraLease Canola Active – Israel), orange juice (Minute Maid Heart Wise containing Cargill CoroWise plant sterols), mayonnaises (Logicol – Australia), milk (Benecol – UK, Logicol – Australia, SereCol – Argentina), yogurt (Logicol – Australia; Benecol – UK), yogurt drinks (Benecol), soy milk (Pacific Foods), meat and soups (Raisio – Finland), and green teas (Chol zero – Korea). Plant sterols are also being sold or developed mixed with other functional ingredients such as: fiber (Unilever Fruit D'or – France); healthy oils (Benecol Olive Spread – UK); non-absorbable diacylglycerol (Kao-ADM Econa Healthy Cooking Oil; Enzymotec MultOil Platform, ArteriCare products – Israel); almonds, soy protein and viscous fibers.[21]

Cognis is now offering an odourless sterol ester to its customers. The plant sterols that have been offered up to now still had an odour, requiring the manufacturers to process the ingredient further in order to mask or remove the smell. The new sterol esters can be incorporated directly into functional beverages, saving time during production.[22]

5.6 Stimulants

Issues related to energy and vitality are of concern to a majority of consumers in Western countries (Natural Marketing Institute Health and Wellness Trends Data Base information). Here, as in other areas of the functional beverages sector, a preference for healthier energy options with increased interest in botanical and natural products can be observed. Thus, caffeine-based drinks alone have lost popularity to combination energy/wellness drinks.

Herbs and botanicals are added to functional beverages to increase mental performance, while extracts such as caffeine are supposed to combat fatigue. Caffeine is a xanthine alkaloid compound that is found in varying quantities in the beans, leaves, and fruit of over 60 plants, where it acts as a natural pesticide that paralyzes and kills certain insects feeding on the plants. It is most commonly consumed by humans in infusions extracted from the beans of the coffee plant and the leaves of the tea bush (*camellia sinensis*), as well as from various foods and drinks containing products derived from the kola nut or from cacao. Other sources include yerba maté, guarana berries, and the Yaupon holly.[23]

Caffeine is a popular additive to energy drinks. It has a bitter taste, requiring masking, e.g. with homoeriodictyol, the main bitter-masking flavanone in the Californian evergreen shrub *Eriodictyon californicu.* Another way to make caffeine more palatable when added to beverages where such a taste is not desirable is microencapsulation. It has the added benefit of having better control over the final caffeine concentration than pure caffeine extract. Most energy drinks contain the same amount of caffeine as in a cup of coffee – around 80 mg.

Popular botanicals used in functional beverages to combat fatigue include bee pollen, royal jelly, ginkgo biloba, guarana and ginseng. For sexual energy, fo-ti, gotu kola, sarsaparilla, saw palmetto, yohimbe and Siberian ginseng are commonly used. Ginkgo biloba is purported to provide mental energy and acuity by increasing blood flow to the brain. While there is no conclusive evidence that any of these botanicals enhance energy, research is currently underway in several countries.

Most botanicals are added to beverages as standardized liquid extracts with water/alcohol or glycerine as solvents. Fully water-soluble spray-dried premixes are also available.

5.7 Botanicals

Adding botanical extracts to functional beverages poses the problem of balance. In order to add the extract in question in a concentration high enough to achieve any effect, issues of solubility, taste or texture must often be faced. Also, many botanical extracts have antioxidant properties, making them sensitive to the presence of oxygen during storage or during the manufacturing process.

5.7.1 Superfruits

The so-called liquid botanicals market, consisting of juices made of superfruits, has experienced steady growth despite otherwise flagging supplement sales. This effect is, to some extent, ported over to the functional foods and functional beverages segments.

The off-mentioned superfruits, filled to the brim with antioxidants, vitamins, and other beneficial substances, are açai, mangosteen, pomegranate, noni, guarana, cupuacu, goji, blackcurrant, blueberry, bilberry, raspberry, and other familiar berries. They are so beneficial, in fact, that some manufacturers are offering whole berry extracts as ingredients, most notably for the cosmeceutical and beverage industry for flavoring purposes and as bases for functional fruity beverages.

The exotic flavour aspect of superfruit extracts is not to be underestimated. Consumers have become ever more subject to what has been called ‘organoleptic boredom’, leading them to be increasingly adventurous in

matters of taste. This opens up opportunities for adding exotic flavors, as they are not only new but also perceived as being particularly healthy.

Of the superfruits, mangosteen deserves special mention because of its status as the current fashion ingredient. It is called the Asian 'queen of fruits', originating in the Sudna Islands and the Moluccas; a tropical evergreen whose fruit look like apricot. Mangosteen started its ascent to popularity as a blend of fruit puree and juice in the spring of 2006. Current mangosteen beverages include liquid botanical supplements with mangosteen juice and aloe vera, but also mainstream products such as tea with mangosteen, or with added pomegranate. The fruit has a bitter flavor, making it necessary to blend it with other fruits in order to mask the taste.

Another popular superfruit, açai, came to popularity in 2006. The açai palm grows in Brazil, in the lower Amazon basin. The ripe fruit resembles as purple grape and has a berry-like, tropical flavor. Açai is typically frozen and exported, but extract formats are also available. The superfruit has since expanded into products such as smoothies and frozen deserts, and even into the ice cream market. It is most commonly combined with other high antioxidant botanicals such as blueberry, pomegranate, or raspberry.

Acerola, another Brazilian superfruit, is found in the West Indies and Northern Brazil. It looks very much like a cherry. Its vitamin C content is 30 times more than that of an orange. Among its supposed health benefits are improved cardiovascular health, boosting the immune system and giving joint support by helping to form natural collagen. Acerola's high antioxidant content is thought to prevent the formation of cancerous cells.

Noni, grown in French Polynesia, is an oval-shaped tropical fruit with a pungent cheesy odour when ripe, requiring blending or masking in formulation. It is loaded with antioxidants and has many medicinal properties.

Sea buckthorn berries, grown primarily in the Himalayas, are rich in a variety of antioxidants including extraordinarily high levels of vitamins C and E, carotenoids, including beta-carotene, omega-3 and omega-6 fatty acids and flavonoids, which have been shown to improve the immune system and cardiovascular and brain circulation. These berries, which have been likened to the flavor of passionfruit, are too acidic to eat fresh for most palates, but the juice is said to mix very well with other juices and the powdered version can be used in bars and other baked goods.

5.7.2 Teas

Tea is a functional beverage in and of itself. Its health benefits as a nutraceutical include antioxidant, antibacterial, antiviral and prebiotic activity. It promotes growth of certain probiotic bifidobacteria and lactobacilli, it inhibits the enzymic activity of cariogenic bacteria and adherence of bacterial cells on tooth surface, and it strongly inhibits the adherence of the bacterium *Porphyromonas gingivalis* (a plaque bacterium).

Green and white teas, as opposed to black tea, all of which are sourced from *C. sinensis* are increasingly regarded as particularly healthy by consumers. Green tea extracts, most notably the catechins, are a popular additive to energy drinks, but also to the so-called wellness drinks. One of the catechins in particular, namely EGCG, has been identified as particularly efficacious in raising the RMR, and has advanced to one of the most popular additives in energy drinks. Green tea extract has a naturally bitter taste, especially in higher concentrations, that needs to be masked.

Teas not sourced from *C. sinensis* include rooibos, yerba maté, and various herbal teas.

Oxygen radical absorbance capacity (ORAC) is a method of measuring antioxidant capacities of different foods. An assay measures the oxidative degradation of a fluorescent molecule (either beta-phicoerythrin or fluorescein) after being mixed with free radical generators such as azo-initiator compounds. Azo-initiators are considered to produce peroxyl free radicals by heating, which damage the fluorescent molecule, resulting in a loss of fluorescence. Antioxidants are able to protect the fluorescent molecule from the oxidative degeneration. The degree of protection is quantified using a fluorometer.[24]

Red rooibos and white Fujian teas have comparable ORAC values to that of green tea, and Darjeeling Makaibari extract boasts almost two times the ORAC value of green tea according to data from Moore Ingredients. Green tea, however, is the most popular because of its high consumer recognition and proven health benefits, according to Tony Moore, owner of Moore Ingredients.[25] He also added that because it is much easier to extract polyphenols, the antioxidant-rich phytonutrient in green tea, suppliers are able to offer green tea at lower prices and maintain higher availability. This has not stopped processors from using other teas, though. For instance, Luna has created a line of Tea Cakes, each featuring a different tea including rooibos, white and green.

Another tea growing in popularity is yerba maté, which is being used in functional beverages not only for its high antioxidant content, but also for its natural energy boost. It has about 90% more antioxidant power than green tea according to studies from Brunswick Laboratories. On top of that it contains three different natural stimulants, caffeine, theobromine and theophylline, which come together to stimulate the body in a more balanced way both mentally and physically.

5.7.3 Other extracts

Hibiscus extract

An extract of the hibiscus flower may have the same health benefits as red wine and tea according to pre-clinical studies.[26] This makes hibiscus an ingredient with a healthy aspect for teas or near-water beverages.

Elderberry extract
Elderberry is a popular functional food ingredient because of its flavour and its health benefits. The ingredient is a concentrated source of anthocyanins. Elderberry anthocyanins enhance immune function by boosting the production of cytokines.[27]

Fruit peel extract
Oils from citrus peels such as grapefruit peel oil in an encapsulated form are offered by manufacturers such as Cargill. These oils add a sensory component to beverages.

Rosehip extract
The sour note contained in rosehip extract is a popular masking agent, for instance in masking the grassy notes of yerba maté.

*Tamarillo extract (*Cyphomandra betacea*)*
Tamarillo, a member of the nightshade family, is native to the Andes of Southern America. Tamarillos have a fruity, sweet-sour taste, mixed with a hint of tomato.

*Kumquat (*Fortunella margarita*)*
A member of the rue family, kumquat resembles a small orange, but is botanically speaking not a citrus fruit. The fruit pulp and peel are usually consumed together, and the combination of the sour pulp and the tart-sweet taste of the peel is a very intense taste experience.

*Cherimoya (*Annona cherimola*)*
This fruit was cultivated for centuries by the Inckas. Its soft, cream-like pulp resembles in taste a mixture of custard and strawberry, with a touch of cinnamon.

*Rambutan (*Nephelium lappaceum*)*
A native of the Malaysian archipelago, rambutan is a tropical relative of the lychee. It is similar in taste to the lychee but much sweeter and more aromatic.

*Grenadilla (*Passiflora ligularis*)*
Belonging to the passionflower family, the grenadilla is indigenous to the Andes. It received its name from the Spanish conqueror. Grenadilla has a very aromatic and stimulating sweet-sour taste, and exotic mixture of peach, apricot, strawberry and raspberry.

*Kiwano (*Cucumis metuliferus*)*
Originating in the steppe of the Kalahari Desert, kiwano has been a popular fruit for over 3000 years. Its taste is reminiscent of melon and cucumber, but also of lime and banana.

*Bitter orange (*Citrus aurantium*)*
This weight-loss ingredient has been suggested as exhibiting thermogenic effects similar to that of ephedrine. Recent studies have identified a selection of specific isomers including *p*-synephrine and *m*-synephrine as the active constituents in citrus aurantium.[28] The primary product channel is in dietary supplements, but soft drinks and functional drinks are becoming more popular.

An example for a soft drink that uses bitter orange is Arkopharma 4321 Shake Up drink launched in the UK in May 2004 by Arkopharma UK Ltd. This drink contains a blend of 10 plant extracts that are designed to aid consumers to lose weight on a calorie-controlled diet.

Korean pine seed oil (containing pinoleic acid)
Pinoleic acid is a fatty acid which is suggested to suppress appetite The product Beautiful Water by Japanese company Suntory is an example for a beverage application of pinoleic acid. Another example is the clinically tested branded ingredient PinnoThin by Lipid Nutrition.

*Ginkgo (*Ginkgo biloba*)*
Standardized on ginkgolides and flavonoids, gingko extract has neuroprotective, antioxidant an anticoagulant properties. A multitude of clinical trials has provided positive evidence for cognitive/memory enhancing effects in older persons.[29] Ginkgo extract is added to wellness drinks such as Bionade (Rhön-Sprudel, Germany).

*Cinnamon (*Cinnamomum verum*)*
Several trials have suggested that cinnamon extract may have blood glucose and lipid-managing activities, making the extract an attractive ingredient for diabetes management treatment.[30] Other studies have reported that cinnamon can reduce risk factors associated with cardiovascular diseases.[31] On the functional beverages side, adding cinnamon to chocolate drinks is not only a tasty, but also an effective option with, in fact, at least several centuries of tradition behind it. Cinnamon extract is offered commercially as Cinnulin PF by Integrity Nutraceuticals International.

*Bitter melon (*Momordica charantia*)*
Another ingredient with a long tradition and claimed efficacy on blood glucose management, bitter melon is used in the beverage industry as tea (e.g. Charantea Ampalaya Bitter Melon Tea).[32]

5.8 Future trends

The global challenges of rising obesity, diabetes, and other health concerns, together with the aging population worldwide, will continue to increase

consumers' interest in healthy food. Functional beverages, positioned between nutraceuticals and foods, will continue to gain ideas from nutraceuticals and pass them on to functional foods and foods in general. An example for this is the use of acerola, which is as yet limited to liquid botanicals, a nutraceuticals sub-category, and to functional beverages, and is awaiting its inclusion in functional foods.

Consumers will demand products that contain fruit. Nutritional benefits aside, fruit are an attractive sugar replacement, provide texture and help with moisture control. Also, they have an inherently 'healthy' image, making consumer education as to their respective benefits particularly easy. Concurrently, the demand for organic ingredients, particularly for organic fruits in functional beverages, will pose challenges to suppliers.

On the juice side, juices not-from-concentrate are expected to rise as compared with concentrate-based juices. These juices, which are not concentrated after pressing but are bottled instead, meet consumer demands for natural products. According to GfK ConsumerScan estimates, the potential of these juices has by no means been fully tapped. They are particularly popular in the United Kingdom, and sales in Germany are growing, while sales of juices made from concentrates are sinking.

The functional beverages market is open to new, exotic ingredients with ethnic histories or proven efficacy in the nutraceutical sectors. The new segment of liquid botanicals will influence beverages, as will cosmeceuticals, both of which will pave the road by making consumers more open to the idea of being healthy to look healthy.

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6

Extended shelf-life beverages

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Abstract: This chapter illustrates how the factors important for food quality and safety are related to processing and packaging concepts for extended shelf-life beverages and liquid food products. It first reviews current processing, sterilisation and filling methods for aseptic and extended shelf-life liquid food products in cold chain distribution. The effects of storage on product quality are then considered. Finally emerging technologies for product processing, package sterilisation and active packaging concepts are described.

Key words: food processing, food packaging, aseptic packaging, extended shelf-life, active packaging, beverages, functional foods.

6.1 Introduction

Increased consumer demands, coupled with ever-widening distribution networks, have driven the development of processes and packaging concepts for extended shelf-life (ESL) liquid foods in cold chain distribution. Such liquid foods include low-acid products, such as milk and milk products, soya beverages and rice drinks; and high-acid products, including juices, nectars and ice tea. In addition, these foods contain emerging sectors such as sports drinks and functional beverages. Production of ESL foods to comply with legislative, including food safety requirements, such that the consumer purchases a product of the highest quality, is a major challenge for the food industry. This chapter provides an overview of the processing methods and packaging technologies which have been developed to extend the shelf-life of liquid foods in cold chain distribution. The relationship of these technologies to those developed for aseptic packaging is also considered. An overview of key concepts and terminology relating to ESL and aseptic packaging is provided in Brody (2005).

It is widely recognised that the term extended shelf-life is imprecise, with different meanings for different products (Rysstad and Kolstad, 2006). A pragmatic definition of ESL refers to processing and packaging solutions

which extend the shelf-life of a chilled product beyond that of the pasteurised product. A characteristic of ESL products, therefore, is the processing methods used to maintain the freshness and nutritional quality of the product. Typically ESL products are not classified as sterile and the activity of residual microorganisms may contribute to the limit of shelf-life.

In contrast, aseptic products are commercially sterile. They differ from conventional packaging solutions in that the product and the package are sterilised separately, followed by filling and sealing of the product under sterile conditions. This process places high demands on the performance of the packaging process.

Three key elements need to be considered for both ESL and aseptic packaging concepts:

1. ***Packaged product***: including microbiological load; physical properties; organoleptic sensitivity to processing methods; and susceptibility to oxidative changes.
2. ***Packaging***: including product processing; packaging sterilisation methodology; filling processes; permeability characteristics; and interactions between product and package.
3. ***Storage conditions***: including shelf-life, as well as temperature profile and light exposure through the distribution chain to the retail display cabinet.

The interactions between these elements (Fig. 6.1) need to be fully understood in order to devise robust packaging solutions.

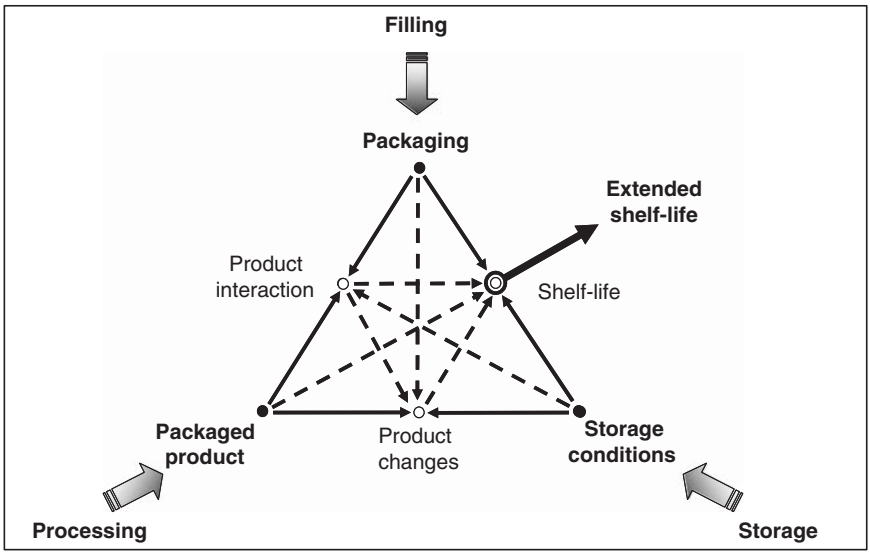

Fig. 6.1 Interactions between factors which influence the shelf-life of liquid foods.

ESL solutions place the highest demand on each of these elements and the interactions between them have increasing significance as shelf-life is extended. During extended storage in refrigerated conditions, it is inevitable that changes in the product will take place which have a negative impact on sensory and nutritional quality, and will therefore limit the shelf-life. Such changes can arise due to (i) the activity of enzymes or microbes present in the packaged product; (ii) chemical changes in the product including oxidation; and (iii) interactions with the packaging material. For these reasons a detailed understanding of all of the components is crucial for the development of an ESL solution, and total systems approaches which take account of all of the contributing factors are essential for success. Critical path analysis of an ESL packaging process can identify the operating criteria which limit shelf-life, thereby facilitating optimisation of control parameters and hence shelf-life.

6.2 Processing methods for aseptic and extended shelf-life products

While the main aim of pasteurisation is to render food safe for human consumption, processing of ESL products must by definition result in food with a shelf-life beyond that achieved by normal pasteurisation. From the processing and packaging perspectives, both pasteurised and ESL products are designed for chilled storage and distribution. Aseptic processing and packaging, on the other hand, is employed for ambient distribution chains and the products must therefore be commercially sterile.

The requirements of processing equipment and conditions are also very dependent on the food to be processed. Low-acid products such as milk and soya beverages support the growth of most microorganisms, including bacterial spores and pathogenic bacteria. Consequently, processing conditions must be chosen to reduce the resistant spore load to an acceptable level, and high safety standards must be implemented in the filling system to prevent any recontamination by pathogenic microorganisms. Since fruit juices and drinks are generally high-acid products in which bacterial spores cannot grow, the processing requirements for this group of products are different. Typical process conditions for producing commercial sterile products are 90–95 °C for 15–30 s in the case of high-acid foods, and 140–144 °C for 2–4 s in the case of low-acid foods.

In the following section a number of processing methods such as indirect and direct heat, as well as physical methods based on microfiltration, are illustrated with examples from a range of products including milk and soya-based products, as well as fruit juices and drinks.

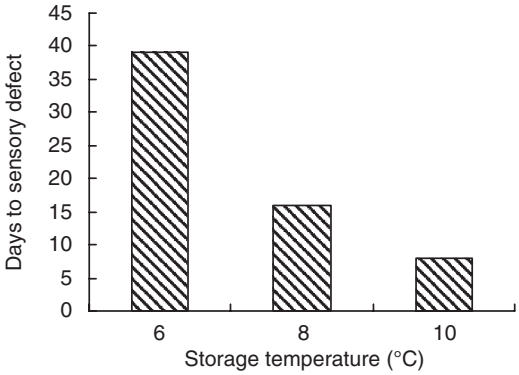

Fig. 6.2 Effect of storage temperature on time for sensory defect to develop in aseptically filled pasteurised skimmed milk.

6.3 Processing of low-acid milk and milk-like products

Various methods can be used to increase the shelf-life of milk or soya-based products in addition, or as an alternative, to standard pasteurisation. This section will give detailed examples of processing of milk, but most of those processes can in principle be used for other low-acid products such as soya or rice-based beverages.

In choosing a method it is important to consider the target shelf-life, the product characteristics, the distribution conditions and the effect of chemical degradation on the flavour profile. In several countries it is extremely difficult to achieve a shelf-life of more than 10–12 days for pasteurised milk at a distribution temperature of up to 8 °C owing to the presence of psychrotrophic spores (of *Bacillus* species) in raw milk which survive pasteurisation and are capable of growth between 6 and 10 °C. As a result, even with no recontamination from aseptic valves, tanks or the filling machine itself, it is difficult to reach a shelf-life of 12 days at 8 °C. To a first approximation every 2 °C reduction of storage temperature results in a doubling of shelf-life (Fig. 6.2).

Methods such as bactofugation and microfiltration can further increase the shelf-life of the milk distributed in a robust cold chain (<6 °C), but if the quality of the cold chain is inferior, some additional heat treatment is normally required. The challenge is to balance the required heat treatment in order to achieve the required microbial kill effect against the effect of temperature on the quality of the product. Reduction in spore load is only 1–2 log for bactofugation and 3 log for microfiltration, while for high-temperature processed milk, the load of critical spore formers may be reduced by more than 8 log (Fredsted *et al.*, 1996).

Current methods used commercially for producing ESL milk are microfiltration, direct heat treatment such as injection or infusion, or in some

Table 6.1 Overview of different treatments of pasteurised and high-temperature pasteurised milk, and the effect on shelf-life and chemical degradation of β-lactoglobulin

Process	Log reduction in aerobic psychrotropic spores	Expected shelf-life (days) stored at 6 °C maximum	Expected shelf-life (days) stored at 10 °C maximum	β-Lactoglobulin (mg/l)
Pasteurisation	0	10–12	3–4	4225
Centrifugation	1	14	4–5	>4000
Microfiltration	2–3	21	6–7	>3500
Pure-Lac ESL Pasteurisation	8	Up to 45	Up to 30**	>3000
Infusion UHT	8*	180	180 at 25 °C**	>1400
High heat process	40	180	180 at 25 °C**	>250

* Thermophilic spores remain.
** Depending on filling solution.

cases indirect heat treatment. A comparison of pasteurised and high-temperature heat treatments is given in Table 6.1.

6.3.1 Microfiltration

The principle of microfiltration is to remove bacterial cells and spores mechanically from the milk using membrane processing where the pore size of the membranes used is much greater than in the cases of reverse osmosis and ultrafiltration (Maubois, 1997). Two constraints limit the application of microfiltration to ESL processing (Kessler, 1997). First, owing to the similar size of cells and spores to milk fat globules, it is not possible to microfilter whole milk; the milk must first be centrifugally separated and the skim milk microfiltered. Second, the overlap of the size distribution of cells and spores with that of casein micelles requires a compromise in the pore size used. In order to minimise changes in milk composition, ceramic membranes with pore diameters of 0.8–1.4 μm are commonly used commercially (Kessler, 1997; Russell, 1998). Pores of this size allow some bacteria to pass through the membrane, and thus the final milk must be pasteurised to ensure the elimination of vegetative pathogens.

With a high-quality cold chain, microfiltration is an excellent process for producing ESL milk with a shelf-life of up to 3 weeks. Although there are constraints concerning viscosity and particles, new technology with inline post-process dosing may open up a wider product portfolio for this technology. Microfiltered milk is marketed in several countries as more ‘pure’ and

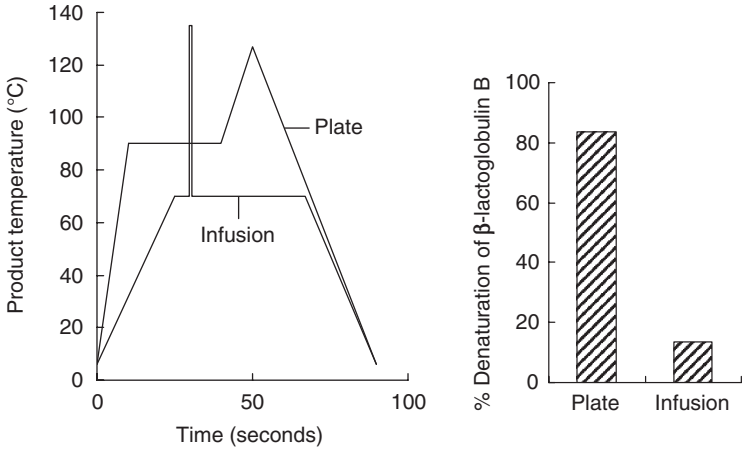

Fig. 6.3 Time–temperature profile for indirect (plate) and direct (infusion) heating in ESL systems and effect of heat exposure on denaturation of β-lactoglobulin.

'natural' than standard heat-treated milk, and has achieved a higher price as a branded product.

6.3.2 Heat treatment

When milk is distributed in a sub-optimal cold chain (>7 °C), a shelf-life of 3–6 weeks can be achieved only by high-temperature pasteurisation. Comparison of the effect of time and temperature on the microbiological kill rate and chemical degradation rate shows that the greatest kill rate and lowest chemical degradation are obtained by combining a high temperature with a short holding time (Kessler, 1989). In order to minimise chemical and sensory degradation at a high processing temperature, indirect heating methods are not ideal. Comparison of temperature–time profiles (Fig. 6.3) shows reduced degradation of milk processed with direct heating. Thus if a long shelf-life (>3 weeks) is required, or the temperature of distribution is above 7–8 °C, direct heating technology is recommended for ESL milk processing. Direct heating by infusion allows high-temperature heating combined with a very short and controlled heating time. This results in a good kill rate, as well as retention of excellent product sensory properties (Fredsted *et al*., 1996).

The choice of method, however, will always depend on a combination of factors including target flavour profile, as well as investment and running cost of the total system. When considering a new ESL processing and packaging system, it is therefore recommended that the following issues are addressed in order:

1. Set target for shelf-life.
2. Analyse raw milk quality (with a focus on psychrotrophic spores).
3. Evaluate product range (e.g. white milk, flavoured, physical structure).
4. Quality of cold chain.
5. Distribution method and channel.

6.4 Processing of juice and drinks (high-acid products)

Although there may be many similarities to processing equipment for pasteurised or ESL dairy products, several key factors must be considered when selecting a processing system for high-acid products:

- The type and range of product to be processed, e.g. pH; viscosity; and presence of fruit cells or particles.
- Shelf-life and distribution conditions.
- Processing, including requirements for homogenisation, special mixing or deaeration.

Based on the above information, the most suitable processing equipment and layout can be designed. Heat treatment of these products is normally achieved with indirect systems based on plate heat exchangers or tubular heat exchangers (Fredsted, 1995).

6.4.1 Plate heat exchangers

Plate heat exchangers allow varying flow patterns, the use of multi-channel passes and asymmetric counter-current and co-current flows. These features allow an optimal balance between thermal transfer and pressure loss in any given situation.

Plates are available with numerous flow profiles, e.g. thermally short (soft) vertical and thermally long (hard) horizontal profiles. The thermally long plate produces greater turbulence and thereby higher transmission rates than the thermally short plate; the latter offers a lower pressure drop. By combining the two, a more optimal heat transfer/pressure drop ratio suitable for the product can be designed. Plates are supplied in a wide range depending on product and required throughput, comprising different plate surfaces, thickness and gaps between the plates for use with products with particle sizes up to 6 mm or fibres up to 12 mm length and a diameter of 1 mm.

There are three major categories of plate type pasteurisers on the market, which normally use product-to-product regeneration, making the plants cost effective with the highest heat regeneration values:

1. Plates with multi-contact points for high efficient heat transfer between the media with various flow configurations.

2. Plates with free flow channels and with metal-to-metal contact lines for products containing pulp, pieces and short fibres.
3. Plates with free flow channels and seal contact lines for products with higher contents of pulp, pieces and long fibres.

Operating pressures depend on the plate and frame construction and range from 6 to 25 bar.

6.4.2 Tubular heat exchangers

Tubular heat exchangers are extensively used in the fruit and drinks industry owing to their flexibility in handling a variety of products with varying viscosities, cell content and particulates. Improvements in hygienic construction, production run times and minimised mixing phases at product change have contributed to their wide acceptance. Three principal types of tubes are available (Fredsted, 1995; Fredsted, personal communication 2007): mono/triple tubes (Fig. 6.4(a)); multi-tubes with a fully welded heat exchanger bundle or with an exchangeable tube bundle (Fig. 6.4(b)); and multi-tubes with corrugated tubes or flow distributors which avoid blocking from fibres, resulting in extended production run times.

Multi-tubes are widely adopted owing to efficient heat exchange values, flexibility, lower investment costs and high regeneration ratios. Intermediate heat transfer with a water loop is normally used, resulting in larger regeneration surface than in ordinary product-to-product regeneration.

6.4.3 Deaeration

Deaeration of juice and juice products/drinks is normally carried out to reduce the oxygen load of the product, to minimise the tendency of blockages in the pasteuriser line; to avoid oxidation of vitamins and other chemical changes in the final product; and to reduce foaming and improve accuracy during filling. When filling transparent bottles with products containing pulp

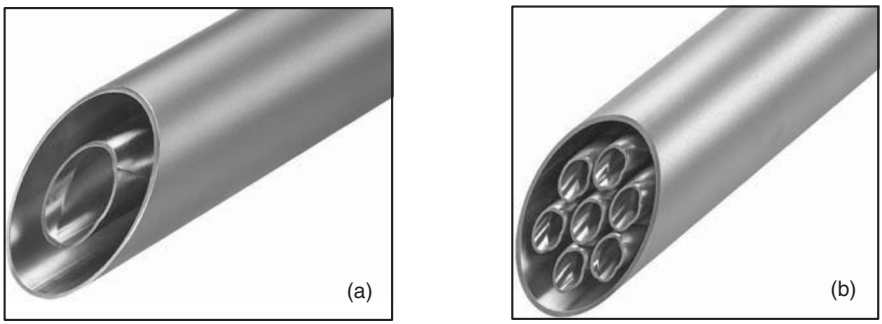

Fig. 6.4 Comparison of structure of monotube (a) and multi-tube (b) heat exchangers. With courtesy of Gea Process Engineering.

and fibres, deaeration avoids floatation of fruit cells. Product stability is enhanced by effective deaeration and the recommended oxygen load after deaeration should be less than 2 ppm.

Ambient deaeration can be used at elevated temperatures to reduce the 'air loads' of products, but efficient oxygen reduction requires vacuum deaeration at 55–65 °C. The vacuum process results in extraction of flavours with the vapour from the product, which must be condensed by means of a suitable heat exchanger and recovered into the product flow.

6.4.4 Homogenisation

Homogenisation has become more important in recent years for premium quality products and products with high viscosities. It can result in taste and colour improvement, reduction of separation effects from pulp as well as micro-fibrillation of fibres. The aim is to achieve a size reduction of the particles to produce a homogeneous product in which the remaining/resulting particles do not separate in the package. Stabilisation is achieved by released cell ingredients (e.g. pectin) and by increased water binding forces due to the large contact surface of particles.

6.5 Filling methods for aseptic and extended shelf-life products

Although there are several potential recontamination sources from liquid food processing to packaged product (Fig. 6.5), it is generally accepted that the most critical step is the filling machine (Eneroth, 1999; Rysstad and Kolstad, 2006). Several critical operations occur in the filling machine and the product may be exposed to the atmosphere from filling until sealing of

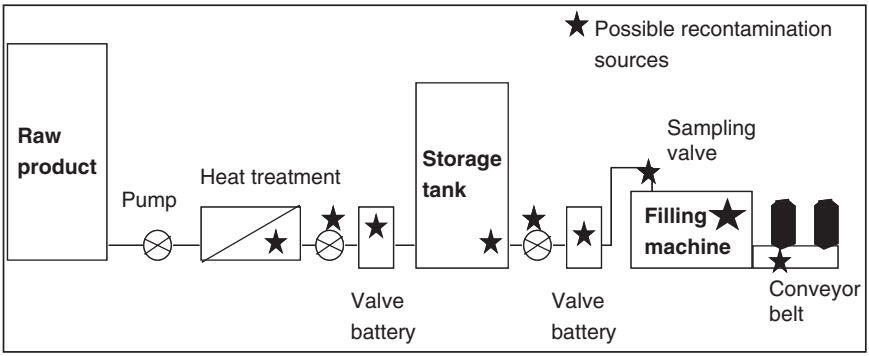

Fig. 6.5 Main sources of recontamination in a standard pasteurised or ESL milk processing and packaging line.

the package. Demand for hygienic filling equipment in the food industry is therefore increasing, and it is the responsibility of the supplier to ensure adequate hygienic design of their equipment. Various legislative and industry standards require that handling, preparation, processing and packaging of food is done hygienically, with hygienic machinery, in hygienic premises. It is, however, left to the industry to decide how to comply with these requirements.

The European Hygienic Equipment Design Group (EHEDG) is a consortium of equipment manufacturers, food industries, research institutes and public health authorities which issues guidelines on hygienic design that are used for standards produced by the European Federation of Standardisation Institutes (CEN). EHEDG actively promotes global harmonisation of guidelines and standards: the US-based organisations NSF and 3-A have agreed to cooperate in the development of EHEDG Guidelines and in turn, EHEDG cooperates in the development of 3-A and NSF standards.

The EHEDG (2001) have described proven methods for testing the performance of the various functions of packaging machines, and thus provide the industry with independent criteria and challenge test methods to compare products from different suppliers. In order for a processor to evaluate different packaging systems, the nomenclature itself is not critical, but both hygienic challenge tests and criteria must be evaluated. The EHEDG has not defined the various filling machine classifications, and there is no common recommendation or legislation. It is therefore important that the supplier states the performance level based on a set of accepted challenge tests, and that the processor can independently compare different systems based on comparable test results.

VDMA (German Engineering Federation) has recently, through expert working groups, recommended standards and procedures for both classification and verification of filling machines for the food industry. One important factor is to choose the correct filling machine concept for the application, taking account of product, shelf-life requirement and storage temperature. In choosing the machinery, attention has to be paid first of all to the systematic implementation of hygienic design criteria. Furthermore, depending on the application, the machines may be equipped with additional functions to improve product protection. At the upper end of the scale are aseptic machines with capabilities described by VDMA (2002). Below this level of performance there has so far been no uniform terminology for machine concepts.

Various terms such as ‘semi-aseptic’ and ‘clean or ultraclean designs’ are used by machine manufacturers for comparable machine concepts. Sometimes, however, the same term is used for different machine concepts by different manufacturers. This led to a working party within the Packaging Machines Division of the VDMA to develop a classification of hygienic filling machines on the basis of the features with which the machines are equipped. By enumerating typical fields of application of each category of

machine, this classification will aid selection of the machine category suitable for packaging of a particular product. Assignment of products to machine categories is often difficult due to the diversity and variability of product characteristics.

VDMA classified filling machines into five different groups based on hygiene features, application and expected shelf-life (VDMA, 2002). Industry standards may differ from this classification, which can make comparison between different suppliers complicated. It is therefore necessary that the suppliers explain and document the hygienic features and performance of the machine and design criteria, as well as challenge test methods and results from tests.

6.6 Aseptic packaging technologies for shelf-life extension

Unlike 'Ultra Clean' or 'ESL' systems, aseptic processing and packaging is fairly well defined, for example by Codex Alimentarius (1993): 'Aseptic processing and packaging means the processing and packaging of a commercially sterile product into sterilised containers followed by hermetically sealing with a sterilised closure in a manner which prevents viable microbiological recontamination of the sterile product' (Fig. 6.6). Similar definitions are provided by FDA (2007) and VDMA (2006). It follows that filling machines for aseptic filling require the highest hygiene levels and control systems.

Aseptic filling machines must be designed to fill low-acid products (i.e. UHT milk and similar) with sufficient safety for ambient distribution. Hence, in order to ensure aseptic operational reliability of aseptic packaging machines, four conditions must be satisfied (VDMA, 2006):

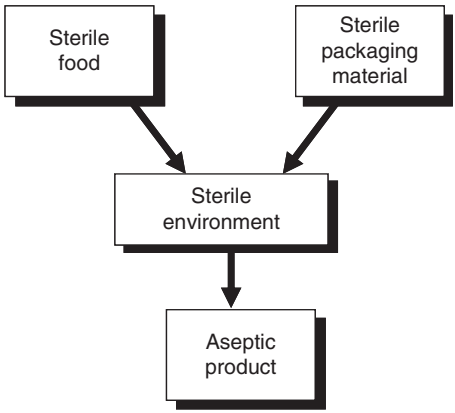

Fig. 6.6 Schematic of aseptic packaging process.

1. The packaging machine must be technically suited to reliably kill micro-organisms including bacterial spores.
2. By means of appropriate control techniques, the machine manufacturer must ensure that the product to be packaged cannot be contaminated as a result of technical faults in the aseptic part of the packaging machine. If, nevertheless, such faults occur, it must be ensured that contaminated packages are either avoided or detected and discarded.
3. The initial microbial count of the packaging material and of the aseptic part of the packaging machine must be minimised to the unavoidable limit by suitable technical and organisational measures.
4. Observance of the preventive organisational measures must be ensured by way of a suitable quality system of the operator of the machine unit.

Detailed requirements of kill rate on packaging material, machine surfaces, quality of air and other critical parameters are given by the FDA (2007) and VDMA (2006).

6.6.1 Aseptic packaging systems

The main aseptic filling systems can be categorised into the following groups:

- Paper-based systems: web-based and blanks-fed.
- Plastic bottles: preformed, sterilised on the machine; blown in line with the filling machine (two-stage poly(ethylene terephthalate) (PET) blow moulding) but sterilised on the filler; and aseptically blow-moulded (high-density polyethylene (HDPE) multi-layer), with closed tops that are cut open inside the filler, still sterilised.
- Pouch systems: flexible pouches and stand-up pouches.

Tetra-Pak's roll-fed system pioneered aseptic packaging technology, and is the leading aseptic packaging system today. It comprises a sterilisation system where the carton web is treated with 35% hydrogen peroxide at high temperature. As the web passes through the peroxide bath, the inside and outside as well as any raw edges of the board are fully exposed to the sterilising agent, ensuring a high kill rate of the web forming the package. Peroxide residues are reduced by means of a 'hot air knife'. The sterilised web is subject to a folding process creating a continuous 'tube' which is filled with sterile product and then subsequently sealed and cut.

One alternative to the Tetra Brik system is a blanks-fed system deployed by SIG Combibloc. Separate flat blanks are individually bottom sealed on the filling machine, followed by sterilisation with 35% peroxide gas in the sealed and preheated carton. Laminar high-efficiency particulate air (HEPA) flow is secured by a relatively small aseptic chamber with an air outlet in the bottom of the chamber.

From an industrial application perspective, it is useful to distinguish between low-acid aseptic filling (for pH >4.5 – typically milk or soya-based products) and high-acid (pH ≤4.5 – typical juice and drinks) systems. An aseptic filling system as described above will fulfil the criteria for both high- and low-acid foods, but there are several systems in the market designed for aseptic packaging of juice and drinks only. FDA requirements are only applicable for low-acid foods, and therefore systems for juice and drinks do not fall under this legislation in the United States. Depending on specification and application, high-acid aseptic filling machines may differ considerably from those approved for low-acid applications with regard to required microbial safety (especially against bacterial spores), the required safeguards against operating error and the level of in-process control.

In Europe there is no legislative body specifying criteria for classification of these types of machinery, but VDMA (2006) has proposed a distinction between Class IV and Class V machines (Table 6.2).

Table 6.2 Examples of differences between Class IV and Class V machines (after VDMA 2006)

Technical characteristics	Class IV	Class V
Microbiological protection of the sterile space with regard to manual intervention by the operating staff	No	Yes
Duplicate monitoring of microbiologically relevant areas	No	Yes
Vapour locks at ends of product-conveying and cleaning in place (CIP) pipes	No	Yes
Control-based protection against possible operating errors having a microbiological risk by operators	Only to a limited extent as agreed with operating company	Maximum technically possible and reasonable protection
Self-check functions and parameters for hygienic operation e.g.: • Sterilisation temperatures • Sterile air volumes • H_2O_2 consumption • Sealing temperatures • CIP temperatures • Valve settings	Only to a limited extent in line with the application and as agreed with operating company	Maximum technically possible and reasonable protection

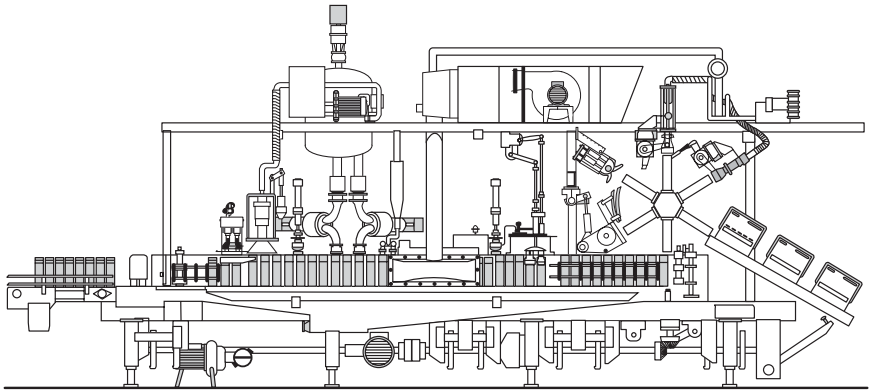

Fig. 6.7 Schematic drawing of the Elopak S-PS80HA filling machine for high-acid aseptic applications.

An example of a high-acid machine for aseptic filling of juice and drinks is Elopak's S-PS80HA (Fig. 6.7) in which carton blanks are fed into the magazine on the right in the illustration, and sealing of the bottom is done in the mandrel section by heating and pressure pads. Then, the package is transferred to a conveyor in the aseptic chamber followed by sterilisation of the packaging material, filling and top sealing of the container before the unit is discharged at the outlet of the sterile chamber. Sterilisation is achieved by the synergistic bactericidal effect of low concentrations of hydrogen peroxide and UV light (Bayliss and Waites, 1979) which typically achieves a 5 log reduction of *Bacillus subtilis* var. *globigii* spores. Use of low concentrations of hydrogen peroxide is beneficial in terms of cost and safety issues. In addition, low residue values in the packages result from efficient drying by hot air and are well below the FDA legislative requirement of less than 0.5 ppm.

There has been a tremendous growth in plastic bottles for liquid foods in recent years and PET or HDPE bottles are available for standard shelf-life, ESL and aseptic applications. Two main principles exist for aseptic bottle systems.

Non-sterile PET bottles sterilised before filling

PET bottles are blown from pre-forms in a non-sterile environment. After blowing, the bottles are conveyed into a sterile chamber which is kept at a slight overpressure with sterile air. PET bottles are sometimes rinsed with an airjet to ensure all possible foreign bodies are blown out. The bottles are sprayed inside and outside with a sterilising solution containing hydrogen peroxide and peracetic acid. The chemical is then evaporated by passing the bottles through a hot air tunnel, after which the bottles are rinsed with sterile water and filled. Alternatively, PET bottles can be sterilised by hydrogen peroxide vapour with heat treatment which does not damage the

PET. A chemically sterilised, heat-sealable closure is applied before the bottles leave the aseptic chamber.

Sterile-blown HDPE bottles
HDPE bottles are extruded, blown with sterile air and sealed under conditions that ensure internal sterility of the container. The sealed bottles are then introduced into an aseptic chamber where the outside surfaces are sterilised with hydrogen peroxide spray. The closed top of the bottle is cut away, the neck trimmed, the bottle filled and a foil cap or heat-sealable closure which has been sterilised outside the chamber is applied.

6.7 Non-aseptic packaging technologies for shelf-life extension

While aseptic processing and packaging will produce products that do not require refrigeration, non-aseptic packaging solutions depend on refrigerated distribution. Such packaging systems for ESL applications are available in a range of formats and capacities. Most systems have clean air in the filling zone, as either a laminar flow of HEPA air, or an overpressure of sterile filtered air, and a decontamination of the packaging material. These machines may be simpler than aseptic machines; they often lack an aseptic chamber, and the requirements for kill rate of internal surfaces and packaging material may be less stringent. While aseptic systems normally have a package sterilisation system capable of a minimum 5 log reduction of resistant spores, the systems for ESL may have package decontamination systems that sometimes are not even verified or documented. In addition, the requirements for control systems are not as strict as for aseptic systems. Although an aseptic filling system may be used to fill a non-sterile ESL product, the cost benefit of an ESL or Ultra Clean filling machine makes this a more logical choice. The packaging material may also be cheaper, as a high-performance barrier necessary for ambient storage over long periods is normally not required. As there are no legal requirements or agreed industry standards for filling systems for ESL products, it may be difficult for a producer to compare products from different suppliers. It is therefore very important that the supplier can document the challenge methods used to test the machine together with the results from individual challenge and product tests as recommended by EHEDG (2001).

The criteria for commissioning and validation of an ESL packaging system must be established by close cooperation between suppliers and the producer. The product is normally not commercially sterile, and is filled on a non-aseptic machine, which requires careful evaluation of the criteria for the test. The commissioning criteria for such a test should specify limits for raw material quality, processing conditions, recontamination rate and storage conditions. It is also quite common to perform an accelerated test

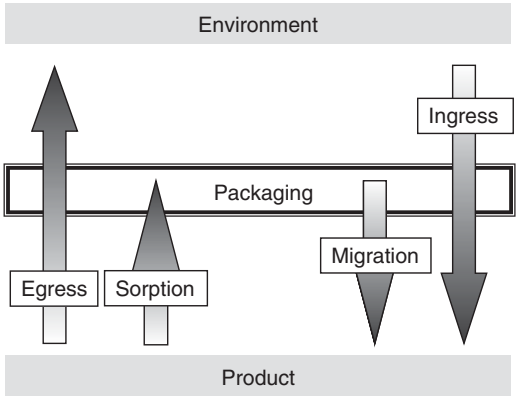

Fig. 6.8 Food–packaging interactions which can influence the shelf-life of ESL and aseptic liquid food products (after Rysstad and Kolstad, 2006).

at room temperature in addition to storage at the agreed refrigerated distribution temperature.

6.8 Effects of storage on product quality

The packaging material can influence the product both directly, as well as indirectly, by influencing the interaction between the product and its environment (Fig. 6.8). Of these interactions, those of particular importance for ESL and aseptic food products are aroma loss due to egress and scalping; migration of monomers and additives from the packaging materials; and ingress of oxygen and light (both UV and visible).

Three key parameters influence the extent of light-induced damage to liquid food products (Fig. 6.9; Borle *et al*., 2001): the light permeability of the packaging which determines the intensity and spectral properties of light ingress; the presence of photosensitiser in the food; and the availability of molecular oxygen which can be converted into reactive oxygen species by the photosensitiser.

In the case of ESL milk and milk products, riboflavin is the most important photosensitiser which, owing to the high levels of dissolved oxygen at filling (5–10 mg per litre), makes milk extremely susceptible to light-induced damage. As well as causing degradation of vitamins, off-taste compounds such as methional and dimethyl disulphide are rapidly formed (Borle *et al*., 2001) at levels of light exposure found in display cabinets. Several studies have demonstrated the importance of reducing light penetration and oxygen ingress to maintain the quality of pasteurised milk and milk products (Rysstad *et al*., 1998; Simon and Hansen, 2001; Simon *et al*., 2001; van Aardt *et al*., 2001; Whited *et al*., 2002; Vassila *et al*., 2002; Moyssiadi *et al*., 2004; Zygoura *et al*., 2004; Mestdagh *et al*., 2005; Gliguem and Birlouez-Aragon,

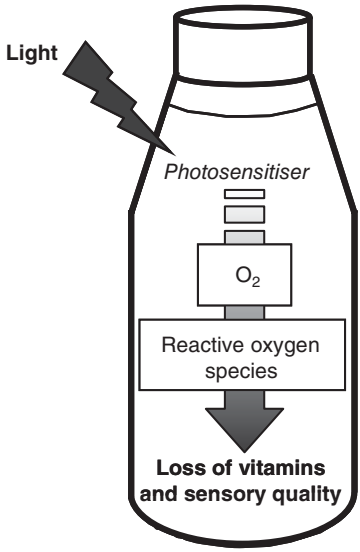

Fig. 6.9 The mechanism of light-induced damage of milk and other liquid food products (after Borle *et al*., 2001).

2005). Oxidative processes can also lead to other changes such as enhanced proteolysis (Wiking and Nielsen, 2004).

Although light is not as damaging to juice as to milk, for certain juices such as high-quality orange juice, light exposure can result in accelerated vitamin C degradation as well as photo-bleaching and the formation of α- and β-terpeniol off-flavours. Functional drinks may contain special antioxidants, vitamins or other light-sensitive ingredients that must be protected from light exposure. Although complete light protection can easily be achieved with aluminium-foil lined cartons (Rysstad *et al*., 2003), several producers prefer non-foil barrier cartons for environmental or other reasons. The importance of limiting oxygen and light ingress into aseptically packaged orange juice has been demonstrated by several studies (Ayhan *et al*., 2001; Ros-Chumillas *et al*., 2007).

Oxygen exposure, which results in vitamin C degradation and browning, is a key determinant of shelf-life for juices and juice drinks. The sum of the oxygen content at the time of packaging and the amount of oxygen ingress will limit the shelf-life of juice products for which there is a specified vitamin C content. The kinetics of such degradation can be modelled based on knowledge of the amount of headspace and dissolved oxygen present in the package after filling, the oxygen permeability characteristics of the package and the storage temperature to allow shelf-life predictions to be made (Fig. 6.10).

Examples given here have been very much based on processing and packaging of milk and juice products. However, there is a tremendous

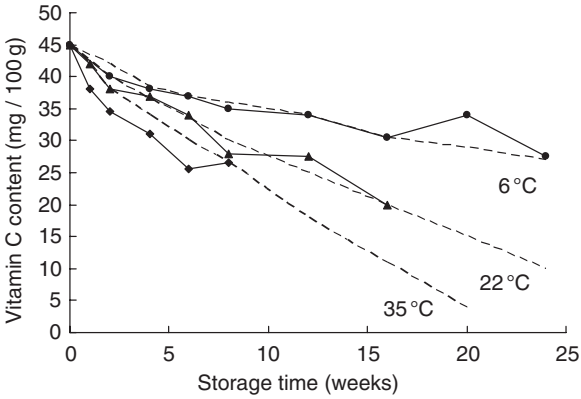

Fig. 6.10 Comparison of real and modelled data for vitamin C degradation.

growth of new ESL and aseptic beverages including functional drinks, sports and performance drinks, enhanced fruit drinks and a large variety of soya-based beverages. These groups of beverages are described in great detail in other chapters of this book. However, as these products will contain functional ingredients such as special vitamins, antioxidants and other sensitive ingredients, a processing and packaging system has to be designed to protect these ingredients through processing until consumed. Some ingredients may be heat sensitive and have special demands for gentle processing, while other may be very sensitive to light and/or oxygen during storage. It is therefore vital that the processor has a good knowledge of the product characteristics and the main factors affecting the quality during processing, storage and distribution as described in Fig. 6.1. Opportunities therefore exist for the development of improved and novel packaging structures for ESL and aseptic food products.

6.9 Future trends

This section reviews emerging technologies which have the potential to further enhance the quality and shelf-life of ESL and aseptic liquid food products. Such technologies can be divided into *process solutions* influencing product treatment, package sterilisation and filling; and *package solutions* influencing the performance of the packaging materials in protecting the product during its shelf-life.

6.9.1 Process solutions

Critical to the success of ESL and aseptic packaging solutions is minimal treatment of the product to retain its freshness characteristics. In addition, for aseptic products, maintenance of product sterility is also essential.

Product processing

Product processing to reduce the microbial load in ESL products or to eliminate the microbial load in aseptic products has conventionally been achieved by heat treatment. Such treatment also has the advantage of reducing the activity of enzymes present in the product thereby extending shelf-life. However, adverse reactions including nutrient degradation occur in conventional thermal processing (Awuah *et al*., 2007) and a number of alternative technologies are emerging to minimise these effects. The majority of these methods employ electromagnetic heating which results in direct heat transfer from the source into the food without using a heat transfer surface. Such methods are claimed to allow more uniform and rapid heating and cooling of the product, resulting in less thermal damage. They include ohmic heating due to electrical resistance (50–60 Hz); radio frequency (RF) heating (10–60 MHz; Zhao *et al*., 2000) and microwave heating (1–3 GHz). The electrical fields can be applied with short pulses (10–100 μs) at high field intensities (20–80 kV cm^{-1}) and have been used at a commercial scale to sterilise tomato juice (Min *et al*., 2003).

Several studies have shown that pulsed field processing of orange juice results in retention of higher vitamin C levels and flavour compounds (Yeom *et al*., 2000; Ayhan *et al*., 2002; Hodgkins *et al*., 2002; Sanchez-Moreno *et al*., 2005; Cortes *et al*., 2006a) as well as improved colour retention (Cortes *et al*., 2006b) compared with thermal pasteurisation. Similarly ultra-high temperature ohmic-treated fresh orange juice maintained higher levels of flavour compounds than juice treated with conventional treatments (Leizerson and Shimoni, 2005). Milk treated with high-intensity pulsed electric fields showed similar shelf-life and nutrient composition (Bendicho *et al*., 2002; Odriozola-Serrano *et al*., 2006) as well as protease inactivation (Bendicho *et al*., 2003) compared with pasteurisation. Furthermore ESL milk has been successfully produced from pasteurised milk by processing with pulsed electric fields (Sepulvida *et al*., 2005). The quality and safety aspect of pulsed electric field applications to milk and milk products are reviewed by Sampredo *et al*. (2005).

Combinations of technologies such as pulsed electric fields and ultrasonication may offer alternative processing solutions in the future (Ross *et al*., 2003). For example ultrasonication and thermal treatment act synergistically to inactivate tomato pectinmethylesterase (Raviyan *et al*., 2004), pulsed electric field acts synergistically with thermal treatment to kill *Escherichia coli* in egg white (Bazhal *et al*., 2005) and ultrasound combined with thermal treatment act synergistically to kill *Staphylococcus aureus* (Ordóñez *et al*., 1987). Detailed reviews of new technologies for thermal food processing (Sun, 2006) and non-thermal food processing (Sun, 2005) are available.

Package sterilisation

The most common package sterilisation procedure involves treatment with high concentrations of hydrogen peroxide. This may be combined with heat

and UV treatment in cartons which synergistically inactive bacterial spores (Bayliss and Waites, 1979). For PET applications, in which high heat treatment cannot be used owing to temperature sensitivity of the PET, peracetic acid is frequently used in combination with hydrogen peroxide as a sterilising agent. A number of alternative methods have been demonstrated to have potential for sterilising food packaging structures including the following:

- ***Pulsed light:*** Surfaces are decontaminated by exposure to extremely short pulses (millisecond to microsecond duration) of high-intensity UV light (which is rich in UV-C) (Gomez-Lopez *et al.*, 2007). This technique is effective against the whole range of contaminating microorganisms including bacterial and fungal spores.
- ***Plasma***: Antimicrobial activity is due to the activity of UV photons and short-lived antimicrobial free radicals present in the plasma gas (Warriner *et al.*, 2004). Low-pressure microwave plasmas have been demonstrated to achieve over 4 log reduction in viability of *Bacillus* spores within 1 s (Schneider *et al.*, 2005). The challenge is to implement this technology at the commercial scale.
- ***UV-excimer:*** High intensity UV-excimer lasers are extremely effective for sterilising the surface of packaging materials, and can cause a 4 log reduction in viability of *Bacillus* spores in less than 1 s (Warriner *et al.*, 2000, 2004). The major limitation to the application of this technology is development of methods to ensure even treatment of the whole of the interior surface of a package.

These methods are of particular interest because they represent chemical-free sterilising solutions for food packaging structures.

6.9.2 Package solutions

The recognition that the light and oxygen barrier properties of the packaging structure are important determinants of shelf-life for ESL products has led to the modification of conventional structure to improve these characteristics. The developments can be broadly divided into new barrier structures and active packaging solutions.

Barrier structures

Owing to their perfect light and barrier properties, Al-lined cartons remain the gold standard for barrier structures for liquid aseptic products. Addition of pigments to transparent packaging structures has been used to filter out the wavelengths of light which cause photosensitive reactions. The broad absorption spectrum of the photosensitiser riboflavin in milk limits the effectiveness of this strategy and multilayer barrier solutions containing a middle black barrier have been devised. Two strategies have been adopted to reduce the oxygen transmission rates of other barrier materials. In the

first, inclusion of biopolymer-layered silicate or clay nanocomposites decreases gas permeability as well as increasing water resistance (Pereira *et al*., 2007; Rhim and Ng, 2007). There is, however, a significant issue of public acceptance and safety of the use of nanotechnology in food packaging materials (Siegrist *et al*., 2007).

Secondly, plasma coating methodologies in which the inside of a PET bottle is coated with an oxygen impermeable layer of silicon oxide or diamond-like carbon (Ikeyama *et al*., 2007) have been demonstrated. Such structures still have the disadvantage of light permeability.

Active packaging solutions

Recently there has been substantial interest in both active and intelligent packaging structures for food products which is driven at least in part by consumer demands (Markarian, 2006). Active packaging materials contain components that are able to influence the chemical properties of the package contents. Of these, a number of oxygen scavenging technologies offer opportunities to protect aseptic and ESL foods from oxidation due to the presence of molecular oxygen at two key stages. First they can function by removal of residual headspace and dissolved oxygen present in juice and juice products at the time of filling. Secondly, they can compensate for defects in packaging structures or for barrier structures such as PET bottles which are not sufficiently high oxygen barriers. The most attractive solutions are polymer based systems which include Amosorb® from BP; Dareval® produced by Kuraray and Darex; Oxbar® and Monoxbar™ from Constar; Shelfplus® from Ciba; and OSP® from Chevron Phillips.

A number of publications and other reports have demonstrated that oxygen scavenging films can extend the shelf-life of orange juice by both rapidly removing the oxygen present at packaging (Zerdin *et al*., 2003) as well as by enhancing the barrier properties of the packaging material (Ros-Chumillas *et al*., 2007). In spite of such demonstrations of technical feasibility, oxygen scavenging technologies have not been widely adopted for ESL and aseptic high-acid liquid foods and food products. Reasons may include production, stability and activation issues; implementation costs; legislative issues; and concerns about impact on the recycling stream. In addition the kinetic of oxidative processes in cold storage is extremely slow. Thus existing packaging structures with high-performance oxygen barriers such as foil-lined cartons remain preferred packaging solutions.

6.10 Sources of further information and advice

A number of institutes and organisations can act as sources of information and advice as well as conducting research and training in relation to packaging structures and processes for ESL and aseptic liquid food products. These include the following.

6.10.1 Campden and Chorleywood Food Research Association Group

Campden and Chorleywood Food Research Association (CCFRA) is the largest membership-based food and drink research centre in the world. It is committed to providing industry with the research, technical and advisory services needed to ensure product safety and quality, process efficiency and product and process innovation. It undertakes R&D for the many industries associated with agriculture, food and drink manufacture, distribution, retailing and food service: in essence those industries which together make up the agri-food chain.

Website: www.campden.co.uk

6.10.2 Center for Advanced Processing and Packaging Studies (CAPPS)

The Center for Advanced Processing and Packaging Studies (CAPPS) supports research that provides the knowledge to assure safety of aseptic and extended shelf-life products; characterise emerging and commercial sterilisation and pasteurisation processes; and enhance the integrity and functionality of aseptic and extended shelf-life packaging.

Website: www.cals.ncsu.edu/food_science/capps.html

6.10.3 European Hygienic Engineering & Design Group (EHEDG)

The EHEDG, which is supported through the European network for Hygienic Manufacturing of Food, provides guidance on the hygienic engineering aspects of manufacturing of safe and wholesome food. This includes production, publication and updating of guidelines; equipment approval through certification to assist equipment suppliers and food manufacturers; and providing advice to legislators and standards groups.

Website: www.ehedg.org

6.10.4 Fraunhofer Institute for Process Engineering and Packaging (IVV)

The Fraunhofer IVV is a competent and professional organisation which carries out contract research and development work for industry. It provides analytical and experimental services in the areas of conformity of polymers and paper; analysis of contaminants; aseptic systems; analysis of food ingredients and foods; expertise in active and intelligent packaging; permeation analysis; material testing; processing and modifying plastics; as well as process engineering solutions.

Website: www.ivv.fraunhofer.de

6.10.5 Leatherhead Food International

Leatherhead Food International has a history of serving the industry for over 85 years and is a market leader in supporting the global food and drink

sectors. Whether reaching into new markets, improving products or innovating, LFI can provide support on all food-related issues from technical analysis and research through to market data and regulatory guidance.

Website: www.leatherheadfood.com

6.10.6 PIRA

PIRA is a leading commercial consultancy, testing and media business which specialises in retail supply chain technologies relating to industries such as packaging, paper, plastics, printing, publishing and consumer goods. PIRA has established a reputation as one of the key knowledge providers in these industry sectors.

Website: www.piranet.com/

6.10.7 TNO Food and Nutrition Research

TNO is a versatile multidisciplinary organisation which provides advice as well as carrying out a wide range of contract research, testing and certification of food and food packaging materials.

Website: www.tno.nl

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Part II

Dairy-based beverages

7

Improving the nutritional quality of milk

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Abstract: The important role of cow's milk in the human diet as a supplier of fatty acids, protein and other key nutrients and relationship with chronic disease is discussed. This is set in the context of trends in milk/dairy product consumption and focuses on the ability to change the composition of milk lipids by animal nutrition, in particular ways of reducing and increasing the proportions of saturated and *cis*-monounsaturated fatty acids respectively. In addition, the potential to improve milk composition by fortification is discussed as is the possibility of reducing components such as lactose by processing.

Key words: milk, chronic disease, fatty acids, animal nutrition, fortification.

7.1 Introduction

Milk is a unique and complex food of great interest, intended to be a complete food for young mammals. The important role of cow's milk in the human diet as a supplier of energy, protein and other key nutrients including calcium is well known. Milk is essentially a complex colloidal system comprising globules of milk fat suspended in an aqueous medium containing lactose, a range of proteins, mineral salts and water-soluble vitamins. Milk from modern Holstein/Friesian cows will typically contain about 40, 36 and 45 g/kg of fat, protein and lactose, respectively, and have an energy content of approximately 2.8 MJ/kg. The fat and protein content of milk varies considerably with the breed and nutrition of the cow. The effect of breed is particularly noticeable in milk from Channel Island breeds which have a higher fat content of typically about 65 g/kg. Although milk is widely consumed, there has recently been increased concern that a high proportion (>50%) of this energy in milk is derived from fat, about 70% of which is made up of saturated fatty acids. For more detail on the foregoing the reader is referred to the review of Givens and Shingfield (2006).

Table 7.1 Trends in consumption of milk (from WHO/FAO, 2003)

Region	Milk (kg/person/year)		
	1964–66	1977–99	2030[1]
World	73.9	78.1	89.5
Developing countries	28.0	44.6	65.8
Transition countries	156.7	159.1	178.7
Industrialised countries	185.5	212.2	221.0

[1] Projected.

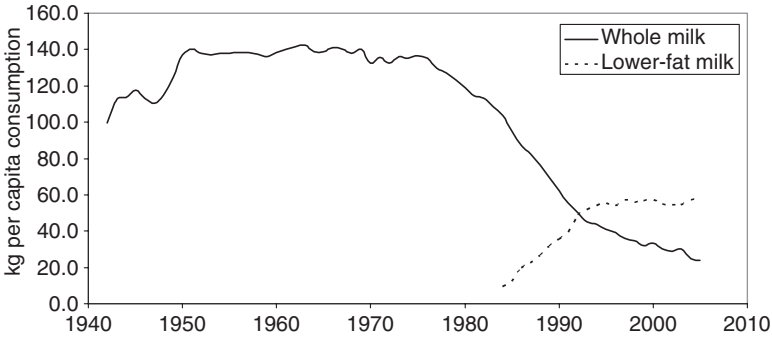

Figure 7.1 Trends in UK milk consumption, 1942–2005. Sources: National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005).

7.1.1 Trends in the consumption of milk and dairy-derived foods

Globally the demand for animal-derived foods in general is growing rapidly, driven by a combination of population growth, urbanisation and rising income. Table 7.1 shows the trends in milk consumption over the past 40 years for various regions of the world. Although the historical and projected trend is upward, in the United Kingdom and other Western countries consumption has shown considerable change over recent decades. During the years immediately following World War II there was an increase in whole milk consumption in the United Kingdom (Fig. 7.1) up to a plateau where consumption remained at around 130 kg per capita per year for over 20 years. In the mid-1970s whole milk consumption started to decline quickly but was partially replaced by the then recently introduced skimmed and semi-skimmed milk (collectively termed lower-fat milk in Fig. 7.1). Consumption of these steadily increased up to a point in the early 1990s when it began to exceed that of whole milk. Between then and 2005, consumption of these reduced-fat milks remained relatively constant, while that of whole full-fat milk continued to decline.

The trend in butter consumption in the United Kingdom is similar to that of whole milk (Fig. 7.2). During the 1950s and 1960s consumption

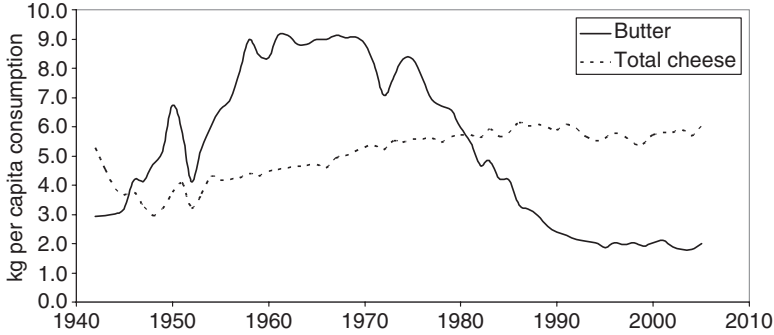

Figure 7.2 Trends in UK butter and total cheese consumption, 1942–2005. Sources: National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005).

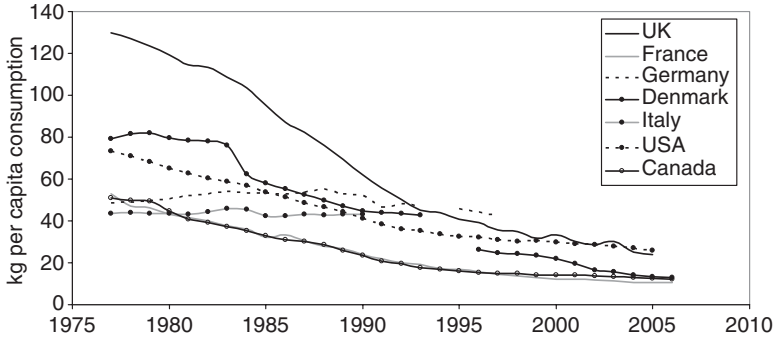

Figure 7.3 Consumption of whole milk in selected countries, 1977–2006. Sources: IDF, personal communication; National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005); CNIEL (2007); ZMP (2007); USDA (2007); Agriculture and Agrifood Canada (2007), Danish Dairy Board (2007).

increased to a plateau, with a decline beginning in the mid-1970s possibly as a result of the increasing availability of other spreads. However the initial decline in total cheese consumption following World War II did not continue, and since the 1950s cheese consumption has increased steadily.

The decline in whole milk consumption in the United Kingdom and the increase in lower-fat milk consumption reflects a general trend in a number of other developed countries (Figs 7.3 and 7.4). Denmark, France, the United States and Canada also show similar trends since the 1970s. Germany, however, has shown a less marked decline in whole-milk consumption and there has been little change in consumption of lower-fat milk up to 1993, although data for recent years were not available. The consumption of liquid milk in Italy has always been much lower than in northern Europe, but between 1977 and 1990 this remained relatively constant for both whole and lower-fat milk (Figs 7.3 and 7.4).

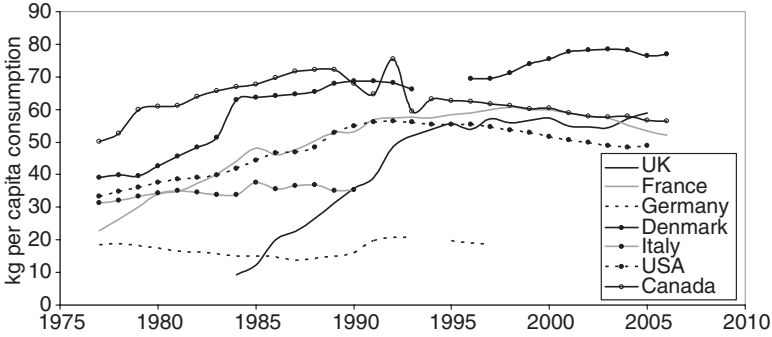

Figure 7.4 Consumption of lower-fat milks (skimmed + semi-skimmed) in selected countries, 1977–2006. Sources: IDF, personal communication; National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005); CNIEL (2007); ZMP (2007); NASS, USDA (2007); Agriculture and Agrifood Canada (2007), Danish Dairy Board (2007).

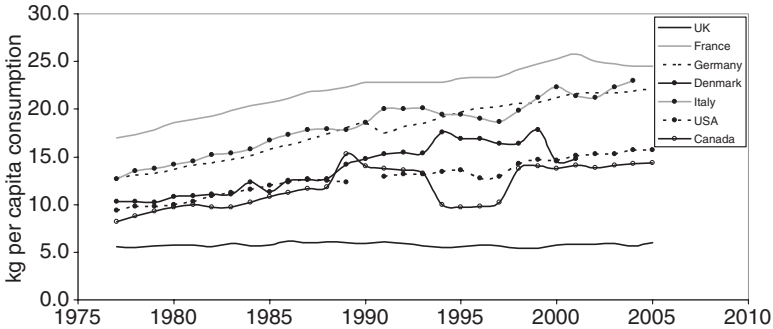

Figure 7.5 Cheese consumption in selected countries, 1977–2006. Source: IDF, personal communication; National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005); CNIEL (2007); ZMP (2007); NASS, USDA (2007); Agriculture and Agrifood Canada (2007). Danish Dairy Board (2007).

Figure 7.5 illustrates the changes in cheese consumption. Of the selected countries, France has the highest consumption followed by Italy and the United Kingdom has always had the lowest. Most countries show a gradual increase in cheese consumption, although that of Canada and Denmark did fluctuate during the 1990s and that of the United Kingdom post-1980s remained relatively constant. Butter consumption appears to have followed several different trends (Fig. 7.6). Consumption in Germany, Italy and the United States, appears to have remained constant while that in the United Kingdom and Denmark has declined, and that of France and Canada has fluctuated.

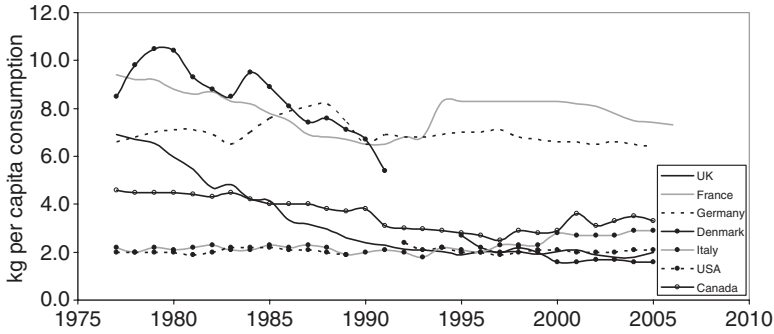

Figure 7.6 Butter consumption in selected countries, 1977–2006. Source: IDF, personal communication; National Food Survey, DEFRA (2001); Expenditure and Food Survey, DEFRA (2005); CNIEL (2007); ZMP (2007); NASS, USDA (2007); Agriculture and Agrifood Canada (2007), Danish Dairy Board (2007).

7.2 The health benefits of milk

7.2.1 Nutrients provided by milk

Milk and dairy-derived foods are available in the retail market in many forms. Based on food intakes assessed by UK National Diet and Nutrition Survey (Henderson *et al*., 2003a,b) over the period July 2000 to June 2001, the contribution of the major milk-derived foods types to energy and nutrient intakes of the UK male population (aged 19 to 64 years) is shown in Table 7.2. Milk and dairy food products are clearly important sources of protein, calcium, phosphorus, iodine, riboflavin and vitamins A and B_{12}. Indeed milk and dairy products alone provide more than the daily recommended intake for vitamin B_{12}. The current nutritional importance of semi-skimmed and skimmed milk is clear and, notably, some 70% of liquid milk is consumed as semi-skimmed milk (Milk Development Council, 2004). Although milk and dairy products provide only about 13% of the recommended folate intake of 200 μg/day (Henderson *et al*., 2003b), there is evidence that the presence of milk in the diet can increase overall folate bioavailability compared with diets containing no milk (Wigertz *et al*., 1997). Also Smith *et al*. (1985) proposed that folate present in milk is more available than folates from other foods, at least in infants. It is probable that these properties of milk are due to the fact that it uniquely contains folate binding proteins. Although their exact role is not fully understood (de Jong *et al*., 2005), it is possible that these proteins will also increase the availability of folate in other foods consumed and also make milk a good candidate for fortification with folate.

Milk and dairy products including butter contribute almost 20% of the total fat consumed but since the lipids in these products are rich in saturated fatty acids they make a major contribution to saturated fatty acid intake. In the United Kingdom the National Diet and Nutrition Survey

Table 7.2 Energy and selected nutrients provided by milk and dairy products to men's diets in the UK (derived from Henderson *et al*; 2003a,b)

Energy/nutrient	Contribution from milk and dairy products	Liquid whole milk	Semi-and skimmed milk	Cheese	Other dairy products	Butter	Total dairy
Energy	Intake (MJ/d)	0.49	nas[5]	0.29	0.10	0.10	0.97
	% of EAR[1]	5	nas	3	1	1	9
Protein	Intake (g/d)	1.8	5.3	4.4	1.8	0	13.2
	% of RNI[2]	3	10	8	3	0	24
Fat	Intake (g/d)	1.7	2.6	5.2	2.6	2.6	14.7
	% of ADI[3]	2	3	6	3	3	17
Calcium	Intake (mg/d)	61	203	112	41	nas	417
	% of RNI	9	29	16	5	nas	60
Phosphorus	Intake (mg/d)	45	165	90	30	nas	330
	% of RNI	8	30	16	6	nas	60
Magnesium	Intake (mg/d)	6.2	19	nas	6.2	nas	31
	% of RNI	2	6	nas	2	nas	10
Zinc	Intake (mg/d)	0.21	0.60	0.64	0.21	nas	1.7
	% of RNI	2	7	7	2	nas	18
Iodine	Intake (µg/d)	15	46	4.4	11	nas	77
	% of RNI	11	33	3	8	nas	55
Vitamin A[4]	Intake (µg/d)	20	31	61	31	41	183
	% of RNI	3	4	9	4	6	26
Riboflavin	Intake (mg/d)	0.12	0.40	0.07	0.09	nas	0.68
	% of RNI	9	31	5	7	nas	52
Vitamin B_{12}	Intake (µg/d)	0.41	1.4	0.34	0.14	nas	2.2
	% of RNI	27	91	23	9	nas	150
Folate	Intake (µg/d)	25	nas	nas	nas	nas	25
	% of RNI	13	nas	nas	nas	nas	13

[1] EAR, estimated average requirement.
[2] RNI, reference nutrient intake.
[3] ADI, average daily intake.
[4] Retinol equivalents.
[5] nas, not available separately, included in total.

Table 7.3 Contribution (% total intake) of animal-derived foods to saturated fatty acid (SFA) and *trans* fatty acid (TFA) intake in selected European countries (from Hulshof *et al*., 1999)

Country	Milk and dairy products		Meat and meat products		Eggs	
	SFA	TFA	SFA	TFA	SFA	TFA
Germany	57.1	71.8	18.2	5.3	2.5	0.8
France	56.7	60.9	20.2	11.4	1.9	1.6
Italy	47.3	62.0	15.3	13.4	2.2	0.7
Iceland	39.8	24.2	18.9	15.0	1.0	0.2
United Kingdom	38.8	24.8	17.1	10.3	1.8	0.9
Greece	27.4	37.5	13.9	14.6	0.6	0.1

(Henderson *et al.*, 2003a) estimated the contribution as 30% of total saturated fatty acid intake although this excluded milk fats in manufactured foods such as cakes, biscuits, etc. A study on fatty acid intake across Europe (Hulshof *et al*., 1999) suggested a higher figure of 40% for the United Kingdom, and milk and dairy foods were consistently the largest source of saturated fatty acids, with the greatest contribution being observed in Germany and France where some 60% of saturated fatty acids were from these foods (Table 7.3). Interestingly, the contribution of butter to saturated fatty acid intake varied widely. In Greece, Spain, the Netherlands and Norway butter provided less than 5%, whereas high contributions were recorded in France (30%) and Germany (39%) with the United Kingdom being intermediate (10%).

Also of note was the fact that across the countries studied, milk and milk-derived foods contributed most of the *trans* fatty acids consumed (Table 7.3). The contributions in Germany, Italy and France were particularly high at approximately 72, 62 and 61% respectively although in the United Kingdom this was lower (25%). The high contributions in Germany arose mainly from butter consumption while in Italy cheese was the main source. In all countries the predominant *trans* fatty acids were *trans* C18:1 and although this study (Hulshof *et al*., 1999) did not report the isomeric profile, other evidence indicates that the primary isomer in foods from ruminant animals is *trans*-11 C18:1 (*trans*-vaccenic acid) unlike that from industrial hydrogenation which is usually mainly *trans*-9 C18:1, elaidic acid (Weggemans *et al*., 2004).

7.2.2 Milk consumption and chronic disease

Because of its major contribution to saturated fatty acid consumption, milk has often been considered to be a major contributor to coronary heart disease. As a result, many have advised that milk consumption should be limited. There is however, good evidence to the contrary. Elwood *et al.*

Table 7.4 Effect of milk consumption on the incidence of the metabolic syndrome in a cohort of UK men over a 20 year period (from Elwood *et al*., 2007)

Milk consumption (ml/day)	Number of subjects	Number of subjects with syndrome	Relative risk for syndrome[1]
Those using a FFQ[2]			
Little or none	139	30	1.00
<570	984	177	0.71
570–1140	868	122	0.56
>1140	140	13	0.38
			P for trend = 0.002
Those using a WDI[3]			
Little or none	150	25	1.00
<570	152	30	1.04
570–1140	150	22	0.76
>1140	151	12	0.43
			P for trend = 0.026

[1] Adjusted for age, energy intake, social class and smoking.
[2] A semi-quantitative food frequency questionnaire.
[3] A 7 day weighed intake record.

(2004) identified 10 prospective cohort studies examining the relationship between milk consumption and vascular disease risk and in all cases except one (with a small number of disease events) there was good evidence of a reduced risk of ischaemic heart disease or ischaemic stroke in individuals who had the highest milk consumption. The authors reported a pooled estimate of the risk of an incident vascular disease event in such subjects, relative to those with a low milk intake of about 0.87 (95% CI 0.74–1.03) for ischaemic heart disease, 0.70 (95% CI 0.55–0.88) for ischaemic stroke and 0.84 (95% CI 0.78–0.90) for either event. More recently, Elwood *et al*. (2007) examined the prevalence of the multifaceted metabolic syndrome (Nugent, 2004) in relation to milk consumption in a cohort of 2375 men in the United Kingdom over a 20-year follow-up period. They found negative relationships between the consumption of both milk and dairy produce and the syndrome. Using a food frequency questionnaire, the adjusted relative risk in subjects who regularly drank 1140 ml of milk or more daily was 0.38 (95% CI 0.18–0.78) and for dairy foods in general was 0.44 (95% CI 0.21–0.91). The data for subjects who used a 7-day weighed intake record was similar (Table 7.4). These data agree with an earlier shorter-term study by Azadbakht *et al*. (2005) who linked the effect to reduced blood pressure in those consuming greatest amounts of milk.

A further reason for increased milk consumption being associated with a reduced incidence of the metabolic syndrome may relate to the effect of milk and dairy consumption on body weight regulation. In the CARDIA

study, Pereira *et al*. (2002) showed that increased consumption of milk/dairy products over a 10 year period reduced the risk of obesity in subjects already overweight. Data from cross-sectional epidemiological studies reviewed by Barba and Russo (2006) support the hypothesis that increased consumption of milk and dairy products leads to less fat accumulation in both adults and children. However other data from prospective and intervention studies showed less consistent effects. Barba and Russo (2006) concluded that while the available data do not fully support the hypothesis that high milk and dairy food consumption results in reduced fat deposition, further research should be conducted to better define the impact of high consumption.

7.3 Optimising the nutritional quality of milk by modifying the diet of the cow

7.3.1 Milk proteins

Protein is the most valuable milk constituent and is influenced by nutritional, physiological and genetic factors (Erasmus *et al*., 2001). Milk protein constitutes about 95% of total milk nitrogen, and comprises caseins (α, β, κ and γ), whey proteins (β-lactoglobulin and α-lactalbumin), serum albumin and immunoglobulins. Even though whey proteins are of high nutritional value, only the casein fraction is important to cheese makers. Casein accounts for between 76 and 86% of total milk protein (DePeters and Cant, 1992) and is essentially independent of nutrition and stage of lactation (Coulon *et al*., 1998).

Milk protein content is dependent on both breed and stage of lactation, as well as nutrition. Breeds that produce milk with a high fat content also have higher protein concentrations, but the ratio of protein to fat is lower for Channel Island breeds compared with the Ayrshire, Holstein or Friesian. Concentrations of milk protein are much less responsive to the effect of the cow's diet than for fat content (Sutton, 1989) although nutrition has an impact. It is known that energy intake is the most important dietary attribute influencing milk protein content. For example Spörndly (1989) estimated for a wide range of diets, that an increase of 1 MJ of metabolisable energy (ME) intake would stimulate 0.002–0.003 proportionate increases in milk protein content. Similarly, Coulon and Remond (1991) estimated that in early and mid-lactation cows each additional MJ of ME intake could be expected to result in respective 0.04 and 0.08 g/kg increases in milk protein content.

While the relationship between energy intake and milk protein synthesis is widely accepted, it is clear that this holds true only when increases in energy are derived from carbohydrates and protein. Increases in dietary fat content often have a negative impact on milk protein concentrations (Sutton, 1989; DePeters and Cant, 1992).

Although increasing energy intake usually provides increases in milk protein content, there do not appear to be accompanying changes in the proportion of casein in total milk protein (Coulon *et al*., 1998). Indeed the effect of diet on the various other proteins in milk and on the potential supply of bioactive peptides resulting from hydrolysis of milk proteins in the human gut is relatively unexplored. However, given the genetic control on proteins synthesised, genetic selection of animals may represent a more effective long-term strategy for enhancing milk protein profile and bioactivity.

7.3.2 Milk fat: the need for change

Notwithstanding the epidemiological evidence for milk consumption discussed above, there remains good evidence that dietary saturated fatty acids do increase the concentrations of plasma low-density lipoprotein (LDL) cholesterol, an identified risk factor for cardiovascular disease and particularly coronary heart disease (Zock, 2006). While in general, saturated fatty acids raise total and low-density lipoprotein (LDL) cholesterol, early studies identified that individual fatty acids have markedly different effects. In particular myristic (C14:0) and palmitic (C16:0) acids have been associated with elevated plasma LDL cholesterol concentrations in human subjects (Katan *et al*., 1995; Temme *et al*., 1996) while the other major saturated fatty acid in foods, stearic acid (C18:0), has been shown to be essentially neutral (Bonanome and Grundy, 1988). Some studies suggest that lauric acid (C12:0) and C14:0 have more potent effects on plasma LDL cholesterol than C16:0 while others suggest that C14:0 and C16:0 are more potent than C12:0. In any event, C16:0 is quantitatively the most important saturated fatty acid in milk fat. Most of the C12:0 and C14:0 in the human diet is derived from milk fat (Gunstone *et al*., 1994), and therefore the consumption of milk and dairy foods would be expected to have adverse effects on plasma LDL cholesterol levels. A recent meta-analysis of 60 selected studies in humans (Mensink *et al*., 2003) confirmed that when replacing dietary carbohydrates with an isoenergetic amount of C12:0 to C16:0 saturated fatty acids an increase in LDL cholesterol occurred but noted that they all also increased the protective high-density lipoprotein (HDL) cholesterol. Mensink *et al*. (2003) argued that the ratio of total to HDL cholesterol provides the most powerful predictor of the effect of dietary fatty acids on risk of coronary heart disease with low values being associated with reduced risk. This interpretation indicates that the effects of C12:0 and C14:0 fatty acids may be somewhat positive as they both reduce the total to HDL cholesterol ratio. The effect of C16:0 remains negative and Mensink *et al*. (2003) concluded that overall the risk of coronary heart disease would be most effectively reduced by the replacement of dietary saturated with either *cis*-monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA). The effectiveness of the two replacement strategies was similar.

Table 7.5 Effect of challenging with saturated fatty acids (SFA) or monounsaturated fatty acids (MUFA) on insulin parameters, plasma glucose and serum lipids in healthy men and women (from Vessby *et al*., 2001)

Measurement	SFA diet			MUFA diet		
	Change[1]	Change (%)	*P* value	Change[1]	Change (%)	*P* value
Insulin sensitivity index (Si)	–4.2	–10.3	0.032	+0.10	+12.1	0.518
Serum insulin (mU/l)	+0.25	+3.5	0.466	–0.35	–5.8	0.049
Plasma glucose (mmol/l)	0.00	±0	0.995	–0.03	–0.60	0.413
Total cholesterol (mmol/l)	+0.14	+2.5	0.018	–0.15	–2.7	0.012
LDL cholesterol (mmol/l)	+0.15	+4.1	0.006	–0.19	–5.2	0.006

[1] Mean change during treatment expressed as least square mean.

The replacement of saturated fatty acids by both MUFA and PUFA not only brings about favourable changes in plasma cholesterol pools but may have other beneficial outcomes. A 3-month intervention study involving 162 healthy subjects (Vessby *et al*., 2001) given diets rich in saturated fatty acids (from butter and margarine) or MUFA (from high oleic sunflower oil) showed that those on the saturated fatty acid diet had significantly impaired insulin sensitivity (–10%) while those on the MUFA diet showed no change (Table 7.5). Also of note in this study was that additional dietary inclusion of *n*-3 fatty acids from fish oil had no effect on insulin sensitivity or insulin secretion and the favourable effects of the MUFA diet were not seen in individuals with a high fat intake (>37% of energy intake). Recent work has also shown that *in vitro* at least, oleic acid may have an important role to play in inhibiting the growth of breast cancer cells (Menendez *et al*., 2005) although this area is far from being clearly understood.

Overall, the evidence summarised above clearly points to the need to reduce the intake of saturated fatty acids and the potential benefits from replacing them with MUFA or PUFA. Given the current contribution of milk and dairy-derived foods to the consumption of saturated fatty acids there seems to be a strong case for creating milk and milk products with an altered fatty acid composition.

7.3.3 Strategies for improving the fatty acid composition of milk

Including a wide range of lipid-rich supplements in the diet of the dairy cow has been the most common means for manipulating milk fatty acid composition. However, both the type and source of the lipid and basal diet influence

the extent of changes that can be achieved. Attempts to increase the concentration of one or more fatty acids may cause changes in other fatty acids which may reduce the potential beneficial effects. For example, feeding diets to enrich milk fat with *cis*-9 C18:1, C20:5 *n*-3 or C22:6 *n*-3 content will usually also result in an increase in *trans* C18:1 concentrations. Although likely to have few negative effects on health, such changes are generally perceived negatively by consumers and some health professionals.

7.3.4 Decreasing the saturated fatty acid content of bovine milk

Supplements of plant oils or oilseeds rich in unsaturated C18 fatty acids can be used to reduce the proportion of short and medium-chain fatty acids (C6:0–C16:0) and increase the concentrations of long-chain fatty acids in milk (Grummer, 1991; Doreau *et al*., 1999). These changes are primarily due to long-chain fatty acids (C16 and above) inhibiting *de novo* fatty acid synthesis in the mammary gland and because lipid supplements increase the amount of circulating long-chain fatty acids available for incorporation into milk fat. In general, feeding plant lipids (other than palm oil rich in C16:0) has no effect on milk fat content of C4:0 or long-chain saturates (C16 and above), but consistently increases C18:0 concentrations at the expense of C16:0 (Palmquist *et al*., 1993; Chilliard *et al*., 2000). Furthermore, comparison of milk fatty acid responses when oils are fed in the diet compared with rumen-protected sources or duodenal infusions of these lipids indicates that the proportion of C6 and C8 fatty acids are lowered when dietary fats are exposed to ruminal metabolism, whereas the increase in milk C18 content during early lactation or in response to duodenal infusions is associated with a reduction in C10–C16 content (Chilliard *et al*., 2000). In all cases, inclusion of plant oils and oilseeds in the diet results in an unavoidable increase in milk *trans* C18:1 content in milk due to extensive lipolysis and biohydrogenation of C18 PUFA in the rumen. A number of studies on the effects of plant lipids on milk saturated fatty acids was summarised by Givens and Shingfield (2006). This summary is shown in Table 7.6.

An example of the effects of reducing the degree of saturation of fatty acids in dairy products was shown by the work of Noakes *et al*. (1996) who studied 33 men and women for 8 weeks, comparing the effects of fatty acid modified and normal dairy products. The modified products resulted in a significant reduction in total (0.28 mmol/l) and LDL cholesterol (0.24 mmol/l) with HDL cholesterol being unaffected. The authors suggest that if these changes were applied to Western populations they would represent a potential strategy to lower the risk of coronary heart disease by about 10% without the need to change normal eating patterns.

7.3.5 Increasing the *cis* monounsaturated fatty acid content of milk

Although C18:0 is the predominant long-chain fatty acid available for incorporation into milk fat, secretion of *cis*-9 C18:1 in milk exceeds mammary

Table 7.6 Effect of plant-based lipids in the diet on the fatty acid composition of bovine milk (from Givens and Shingfield, 2006)

Lipid source	Intake (g/d)	Milk fatty acid composition (g/100g total fatty acids)														Source
		C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	*cis*-9 C18:1	*trans* C18:1	C18:1	C18:2 *n*-6	C18:3 *n*-3	CLA	
Control	0	2.9	2.5	1.6	3.7	4.2	12.5	30.1	11.2	19.4	1.6	21.7	1.3	0.40	0.46	Ryhänen *et al.*, (2005)
Rapeseed oil	500	2.6	1.9	1.2	2.5	2.7	10.1	22.6	14.3	25.8	4.3	31.4	1.4	0.50	1.02	
Control	0	4.6	2.5	1.3	2.9	3.2	12.4	31.4	15.4	13.0	4.1	17.1	0.9	0.43	0.31	Shingfield *et al.*, unpublished
Rapeseed oil	500	4.7	2.2	1.1	2.1	2.3	10.0	23.4	21.0	17.6	6.8	24.4	0.8	0.40	0.42	
Control	0	5.0	2.3	1.3	3.1	4.0	11.6	30.7	8.3	18.1	2.0	20.1	2.1	0.45	0.60	Givens *et al.*, (2003)
Whole cracked rapeseeds	2530	3.2	1.1	0.6	1.3	1.9	7.9	19.8	14.1	34.7	2.6	37.3	2.4	0.48	1.02	
	4100	2.7	1.0	0.4	1.0	1.4	6.0	18.0	15.8	39.3	2.0	41.3	2.8	0.60	0.74	
Control	0	3.5	2.3	1.5	3.2	3.5	10.0	25.9	9.9	NR	NR	18.5	1.8	0.20	0.35	Chouinard *et al.,* (2001)
Ca-salts of rapeseed oil	924	3.0	1.5	0.8	1.6	2.0	7.6	16.4	12.9	NR	NR	32.5	1.9	0.16	1.32	
Control	0	1.9	1.9	1.4	3.6	4.4	13.5	33.9	9.5	NR	NR	23.2	2.6	0.25		Jenkins (1998)
Oleamide [a]	350	1.4	1.0	0.5	1.3	1.7	7.8	20.4	9.4	NR	NR	48.2	3.8	0.12		
Control	0	5.1	3.7	1.8	5.3	4.7	14.0	32.1	7.9	15.8	1.5	17.3	2.6	0.50	0.50	Loor *et al.,* (2002)
Canolamide [a]	300	5.4	3.3	1.4	3.4	2.9	10.7	21.4	13.0	27.5	2.9	30.4	2.7	0.70	0.7	
Control	0	4.6	2.5	1.3	2.9	3.2	12.4	31.4	15.4	13.0	4.1	17.1	0.9	0.43	0.31	Shingfield *et al.*, unpublished
Soyabean oil	500	4.8	2.2	1.1	2.2	2.4	9.8	24.3	20.0	15.8	7.7	23.5	1.1	0.55	0.53	
Control	0	4.0	2.4	1.2	2.8	3.0	11.9	38.2	13.4	11.1	2.4	14.1	0.9	0.41	0.36	Shingfield *et al.*, unpublished
Sunflower oil	500	4.4	2.2	1.1	2.1	2.8	9.3	25.5	23.0	13.8	7.0	21.8	1.3	0.26	0.71	

Table 7.6 *Cont'd*

Lipid source	Intake (g/d)	Milk fatty acid composition (g/100g total fatty acids)														Source
		C4:0	C6:0	C8:0	C10:0	C12:0	C14:0	C16:0	C18:0	*cis*-9 C18:1	*trans* C18:1	C18:1	C18:2 *n*-6	C18:3 *n*-3	CLA	
Control	0	3.9	2.5	1.5	3.5	4.0	12.1	29.4	10.4	16.1	1.8	18.3	2.6	0.54	0.40	AbuGhazaleh *et al.*, (2002)
Extruded soyabeans	454	3.9	2.3	1.3	2.8	3.0	10.1	24.0	12.1	18.9	3.8	23.1	4.5	0.87	0.87	
Control	0	3.5	2.3	1.5	3.2	3.5	10.0	25.9	9.9	NR	NR	18.5	1.8	0.20	0.35	Chouinard *et al.*, (2001)
Ca-salts of soyabean oil	848	3.4	1.6	0.9	1.6	1.8	6.9	16.4	13.3	NR	NR	31.8	2.2	0.16	2.25	
Control	0	1.1	1.0	0.8	2.7	5.0	14.4	37.5	9.8	20.4	1.7	22.1	3.6	NR	NR	Jenkins *et al.*, (1996)
Butylsoyamide[b]	350	1.3	1.1	0.8	2.5	4.5	13.5	35.5	10.2	19.6	2.7	22.3	6.3	NR	NR	
Control	0	1.8	1.0	0.6	1.8	2.7	9.9	40.2	12.3	NR	1.1	21.0	2.0	0.72	0.16	Offer *et al.*, (1999)
Linseed oil	250	1.8	1.0	0.6	1.5	2.1	8.8	34.0	15.6	NR	2.1	27.2	1.8	0.84	0.28	
Control	0	4.6	2.5	1.3	2.9	3.2	12.4	31.4	15.4	13.0	4.1	17.1	0.9	0.43	0.31	Shingfield *et al.*, unpublished
Linseed oil	500	4.5	2.2	1.1	2.3	2.4	10.0	22.2	20.2	17.3	7.7	25.0	0.7	0.57	0.49	
Control	0	3.9	2.3	1.6	3.0	3.7	11.3	28.9	10.2	21.4	2.9	25.8	2.2	0.32	0.51	Offer *et al.*, (2001a)
Crushed linseeds	1500	3.9	2.0	1.3	2.4	2.9	9.9	23.9	12.6	26.1	3.4	31.2	2.8	0.87	0.62	
Control	0	3.5	2.3	1.5	3.2	3.5	10.0	25.9	9.9	NR	NR	18.5	1.8	0.20	0.35	Chouinard *et al.*, (2001)
Ca-salts of linseed oil	896	3.4	1.9	1.0	2.0	2.1	7.4	16.2	13.2	NR	NR	28.5	2.4	0.28	1.95	

[a] Prepared by reacting rapeseed oil with ethanolamine.
[b] Prepared by reacting soyabean oil with butylamine.
NR: not reported.
CLA refers to *cis*-9, *trans*-11 C18:2.

C18:0 uptake due to the activity of stearoyl CoA (Δ-9) desaturase activity in mammary secretory cells. Conversion of C18:0 to *cis*-9 C18:1 is the predominant precursor:product of the Δ-9 desaturase, transforming about 40% of C18:0 uptake by the mammary gland (Chilliard *et al.*, 2000). It is therefore theoretically possible to exploit the ability of the mammary gland to enhance milk fat *cis*-9 C18:1 by supplementing diets with lipids rich in C18:0 such as tallow or hydrogenated oils. However, this approach does not change the *cis*-9 C18:1 : C18:0 ratio in milk fat and the feeding of tallow to dairy cows is not permitted within the European Union (Chilliard *et al.*, 2000). Feeding plant oils or oilseeds rich in *cis*-9 C18:1 can be used to enhance milk fat *cis*-9 C18:1 content, but unless these sources are effectively protected from ruminal metabolism, this strategy will also increase the concentrations of *trans* C18:1 in milk (Table 7.6). Supplements of *cis*-9 C18:1 acyl amides (Jenkins, 1998; Loor *et al.*, 2002) or high levels of rapeseeds or rapeseed oil in the diet (Murphy *et al.*, 1987; Givens *et al.*, 2003; Ryhänen *et al.*, 2005) have been shown to substantially increase milk fat *cis*-9 C18:1 content (Table 7.6), but both approaches can cause significant reductions in feed intake that can result in lowered milk production. Recent unpublished studies in this laboratory have, however, shown that rapeseed prepared by careful milling with wheat has substantial potential to be used in dairy cow diets to produce milk with lower saturates and higher *cis*-MUFA in a sustainable way.

7.3.6 Increasing the polyunsaturated fatty acid content of milk

Owing to extensive biohydrogenation in the rumen and the inability of ruminant tissue to synthesise PUFA, typically concentrations of C18:2 *n*-6 and C18:3 *n*-3 in milk fat are extremely low (Table 7.6). Even when high amounts of PUFA from plant oils and oilseeds are included in the diet, absolute increases in C18:2 *n*-6 and C18:3 *n*-3 are relatively small (Table 7.6). It has often been thought that feeding intact oilseeds rather than the extracted oil would enhance milk fat PUFA concentrations to a greater extent as result of the seed coat providing protection to the lipids from lipolysis and subsequent biohydrogenation in the rumen. Thus far, few direct comparisons have been made, and there is little consensus in the literature to suggest that oilseeds offer significant advantages over plant oils for enhancing milk fat PUFA concentrations (Chilliard and Ferlay, 2004). Indeed it is known that for rapeseed, retaining an intact seed coat can lead to low digestibility of the seeds and hence little oil release (Murphy *et al.*, 1987).

There is currently considerable interest in the potential of diets based on fresh grasses and legumes for increasing the C18:3 *n*-3 content of milk fat. This subject has been reviewed by Dewhurst *et al.* (2003) but essentially, although grasses and legumes contain only low concentrations of lipids, these lipids contain high concentrations of C18:3 *n*-3 which, when the plants

Table 7.7 Effects of forage conservation on contents of C18:3 *n*-3 in milk fat (compiled from Dewhurst *et al*., 2003, 2006)

Forage type	C18:3 *n*-3 in milk (g/100 g fatty acids)		Conservation method	Source
	Fresh forage	Conserved forage		
Grass/wheat	0.84	0.36	Silage	Timmen and Patton (1988)
Grass	1.97	1.46	Hay	Aii *et al*. (1988)
	1.34	1.13	Hay	Aii *et al*. (1988)
Grass	2.31	0.45		Hebeisen *et al*. (1993)
Maize/legume	0.95	0.25	Silage	Kelly *et al*. (1998)
Grass/clover	2.02	0.81	Hay	Dhiman *et al*. (1999)
Maize/legume	0.71	0.38	Silage	White *et al*. (2001)
Grass	0.76	0.41	Silage	Offer (2002)

are consumed in large quantities, can have a substantial effect on the C18:3 *n*-3 content of milk fat. The benefits of this are much reduced if the fresh forage is conserved by ensiling (Table 7.7). The possibility of improved fatty acid composition of milk when produced from organic systems has generally focused on C18:3*n*-3. Ellis *et al*. (2006) compared the fatty acid composition of milk from 19 conventional and 17 organic farms in the United Kingdom and showed overall that organic milk had significantly higher total *n*-3 fatty acids that from conventional systems (1.11, 0.66 g/100 g fatty acids respectively). Although not reported, most of the *n*-3 fatty acids are likely to have been as C18:3 *n*-3; such increases are likely to stem from the increased use of fresh forages and legumes in diets for cows on organic systems and little to do with the adoption of organic production standards per se. In any case the impact on the national diet over the year is likely to be very small.

There is also current interest in the possibility that more botanically diverse forages can be used to enhance C18:3 *n*-3 in milk (Leiber *et al*., 2005). One recent study (Lourenço *et al*., 2005) showed that feeding cows silages made up of 60% (dry matter, DM, basis) from a species-rich semi-natural pasture-produced milk with significantly higher C18:3 *n*-3 content that when the silage included contained 60% DM from a species poor semi-natural pasture (Table 7.8) although the difference was not large.

In addition to increasing concentrations of C18:3 *n*-3 in milk fat, there is also interest in enhancing the levels of the long chain *n*-3 fatty acids C20:5 *n*-3 (EPA) and C22:6 *n*-3 (DHA). The beneficial effects of these fatty acids

Table 7.8 Effect of plant species diversity on *n*-3, *trans*-11 C18:1 and conjugated linoleic acid in milk fat (from Lourenço *et al*., 2005)

Fatty acid	Diet based on silages from forages			
	100 IM[1]	20 SPP, 80 IM	60 SPP, 40 IM	60 SPR, 40 IM
C18:3 *n*-3	0.612	0.604	0.549[a]	0.590[b]
trans-11 C18:1	0.469	0.478	0.497[a]	0.822[b]
cis-9, *trans*-11 CLA	0.259	0.240	0.264[a]	0.438[b]
trans-10, *cis*-12 CLA	0.014	0.009	0.013[a]	0.033[b]
C20:5 *n*-3	0.095	0.105	0.075	0.067
C22:6 *n*-3	0.039	0.032	0.040	0.033

[1] IM, intensively managed grassland; SPP, species poor semi-natural grassland; SPR, species rich semi-natural grassland; number refers to percentage of forage dry matter from each silage.
[a,b] orthogonal contrast between 60 SPP and 60 SPR, values with different superscripts are significantly different ($P < 0.05$).

have been well documented and include anti-atherogenic, anti-thrombotic and anti-inflammatory effects and overall, increased intake leads to a reduced risk of coronary heart disease (see review of SACN/COT, 2004). It is now established that *in vivo* synthesis of EPA and DHA from dietary C18:3 *n*-3 is very limited especially in men (Burdge *et al*., 2003; Burdge and Calder, 2005), suggesting that a dietary supply is needed. Recent studies have shown that in many countries dietary intakes of EPA and DHA are substantially sub-optimal (UK, Givens and Gibbs, 2006; Belgium, Sioen *et al*., 2006; various parts of the world, Vermunt and Zock, 2007).

In milk from cows fed conventional diets based on forages and cereal-based concentrates, the concentrations of C20:5 *n*-3 and C22:6 *n*-3 are extremely low (typically less than 0.1 g/100 g fatty acids). It is possible to increase levels of C20:5 *n*-3 and C22:6 *n*-3 in milk by feeding sources of these fatty acids such as fish oil and marine algal lipids (see review of Givens and Shingfield, 2006) but the level of enrichment in milk fat is low with a typical efficiency of transfer of C20:5 *n*-3 and C22:6 *n*-3 from the diet into milk of 0.026 (±2.2) and 0.041 (±5.7), respectively (Chilliard *et al*., 2001). These values are much lower than the transfer efficiencies of 0.18–0.33 and 0.16–0.25, for C20:5 *n*-3 and C22:6 n-3, respectively, when fish oil was infused post-ruminally (Chilliard *et al*., 2001). The poor transfer of C20:5 *n*-3 and C22:6 *n*-3 from diet into milk is partly a result of extensive (between 74 and 100%) biohydrogenation in the rumen (Doreau and Chilliard, 1997; Scollan *et al*., 2001; Shingfield *et al*., 2003) and partly due to the preferential partitioning of these fatty acids into plasma phospholipids and cholesteryl esters for which the mammary lipoprotein lipase has only a low affinity (Offer *et al*., 1999, 2001a; Rymer *et al*., 2003).

There have been a number of approaches developed which attempt to protect plant or marine lipids from metabolism in the rumen and hence

provide a greater supply to the mammary gland. These include encapsulation of oils and oilseeds with a formaldehyde casein complex, calcium salts of fatty acids or fatty acyl amides. In many cases these approaches have been developed to reduce the negative effects of feeding large amounts of lipid on rumen function and animal performance, but they can also lead to significant and strategic changes in milk fatty acid composition, depending on the degree of protection from rumen metabolism.

7.3.7 Increasing the conjugated linoleic acid content of milk

Milk and dairy products are the main source of conjugated linoleic acid (CLA) in the human diet (Lawson *et al*., 2001; Parodi, 2003) with the *cis*-9, *trans*-11 isomer representing some 70 to 90% of the total. Over recent years there has been much interest in the potential beneficial effects of CLA on human health since studies involving the feeding a mixture of CLA isomers to small laboratory animals has been shown to reduce the rate of growth of chemically induced tumours as well as reducing atherogenesis, limiting the development of diabetes and reducing body fat content (see reviews of Roche *et al*., 2001; Yaqoob *et al*., 2006). However many of the studies used mixtures of CLA isomers, mainly equal quantities of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA, making it difficult to understand the relative bioactivity of each isomer. In addition, as pointed out by Roche *et al*. (2001), most animal studies have used relatively high doses of CLA (5 to 10 g CLA/kg diet) over short periods of time (4 to 8 weeks). Such doses would be equivalent to about 5 to 10 g CLA/d for humans. In the United Kingdom, milk and dairy products currently provide on average about 50 mg of *cis*-9, *trans*-11 CLA per day to adults (Givens and Kliem, unpublished) which indicates that milk fat would need to be enriched very substantially to provide intakes equivalent to those used in the small animal studies. There is some evidence from studies with mice, hamsters and pigs to suggest that the *trans*-10, *cis*-12 isomer in particular, causes hyperinsulinemia and insulin resistance, which may be related to the inhibitory effects of this isomer on Δ-9 desaturase activity (Terpstra, 2004). However, a recent controlled intervention study with healthy men consuming relatively pure supplements of *cis*-9, *trans*-11 or *trans*-10, *cis*-12 CLA across a wide range of intakes (0.59–2.38 and 0.63–2.52 g/d) indicated no significant effects on plasma insulin concentrations, the homeostasis model for insulin resistance or on indices of insulin sensitivity (Tricon *et al*., 2004). Furthermore, the concentration of *trans*-10, *cis*-12 CLA in milk fat is extremely low across a wide range of dairy cow diets (Shingfield *et al*., 2003; 2005), indicating that the contribution of milk and dairy products to *trans*-10, *cis*-12 CLA intake in the human population is extremely small.

In light of the potential beneficial effects on human health, numerous studies have examined the impact of nutrition and other factors on milk fat CLA concentrations. This topic has been extensively reviewed in recent

years (Griinari and Bauman, 1999; Bauman *et al*., 2001, 2003; Chilliard *et al.,* 2001; Chilliard and Ferlay, 2004). Diet is the main determinant of milk fat CLA content, as compared with the effect of breed, stage of lactation or parity, but there can be considerable variation (approximately three-fold) between individual animals fed the same diet (Peterson *et al*., 2002; Lock and Garnsworthy, 2003; Kelsey *et al*., 2003) possibly indicating a genetic component. Concentrations of CLA in milk can be enhanced using whole oilseeds or plant oils (Table 7.6), but greater increases have been reported when marine lipids are fed (Offer *et al.*, 1999, 2001b; Chouinard *et al*., 2001). The reasons for the higher increases in milk fat CLA content, when fish oil or marine algae are included in the diet compared with an equivalent amount of plant oil, appear to be related to the inhibitory effects of marine lipids on the final reduction of *trans*-11 C18:1 in the rumen. Shingfield *et al*. (2003) showed that feeding only 250 g/cow per day of fish oil had no effect on *cis*-9, *trans*-11 CLA synthesised in the rumen, but dramatically increased the amount of *trans*-11 C18:1 leaving the rumen from 17.1 to 121.1 g/day. As a result this significantly increased the supply of *trans*-11 C18:1, the substrate for endogenous *cis*-9, *trans*-11 CLA synthesis in the mammary gland. Plant oils rich in C18:2 *n*-6 and C18:3 *n*-3 also cause *trans*-11 C18:1 to accumulate in the rumen, but between two to three times as much vegetable lipid needs to be fed to produce the same response reported as fish oil (Loor *et al*., 2004; Shingfield *et al*., 2004a). As for C18:3 *n*-3 discussed above, concentrations of CLA are also known to be higher in milk from cows fed fresh pasture compared with dried grass, maize, grass or legume silages (Kelly *et al*., 1998; Stanton *et al*., 2003). Under UK conditions, milk fat CLA content is higher during the spring and summer months as a result of higher intakes of fresh grass (Lock and Garnsworthy, 2003).

As for C18:3 *n*-3, there is current interest in the possibility that more botanically diverse forages can be used to enhance the CLA content of milk. A number of studies with botanically diverse highland and mountain pastures have shown that these have potential to enhance milk fat CLA concentration (Collomb *et al*., 2001, 2002; Leiber *et al*., 2005). A recent study (Lourenço *et al*., 2005) showed that feeding cows silages made up of 60% (DM basis) from a species-rich semi-natural pasture produced milk with roughly double ($P < 0.05$) the concentrations of *trans*-11 C18:1, *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA than when the silage included contained 60% DM from a species poor semi-natural pasture (Table 7.8). The authors propose that the increased milk *cis*-9, *trans*-11 CLA seen from the species rich forage is likely to have been the result of an increased supply of *trans*-11 C18:1 to the mammary gland. This was supported by greater concentrations of biohydrogenation intermediates (including *trans*-11 C18:1) in milk and rumen fluid. Overall the data suggest that some factors in the species-rich forage may have inhibited rumen biohydrogenation. Lee *et al*. (2003) have identified lipase inhibitors in clover but clearly this is an area needing further study.

Table 7.9 Effect of fish oil in the diet on the isomeric forms of *trans* C18:1 fatty acid in milk (from Shingfield *et al*., 2003)

Trans C18:1 isomer	Control diet		Fish oil containing diet	
	g/100 g fatty acid	% of total *trans* 18:1	g/100 g fatty acid	% of total *trans* 18:1
C18:1 *t*-4	0.019	0.42	0.038	0.26
C18:1 *t*-5	0.014	0.31	0.038	0.26
C18:1 *t*-6,7 + 8	0.26	5.8	0.41	2.9
C18:1 *t*-9	0.26	5.8	0.57**	4.0
C18:1 *t*-10	0.21	4.7	1.01*	7.0
C18:1 *t*-11	1.80	40.1	9.39**	65.2
C18:1 *t*-12	0.34	7.6	1.19***	8.3
C18:1 *t*-13 + 14	0.63	14.0	1.05*	7.3
C18:1 *t*-15	0.50	11.1	0.47	3.3
C18:1 *t*-16	0.46	10.2	0.24*	1.7
Totals	**4.5**	**100**	**14.4**	**100**

*,**,*** Significantly different from control $P < 0.05$, 0.01 and 0.001 respectively.

7.3.8 *Trans* fatty acids in milk fat

As discussed above in relation to CLA, inclusion of plant oils rich in C18:2 *n*-6 and C18:3 *n*-3 and fish oils in dairy cow diets causes *trans*-11 C18:1 to accumulate in the rumen and this subsequently leads to increased concentrations in milk. While *trans*-11 C18:1 normally represents the major *trans* fatty acid, increases in it are usually accompanied by increases in other *trans* fatty acids. The effect on the various C18:1 *trans* isomers is illustrated in Table 7.9 for milk from a study which included fish oil in the diet of the cow.

There has been concern for many years about the negative health effects of dietary *trans* fatty acids since high intakes have been associated with a substantially increased risk of coronary heart disease (Willett *et al*., 1993; Kromhout *et al*., 1995; Ascherio *et al*., 1999) and the more recent meta-analysis of Mensink *et al*. (2003) indicated that *trans* fatty acids represented a greater risk to coronary heart disease than saturated fatty acids. Early studies in the United Kingdom identified an association between consumption of hydrogenated vegetable and marine oils and deaths from ischaemic heart disease (Thomas *et al*., 1983; Thomas, 1992) and by the mid-1990s it was clear that not only did epidemiological data highlight the increased coronary heart disease risk, but unique adverse effects on blood lipids were also evident (Ascherio *et al*., 1999).

Despite the concern about dietary *trans* fatty acids it seems that the metabolic response to different *trans* fatty acids can be quite different and it is of note that the profile of *trans* fatty acids in milk fat is quite different from that in industrially hydrogenated foods (Fig. 7.7). Four prospective

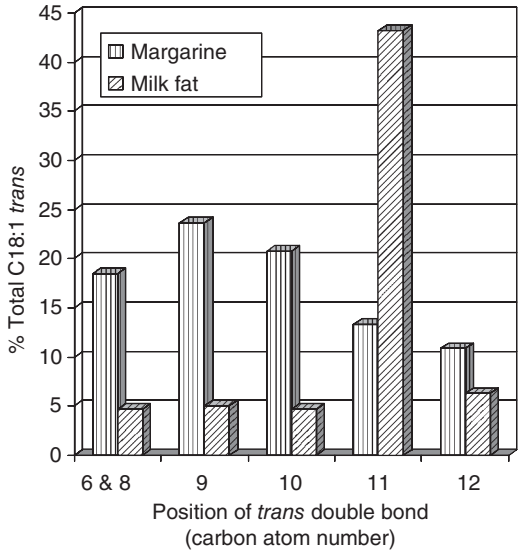

Figure 7.7 Distribution of main C18:1 *trans* isomers in margarines (46 samples) and butter (1765 samples) (from Precht and Molkentin, 1995).

epidemiological studies have examined the relationship between the intake of *trans* fatty acids from ruminant-derived foods and the risk of coronary heart disease (Willet *et al*., 1993; Pietinen *et al*., 1997; Oomen *et al*., 2001; Jakobsen *et al.,* 2006). None of these studies found a significant positive relationship and indeed in three of the studies there was a non-significant trend towards a negative relationship. These findings are supported by the overarching evidence discussed earlier, that increased milk consumption is associated with a reduction in the risk of ischaemic heart disease (Elwood *et al*., 2004). Mozaffarian *et al*. (2006) proposed that lack of an increased risk of coronary heart disease associated with the intake of *trans* fatty acids from ruminant-derived foods relative to the substantially increased risk from industrially produced *trans* fatty acids may be a result of lower intakes, different bioactivities or the fact that dairy and meat products contain some other factors that negate any negative effects that the *trans* fatty acids present cause. It is noteworthy, however, that since comparisons of ruminant and industrial *trans* fatty acids have been based on few studies using relative intake data (e.g. quintiles of intakes), the review of Weggemans *et al*. (2004) examined the relationship between absolute intake (i.e. grams consumed per day) of ruminant and industrial *trans* fatty acids and risk of coronary heart disease. They reported that where direct comparison was possible, there was no difference in risk between total, ruminant and industrial *trans* fatty acids for daily intakes up to 2.5 g. At higher daily intakes (>3 g) total and industrial *trans* fatty acids were associated with an increased risk of coronary heart disease but there were insufficient data available on

ruminant *trans* fatty acids at this level of intake. Weggemans *et al*. (2004) therefore concluded that based on the small amount of data available there was no case to discriminate between ruminant and industrial *trans* fatty acids in dietary recommendations or legislation although, subsequently, Lock *et al*. (2005) challenged this interpretation.

Because of the small amount of data available and since most human intervention studies have evaluated monounsaturated *trans* fatty acids from industrial sources, a study (TRANSFACT) is currently underway to directly compare the effects of *trans* fatty acids from milk and industrial sources on risk factors for cardiovascular disease in healthy humans (Chardigny *et al*., 2006).

7.4 Fortification of milk

Milk is widely consumed in many parts of the world including both the developed and developing regions. It is usually affordable, consumed regularly and centrally processed so quality control can be implemented easily, and it is for these reasons that it is seen as an efficient vehicle for delivering additional nutrients to the consumer via fortification. Milk is fortified for many reasons, to remedy processing losses, to address or reverse nutrient deficiencies, to aid absorption and utilisation of other fortified ingredients and, overall, because it is a widely consumed food. The fortification of milk is not a new idea. The discovery in the 1920s that increasing vitamin D intake prevented the onset of the childhood disease rickets led to the fortification of milk with vitamin D in several countries, including the United States and the United Kingdom. It was realised that milk could be used as a means of improving public health. Since then changes in processing and patterns of consumption of milk have meant that fortification has been adapted to ensure the nutritional needs of the population can still be met using milk as a vehicle. In some countries milk fortification with some nutrients is mandatory; in Canada for example, whole milk is fortified with vitamin D at a rate of 0.9 to 1.2 μg (35 to 47 IU) per 100 ml (Health Canada, 2005). In other countries it is voluntary, and milk can be fortified with more than one nutrient.

Table 7.10 shows a selection of nutrients in milk which can and have been used in fortification. Technically the word 'fortification' applies to the addition of an essential nutrient to give concentrations higher than those naturally found in a food and therefore includes nutrients which are naturally present in milk (e.g. calcium) and those that are normally not present (e.g. long-chain *n*-3 fatty acids).

7.4.1 Fortification with fatty acids

Increasing milk *n*-3 fatty acids has been seen as a way of combating the imbalance in *n*-6/*n*-3 ratio (Simopoulos, 2000) or inadequate intakes of

Table 7.10 Nutrients used in commercial milk fortification together with example countries and doses.

Nutrient	Typical concentration in whole milk* (per 100 ml)	Country where fortification occurs	Total amount in fortified product (per 100 ml)
n-3 fatty acids (long chain)	Trace, depends on cow diet	UK	45.2 mg EPA + 25.2 mg DHA
Conjugated linoleic acid	<70 mg, depends on cow diet	Spain	600 mg
Vitamin A (retinol)	~30 μg	Canada	>120 IU (semi-skimmed/ skimmed)
Folate (Vitamin B_9)	~5–7 μg	New Zealand	170 μg
Vitamin D	Trace	US	42 IU
Vitamin K	Trace	New Zealand	20 μg
Calcium	100 mg	US	208 mg
Iron	28 μg	New Zealand	600 μg

* Assuming whole milk = 3.5% fat.

long-chain *n*-3 fatty acids seen in many Western societies (Givens and Gibbs, 2006; Sioen *et al*., 2006). Milk naturally contains *n*-3 fatty acids, predominantly in the form of C18:3 *n*-3 (α-linolenic acid), which is the predominant fatty acid in green forages. However it is only present in milk in small amounts (less than 1.0 g/100 g total fatty acids; Givens and Shingfield, 2006) and varies considerably depending on diet of the cow. α-Linolenic acid is the *in vivo* precursor for the long chain *n*-3 fatty acids, EPA and DHA, although conversion in humans is poor (Burdge *et al*., 2003; Burdge and Calder, 2005). Milk from cows on normal diets naturally contains very little EPA and DHA (usually less than 0.1 g/100 g total fatty acids, Givens and Shingfield, 2006). Accordingly, fortification of milk with *n*-3 fatty acids usually involves the addition of fish oils to provide EPA and DHA.

Over the last 10 years milk fortified with EPA and DHA has been available commercially in several countries. In the United Kingdom for example, the St Ivel Advance® brand markets a fortified whole milk and semi-skimmed milk containing 113 and 63 mg EPA + DHA per 250 ml respectively (St Ivel, 2007). In Spain a product exists that provides around 66 mg EPA + DHA per 100 ml (Puleva Omega 3; Puleva Food SL, 2007) and the effect of consuming such a product on cardiovascular risk factors has shown positive effects (Carrero *et al*., 2004) although the milk was also fortified with oleic acid, folic acid and other vitamins and had its saturated fatty acid content reduced substantially. Although most of these products provide relatively small increases in EPA + DHA intakes at normal levels of consumption, as noted earlier, many populations have substantially sub-optimal

intakes and for these the availability of fortified milk may be very valuable.

There have also been efforts to fortify milk with CLA. As noted earlier, dairy cow diets which are high in polyunsaturated fatty acids can increase the CLA concentration in milk (e.g. Shingfield *et al*., 2003; Collomb *et al*., 2004), although concentrations rarely exceed 2.0 g/100 g total fatty acids. At this concentration, milk and milk products would contribute about 230 mg CLA per day to the average UK adult diet (Givens and Kliem, unpublished). Since much higher intakes have been associated with beneficial properties (e.g. Ip *et al*., 1994), studies have been conducted into directly fortifying milk with CLA. Campbell *et al*. (2003) fortified milk with CLA (added to skimmed milk with/without cream to create 2% total fat milk; CLA profile: *cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA formed 36.9% and 36.7% total fatty acid methyl esters (FAME) respectively) and included antioxidants to prevent oxidation. These were found to be ineffective and the *cis*-9 *trans*-11 CLA isomer decreased by 16% after 3 weeks' storage. However in a more recent study, Rodríguez-Alcalá and Fontecha (2007) compared the effects of storage on the CLA content of various fortified foods and found that the major isomers in CLA fortified milk did not decrease even after 10 weeks of refrigerated storage. The work of Campbell *et al.* (2003) also showed that in sensory tests CLA fortified milks overall scored slightly lower ($P < 0.05$) than control milks. Despite this, CLA fortified milks are commercially available in a number of countries (e.g. Spain, 'Naturlinea', Central Lechera Asturiana, 2007).

7.4.2 Fortification with vitamins

Vitamins A and D are fat-soluble vitamins and in milk are therefore primarily found in the fat fraction. However, in many developed countries consumption of full-fat milk has decreased in recent times and there has been an increased consumption of semi-skimmed and skimmed milk. A consequence therefore has been a reduction in the intake of fat-soluble vitamins and so steps have been taken to reverse this to prevent deficiencies of these vitamins. Currently in the United Kingdom, although dairy products provide about 26% of the recommended intake of vitamin A (Table 7.2), vitamin D from the same source only represents about 3% of total dietary vitamin D.

Fortification with vitamins A and D started in the United States in the 1920 and 1930s to reduce the incidence of vitamin deficiencies with vitamin D being added to compensate for the lack of exposure to sunlight. The elimination of rickets in the United States in the 1930s marked the success of milk vitamin D fortification. At present vitamin D fortification is conducted in several countries including the United States, Canada and Argentina. Calvo *et al*. (2004) suggest that current levels of fortification in the United States are inadequate for the adult population, and that efforts

need to be made to increase vitamin D intake of vulnerable sub-populations that may not consume a large amount of milk. In most places vitamin A fortification of whole milk is optional, but in several countries (e.g. United States, Canada), it is mandatory for semi-skimmed and skimmed milks.

The B group vitamins are water soluble and include eight individual vitamins, B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6 (pyroxidine), B_7 (biotin), B_9 (folate) and B_{12} (cobalamin). Many of these vitamins are present in significant amounts in milk, typically a 200 ml serving of semi-skimmed milk will provide an adult with 100% of the daily recommended nutrient intake of vitamin B_{12}, 15% B_1, 45% B_2 and 9% B_9 (Dairy Council, 2007).

Of the B vitamins that are present in lower amounts in milk, folate (B_9) has been highlighted as a possible candidate for milk fortification. Adequate folate intake is extremely important for foetus development in the early stages of pregnancy, as well as having a key role in processing homocysteine, elevated levels of which can lead to increased risk of cardiovascular disease and may be involved in dementia progression in older people. It has been shown that in countries where folate fortification of foods is not permitted, folate intake is usually lower than the recommended amount (de Bree *et al*., 1997). Whole milk contains between 5 and 9 μg folate per 100 g (Forssén *et al*., 2000) and the concentration of natural folate in milk has been found to vary depending on season (Wigertz *et al*., 1997). In the United Kingdom, milk/dairy products provide only about 13% of the dairy recommended intake (Table 7.2) but, as pointed out by Smith *et al.* (1985), folate in milk may be more bioavailable than that in other foods, owing to the presence of folate-binding proteins in milk. In pasteurised milk, folate-binding proteins still remain bound to folate and are thought to prevent microbial digestion of the folate, so increasing the chances for absorption (Ford, 1974). In addition folate-binding proteins may facilitate uptake of folate by the gut from other foods, although there are conflicting reports on this. The presence of folate-binding proteins has led to milk being fortified with additional folate. de Jong *et al.* (2005) found that milk fortified with folic acid (the synthetic form of folate; 200 μg in 500 ml milk) led to increased circulating folate. Since then fortified milks have been made commercially available (e.g. Anmum™, Fonterra™, 2007), targeted specifically at pregnant women.

Vitamin C is another water-soluble vitamin, and cannot be synthesised by humans. Milk contains only around 1 μg per 100 ml milk. Studies have shown encapsulated vitamin C (to prevent degradation) can be usefully added to milk (at 100 or 250 mg/L) already fortified with iron to enhance iron absorption (Lee *et al*., 2004).

Milk contains very little or no vitamin K, and within the last few years it has been added to milk to help maintain bone strength and prevent bone degradation (Fonterra™, 2007). Such fortified milks are also fortified with calcium, vitamin D and other minerals (e.g. Anlene™, Fonterra™, 2007).

7.4.3 Fortification with minerals

Milk and dairy products typically provide some 60% of calcium requirements (Table 7.2) and are the major source of calcium in the diet. Calcium in dairy products has a high bioavailability, but an overall decrease in milk consumption is leading to inadequate calcium intakes in many countries. Other food commodities have been fortified with calcium (e.g. fruit juices) but milk is probably the most universal vehicle for additional calcium and other minerals. Very often milk fortified with calcium is also fortified with iron and zinc, as calcium can reduce the bioavailability of these minerals (e.g. Wood and Zheng, 1997; Perales *et al.*, 2006).

7.5 Removal of undesirable compounds

7.5.1 Removal of lactose

In the human small intestine, lactose is normally hydrolysed by a lactase enzyme and although a declining ability to digest lactose after childhood is normal, some degree of lactose intolerance in adults is common in many populations. The incidence of acute lactose intolerance is uncommon in the Nordic countries, the United Kingdom, Ireland and Australasia although in Asians, lactase decline usually starts at 2 to 3 years of age and acute lactose intolerance is seen in 95% or more of adults (Vesa *et al.*, 2000). As a result, various approaches have been proposed to reduce the lactose content of milk and milk products.

There is general agreement that fermented dairy products including yoghurt and milk with additions of *Lactobacillus acidophilus* and *Lactobacillus casei* are much better tolerated than standard milk (Kolars *et al.*, 1984) due to the fact that the fermentation process will remove much of the lactose. It has also been proposed that especially in yogurt, the activity of β-galactosidase can further reduce the problems associated with lactose intolerance. Another approach has been to hydrolyse the lactose in the milk enzymatically. For instance in long-life milk, lactase is added to the milk after sterilisation and the product is released for sale after a period of time when lactose concentration will have decreased substantially (Saxelin *et al.*, 2003). Such processes can make milk taste much sweeter as the hydrolysis products of lactose, glucose and galactose are much sweeter than lactose. However Saxelin *et al.* (2003) report that this problem has been largely solved by Valio Ltd, with lactose being removed from milk by a Valio patented physical separation method. This lactose-free milk drink is presently available in Finland, Sweden, Switzerland, South Korea, Spain and Belgium (Valio, 2007).

7.5.2 Removal of fat

As noted above there has been an increasing demand for reduced fat milk products. Much of this is produced by processing whereby the fat is removed

mechanically. There has also been some renewed interest in producing milk of low-fat content by manipulating the diet of the cow. The so-called low-fat milk syndrome where diet induces milk with a low-fat content to be produced was first described over 100 years ago. Many theories have been proposed to explain the mechanisms involved in this process. These have included low-fibre diets which give rise to a limited supply of lipid precursors and more recently factors which directly inhibit lipid synthesis in the mammary gland. Bauman and Griinari (2001) have reviewed this topic and confirmed that diet-induced low-fat milk contained increased concentrations of *trans*-10 C18:1 and its rumen precursor, *trans*-10, *cis*-12 CLA. Bauman and Griinari (2001) showed that for a wide a range of diets there was a curvilinear relationship between the reduction in milk fat yield and the increase in the concentration of *trans*-10, *cis*-12 CLA in milk fat. They also reported that postruminal infusion of *trans*-10, *cis*-12 CLA brought about a sharp inhibition of milk fat synthesis and a change in the fatty acid composition of milk similar to that seen in diet-induced milk fat depression. It therefore seems that diets which induce low-fat milk change the rumen biohydrogenation process in a way that favours the production of *trans*-10, *cis*-12 CLA, which appears to be a potent inhibitor of milk fat synthesis.

This knowledge has given rise to interest in the development of rumen-protected CLA products which may be added to the diet of the cow to rapidly induce the production of low-fat milk. An example of this is the study reported by Shingfield *et al*. (2004b) in which supplements of casein-formaldehyde treatment of CLA methyl esters containing equal amounts of *cis*-9, *trans*-11 and *trans*-10, *cis*-12 were given to early lactation dairy cows. The treatment diet supplied 14.3 g *trans*-10, *cis*-12 CLA/cow per day and this rapidly reduced milk fat content with the milk on average containing only 19.2 g fat/kg compared with 34.9 g/kg in milk from the control diet. It remains to be seen if this approach leads to a commercial and sustainable means of producing low-fat milk.

7.6 Future trends

Development of milk and dairy food products containing reduced concentrations of saturated fatty acids seems to be a consistent and worthwhile objective with respect to reducing the risk factors for chronic disease. This is despite the epidemiological evidence that increased milk consumption reduces the relative risk of many aspects of chronic vascular disease. This apparent paradox does, however, need thorough investigation so that the positive effects of milk can be understood and exploited fully. Changes in milk lipid composition must be fully assessed in human studies of sufficient duration for effects to be manifested; so far, few studies of this type have been done.

It seems likely that concerns about the relationship between diet and chronic disease will continue to increase, not least because of increasing cost to national health-services for treating such conditions. This will increase the urgency to fully understand the health-related aspects of staple foods such as milk and the need for clear nutritional messages to be given to the public.

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8

Improving the sensory quality, shelf-life and functionality of milk

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Abstract: This chapter provides an account of the technological advances in the dairy industry aimed at (i) improving the shelf-life and safety of milk; (ii) enhancing the sensory qualities of milk; (iii) the development of ingredients from milk and expansion of their applications base; and (iv) the development of modern milk-based beverages, notably those targeted at the functional foods market. The chapter concludes with future trends anticipated in the dairy beverage sector and sources of further information.

Key words: milk safety, milk shelf-life, processing of milk, ingredients from milk, milk beverages.

8.1 Introduction

Milk is a complex mixture of fats, proteins, carbohydrates, minerals and minor components that is essentially complete for the growth and development of the neonate. Milk is one of the oldest established beverages consumed, either in natural or fermented state, for its nutritional, refreshment and enjoyment value. Perhaps milk's one major drawback is its perishable nature. Consequently, traditional preservation techniques have been applied in order to prolong storage life and ensure safety. The evolution of sour or fermented milk consumption has been associated more with countries with warmer climates where fermentation-induced acidification functioned both as a form of product preservation and also because it conferred unique sensory properties. In-can and in-bottle heat sterilisation processes marked the arrival of 'long-life' milk as a new product concept, although at the expense of compromising the milk's original fresh flavour characteristics. This technology was later to be applied to concentrated milks. A variant of the latter was sweetened condensed milk, where high sugar concentration

provides the preservation effect instead of heat. As the chapter unfolds, we will see how modern-day technological processes may be used to preserve milk with varying levels of success in terms of shelf-life extension and the retention of milk's fresh characteristics.

Technological developments have allowed a greater understanding of the components that make up milk, their biological properties and physical functional properties as food and beverage ingredients. With the development of technologies to concentrate and isolate these components, manufacturers have added significant value to liquid milk by producing a range of valuable milk-derived food and beverage ingredients. As manufacturers strive for greater value from milk, the number and variety of milk-derived ingredients is likely to increase, giving traditionally non-dairy beverage manufacturers the opportunity to develop beverages that meet consumer demands with the added benefits of dairy components.

As a beverage, milk's unique market position has been eroded over the years with the emergence of new drink products which are heavily marketed to appeal to younger age categories. However, with changing lifestyles and consumers becoming more selective about the quality of such drinks, the dairy industry worldwide is responding to new market challenges by configuring milk in a variety of formats in order to link particular nutritional content with functional benefits. For example, low-fat milks as well as milks fortified with ingredients, including calcium (bone health), vitamins A and D, folic acid (foetal development), omega-3 fatty acids (cardiovascular disease) and other bioactives with targeted health benefits. Furthermore, beverages may also be formulated using selected functional constituents of milk which today can be sourced in the form of commercial ingredients. This approach enables the functional benefits of milk to be experienced while consuming modern-style beverages.

The chapter provides an account of the technological advances in the dairy industry aimed at (i) improving the shelf-life and safety of milk; (ii) enhancing the sensory qualities of milk; (iii) the development of ingredients from milk and expansion of their applications base; and (iv) the development of modern milk-based beverages, notably those targeted at the functional foods market.

8.2 Improving the safety and shelf-life of milk and milk derivatives

Microbial quality and safety go hand-in-hand up to a point when handling milk. In other words, adherence to good hygienic practices and maintenance of low bacterial numbers will serve both objectives well. This begins with the health status of the lactating dairy cow, the conditions under which milking is carried out, and logistics involving immediate refrigeration, storage and transportation to milk processing plants. Compromises in

microbial quality may result in immediate sensory defects, undermine processability of milk (e.g. protein coagulation on heat exchangers and generation of heat-stable proteolytic and lipolytic enzymes which affect long-life products at a later stage in their storage life). Where quality and safety differ is that the presence of low bacterial counts in milk is virtually meaningless if it happens that some of those microorganisms compromise public health. Hence, the success of pasteurisation as a thermal step for the destruction of virtually all pathogenic microorganisms has become a critical control point for this purpose with the result that the frequency of milk-borne disease is now very low (Muir, 1996a).

Familiar pathogenic bacteria such as *Mycobacterium tuberculosis* (tuberculosis-causing micoorganism), *Staphylococcus aureus* (frequently associated with mastitis infection), *Brucella*, *Campylobacter*, enterotoxin and verotoxin-producing strains of *Escherichia coli* and *Listeria monocytogenes* and others are destroyed by pasteurisation. Recent results of a survey of over 5000 retail milk samples taken across the United States (Frye and Donnelly, 2005) confirm a low frequency of *Listeria monocytogenes* contamination (0.018%) of pasteurised fluid milk products (whole milk, non-fat milk, chocolate milk and other [reduced fat and low fat]). Much higher values figured in earlier estimates by US regulatory agencies following the emergence of this pathogen during the 1990s. The current improvements testify to the benefits of operational and process plant upgrades that have been implemented in the intervening years. More recently, the incidence of *Enterobacter sakazaki* in dried infant milk formula has given rise to concerns about this pathogen. The bacterium is destroyed by pasteurisation; however, it is proving to be quite challenging to control in-process contamination during the advanced stage of milk spray drying and subsequent powder handling. This pathogen is regarded as low risk in relation to the production of liquid milk beverages.

8.2.1 Heat treatment approaches to improve safety and shelf-life

Pasteurisation

A traditional dairyman's approach to the processing of milk was to preserve its wholesomeness in the chain from farm to consumer. Nothing was to be added, and indeed little was taken away. The advent of the high-temperature, short-time (HTST) process for milk pasteurisation guaranteed the consumer a safe product from a public health point of view while at the same time scarcely tampering with milk's natural 'fresh' flavour. However, a modest shelf-life (typically 7–10 days under refrigerated conditions) was to be expected.

While developments in thermal and other technological processes are central to the production of extended shelf-life (ESL) milks, considerable progress has also been made with improvements to packaging in order to minimise post-pasteurisation contamination. ESL packaging refers to a

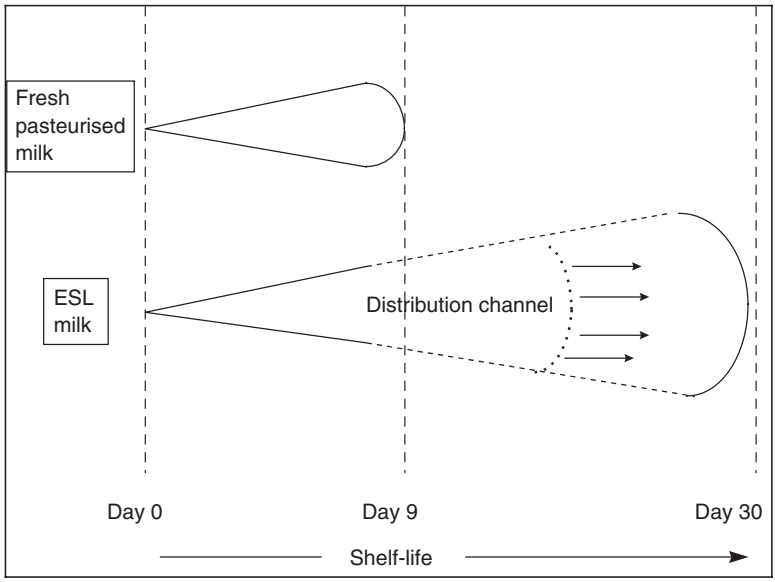

Fig. 8.1 Concept diagram illustrating the expansion of distribution channels and market reach afforded by ESL milks.

standard pasteurised, HTST product packaged into 'standard' dairy cartons in a sterile environment utilising advanced sanitation features such as high-efficiency particulate air (HEPA) filtration. Extended long life (ELL) packaging refers to ultra-pasteurised (ultra-high temperature, UHT) product packaged using all 'ESL' technologies plus hydrogen peroxide packaging sterilisation; exposure to ultraviolet radiation and optional use of advanced barrier cartons. A shelf-life of up to 90 days is claimed for dairy products depending on milk quality, carton type and distribution controls.

ESL milk appeals to consumers whose centralised shopping (e.g. a once-per-week or less frequent basis) simplifies the organisation of grocery requirements in individual households. Milk purchases that fit with such domestic routines are equally appealing. The downside, however, is that consumers may be under greater pressure to store such bulk purchases of ESL milk in a domestic refrigerator that may be already limited in capacity. In this respect, UHT milk has an advantage in that it may be stored under ambient conditions. The appeal of ESL milk to the processor/wholesaler is that less frequent deliveries are required to retail outlets; logistics are better managed through depot-based distribution centres located in target markets, and greater geographical reach is afforded (Fig. 8.1).

Traditionally, pasteurised milk has been consumed within a few days of production. However, as the storage life is being extended there is greater awareness of the opportunity for growth in milk of microorganisms which

contaminate the product post-pasteurisation and, indeed, the possibility of spores of sporeforming bacteria which survive pasteurisation becoming vegetative once more. *Bacillus* spp. (*B. cereus*, *B. licheniformis*, *B. coagulans*) are of greatest significance because of their ability to grow under refrigerated conditions. *B. cereus* has long been associated with quality defects and as regards its pathogenicity, emetic and diarrheogenic strains have only been found in low numbers and on a limited number of occasions (Muir, 1996a). Refrigerated milk storage (<6 °C) will, however, limit the germination and growth of *Clostridium perfringens* spores. *Bacillus* spore germination post-pasteurisation is affected by pasteurisation temperature with activation being greater within the range 72 °–76 °C, 15 s (Hanson *et al*., 2005). Higher pasteurisation temperatures appear to cause cell injury, but after a period of recovery growth resumes. Hanson *et al*. (2005) also found that *Bacillus mycoides* more than *B. cereus* occurred in the isolates prepared during their study and suggested that the former bacterium may be implicated in sweet curdling of milk more than previously reported.

An experimental model relating shelf-life of pasteurised milk to temperature and pre-incubation count is outlined by Muir (1996b):

$$\text{Shelf-life (h)} = \{0.00621[T - (269.55 - 0.74(\text{CFC}_{15}) - 0.11(\text{CFC}_{15})2]\}^{-2}$$

where T = storage temperature in K; $\text{CFC}_{15} = \log_{10}$ count after pre-incubation of pasteurised milk at 15 °C for 24 h and enumeration on milk agar containing cetrimide-fucidin-cephaloridine (CFC) as an inhibitor selective for the growth of *Pseudomonas*. It is claimed that shelf-life of pasteurised milks stored within a temperature range of 6–14 °C may be predicted to within 1 day for between 60 and 90% of samples (Muir, 1996b).

Ultra-high temperature

Until recently, the prospect of achieving a much longer shelf-life of milk depended on the use of extensive heat treatments. During the 1960s, UHT processes were developed in conjunction with aseptic carton filling as a means of virtually sterilising milk by continuously heating milk within the temperature range 135–150 °C to prolong shelf-life to at least 12 months (Burton, 1988). More specifically, high temperatures of 140 °C or so with short holding times in the order of 4 s constituted the innovative element of UHT systems. These represented a significant development over traditional retort sterilisers where milk was heated much more severely, to the point of discoloration, in bottles at ~120 °C for 15 min. In terms of microbe destruction, UHT processing generally succeeds in achieving in the order of a 9 log reduction of thermophilic spores (Hinrichs and Kessler, 1995). The ultimate objective from a food safety perspective, however, is to ensure that *Clostridium botulinum* spores do not survive the process because of the pathogenicity of the toxins produced during subsequent growth (Burton, 1988).

A number of physical indices are used to express the microbiocidal effect of UHT processes (e.g. D = decimal reduction time – rate of thermal death at a given temperature; z-value = variation of thermal death rate with temperature; Q_{10}-value = amount by which thermal death rate changes for a temperature change of 10 °C). The lethal rate, L, represents the relationship between the D-values at reference and designated temperatures, while the sterilisation value, F, is the sum of L and t (processing time) and can be calculated from the temperature–time course of a process and the z-value (van Boekel and Walstra, 1995). A downside of UHT processing by comparison with pasteurisation, however, is a so-called 'cooked flavour' remaining in the milk. Many European countries, other than the United Kingdom and Ireland, adopted this technology where UHT milks to this day account for almost the entire retail trade in domestic milk. Such was the concentration of UHT processing in mainland Europe over the years that strains of high heat-resistant spores (HRS) began to survive UHT processing conditions and became a process contaminant that eventually contributed to UHT product spoilage during storage. This problem was eventually resolved by increasing UHT temperatures and/or holding times.

Ultra-pasteurised is a term commonly used in the United States since the late 1980s to define milk that has been heated at or in excess of 138 °C for at least 2 s (before or after packaging), in order to confer extended shelf-life on the product under refrigerated storage conditions. Undoubtedly, temperatures in between HTST pasteurisation and UHT conditions have been exercising the minds of dairy technologists when challenged with extending shelf-life of milk while at the same time hoping to avoid compromising sensory aspects. The study of Blake *et al.* (1995) provides some insights into the usefulness of sub-UHT conditions based on direct steam injection (120, 130 or 140 °C for 4 or 12 s) for extending the shelf-life of thermally processed milks. No microorganisms survived temperatures of 134–140 °C when milks with high initial total plate count (4.4×10^8 cfu/ml) were heated. High total and psychrotrophic counts were obtained after 15 days' storage (7 °C) from milk processed at 100 or 110 °C for 4 s. Spore-forming *Bacillus* spp. isolated at ≤128 °C gave the authors (Blake *et al.*, 1995) some concern about the potential pathogenicity of *B. cereus* in ESL milks processed at such temperatures, especially given their propensity to grow if storage conditions rose to 10 °C

Novel heating technologies

The Pure-Lac™ concept, introduced by APV/Elopak, is based on the use of the APV Nordic's novel steam infusion heating technology which heats milk to between 130 and 145 °C for little over 1 s, and Elopak's know-how in milk packaging technology. Sensory quality equal to or better than pasteurised milk with a shelf-life of up to 45 days, depending on packaging method, is claimed.

The *Milk Marque* process is based on a patent taken out by the former Milk Marketing Board (Surrey, England) in 1980. The patent covers a system of high-temperature pasteurisation up to 130 °C followed by rapid cooling to 5 °C and packing aseptically. The technology, which is available via Charles Wait (Process Plant) Ltd, features a two-stage preheater, a super-heater, rapid cooling and storage in an aseptic tank prior to aseptic filling. A storage life of 28–35 days at 7 °C is claimed without impairing flavour. Investment costs are claimed to be lower since the process exploits conventional plate heater technology.

8.2.2 Innovative and emerging technologies to improve safety and shelf-life

It appears certain now that the gap between pasteurised and UHT milks will be bridged in the future by a new generation of processed 'fresh' milk products manufactured using new technologies in areas such as thermal and non-thermal processes, membrane filtration and packaging systems. Adoption of these technological developments will be beneficial in terms of maintaining the wholesome flavour of a typical pasteurised milk for up to a 1 month period under chilled storage conditions.

In many cases new non-thermal technologies have been developed to provide microbial inactivation in foods as an alternative to traditional thermal processes such as pasteurisation or sterilisation. From a simple microbial perspective, these newer technologies are generally cost prohibitive when compared with the more established processing technologies. However, scientific and commercial interest in these emerging technologies is increasing because they provide additional value to food products beyond that achievable by more conventional, often heat-based, processes. The science and patent literature reveals that for milk and dairy-based foods these alternative technologies can be used (often in combination with the components in milk) to create value added products for niche markets.

Microfiltration-based approaches

Advances in membrane-based processing technologies that have occurred over the past 30–40 years have resulted in new options for the dairy processor to achieve enhanced quality and extended shelf-life of liquid milk and milk derivatives (Cheryan, 1998). In particular, microfiltration (the most porous of the typical membrane-based techniques used in the dairy industry), sometimes in combination with other thermal and non-thermal processes, provides for removal of bacteria and spores, and an ESL milk product (Pedersen, 1992). Additionally, in regions of the world where somatic cells represent a problem, large-pore microfiltration provides the processor with an option for removal of these cells. In other parts of the world, vegetative spores associated with animal feeds can pass directly through the animal and into the milk supply. Some of these spores survive pasteurisation and

can cause shelf-life problems in the final products produced. Large-pore microfiltration will allow milk to pass through the membrane and reject the problematic bacteria, spores and somatic cells which are too large to pass through the membrane (Gregory, 2002). This approach can also be used as a precursor to pasteurisation, allowing for ESL of products with standard pasteurisation techniques and equipment.

Increasing the shelf-life of liquid milk from 16 to 42 days can be achieved by a combination of microfiltration and thermal processes embodied in the Tetra Therm ESL™ system, a technological development of the late 1980s. Tetra Pak claimed that the process replicated the sensory and chemical properties of pasteurised milk (72 °C, 15 s) with the added benefit of achieving a significantly lower bacterial content. With bacteria reduction in the order of 90–97% (1–2 log units) in normal pasteurised milk, the Tetra Therm ESL™ process improves this outcome 3–5 log units (i.e. 1000 to 100 000 times better). The process is based on splitting milk into its cream and skim milk phases in order to process each stream separately: skim milk is microfiltered using membranes with a high bacterial removal efficiency; that part of the skim milk (retentate) which is retained by the microfiltration membrane is combined with the cream before heat treatment of the mixture to 120–130 °C for 2–4 s. The final process steps: mixing of all process streams; homogenisation, and pasteurisation are performed in an enclosed system in order to minimise post-pasteurisation contamination. The claims for the Tetra Therm ESL™ system are based on milk with an initial bacterial contamination of 30 000 cfu/ml, and this may be improved further if the microbiological load in the raw milk is initially lower.

Alternative processes based on the use of microfiltration only (i.e. without pasteurisation) have been suggested mainly as a novel approach to present liquid milk with an even more authentic fresh flavour. However, a concern regarding the survival of pathogenic microorganisms, no matter how little, makes it almost mandatory to use microfiltration in combination with a thermal technique such as pasteurisation. Continuing developments in membrane technology such as increasing the efficiency of bacterial removal from 99.9% to 99.99% is helping to provide even greater safety assurance, shelf-life extension and enhanced organoleptic outcomes.

Packaging and filling improvements

The evolution of Tetra Pak's filling systems based on their Tetra Rex cartons takes in such milestones as Tetra Rex Ultra Clean (1987), Tetra Rex ESL (1992) and Tetra Rex Advanced ESL (1998). Package configuration and seal quality features in the current generation of ESL cartons include the avoidance of paper in contact with product, improved tightness of package seals in order to avoid re-contamination, and the development of barrier materials for oxygen-sensitive products. The product protection components of the filling machines include automatic cleaning boxes, steam sterilisation of

filling systems, complete aseptic product valve, and a newly developed carton sterilisation system.

In recognition of system vulnerability associated with post-pasteurisation contamination, Tetra Pak responded with their 'concept' dairy (Dairy Design D2) at the 25th International Dairy Congress, held in Aarhus, Denmark, during September 1998 (Harrysson, 1998). A revolutionary revision of traditional milk standardisation approaches culminates in fat standardisation now being moved from the process to the package. A dual stream filling system is utilised which combines cream and skim milk inside the pack. The claimed benefits include improved product quality as well as reduced operating costs (fewer storage capacity requirements, etc.).

High-pressure processing

High-pressure processing (HPP) is a non-thermal technique that relies upon the application of ultra-high hydrostatic pressure (up to 700 MPa) to food streams and products, such as milk, to achieve safety, preservation and enhanced quality. HPP leads to microbial (and some enzyme) inactivation without off-flavours, colour degradation (usually), and other quality loss associated with heat. HPP is inherently a batch technique, or at best semi-continuous, and is thus not well suited to the commercial processing and throughput of large volumes of milk or other dairy streams. Rather, the technique may well come to the fore in value addition to some products targeted at niche, high-value market segments, or to novel modulation of functionality for improved organoleptic and quality outcomes.

There is considerable interest in the use of HPP for the inactivation of bacteria, spores, and other pathogens and spoilage organisms in milk and related products. Numerous studies can be found in the literature comparing the effects of pressure, a combination of pressure and heat, and thermal processing on cell counts in milk and milk products (Adams *et al*., 2006; Arques *et al*., 2006; Lopez-Pedemonte *et al*., 2006, 2007; Sarkar, 2006; Scurrah *et al*., 2006; Usajewicz and Nalepa, 2006). Overall, while milk safety and shelf-life, equivalent to both pasteurisation and sterilisation, can be achieved (e.g. using a combined pressure/temperature treatment), it is not commercially practical to use HPP for microbial inactivation alone. High-pressure treatment, however, affects not only the microbial activity of milk and dairy systems, but also the functional properties of the components present in milk, as well as the biochemical (enzymatic) processes that take place in milk. Thus, HPP offers the processor the potential for quality enhancements not achievable using heat-based techniques alone.

HPP has recently been developed to increase the shelf-life of yoghurts to ~90 days with no loss of active cultures (Carroll *et al*., 2006). Complementing the benefit in shelf-life extension, it has been reported that HPP can also increase the viscosity of yoghurt formulations compared with control, allowing either improvements in quality or relative reductions in formulation and ingredient costs (Walker *et al*., 2006). The Japanese

food company Meidi-Ya has also developed dairy-based desserts using HPP.

Perhaps one of the most exciting opportunities for HPP in dairy systems is the potential to modulate the functionality of dairy proteins using pressure. When subjected to high pressure, the milk proteins behave quite differently. Casein micelles, for example, have been shown to dissociate with increasing pressure, followed by some re-association upon pressure release (Huppertz *et al*., 2006; Orlien *et al*., 2006). By contrast, whey proteins have been shown to reversibly unfold at relatively low pressures, followed by aggregation and gelation at higher applied pressures (Kresic *et al*., 2006; Lee *et al*., 2006). Indeed, the two major whey proteins behave quite differently to applied pressure, α-lactalbumin being quite stable to denaturation at pressures up to 600 MPa for 10 min, while by contrast under these conditions β-lactoglobulin is >75% denatured (R. Stockmann, personal communication). In a milk system, increasing applied pressure leads to association of whey proteins with casein micelles. Thus, the physicochemical behaviour of the milk proteins under pressure is quite different from that observed for heat treatment, thus providing the processor and dairy technologist with a further lever to influence the functional quality of milk and dairy products.

The science underpinning the high-pressure-modulated structural behaviour of milk proteins provides an opportunity to design food systems with novel or improved properties. A number of potential commercial applications have been suggested, including (i) yoghurt with enhanced sensory properties and minimal syneresis; (ii) functionally enhanced milk powders; and (iii) flavour-enhanced milk (Kresic *et al*., 2006; Kuehn *et al*., 2006; Lee at al., 2006; Walker *et al*., 2006).

Ultra-high-pressure homogenisation

While researchers worldwide in recent years have demonstrated some interesting structural and functional changes in milk as a result of HPP, it looks increasingly unlikely for economic reasons that there will be, in the short to medium term, commercial application of HPP in regular dairy processes. However, a related emerging technology, ultra-high-pressure homogenisation (UHPH) is proving to be quite effective in bactericidal terms – even more so than HPP where milk is concerned. Furthermore, UHPH (also referred to dynamic high pressure) offers the benefit of continuous operation and may well be more affordable than HPP.

UHPH using the Stansted device (Stansted Fluid Power, Essex, UK) is based on forcing milk through a high-pressure ceramic valve capable of supporting 350 MPa and a second pneumatic valve, located after the first, that operates at lower pressures <50 MPa. High pressure is provided by two intensifiers driven by a hydraulic pump. Hence, the pressures typically studied (100, 200 and 300 MPa) during UHPH are broadly in line with those of HPP. Pereda *et al*. (2007) found that UHPH at 200–300 MPa in a single

pass was as efficient (99.99%) in reducing psychrotrophic, lactococci, and total bacteria as high temperature treatment (90 °C, 15 s) with up to 3.5 log reductions in cfu/ml. Coliforms, lactobacilli and enterococci were eliminated. The microbial shelf-life of milks during storage at 4 °C from both treatments was similar over a 14–18 day period. While instrumental techniques could detect physical changes in milk such as colour lightness and viscosity decrease post-treatment, the authors (Pereda *et al*., 2007) were unable to differentiate these attributes subjectively and believed that consumers would respond similarly.

Earlier, Vachon *et al*. (2002) reported on the effectiveness of the Avestin Emulsiflex C5 homogeniser device for the inactivation of pathogens. There was complete elimination of *E. coli* O157 : H7 in milk which had been spiked with a starting count of 10^8 cfu/ml by operating the UHPH system at 200 MPa using five passes. *L. monocytogenes* proved more difficult to destroy and a 5.6 log reduction in viable count could only be achieved after increasing pressure to 300 MPa with five passes – the difference in performance being explained by possible variation in cell membrane properties of the respective pathogens (Vachon *et al*., 2002). The lethality effect of UHPH was also enhanced by operating at temperatures within the range 45–60 °C. Overall, the optimised level of pathogen inactivation achieved by UHPH under the above conditions was in the order of 2 log reduction greater than for HPP (Vachon *et al*., 2002).

Pulsed electric field processing

Pulsed electric field (PEF) processing is an emerging technology that relies upon the application of high-voltage pulses (up to 50 kV/cm) to the target product or liquid to mainly inactivate vegetative cells. In this technology, the product or liquid becomes part of the 'electrical circuit', and is subjected to several short pulses, with treatment times of microseconds. PEF leads to some concurrent heating; thus temperature control is important. In contrast to HPP, PEF is suitable for liquid streams and continuous operation (Qin *et al*., 1995).

The maximum effect of PEF, as an emerging non-thermal processing technology, appears to be limited to a 1.4 log reduction of total microflora as well as *Salmonella enteritidis* in milk when studied at temperatures <50 °C (Floury *et al*., 2006). Processing parameters (electric field and pulse width) were chosen as follows: 45 kV/cm/500 ns and 55 kV/cm/250 ns, with increasing pulse frequencies from 40 to 120 Hz, that correspond to an energy input varying in range from 0 to 100 kJ/kg. These workers challenged previous research findings by showing that some physicochemical characteristics of milk were affected by PEF, e.g. viscosity decrease and enhanced coagulation for high field levels (45–55 kV/cm) with 2.1 to 3.5 μs cumulated treatment time. Up to 25% of milk protein is denatured by PEF treatment at 20 °C using an electric field intensity of 22 kV/cm and 80 pulses.

Earlier, Michalac *et al.* (2003) reported a 0.3–3.0 log reduction in the total bacterial count of raw milk as well as specific microorganisms (*P. fluorescens*, *L. lactis* and *B. cereus*) inoculated in UHT milk using a continuous PEF bench-scale system set to deliver 35 kV/cm field strength with 64 pulses of bipolar square wave for 188 μs, but could find no significant effect on protein, total solids, colour, pH, particle size, density or electrical conductivity. Lowering pH from 6.6 to 3.8 increased the log reduction of *Listeria innocua* to 3.0, but had no effect on *E. coli* (log reduction = 1.0 or less) (Geveke and Kozempel, 2003). For the present, it would appear that PEF in combination with thermal treatment offers the best prospect of achieving better quality milk in microbiological terms over the duration of a 30-day shelf-life. Synergism between the two technologies was evident when thermal treatments of 73 and 80 °C with 6 s holding time was combined with PEF (Fernandez-Molina *et al.*, 2005). However, a recent paper (Alkhafaji and Farid, 2007) reveals that 6 log reductions in *E. coli* suspended in simulated milk ultrafiltrate was achieved using electric field intensities of 37.2–49.6 kV/cm at temperatures not exceeding 38 °C. This was achieved through the design of a new PEF treatment chamber that operates at high electric field intensities with limited increase in temperature and fouling.

Recent research in Australia has shown that for the major milk spoilage and pathogenic organisms (*Pseudomonas fluorescens*, *Enterobacter faecalis*, *Salmonella typhimurium* and *Listeria monocytogenes*), PEF in combination with mild heat (≤60 °C) leads to a minimum 5–6 log reduction in these microbes, and a concomitant enhancement in milk organoleptic quality due to the lower heat exposure (J. Wan, personal communication).

Other novel technologies

Pulsed UV light (248 nm) with an energy intensity of 25 J/cm^2 has been demonstrated at laboratory level to arrest bacterial growth. Further exploitation of this technology will require further sensory and chemical analyses (Smith *et al.*, 2002).

Adding CO_2 to raw whole milk (44–58 mM) reduced the number of surviving standard plate count organisms in milk heated over the temperature range 67–93 °C (Loss and Hotchkiss, 2002). A decrease in thermal survival rates (*D*-values) of *Pseudomonas fluorescens* R1–232 and *Bacillus cereus* ATCC 14579 spores in milk was positively correlated with CO_2 concentrations (1–36 mM). The authors believe that a pasteurisation process modification may involve introduction of CO_2 during heating up time in the plant's regeneration section or, alternatively under pressure during homogenisation. CO_2 could be removed later before packaging using vacuum treatment.

Addition of the antimicrobial agent, nisin, along with an increase in temperature reduces germination of *B. cereus* spores with a *D*-value of 4.7 h (Vessoni Penna *et al.*, 2002). Both nisin and subtilisin are able to inhibit the

outgrowth from bacterial germinated spores. It is believed that the antimicrobial peptides covalently modify the sulphydryl groups of protein components of freshly germinated spores and hence exert a profound bacteriostatic effect, resulting in the inhibition of subsequent cell outgrowth (Vessoni Penna *et al*., 2002). Nisin is particularly beneficial in arresting the growth of *B. cereus*, which, at concentrations of 10^5 cells per ml or greater, leads to enterotoxin production – a development that would be highly undesirable in applications such as infant milk beverages. Nisin addition (75 and 150 IU/ml) also works effectively in combination with reduced UHT heating (RHT) conditions (117 °C, 2 s) – conditions which may apply in certain ESL processing situations where milk heating temperatures intermediate between pasteurisation and UHT are selected. RHT-nisin treated milk samples stored at 30 °C showed very low spoilage rates during 150 days, although not low enough to satisfy requirements for commercial sterility. RHT-nisin samples were preferred to the UHT control. The combination of RHT-nisin and low storage temperature is effective against Gram-positive sporeforming bacteria (Wirjantoro *et al*., 2001).

Phytosterols are frequently being added to dairy and other food products because of consumer interest in their ability to reduce serum cholesterol. Monu *et al*. (2008) examined the antimicrobial activity of dispersible forms of phytosterols in milk. They found that these dispersible forms of phytosterols (containing sodium stearoyl lactylate) appeared to affect total plate count and psychrotrophic bacteria in refrigerated milk. Ironically, *Pseudomonas* spp. were not among those inhibited. This particular functional ingredient may benefit shelf-life extension when incorporated into pasteurised milks.

8.2.3 Labelling and food legislation

Many issues arise in the context of product labelling and legislation. For instance, product definition needs to be addressed, and use of the term 'fresh' outside its traditional context for pasteurised milk is currently causing some unease. Additionally, there are challenges associated with the identification of chemical markers which will differentiate between technological approaches. Glaeser (1994) considered the impact of a technical innovation in steam infusion heating technology for the production of pasteurised milk with improved keeping qualities in the context of current legislation. The term 'highly pasteurised' would be acceptable provided that the treatment was licensed by the appropriate authorities in EU member countries. It would be important to distinguish this 'highly pasteurised' milk from UHT through the identification of suitable chemical markers (e.g. lactulose and β-lactoglobulin) that would make a clear distinction between UHT and 'highly pasteurised' milks. Licensing should be conditional on the monitoring of stored samples for bacterial counts and microbial flora.

8.2.4 Emerging milk safety threats

Mycobacterium avium para-tuberculosis *(MAP)*

The effectiveness of pasteurisation in destroying MAP has come under the spotlight in recent years. This pathogen causes Johne's disease in dairy cows, and, though not certain, is tentatively associated with Crohn's disease in humans. The results of initial laboratory pasteurisation tests raised doubts about its effectiveness in controlling such a microorganism that could be of major public health concern. However, follow-up studies using processes which mimicked process plant conditions, i.e. achievement of turbulent flow conditions and better process control, reveal that it has not been possible to detect MAP survivors in test conditions where raw milks were spiked with the pathogen at levels of 10^4 cfu/ml (Lynch *et al*., 2007). In the meantime, dairy companies in many countries have cooperated with their respective local regulatory agencies to extend holding time following achievement of pasteurisation temperature in HTST processes from 15 s to 25 s in order to increase the effectiveness of thermal destruction and provide extra assurance from a milk safety perspective. A US group reached a similar conclusion after investigating a range of temperatures using batch and HTST heating systems. Five temperature–time combinations were evaluated: 62.7 °C, 30 min; 65.5 °C, 16 s; 71.7 °C, 15 s; 71.7 °C, 20 s; and 74.4 °C, 15 s. Treatment of milk regardless of bacterial strain or pasteuriser unit resulted in an average 5.0 and 7.7 log kill for low and high concentrations of inoculum, respectively (Stabel and Lambertz, 2004). The authors conclude that the current US minimum standards (71.7 °C) for batch and HTST pasteurisation of grade A milk significantly reduces the survivability of MAP.

Shedding of MAP (i.e. transmission via the mammary gland by an infected animal into the milk), accounts for low numbers of the pathogen. Considerable dilution of an individual cow's infected milk follows in the chain from farm to factory, especially during storage in milk silos following reception at the dairy plant. A much greater threat of MAP contamination from an infected animal is via faecal discharge. Hence, adherence to good hygienic practices during pre-milking preparation of the animal's udder ensures that in the event of undetected animal infection contaminating MAP numbers in raw milk are kept low. Thus, in the event of MAP infection, a combination of low numbers in bulked raw milk and assured levels of destruction in the pasteurisation plant reduces public health risk to low levels.

Cryptosporidium parvum

Cryptosporidiosis is an infectious condition of humans more frequently associated with the ingestion of the protozoan parasite *Cryptosporidium parvum* in contaminated water. Unlike bacteria, protozoa are not destroyed

by chlorine-based water disinfection technologies and require alternative technological approaches to ensure control. *C. parvum* is transmitted by a faecal–oral route and oocysts of the parasite are commonly found on dairy farms and may easily contaminate water, raw milk and food contact surfaces. The microorganism is readily destroyed by heat treatment so it should not pose a threat in pasteurised milk products. An immunomagnetic separation-polymerase chain reaction assay for direct detection of *C. parvum* in milk has been developed (Di Pinto and Tantillo, 2002).

Enterobacter sakazaki

This microbe is a member of the Enterobacteriaceae family, and is a non-sporeforming, Gram-negative rod formally identified as a new species in 1980 (Farmer *et al*., 1980). Awareness of its pathogenicity is relatively recent since it has been associated with infant illness and two instances of infant mortality as a result of the consumption of contaminated powdered baby milk. *E. sakazaki* is susceptible to thermal destruction with 11 log reduction achieved during pasteurisation at 72 °C for 15 s holding time. The organism has strong biofilm-forming characteristics and it is speculated that its adherence to a wide range of manufacturing surfaces may enhance resistance to sanitisers (Farmer *et al*., 1980), and build up of critical cell numbers in baby milk preparation utensils (Forsythe, 2005). It survives in infant milk powder for extended periods of time, and this gives rise for concern about its presence in dried dairy ingredients that are used during infant formula processing as well in the preparation of other beverage products.

8.3 Improving the sensory qualities of milk

The flavour of fresh bovine milk is attributed largely to a collection of compounds that fall into chemical categories such as carbonyl, sulphur, alcohols and free fatty acids. Specific volatile compounds associated with milk flavour include 2-butanone, ethyl caproate, heptanal, heptanone, hexanal, nonanal, octanal and pentanal. Milk is susceptible to a number of microbial- and chemical-induced flavour changes during the course of handling and processing, but other background factors are also emerging. While many people prefer to drink milk cold ex-refrigerator, a recent research study would seem to suggest that the temperature at which milk is tasted is not a factor in flavour perception, because it is largely overshadowed by fat content and other compositional factors dominating the aftertaste sensation (Francis *et al*., 2005). Non-fat milk with its greater concentration of hexanal and lesser amounts of benzaldehyde, ethyl caproate, heptanal, 2-heptanone and nonanal than whole milk was regarded as having more sour aromatics, bitter, cooked and fat character, and as being slightly more chalky and flat (Francis *et al*., 2005).

8.3.1 Microbiological effects on sensory properties

Feed and animal husbandry practices

Distinct flavour differences in batch-pasteurised and homogenised milks from cows fed on pasture-based and conventional total mixed ration diets can be established by instrumental and expert sensory analyses (Croissant *et al*., 2007); however, consumers were unable to differentiate between the two. Trained sensory panellists identified greater intensities of grassy and cowy/barny flavours in the milks produced from pasture-based diets.

Olfactometric analysis has to date identified approximately 75 aroma active compounds that occur in both 'off-flavour' and 'good flavour' quality milks drawn from cows subjected to a variety of feeding regimes. Mounchili *et al*. (2005) concluded that concentration differences among a common set of compounds is more important in determining flavour than the absence or presence of specific compounds. The same authors also found that baled grass silage contributed most to 'feed' off-flavour. The roles of few specific flavour compounds have been investigated in more detail during the past decade. A single aroma compound, hept-*cis*-4-enal, occurring in minute concentrations (pictogram level) has been identified as making an important contribution to the flavour of fresh/homogenised/pasteurised milk (Bendall and Olney, 2001). Gamma-12:2 lactone is an odour active compound present in milk from cows fed a concentrate-supplemented diet, but not in milk from cows fed on pasture (Bendall, 2001).

A Canadian study which investigated an incidence of milk off-flavours in bulk tank milk from the Province of Prince Edward Island in the late 1990s identified transmitted flavours (feed related) as a prominent herd-level contributory risk factor associated with the event (Mounchili *et al*., 2004). Individual factors within this classification of transmitted flavours were identified as air-quality in dairy cow housing, use of baled silage as the main forage source and animal access to roughage before milking. Frequency of milking does not appear to affect milk flavour (Klei *et al*., 1997) especially where three times a day milking is practised.

Storage of milk prior to processing

Working with high-quality raw milk, Wiking *et al*. (2002) showed that most flavour changes occur in the 24 h period *post partum*. Duration of storage affected the sensory properties of milk such as cream aroma, cream flavour, boiled milk flavour, metallic flavour and creaminess attributes.

Post-thermal handling and contaminants

Factors affecting the deterioration of pasteurised milk include storage temperature, post-pasteurisation contamination, growth behaviour of contaminating bacteria and incidence of spores of *B. cereus* in the original milk (Walstra *et al*., 1999). Detectable changes to pasteurised milk become noticeable when the respective counts of total bacteria and *B. cereus* reach $5–20 \times 10^6$ cfu/ml.

Microorganisms that survive pasteurisation or contaminate the milk subsequently can give rise to off-flavour development. Growth of psychrotrophs (*Pseudomonas* spp.) gives rise to unclean or putrid flavours. *B. cereus*, a bacterium traditionally associated with the formation of bitty cream, also causes unclean flavour. Proteolytic enzymes such as plasmin cause bitter flavour in UHT, while lipolytic enzymes such as heat resistant microbial lipases cause rancidity in pasteurised milk – the secretion of the latter enzymes taking place as a result of microbial growth during pre-pasteurisation storage. *B. sporothermodurans* was identified during the 1990s as a particularly heat-resistant sporeformer which was becoming an increasingly prevalent survivor capable of spoiling UHT milks. Following its identification, many UHT processors increased the thermal treatment of their plants in order to ensure outright destruction of the microorganism.

The development of an ultra-clean packaging system to prevent post-pasteurisation contamination of liquid milk in Moorepark during the late 1980s showed that by maintaining Gram-negative rods at <1 per 100 ml milk, the shelf-life of milk could be extended by 10 days during chilled (4 °C) storage (J.J. Tuohy, personal communication). Various measures have been designed into the more advanced milk packaging systems in order to prevent recontamination. These include hydrogen peroxide treatment of packaging materials; ducting of sterile (HEPA filtered) air to critical areas where product may be exposed (e.g. feed tank, filling line enclosure and surrounds of the metering pistons). These approaches and others are elaborated by Henyon (1999) in the course of outlining developments in ESL milk in North America.

8.3.2 Thermal treatment effects on sensory properties

Milk flavour differentiation according to heat treatment history

Analysis of volatile flavour compounds in milk (acetone, butanone, 2-pentanone, 2-heptanone, 2-nonanone, acetic acid and hexanal) in combination with cluster analysis has been shown to successfully differentiate according to three categories: (i) raw and pasteurised milks, (ii) UHT milks and (iii) sterilised and in-bottle sterilised milks (Senorans *et al.*, 1996).

Recent research (Blake *et al.*, 1995) conducted on ESL milk examined sensory and microbial quality information based on the direct steam injection process. There were no significant differences noted in cooked or off-flavours between 132 and 140 °C. Psychrotrophic *Bacillus* species were isolated from milk processed at and below 132 °C while no organisms were isolated from milk processed at temperature at or above 134 °C. Taste panels preferred the milks in the order HTST > ESL > UHT.

Pasteurisation

A shelf-life survey of three commercial pasteurised milk samples, collected from three commercial dairy plants identified the growth of heat-resistant

psychrotrophic microorganisms (Gram-positive rods including *Paenbacillus*, *Bacillus* and *Microbacterium*) as largely responsible for developing spoilage off-flavours (Fromm and Boor, 2004). When representative microorganisms were inoculated into pasteurised skim and whole milks, skim milks produced mainly bitter off-flavours due to greater proteolytic activity, while lipolysis was prevalent in the contaminated pasteurised whole milks where sour-type off-flavours were evident (Deeth *et al*., 2002).

Oxidation and packaging

Volatile compounds identified as potential markers of fresh milk quality include dimethyl disulphide, pentanal, hexanal and heptanal because of good correlation with sensory attributes (Karatapanis *et al*., 2006). These authors distinguished between light-induced oxidative effects and autoxidative effects related to packaging material. A study of milks ranging in fat contents (whole, reduced fat-content and non-fat milks) found that milk fat protects against vitamin A degradation, but adversely affects milk flavour after exposure to light (Whited *et al*., 2002). Oxygen-scavenging film reduces dissolved oxygen content and stale flavour volatiles including three methyl ketones and two aldehydes (Perkins *et al*., 2007). However, a consumer panel was unable to establish a significant difference in odour between the treatment and control samples. Antioxidants such as alpha-tocopherol, ascorbic acid and combinations of the two are somewhat effective in preventing light-induced oxidative off-flavour development. By conducting sensory testing for difference, Aardt *et al*. (2005) showed a significant difference in oxidation off-flavour between light-exposed control milk and light-exposed milk with added alpha-tocopherol and ascorbic acid. Alpha-tocopherol on its own was ineffective.

Ultra-high temperature

It is well established that UHT milk comprises 'cooked' flavour (sulphydryl group release), ketone flavour and slight caramelisation associated with near sterilisation temperatures. It is also accepted that the intensity of these flavours is specific for the individual UHT process that may be involved. Indirect heating technology has become the workhorse of the dairy industry over the years for manufacturing UHT milk, despite the appeal of a more energy efficient direct steam-based injection technology, ensuring rapid condensation of steam in the immediate moments following injection. In the meantime, a reversal of heating technology whereby milk is sprayed on to steam vapour in chamber (i.e. infusion heating), excited the dairy industry for a period as an alternative approach to UHT heating with assurances of improved flavour quality. Further refinements in this technology emerged in the 1990s whereby a new generation of infusion heaters featured heating residence times down to 1 s or less, better evacuation of gases from the heating chamber and minimum contact of milk with hot metal surfaces

(examples include Den Hollander Engineering Falling Stream Heater (FSH) and APV infusion heating).

The results of sensory analysis published by Japanese workers (Iwatsuki *et al*., 2001), indicates that heating milk to 130 °C during UHT processing was preferred instead of heating to either 120 or 140 °C. 'Milk body' (a term used by the authors to encompass milk flavour, viscosity and fattiness) became more pronounced as UHT processing temperature increased. Purchasing habit more than flavour appeared to influence consumer choice when deciding to purchase either pasteurised or UHT milks according to an Australian survey conducted by Perkins and Deeth (2001), though some of the survey respondents conceded that negative perceptions of UHT in terms of poor nutritional value, poor flavour and not real/pure milk were other influencing determinants. This study was conducted in a market where more than 80% of respondents used pasteurised milk as their main milk type.

Combining developments in packaging (Intasept™ aseptic pouches with oxygen-scavenging film) with UHT processing was recently shown (Perkins *et al*., 2007) to be effective in restricting the development of a number of chemical markers (dissolved oxygen content, stale flavour volatiles, e.g. selected methyl ketones and aldehydes and free fatty acid development) associated with off-flavour development. Notably, the taste panel failed to differentiate the UHT milks (packaged using either the oxygen-scavenging or standard films) on the basis of odour.

8.3.3 Innovative and emerging technologies and how they influence sensory qualities

Microfiltration

Lactantia PurFiltre™ milk was introduced to the Canadian market by Ault Foods in January 1995 (Eino, 1997). The company seized on the opportunity associated with developments in microfiltration technology to provide consumers with a premium branded fresh milk with an extended shelf-life. Market research conducted by the company prior to launch convinced them that consumers considered the taste of PurFiltre™ milk to be fresher than their regular brands. Thus, the company felt confident that they could justify asking for a premium of 10 cents (CAN)/litre higher than that for regular pasteurised milk. It is claimed that the concept helped energise the market and stopped a decline in liquid milk sales. Ault's market share grew from 25.5% to 29.5% which was attributed in part to a positive consumer response to fresher taste.

Market entry of other microfiltered milks, including Neilson Dairies' TruTaste™ and Natrel's double-centrifuged UltraLait™, appear to have contributed to an expansion of the market for retail fresh milks, processed by novel technologies, to 10% in Ontario and 20% in Quebec. Notably, market feedback suggests that the dominant consumer reason for purchase

of these ESL milks is flavour. It appears that 'purity' is not an issue as the perception is that all milk is 'pure', and that the extension to shelf-life is also less of an issue since milk shopping habits have not changed.

High-pressure processing
HPP has been promoted as a potential alternative to conventional thermal processing of milk on the basis of its bactericidal effects at moderate temperatures. However, there are few studies elaborating the expected benefits to the sensory properties of milk which has been subjected to HPP. From a chemical olfactory perspective, HPP (482 and 586 MPa) at low temperature (25 °C) causes minimum change of the volatile composition of milk (Vazquez-Landaverde *et al*., 2007), while under extreme pressure and temperature conditions, volatile compound formation is different from that under atmospheric pressure conditions. Heat treatment at high temperature (60 °C) promotes the formation of both aldehydes and methyl ketones, whereas high pressure (620 MPa) at high temperature (60 °C) favours the formation of aldehydes (Vazquez-Landaverde *et al*., 2007).

Ultra-high-pressure homogenisation (UHPH)
Based on the limited research undertaken to date, the belief is that UHPH should give new opportunities to develop retail milks with an equivalent shelf-life to that of high-temperature pasteurised milk (90 °C, 15 s) in terms of microbial and physicochemical characteristics. Commercial realisation of UHPH is being brought a step closer with the introduction by GEA Niro Soavi – a major international process equipment manufacturer with strong associations with the dairy industry, of its pilot-scale prototype NS3110 UHPH unit. The designers claim that the NS3110 UHPH is capable of achieving 100% cell disruption at 400 MPa in a single pass. However, there is a need for further studies on sensory aspects of UHPH-treated milk post-processing and throughout storage. Reduction in fat globule size is likely to impact on the body and mouthfeel characteristics – this may well be positively influenced when attempting to retain such characteristics when developing reduced-fat variants of milk. Certainly, the loss of activity of the indigenous enzyme plasmin and its activator, plasminogen, during UHPH should have a positive influence in containing potential proteolytic activity (Hayes and Kelly, 2003). However, alkaline phosphatase – a marker enzyme that should not be detectable when pasteurisation is effective – is unaffected by UHPH.

8.4 Ingredients from milk and their applications

A greater understanding of the components in milk, along with the development of technologies to concentrate and isolate these components, has enabled the development of new ingredients and novel beverages that

contain milk-derived ingredients. These ingredients are employed in a range of foods and beverages to improve the nutritional quality and impart desirable organoleptic characteristics.

8.4.1 Whole milk preparations

Concentrated milk

Concentrating, packaging and heating milk to produce a shelf-stable concentrated product has been undertaken for over 100 years (Hunziker, 1918) and remains popular today. The addition of sucrose to this concentrate to further extend shelf-life has also been widespread. These shelf-stable canned products are employed as milk substitutes in countries where fresh milk is unavailable or refrigeration is inadequate for fresh milk distribution, and also as ingredients in various foods including desserts.

Milk powder

Extending the shelf-life and transportability of milk has been achieved by manufacturing powders from full-cream and skim milk. These powder products are made by standardising milk to desired fat and protein contents followed by heating, concentrating and spray drying using selected conditions to achieve desirable physical functional properties (Walstra *et al*., 1999). Whole and skim milk powders are stable at ambient temperature when heated adequately and packaged in oxygen barrier materials, relatively inexpensive to transport due to their low water content, and easy to reconstitute with water to give a product similar to fresh milk. Owing to their desirable characteristics, full-cream and skim milk powders are often employed to reconstitute milk in countries where fresh milk is unavailable. This was the basis of the so-called recombination industry (i.e. the construction of recombined milk plants in locations such as the Middle East, South East Asia and parts of Latin America) where imported raw materials such as milk powders and butteroil were processed to produce consumer dairy products to suit local market tastes. The term recombination is less used today mainly because of the increasing sophistication with which newly developing ingredients are used to formulate modern dairy-based foods and beverages. They are also a major ingredient in infant formula, protein-enriched milk, and a wide range of manufactured foods such as bakery products, confectionery, cheese, yoghurt and ice cream. As beverage ingredients, milk powders are employed to impart a creamy flavour, to increase the solids content, and to improve the body and mouthfeel of gelled dairy products.

Milk protein concentrate

Concentration of milk using membranes followed by drying has been employed to produce milk protein concentrates. The membrane process allows removal of lactose and minerals, while retaining and concentrating

the larger molecular weight proteins. Depending on the extent of membrane processing, milk concentrate ingredients can be produced which vary in protein, carbohydrate and mineral content. Similar to milk powders, milk protein concentrates are employed in a range of beverages and manufactured food products to impart dairy flavour, to increase the protein content and to increase body and mouthfeel. Typical processed foods include dairy beverages, bakery products, desserts, yoghurt and ice cream.

8.4.2 Isolated milk proteins

Caseins and caseinates

Casein phosphoproteins are the major proteins found in milk, accounting for approximately 80% of all milk proteins (Madureira *et al*., 2007). The caseins are designated alpha-S1 (45%), alpha-S2 (12%), beta (34%) and kappa (10%) (Modler, 1985). Owing to their hydrophobic nature, caseins form micelles in milk which are held together by calcium ions, hydrophobic bonding and a surface cover of hydrophilic kappa-casein protein. During cheese making, the enzyme chymosin (EC 3.4.23.4) hydrolyses the kappa-casein between the phenylalanine 105 and methionine 106 amino acids, leading to destabilisation of the casein micelle and casein aggregation. As the isoelectric point of caseins is approximately 4.6, addition of acid (lactic, hydrochloric or sulphuric) to milk also results in casein micelle destabilisation and precipitation of the caseins. Caseins manufactured in this manner are washed and neutralised with sodium, potassium, ammonium or calcium hydroxides to between pH 6.8 and 7.2 (Modler, 1985).

One of the most promising and under-utilised microfiltration techniques for milk processing is that used for separating casein from the serum milk proteins or whey proteins. This application produces a casein-rich milk concentrate that can be used for cheese-making or other applications where native casein proteins provide enhanced functionality, and a fat-free serum milk protein stream that resembles acid whey without the acid, that can be further processed using ultrafiltration to make whey protein concentrate products (Saboya and Maubois, 2000; Vadi and Rizvi, 2001).

Caseinates made using precipitation techniques do not resemble the casein micelles in milk, as the colloidal phosphate structure has been destroyed and aggregates formed (Modler, 1985). The properties of caseins are dependent on the manufacturing method and also the properties of the food system in which they are employed. For example, the sodium, potassium and ammonium forms are soluble above pH 5.5, whereas calcium caseinates form colloidal dispersions (Modler, 1985).

Owing to their surface-active properties, caseins have good emulsifying, whipping and hydrating properties (Modler, 1985). Caseins are employed in a range of beverages including instant breakfasts, milk analogues, and as coffee whiteners. They are also employed in various manufactured foods including formulated meat products, margarine, whipped foods, desserts,

puffed snacks and processed cheese. Enzymatic and chemical modification of caseins has been undertaken to improve physical, sensory and nutritional properties. For example, enzymatic hydrolysis of casein has been employed to produce casein phosphopeptides, an ingredient employed in various food, beverage and pharmaceutical formulations to remineralise carious lesions in dental enamel (Cross *et al*., 2007; Reynolds, 1999).

Whey protein concentrates and isolates

Whey is the by-product of cheese or casein making processes and contains as a percentage of dry matter approximately 13% protein, 75% carbohydrate (mainly lactose) and 10% ash. The proteins found in whey are β-lactoglobulin, α-lactalbumin, bovine serum albumin, glycomacropeptide and other minor components. To concentrate the protein components in whey, hollow fibre or spiral wound ultrafiltration membranes of selected size are employed. These membranes allow the smaller molecular weight components (minerals and lactose) to pass through, concentrating the protein components to produce a range of whey protein concentrate ingredients with protein contents ranging from 30% to 89% by weight. Whey protein concentrates have a full complement of essential amino acids, making them a nutritionally valuable source of protein. They also have a high proportion of β-lactoglobulin, a protein which forms gels when hydrated and heated (Dumay, 1988).

An isolate of whey protein is produced when the whey proteins are concentrated to greater than 90% purity. To achieve this protein concentration, the major whey proteins β-lactoglobulin and α-lactalbumin are isolated using precipitation or ion-exchange chromatography techniques. Whey protein isolates have similar nutritional and physical functional properties to whey protein concentrates (Lupano *et al*., 1992). However, whey protein isolates are reported to form firmer gels and be more sensitive to ion concentrations and pH changes than whey protein concentrates (Lorenzen and Schrader, 2006).

Whey protein isolates and concentrates are manufactured by a number of dairy ingredient companies using membrane and ion-exchange processes. These whey protein products are employed in a range of beverages for both their nutritional proprieties and physically functional characteristics (Jayaprakasha and Brueckner, 1999). For example, whey protein concentrates are often employed in infant formula (Jost *et al*., 1999) and sport supplement products such as Myopure™ (Australia) and Ascend (Murray Goulburn Cooperative, Australia) which are readily reconstituted with water into beverages. They are also employed in processed meat products (Hongsprabhas and Barbut, 1999), breakfast bars and sport nutrition bars. Whey proteins are also reported to positively influence satiety and are employed as ingredients in dietary beverage products (Luhovyy *et al*., 2007).

Beta-lactoglobulin

Beta-lactoglobulin is found in bovine milk and whey at a concentration of approximately 3.3 g/l (Konrad *et al*., 2000), accounting for approximately 58% w/w of all bovine whey proteins (Madureira *et al*., 2007). Several variants of beta-lactoglobulin exists, of which the A variant is most common. The quaternary structure of beta-lactoglobulin depends on the pH. Between pH 5.2 and 7 it exists as a dimer with a molecular weight of approximately 36 700 Da, at pH 3.5 to 5.2 as an octamer with a molecular weight of approximately 140 000 Da, and at pH 3.0 or above 8.0 as a monomer with a molecular weight of 18 277 Da (Madureira *et al*., 2007).

Beta-lactoglobulin is thought to transfer passive immunity to the newborn, to regulate phosphorous metabolism in the mammary gland (Farrell *et al*., 1987) and to be an important source of amino acids for neonates (Kontopidis *et al.*, 2004). As it is rich in cysteine, an important amino acid in the synthesis of glutathione, it is thought to be good for fuelling muscle growth (Wit, 1998). Beta-lactoglobulin binds to small hydrophobic ligands such as retinol, fatty acids, vitamin D, cholesterol, aromatic compounds and triacylglycerols (Madureira *et al*., 2007) and can be employed as a carrier of these molecules. When destabilised by heat, beta-lactoglobulin forms strong gels (Mulvihill and Kinsella, 1987) and is one of the major proteins responsible for the gel-forming characteristics of whey protein concentrates and isolates (Dumay, 1988).

Beta-lactoglobulin can be isolated from bovine whey by lowering the pH with or without heat to selectively precipitate whey proteins (Aschaffenburg and Drewry, 1957; Pearce, 1983; Konrad *et al*., 2000), selective salting out of whey protein using ammonium sulphate or sodium chloride (Konrad *et al*., 2000; Lozano *et al*., 2008), by ion-exchange chromatography (Imafidon and Ng Kwai-Hang, 1992), or by pepsin digestion of all whey proteins except beta-lactoglobulin followed by membrane isolation (Konrad *et al*., 2000). Ion-exchange membrane processes have recently been investigated to capture and elute beta-lactoglobulin (Goodall *et al*., 2008). With further development, these membranes maybe employed for the commercial isolation of beta-lactoglobulin. Although there are a number of techniques available to isolate beta-lactoglobulin, there are limited suppliers of commercial quantities that allow its use as a food ingredient. The major commercial source of beta-lactoglobulin is currently whey protein concentrate and whey protein isolate. With further research and development to understand beta-lactoglobulin's nutritional and functional benefits in food and beverage formulations, the commercial manufacture of this ingredient is likely in the future.

Alpha-lactalbumin

Alpha-lactalbumin is found in bovine milk at approximately 1.5 g/l (Levieux and Ollier, 1999). It is the second most abundant protein in whey,

accounting for approximately 20% w/w of the total whey proteins (Madureira *et al*., 2007). The molecular weight of alpha-lactalbumin is 14 175 Da, and three genetic variants of alpha-lactalbumin have been identified. Between pH 5.4 and 9.0 its globular structure is stabilised by four disulphide bonds.

Alpha-lactalbumin's main biological role is reported to be in the synthesis of lactose in the mammary gland which facilitates milk production, and is employed as a source of energy in the newborn (Wit, 1998; Lonnerdal and Lien, 2003). Alpha-lactalbumin is also reported to have anticarcinogenic activity. In mammalian cancer cell line cultures it has been shown to constrain cell division (Ganjam *et al*., 1997) and induce apoptosis (Hakansson *et al*., 1995). Owing to alpha-lactoalbumin's high tryptophan content (~6% w/w), it is suggested to be a good source of tryptophan (~4.8% w/w) for the synthesis of the neurotransmitter serotonin (Madureira *et al*., 2007). In addition, a diet rich in alpha-lactalbumin has been shown to increase plasma tryptophan-large neutral amino acids ratios and cognitive performance in stress-vulnerable subjects (Markus *et al*., 2005). Alpha-lactalbumin has good physical functional properties, as it improves the heat-induced gel formed by beta-lactoglobulin (Matsudomi *et al*., 1993).

Alpha-lactalbumin can be isolated from whey by ultrafiltration followed by selective acid precipitation (Marshall *et al*., 1976; Muller *et al*., 2003), ion-exchange chromatography (Ye *et al*., 2000) or tryptic digest of contaminating proteins followed by ultrafiltration (Konrad and Kleinschmidt, 2008). A number of companies manufacture and supply alpha-lactalbumin including Friesland Foods (Netherlands), Arla Foods (Denmark), Amor Proteins (France) and Davisco Foods International (USA). As alpha-lactalbumin is found at higher concentrations in human milk (~2.8 g/l) than in bovine milk (~1.2 g/l), bovine alpha-lactalbumin is often employed to supplement infant formula. Owing to its high nutritional quality, alpha-lactalbumin preparations are also employed in protein-fortified beverages and nutrition bars.

Glycomacropeptide

Glycomacropeptide (GMP) is the hydrophilic 63 amino acid C-terminal peptide released into whey at a concentration of approximately 1.2–1.5 g/l during cheese making when kappa-casein is cleaved between phenylalanine 105 and methionine 106 by chymosin (Swaisgood, 1974; Manso and Lopez-Fandino, 2004). GMP exists in several forms due to extensive post-translational glycosylation, phosphorylation at serine residues and genetic variances (Manso and Lopez-Fandino, 2004). GMP may have five different saccharide chains covalently attached through *O*-glycosidic linkages at serine or threonine residues (Dziuba and Minkiewicz, 1996; Brody, 2000).

Extensive research has been conducted on GMP and various nutritional advantages and biological activities have been reported. As GMP does not contain the aromatic amino acids phenylalanine, tyrosine and tryptophan,

GMP can be employed as a protein source for people with metabolic disorders such as phenylketonuria (Nielson and Tromholt, 1994). It is reported that GMP inhibits splenocyte proliferation induced by *Salmonella typhimurium* lipopolysaccharide (Otani *et al*., 1992) and that it is a potent immune-enhancer, increasing macrophage like cell phagocytic activity (Li and Mine, 2004). GMP is also reported to bind a number of microbial toxins (Kawasaki *et al*., 1992; Isoda *et al*., 1992) and have antiviral and anticariogenesis properties (Kawasaki *et al*., 1993a; Schupbach *et al*., 1996; Aimutis, 2004). A very interesting reported activity of GMP is its ability to induce satiety after consumption by promoting the release of cholecystokinin from gastrointestinal cells (Yvon *et al*., 1994; Corring *et al*., 1997; Dunshea *et al*., 2007).

GMP can be isolated from cheese whey by ultrafiltration (Kawasaki *et al*., 1993b), selective precipitation of other whey proteins (Nielsen and Tromholt, 1994; Metwally *et al*., 2001), anion exchange chromatography (Xu *et al*., 2000; Nakano and Ozimek, 2000), or using a combination of cation and anion exchange chromatography (Doultani *et al*., 2003). GMP is currently produced commercially by a number of manufacturers including Davisco Foods International (USA), MG Nutritionals (Australia) and Arla Foods (Denmark). Owing to its nutritional properties and reported biological activities, it finds use in a range of products including infant formula and weight loss beverages.

Lactoferrin

Lactoferrin is a minor protein component of milk, found at a concentration of between 31 and 350 μg/ml, depending on the stage of lactation, amount of milk produced and somatic cell count (Cheng *et al*., 2008). Lactoferrin is an 80 000 Da molecular weight iron-binding glycoprotein of the transferrin family found in most biological fluids and involved in mammals' innate immune system. Lactoferrin is found as either native-, apo- without iron and holo-lactoferrin saturated with iron.

Lactoferrin has a vast number of biological properties that make it useful as a food and beverage ingredient. It has antibacterial activity against a number of pathogenic and spoilage organisms such as *Pseudomonas* (Kim *et al*., 2008), viruses such as *Herpes simplex* (Pan *et al*., 2006; Ammendolia *et al*., 2007) and fungi (Farnaud and Evans, 2003). The mechanism of action is through the binding of free iron, making in unavailable to the microorganisms, and through direct binding to the microbial membrane, disrupting membrane potential and integrity (Madureira *et al*., 2007). Lactoferrin has also been shown to act synergistically with lysozyme, antibodies, immunoglobulins, lactoperoxidase and antibiotics against bacteria (Farnaud and Evans, 2003). In addition to lactoferrin's antimicrobial properties, it is also reported to have immune system stimulating properties. Lactoferrin's ability to enhance the immune system is thought to be due to its ability to increase macrophage activity, induce inflammatory cytokines including

interleukon-8, tumour necrosis factor-alpha and nitric oxide, and stimulate proliferation of lymphocytes, active monocytes, natural killer cells and neutrophils (Madureira *et al*., 2007; Legrand *et al*., 2005). Lactoferrin is also reported to have anti-carcinogenic properties (Parodi, 2007), to enhance osteoblast calcium deposition (Takayama and Mizumachi, 2008), and also to improve the growth performance of farmed animals (Madureira *et al*., 2007; Wang *et al*., 2008).

Lactoferrin is currently isolated from whey or milk using membrane (Ulber *et al*., 2001) and ion-exchange chromatography processes (Andersson and Mattiasson, 2006). It is available commercially from a number of milk processors including Murray Goulburn Cooperative Limited (Australia), DMV International (Netherlands), Tatua Nutritionals (New Zealand), Morinaga Milk Industries Co. Limited (Japan), Friesland Foods (Netherlands) and Glambia Nutritionals (Ireland). As lactoferrin is naturally high in human milk (2–4 g/l), bovine lactoferrin is often employed to supplement infant formula. As it has a number of beneficial biological properties, it is also employed in sports supplements, nutritional beverages, cosmetics and oral hygiene products.

Lactoperoxidase

Lactoperoxidase (E.C. 1.11.1.7) is an important part of the mammal natural defence system, and is found in a number of secretions including tears and saliva, and in milk at a concentration of approximately 30 mg/l, representing approximately 1% w/w of the whey proteins (Seifu *et al*., 2005; Madureira *et al*., 2007). Lactoperoxidase is a member of the peroxidase family, a group of enzymes widely found in nature that utilise hydrogen peroxide to oxidise thiocyanates to hypothiocyanate (Seifu *et al*., 2005). The molecular weight of lactoperoxidase is approximately 78 000 Da and it contains a number of carbohydrate groups (Seifu *et al*., 2005). When lactoperoxidase, hydrogen peroxide and thiocyanate ion are present together, the lactoperoxidase-catalysed reaction yields intermediary oxidation products of thiocyanate, notably hypothiocyanate, which exhibit antimicrobial effects against bacteria, fungi and viruses (Seifu *et al*., 2005; Madureira *et al*., 2007). The combination of lactoperoxidase, hydrogen peroxide and thiocyanate is termed the 'lactoperoxidase system'. In biological fluids, the thiocyanates are present in mammal secretion from the ingestion of glucosinolate and cyanogenic glycoside containing feeds and hydrogen peroxide is present due to production by polymorphonuclear leucocytes during phagocytosis, production by other microorganisms and as a product of specific oxidation reactions (Madureira *et al*., 2007).

Lactoperoxidase can be isolated from milk or whey using ion-exchange technologies (Yoshida and Ye, 1991; Morrison *et al*., 1957) and is available commercially from a number of milk processors including DMV International (Netherlands) and Tatua Nutritionals (New Zealand). The most

widely recognised application of the lactoperoxidase system is in the preservation of raw milk during storage and transport. The lactoperoxidase system can also be employed as an antimicrobial pre-treatment to allow low-temperature thermal treatment of heat-sensitive foods and beverages (Seifu *et al*., 2005). The lactoperoxidase system has also been reported to be beneficial in the eradication of microorganisms from fresh meat surfaces, in the preservation of cosmetic products, in reducing plaque accumulation, gingivitis and carious lesions (Seifu *et al*., 2005).

Immunoglobulins

Immunoglobulins (Ig) are globular glycoproteins found in bovine milk and colostrum, each comprising two glycosylated 'heavy' and two 'light' chain subdomains designed to bind antigens and elicit host defence processes, and thereby offer passive immunity to the consumer. The four immunoglobulins found in bovine milk are IgG1, IgG2, IgA and IgM at concentrations of approximately 0.60, 0.12, 0.13 and 0.04 g/l, respectively (Korhonen *et al*., 2000; Gapper *et al*., 2007). Much higher concentrations of immunoglobulins are found in blood and colostrum. Immunoglobulins are present in colostrum and milk to protect the gut mucosa against pathogenic microorganisms. In colostrum, they are present at higher concentrations to confer passive immunity to the ruminant neonate until their immune system matures.

Immunoglobulins are intrinsically heterogeneous owing to their physiological function particularly in the primary sequence responsible for antigen binding and are nutritionally valuable proteins because of their richness in sulphur-containing amino acids. Colostrum, which is the richest source of bovine immunoglobulins, is under particular development as a food and beverage ingredient, targeting lucrative and burgeoning global markets (e.g. sports nutrition), particularly in North America and Europe.

Immunoglobulins are prized either for their polyclonal binding activity raised against specific antigens through hyperimmunisation or as non-specific polyclonal antibodies. Colostrum-derived immunoglobulins have been raised against both bovine and human pathogens through hyperimmunisation methods, with the intended application for passive immunisation of the consumer. Non-specific polyclonal antibodies in bovine colostrum appear to offer protection against human forms of selected pathogens such as *Cryptosporidium parvum* (Okhuysen *et al*., 1998). Examples of antibodies raised for their specific passive immunity activities and with demonstrated efficacies include (i) rotavirus (Scammell, 2001); (ii) cariogenic bacteria (Wei *et al*., 2002); and (iii) *Shigella flexneri*, *Escherichia coli*, *Clostridium difficile*, *Streptococcus mutans*, *Cryptosporidium parvum* and *Helicobacter pylori* (Korhonen *et al*., 2000).

Immunoglobulins derived from bovine milk and colostrum are often added to infant formula and other food products to help reduce viral and

microbial infections and improve consumer passive immunity (Gapper *et al*., 2007). Colostrum is a very rich source of immunoglobulins and is being marketed as a sports supplement to improve performance.

Other minor proteins

There are many other components in milk that may be employed in the manufacture of novel beverages. Some of these are manufactured commercially, while others are being discovered, characterised and methods of isolation being developed. Below are a number of components from milk that maybe available in the near future for the development of novel beverages.

Bovine serum albumin (BSA) is found in milk at a concentration of approximately 0.13 g/l (Levieux and Ollier, 1999). It is not synthesised by the mammary gland, but leaks from the bloodstream into the milk. It has a molecular weight of 66 432 Da with 17 intermolecular disulphide bonds and 1 free sulphydryl group. BSA is reported to be a very functional protein, binding lipids, free fatty acids and flavour compounds (Kinsella and Whitehead, 1989). It also forms firm gels similar to those of beta-lactoglobulin (Matsudomi *et al*., 1991) and the firmness of the gel is increased when proteins such as alpha-lactalbumin and beta-lactoglobulin are present (Matsudomi *et al*., 1992, 1994). BSA is also reported to have good emulsification properties (Waniska *et al*., 1981; Saito *et al*., 2006) and antioxidant properties that protect lipids against phenolic-induced oxidation (Tong *et al*., 2000).

There are reports that BSA can be isolated from milk using combinations of gel filtration and anion-exchange chromatography (Neyestani *et al*., 2003) and immunoaffinity chromatography (Losso *et al*., 1998). Even though BSA has a number of properties that would benefit beverage manufacturers, commercial quantities of BSA from milk and whey are limited. This could be due to the lack of published commercially viable BSA manufacturing methods.

Mucins are a family of large, heavily glycosylated proteins (glycoconjugates). The dense 'sugar coating' of mucins gives them considerable water-holding capacity and also makes them resistant to proteolysis, which may be important in maintaining mucosal barriers, and also provides a basis for their potential functionality in products. Mucins are secreted as massive aggregates of proteins with molecular weights of ~1–10 million Da. Within these aggregates, monomers are linked to one another primarily by non-covalent interactions, although intermolecular disulphide bonds may also play a role in this process. Scientific attention is being paid to these proteins, and others derived from the milk fat globule membrane, in relation to their biological functionality and role (Mather, 2000; Peterson *et al*., 2001). Mucins appear to have antimicrobial and anti-infectivity functionality, and as such may be useful bioactive agents in functional beverages as long as

cost-effective processes can be developed and commercialised for their isolation.

Milk, colostrum and whey contain a number of small proteins and peptides that serve as potent growth stimulants and mediators for a variety of mammalian cells, both *in vivo* and in culture (Donovan and Odle, 1994; Belford *et al*., 1995; Francis *et al*., 1995; Rogers *et al*., 1995, 1996; Smithers *et al*., 1996; Pakkanen and Aalto, 1997; Smithers, 2004). These *growth factors* influence cell growth and differentiation by promoting DNA and protein synthesis and by inhibiting protein breakdown. Growth factor activities present in dairy fluids include insulin-like growth factor (IGF)-I, IGF-II, platelet-derived growth factor, acidic and basic fibroblast growth factors, transforming growth factor-β (Belford *et al*., 1995; Rogers *et al*., 1995, 1996), and possibly other, as yet unidentified, factors. A simple process for isolation of these factors from dairy fluids, employing membrane and chromatographic techniques, has been developed and patented (Ballard *et al*., 1991; Francis *et al*., 1995; Regester and Belford, 1999). Dairy-derived growth factors have potential applications in a number of lucrative biotechnological, biomedical and functional food areas. These include the topical treatment of chronic wounds such as venous and other skin ulcers (Belford *et al*., 1995, 1997; Regester and Belford, 1999), and oral prophylactic or therapeutic treatment of some gastrointestinal diseases (Regester and Belford, 1999). Indeed, dairy-derived growth factors are efficacious in delaying the onset of mouth ulcers in an animal model of oral mucositis (Clarke *et al*., 2002), and an extract of these growth factors is currently in human clinical trials as a natural prophylactic and potential therapeutic agent against mucositis in cancer patients (L. Read, Personal communication). Advitech Inc. (Canada) have recently released Dermylex™, a dairy-derived growth factor extract that when taken orally has been shown to reduce symptoms of moderate psoriasis (Poulin *et al*., 2005, 2007).

Commercial production of isolated dairy-derived growth factors has recently been reported by MG Nutritionals in Australia (Rowney *et al*., 2005). Colostrum-based products containing growth factors are available from a number of commercial suppliers globally including MG Nutritionals (Australia), Nutricia (Australia), and Tatua Biologics (New Zealand). Rapid expansion of the sports nutrition market in North America has seen some dairy products promoted for their content of whey proteins and peptides, including growth factors. The properties and lucrative potential applications of these growth factors, particularly in new functional clinical foods and sports and health products, will ensure their longer-term widespread use.

8.4.3 Hydrolysed milk proteins

Milk proteins are hydrolysed by microbial or pancreatic enzymes to decrease allergenicity, increase adsorption and improve functional properties in a range of food and beverage products. Milk proteins also contain biological

active peptides that are released by microbial fermentation or hydrolysis with microbial or pancreatic proteases. The bioactive peptides that are released contain from 2 to 20 amino acid residues, and when consumed have an impact on bodily functions or conditions, and may influence health (Haque and Chand, 2008). The peptides released are reported to include (i) anti-hypertensive peptides that inhibit angiotensin-converting enzyme and lower blood pressure (Lopez-Fandino *et al*., 2006); (ii) antimicrobial peptides that interact with bacterial membranes to give both bacteriostatic and bactericidal activities (Haque and Chand, 2008); (iii) immunomodulatory peptides that influence immune function (Gauthier *et al*., 2006); (iv) anti-tumour peptides that trigger apoptosis of cancer cells (Lopez-Exposito and Recio, 2008); and (v) antioxidant peptides that have radical scavenging properties (Pihlanto, 2006).

Milk-derived peptides are produced by the hydrolysis of whole milk proteins with microbes or isolated proteolytic enzymes (e.g. trypsin), often in combination to maximise the release and activity of the peptides (Korhonen and Pihlanto, 2007). Hydrolysis with isolated proteolytic enzymes can be achieved using either a batch method or using continuous membrane or chromatography reactors (Korhonen and Pihlanto, 2007). Without further purification or separation, the concentration of milk-derived bioactive peptides in the hydrolysed mixture is often low. Membrane separation techniques including ultrafiltration and nanofiltration have been developed to enrich peptides within specific molecular weight ranges (Korhonen and Pihlanto, 2003). In addition, biphasic extraction techniques with organic solvents have been employed (Froidevaux *et al*., 2001). Future developments in the production and isolation of bioactive milk peptides are likely to include continuous proteolysis and separation chromatography (Recio and Visser, 1999), ion-exchange chromatography membranes (Ghosh, 2001) and recombinant procedures (Jiang *et al*., 2004).

There are many fermented and hydrolysed milk products manufactured and marketed for their reduced allergenicity (in infant formula), improved functional properties, improved absorption rate and desirable health benefits. There are many manufacturers of hydrolysed milk proteins, including DMV International (Netherlands), MG Nutritionals (Australia), Arla Foods (Denmark) and Davisco Foods International (USA).

Of the milk protein hydrolysates that are reported to improve human health, there are relatively few that have undergone human clinical trials to prove efficacy. The casein-derived peptides isoleucine–proline–proline (IPP) and valine–proline–proline (VPP) are found in a number of commercial products including 'TensGuard' manufactured by DSM Nutritional Products (Netherlands) and 'Ameal' manufactured by Calpis Corporation (Japan). Human clinical trials suggest that these peptides reduce blood pressure (Mizuno *et al*., 2005) and improve vascular endothelial function (Hirota *et al*., 2007). However, a recent study indicates that these peptides have minimal influence on blood pressure (Engberink *et al*., 2008). The

casein-derived C12 peptide manufactured by DMV International (Netherlands) and sold as C12 Peption™ has also been shown in a human clinical trial to reduce blood pressure (Cadee *et al.*, 2007).

Lactium® is a novel trypsin hydrolysed alpha-casein product produced by Ingredia (France) that is sold as a beverage and in other formats such as powders and tablets. This product is proposed to have relaxing properties that reduce stress and habits associated with stress. A double blind, randomised, crossover, placebo-controlled study with 63 women with stress-related symptoms has been undertaken with Lactium®. Results suggest that after 30 days of supplementation, stress-related symptoms such as digestion, intellectual, emotional and social problems decrease (Kim *et al.*, 2007). There is an increasing need for clinical evaluation and substantiation of the health benefits of milk derived protein hydrolysates and peptides. With evidence that these products positively influence human health, the use of milk-derived protein hydrolysates and peptides in beverages is likely to increase.

8.4.4 Milk lipids

The major lipids found in milk are the triglycerides with myristic, palmitic, stearic, and oleic long-chain fatty acids and small amounts of butyric, caprioic, caprylic and capric short-chain fatty acids. Other lipids in milk include mono- and diglycerides, free fatty acids, phospholipids and cholesterol (Jensen, 2002). The phospholipids, glycolipids and glycosphingolipids are minor components and are mainly associated with the fat globule membrane with proteins, where they help stabilise the triglycerides and prevent lipid coalescence, flocculation and separation.

Perhaps some of the most interesting and functionally useful fats from milk and other dairy streams are the polar lipids, including the phospholipid sphingomyelin and the glycolipid cerebrosides. Sphingomyelin from milk contains a large fraction of long and saturated acyl groups, which results in a high gel-to-liquid crystal transition temperature. As such, sphingomyelin from milk forms liposomes readily in the presence of cholesterol, and also forms stable oil-in-water emulsions with soybean oil (Malmsten *et al.*, 1994). This surface activity of sphingomyelin provides opportunities for this polar lipid in drink delivery systems applications where the novel emulsions and liposomes formed with sphingomyelin may serve as carriers for bioactives (Singh, 2006). In addition, literature reports suggest that dietary supplementation with sphingomyelin isolated from milk reduces the number of aberrant crypt foci (possible precursors to colon tumours) and the appearance of colon adenocarcinoma in a mice model of the disease (Schmelz *et al.*, 1996).

Conjugated linoleic acid (CLA) occurs naturally in milk fat and has come to prominence within the past two decades or so after it was discov-

ered to have anti-cancer activity initially. Since then, it has been a 'beacon of hope' from the point of view of repositioning milk fat in markets which have suffered from many negative consumer perceptions along the way because of its more saturated nature and association with an increasing incidence of cardiovascular disease. CLA occurs in the form of several isomers of conjugated linoleic acid (at least 13 have been reported) – the more significant of which is *cis*-9, *trans*-12 CLA. The fact that CLA is more prevalent in milk produced from pasture-fed cows (e.g. milk and dairy products manufactured in Australia, New Zealand and Ireland) is a plus from the perspective of creating a natural CLA-enriched milk-based beverage through careful dietary management of lactating dairy cows. Meanwhile, an increasing body of scientific evidence is revealing the extent of the biological activities that are associated with the *cis*-9, *trans*-11 CLA (*c*9, *t*11 CLA) and another isomer *trans*-10, *cis*-12 CLA (*t*10, *c*12 CLA) which include anti-cancer, anti-atherosclerotic, anti-diabetic and immune-enhancing properties, and positive effects on body composition and bone formation (Belury, 2002; Pariza *et al*., 1999, 2000). In expanding the functionality of CLA, the *t*10, *c*12 CLA isomer has been shown to alter body composition by reducing fat content and increasing lean body tissue in animal models, and also in some studies with humans (Blankson *et al*., 2000; Smedman and Vessby, 2001; Thom *et al*., 2001). Human studies show a tendency for reduced body fat, particularly abdominal fat, changes in serum total lipids and decreased whole body glucose uptake (Thom *et al*., 2001) with maximum reduction in body fat mass being achieved with a 3.4 g daily dose. In recent years, strains of a number of dairy starter and probiotic cultures have been identified as possessing the ability to biosynthesise CLA, e.g. strains of *Lactococcus*, *Streptococcus*, *Enterococcus*, *Lactobacillius*, *Bifidobacterium* and *Propionibacterium.* Coakley *et al*. (2003) identified *B. breve* and *B. dentium* as efficient producers of CLA, with the majority in the form of *c*9, *t*11 CLA.

Bifidobacteria are natural inhabitants of the human gastrointestinal tract and have been associated with a large number of probiotic properties, including the prevention or alleviation of a number of human gastrointestinal conditions (O'Mahony *et al*., 2005). Given that certain species of *Bifidobacteria* as well as other food-grade lactic acid bacteria are known to produce CLA during fermentation, this opens up another possibility for the generation of fermented beverages containing CLA. Working with recombinant strains, Rosberg-Cody *et al*. (2007) have recently heterologously expressed linoleic acid isomerase in *L. lactis* and *E. coli*, and in so doing generated cultures producing *t*10, *c*12 CLA that exhibited an anti-proliferative effect. The authors believe that this demonstrates the potential for using recombinant strains to deliver bioactive CLAs to (or produce within) the mammalian intestine, where they exert an anti-proliferative activity.

8.4.5 Milk carbohydrates

Lactose

Lactose is the major carbohydrate in bovine milk, found at a concentration of approximately 48 g/l. It is a disaccharide made from galactose and glucose that is synthesised in the mammary gland in a reaction catalysed by galactosyl transferase and alpha-lactalbumin. After ingestion, lactose is hydrolysed in the intestinal lumen to its monosaccharide components by lactose/phlorizin hydrolase (Adam *et al*., 2004). The glucose and galactose released provide the newborn with a readily available source of energy. In humans, lactose intolerance is caused by the loss of lactose/phlorizin hydrolase activity and can result in abdominal pain, gas, nausea and diarrhoea (Adam *et al*., 2004). Lactose intolerance can be due to digestive diseases and damage to the small intestine, resulting in reduced lactose/phlorizin hydrolase production or an inability to produce the enzyme from birth. In most cases, the ability to produce lactose/phlorizin hydrolases decreases naturally over time (Adam *et al*., 2004).

The production of cheese from bovine milk generates a significant amount of whey with a lactose concentration similar to that of milk. Methods to isolate lactose from whey and whey filtration permeates have been developed, along with applications of lactose in a range of food and pharmaceutical products. The most common method to manufacture lactose from whey permeate is by supersaturated crystallisation followed by fluidised bed-, roller- or spray-drying (Yang and Silva, 1994). Other methods include the precipitation of lactose by alkali earth metals or the addition of alcohols or other solvents to reduce lactose solubility (Yang and Silva, 1994). The temperature of heating and crystallisation gives rise to different forms of lactose, notably the non-hydroscopic α-monohydrate, the hydroscopic α-anhydride, and the hydroscopic β-anhydride forms (Yang and Silva, 1994). Not all of the lactose is recovered from whey using crystallisation processes. To increase recovery, minerals can be removed by nanofiltration, electrodialysis and ion-exchange before spray drying (Yang and Silva, 1994). Processes based on ion exclusion chromatography and other techniques are likely to be commercialised in the future to enhance lactose recovery and purity from whey (Durham *et al*., 2004).

Lactose is manufactured by a large number of dairy companies including Friesland Foods Domo (Netherlands), DMV (Netherlands), Murray Goulburn Cooperative (Australia), Arla Foods (Denmark) and Davisco Foods International (USA). Owing to its inertness, price, toxicology and physical properties lactose is employed in the pharmaceutical industry as an excipient in capsules, sachets, pellets and inhalers. It is also widely employed in the food and beverage industry to carry flavour and colour, increase Maillard browning reactions, reduce caking of dry powders employed to make beverages, reduce formulation costs, improve the texture of processed fruit products, and to reduce the sweetness of soups, sauces, cocoa, confectionery,

meats and sausages (Yang and Silva, 1994). Lactose is also employed in infant formula to increase the lactose content to that of human milk, and to manufacture lactose derivatives such as lactulose, lactitol, lactobionic acid and galacto-oligosaccharides.

Oligosaccharides

While lactose is the dominant carbohydrate in milk, there is now increasing interest in the role of oligosaccharides which occur in much smaller concentrations. Human milk contains a larger variety of oligosaccharides than cows' milk and have the potential to modulate gut flora, affect different gastrointestinal activities and influence inflammatory processes of the neonate. Their pattern of secretion in milk ties in with the period of greatest biological demand by the neonate, i.e. greater concentrations of oligosaccharides occur in the period *post-partum.* Oligosaccharides are saccharide polymers containing a small number (typically three to ten) of linked component sugars, and also occur widely in nature as fructo-, galacto- and mannan-oligosaccharides. Generally classified as soluble fibres, the health benefits of oligosaccharides have emerged as their prebiotic role has been elucidated.

The presence of both acidic (sialic acid-containing) and neutral oligosaccharides in milk distinguishes them from other types – the sialyl attachments of the former function as competitive inhibitors for binding sites on internal epithelial surfaces when challenged by bacterial and viral infections (Mehra and Kelly, 2006). Neutral milk oligosaccharides act as prebiotics (i.e. following consumption, the undigested portion serves as food for the intestinal microflora) and, depending on the type of oligosaccharide, different bacterial groups are stimulated or suppressed. Clinical studies have shown that administering fructo- or galacto-oligosaccharides increases the number of friendly probiotic bacteria in the colon while simultaneously reducing the population of harmful bacteria (prebiotic function) (Kunz *et al.*, 2000). Other benefits associated with consumption of fructo- or galacto-oligosaccharides include increased production of beneficial short-chain fatty acids such as butyrate, and increased absorption of minerals, including calcium and magnesium. In animal studies fructo-oligosaccharides with a low degree of polymerisation (2–8) generated the highest levels of butyric acid all along the hindgut of rats, whereas fructo-oligosaccharides with a high degree of polymerisation (10–60) produced the highest levels of propionic acid. These specific differences were also generally reflected in the caecal pools and molar proportions of short-chain fatty acids (Nilsson and Nyman, 2005). In relation to mineral absorption, the effects seem to be specific for the type of carbohydrate, ingested dose and rate of fermentation by the intestinal flora (Scholz-Ahrens *et al.*, 2001). The underlying mechanisms are manifold: increased solubility of minerals because of increased bacterial production (owing to greater supply of substrate) of short-chain fatty acids; an enlargement of the absorption surface by promoting pro-

liferation of enterocytes mediated by bacterial fermentation products – predominantly lactate and butyrate; increased expression of calcium-binding proteins; improvement of gut health; degradation of mineral complexing phytic acid; release of bone-modulating factors such as phytoestrogens from foods; stabilisation of the intestinal flora and ecology; stabilisation of the intestinal mucus; and impact of modulating growth factors such as polyamines (Scholz-Ahrens *et al.*, 2007).

Clinical intervention studies are not possible for the moment as the large quantities of human oligosaccharides needed for such studies are not currently available. Once the functional equivalence of oligosaccharides derived from other sources (e.g. cow's milk) has been established with that of human oligosaccharides then their addition to infant formula may be useful in the context of mimicking biological functions of human milk constituents, and developing a more 'human-like' product (Kunz and Rudloff, 2006). In this respect, Vivinal GOS is a dairy-based product rich in galacto-oligosaccharides manufactured by Friesland Foods in the Netherlands. According to the manufacturer, addition of Vivinal GOS to infant formula yields a composition that more closely resembles that of human milk. Two of the galacto-oligosaccharides present in Vivinal GOS (i.e. β-(1–4)-galactosyllactose and β-(1–6)-galactosyllactose) occur naturally in human milk. Vivinal GOS also stimulates the growth of bifidobacteria. Research has shown that breast-fed infants have higher levels of the health-promoting bifidobacteria than bottle-fed infants. This difference in colonic flora is attributed to the presence of prebiotic/bifidogenic factors in human milk, including galacto-oligosaccharides. Bifidobacteria are important for infants because they are associated with such beneficial effects as protection against infection and diarrhoea. Acid-stable, fruit-based beverages containing Vivinal GOS are now marketed by Friesland Foods Domo under their 'Cool Best' brand. It is claimed that the galacto-oligosaccharides of Vivinal GOS are stable during heating and storage at pH 3.0, resulting in a beverage of consistent quality and composition.

8.5 Milk-based beverages, and beverages that utilise ingredients from milk

Pasteurised milk is a beverage which is usually retailed in cartons and bottles intended for consumption within several days. This 'freshness' aspect of milk has been a particularly strong selling point, and the logistics of doorstep delivery that evolved around the notion of delivering a high-quality, nutritional product to the consumer was for a long period a feature of 'liquid' milk markets within the United Kingdom, Ireland, Australia and New Zealand in particular. It has often been argued that the relatively high per capita consumption of milk was due in part to the unique marketing support afforded by doorstep delivery. With the increase in supermarket

dominance over the past 20 years, most milk products are now distributed to and sold through supermarket outlets.

There are a range of beverages on the market that are made from milk, milk products or isolated ingredients from milk. These include pasteurised milk, pasteurised milk with added nutrients, infant formula, weight loss drinks, high-protein beverages, mixed fruit and milk beverages, and beverages that improve health and sports performance. The challenges of incorporating milk-derived ingredients into beverages can be difficult to overcome and include maintaining biological activity and physical properties (e.g. solubility) during thermal processing, at a range of product pH values and throughout product distribution and extended shelf-life. These challenges are being overcome by manufacturers, resulting in a range of popular beverages that contain milk and milk ingredients.

As consumers look to food and drink to improve their health and well-being, and with consumers' increasing acceptance of functional foods and a desire to self-medicate, the functional drinks market has seen recent high growth. In Europe and the United States alone this category is worth in excess of US$20 billion, and continues to grow at >4% annually. As this category expands, the industry is increasingly adopting 'pharma technologies' in order to create more sophisticated and personalised health-promoting products. These technologies include genomics, transcriptomics, metabolomics and nanotechnology. However, a word of caution in that the food industry must always be conscious of cost as margins in foods and beverages are considerably smaller than in the pharma space. Pressure on labelling and health claim regulations is likely to increase and to change globally as a result of the evolution of functional foods and beverages. These changes are likely to include issues such as harmonisation of regulatory guidelines and legislation, and the need for more extensive clinical trials, both on ingredients and final foods.

8.5.1 Flavoured and fortified milk beverages

Flavoured milk beverages

The addition of flavour, colour and sucrose to milk has increased the popularity of milk as a beverage to consume with a meal, as a hydration product or as a snack. The addition of stabilisers to help maintain flavour and colour distribution throughout the product during storage and transport is common. As consumers move towards healthier products, milk manufacturers are developing reduced fat and reduce carbohydrate flavoured milk beverages. Additional developments in the flavoured milk category include UHT processing to produce shelf-stable, long-life flavoured milks (e.g. Natrel flavoured milk), the addition of hydrocolloids to increase the viscosity and produce 'thick shake' milk products, and reducing the sugar content or replacing the sugar with artificial sweeteners to produce low-carbohydrate milks.

Calcium fortified milk

There are a number of milk-derived beverages fortified with calcium, and in some countries claims on beverage labels linking high calcium intake with strong bones, and reduced risk of osteoporosis are allowed. The extent of such claims are governed and controlled by food regulatory bodies specific to the country of sale. Beverages fortified with calcium have been developed based on studies showing that consumption of milk and dairy products throughout childhood and adolescence is associated with improved bone mass and reduced risk of osteoporosis (Stracke *et al.*, 1993). In addition, numerous studies have shown that increased consumption of calcium with and without vitamin D is associated with a decreased risk of osteoporosis and bone fracture (Shea *et al.*, 2002; Rizzoli *et al.*, 2008). Examples of milk beverages fortified with calcium include Natrel Calcium (Canada), Calcium Plus (France), Calci Kids (New Zealand) and PhysiCAL (Australia).

Magnesium fortified milk

There are a number of beverage manufacturers fortifying milk products with magnesium. Such products are benefiting from published research indicating that increased dietary magnesium intake is associated with a decreased risk of gallstone formation, diabetes, metabolic syndrome and hypertension (Bo and Pisu, 2008). Examples of products fortified with magnesium include Candia Viva Magnesium (France) and Magnesio (France).

Lipid fortified milk

Omega-3 fatty acids are a class of unsaturated lipids that contain 18 to 22 carbons and a double bond at the third position from the methyl end. These fatty acids cannot be synthesised by humans and must be obtained from the diet. Increased omega-3 fatty acid consumption is reported to reduce the risk of cardiovascular disease and play a role in the prevention of other diseases (Ruxton *et al.*, 2007; Harris *et al.*, 2008). Owing to the reported health benefits, omega-3 fatty acids have been added to a range of beverages including milk. In clinical trials, consumption of milk fortified with omega-3 fatty acids has been shown to influence markers of cardiovascular disease (Visioli *et al.*, 2000; Baro *et al.*, 2003). Omega-3 fatty acids are also added to infant formula and probiotic beverages. The omega-3 fatty acids employed in beverages are derived from plants (mainly alpha linolenic acid) or fish (eicosapentaenoic acid [EPA] and docosahexaenoic acid [DHA]). As very little alpha-linolenic acid is converted to DHA and EPA, supplementation with the latter two from fish oil is preferred. As omega-3 fatty acids have solubility and flavour issues when employed in beverages, the development of emulsion and packaging technologies has enabled filling and storage of omega-3 fatty acid fortified milks with minimal oxidation. There are numerous milk beverages on the market that are fortified with omega-3 fatty acids including Candia Omega-3 (France), Natrel

Omega-3 (Canada), Heart Plus (Australia), Dawn Omega Fresh Milk (Ireland) and NaturLinea (Spain).

Saturated fatty acids and cholesterol are naturally present in bovine milk, and excessive consumption of these fats and cholesterol has been associated with increased risk of coronary heart disease (Hu *et al*., 1999; Kromhout *et al*., 1995). Skimming of milk to remove the majority of the fat and cholesterol is standard practice within dairies and allows the standardisation of milk fat content, and also the production of low-fat skim milk beverages. Although these products are healthier alternatives owing to their lower fat and energy content, they have a watery appearance and flavour differences when compared with un-skimmed milk products. To produce full-fat flavoured milk products with improved appearance and flavour, manufacturers have developed the technology to enable the milk fat and cholesterol to be removed and replaced with unsaturated fats derived from plants (e.g. Canola and Sunflower). An example of such a product is Farmers Best, manufactured by Dairy Farmers in Australia.

Sterol fortified milk

Phytosterols (sterols and stanols) are naturally occurring compounds found in various plants that are structurally similar to cholesterol. Consumption of these compounds has been reported to reduce cholesterol absorption and reduce plasma LDL cholesterol concentrations in people with hypercholesterolemia (Jones *et al*., 2000). As elevated plasma LDL cholesterol concentrations have been linked to an elevated risk of cardiovascular disease, phytosterol supplementation has been recommended as a strategy to reduce cardiovascular disease risk (Cleeman *et al*., 2001). Technologies to incorporate sterols and stanols into milk beverages have been developed (Masutake, 2006) and clinical trial results indicate that sterol-enriched milk is effective at reducing serum cholesterol concentrations (Noakes *et al*., 2005; Gonçalves *et al*., 2007). Sterol enriched milk products have gained regulatory approval in a number of countries and products such as Pura Heart Active (Australia), Becel ProActive (worldwide), and Devondale Reduce (Australia) are available in the marketplace.

Vitamin fortified milks

An increasing trend in some countries is to legislate and regulate for the addition of key vitamins in both non-fat and regular milk. Notable is the United States where a milk ordinance requires vitamin A fortification of all reduced fat fluid milk products (Murphy *et al*., 2001). In the United States, the addition of vitamin D is optional in all milk products, but the trend has been for manufacturers to supplement their processed milks with vitamin D to meet consumer demands. Such vitamin concentrations in fortified milks are expected to be 2100–3200 IU/l for vitamin A, and 420–630 IU per quart for vitamin D (Murphy *et al*., 2001).

Milk has been identified as an ideal vehicle to enhance the folate status in women of childbearing age, and thus serve as a means of preventing neural tube defect in children. Daily consumption of ~400 μg of folic acid prior to conception and during early pregnancy is recommended for the prevention of such a defect in the neonate (Green *et al*., 2005). A recent clinical study demonstrated that daily consumption of milk fortified with 375 μg folic acid increases blood folate and lowers homocysteine concentrations in women of childbearing age, and as such would be expected to reduce the risk of neural tube defect (Green *et al*., 2005). While this and other studies are compelling, many countries (e.g. in Europe) still ban the supplementation of milk with folic acid, although there are manufacturers in some parts of the world (e.g. Australia) who have been brave enough to develop and market milk containing added folate.

8.5.2 Whey-derived beverages

There are a number of beverages on the market that utilise concentrated or isolated whey proteins as major ingredients. For example, whey proteins are regularly employed as a major ingredient in sports supplements, weight loss and health-promoting beverages. A unique carbonated beverage manufactured from whey in Switzerland and sold throughout Europe is Rivella®. The product is manufactured using a combination of protein precipitation, lactose fermentation, flavour addition and sweetening procedures, giving rise to a number of varieties of Rivella® with different flavours and energy contents.

8.5.3 Infant formula

Mothers who cannot breast feed, or choose not to, rely on the use of another mother (wet nursing), prepared foods (dry nursing) or animal milks. Animal milk products for infants are termed infant formulas and are made predominately from bovine milk ingredients, and other protein sources such as soy.

The composition of bovine milk varies significantly from that of human breast milk, including the protein quantity and types, lipid structures, and carbohydrate quantity and structures. For example, human milk contains approximately 2–4 g/l of lactoferrin whereas bovine milk under normal conditions contains a maximum of approximately 0.35 g/l (Madureira *et al*., 2007). A better understanding of breast milk composition and infant needs, along with developments in bovine milk fractionation is facilitating the development of infant formula products with energy, protein, lipid and carbohydrate contents similar to that of breast milk. The process of 'humanising' infant milk formula, i.e. realigning the composition of bovine milk closer to that of human milk, has been facilitated by technological developments such whey demineralisation which provides an ingredient

that enables (i) the casein/whey protein ratio to be virtually inverted, (ii) elevates lactose content, and (iii) reduces ash content to minimise the renal load on the neonate. New generation infant formula now goes even one step further by substituting total whey protein with alpha-lactabumin-enriched whey protein concentrate in recognition of the latter's prominence in the whey protein complement of human milk. There is a continuous effort to understand the biologically active components in human milk and their role in infant growth and development, and to develop new generation infant formulas that mimic human milk, and provide optimal infant growth and development. While most infant formula is produced in spray-dried form, a certain proportion is also processed in liquid form using either UHT or retort sterilisation technologies as ready-to-drink (RTD) beverages.

8.5.4 Lactose-hydrolysed and lactose-free beverages

Milk and milk-derived products represent a long-established and essential part of the diet of millions of people around the world. However, a large number of potential dairy consumers, notably in Africa, the Americas and Asia, cannot partake of such products due to lactose sensitivity or intolerance. These sensitive individuals do not have sufficient β-galactosidase ('lactase') in their small intestines to digest the lactose found in milk and milk products. Such maldigestion leads to intestinal disorders such as flatulence, bloating and abdominal pain (Heyman, 2006). These symptoms of lactose intolerance can be reduced or eliminated by using lactose-hydrolysed or lactose-reduced/free milk and dairy products. Such products, in which the lactose has effectively been 'removed', offer an opportunity for the dairy industry to gain new milk consumers, who would otherwise avoid milk and dairy products all together.

A number of technologies for the effective 'removal' of lactose from milk and other dairy streams have been developed and some commercialised (Gekas and Lopez-Leiva, 1985; Harju, 2004). Lactose in milk has traditionally been hydrolysed in batches using a yeast β-galactosidase, while in fermented dairy products, β-galactosidase derived from mould sources has been an alternative. β-Galactosidases from other sources have only generated passing scientific interest because their safety has yet to be demonstrated for approved use in food processing. The cost of the β-galactosidase enzyme represents a critical factor in any lactose hydrolysis process. Thus, immobilisation of β-galactosidase represents an approach to dramatically reduce the cost of this enzyme-based processing (Ladero *et al.*, 2002). However, while cost of the enzyme can be addressed using such an approach, hygiene and sanitation of the process remain problematic. For this reason, immobilised β-galactosidases have mainly been used in cheese whey processing.

Enzyme-catalysed hydrolysis of lactose in milk leads to the generation of glucose and galactose and, as such, to an extremely sweet milk product

when compared with normal milk. This sweetness represents the main drawback to lactose-hydrolysed milks and dairy products, and has resulted in poor market performance. To address this shortcoming, a chromatographic processing technology has been developed to specifically remove lactose from milk rather than to chemically alter it via hydrolysis (Harju, 2004). This process has been based on the use of ion-exclusion chromatography, and inherited from a similar process used in the sugar industry. This patented process, developed and refined by the Valio Company in Finland, relies upon the physical removal of a large percentage of the milk lactose via chromatography, with the remainder being hydrolysed. In this way it is possible to manufacture a lactose-free milk with the same taste and sweetness of fresh milk (Harju, 2004). The process has been commercialised, and a range of lactose-free milks and other products are available from Valio in Scandinavia, including creams, yoghurts, fermented milks, quarks, cottage cheese and sour cream.

Technologies to manufacture lactose-hydrolysed and lactose-free milks and other dairy streams with the same organoleptic quality as normal fresh milk and whey are available, and successful consumer dairy products based on such technologies now have an established place in the market.

8.5.5 Functional milk-based beverages

Weight loss beverages

Very low-calorie diets (VLCD) are nutritionally complete diets that contain less than 3350 kJ (800 calories) per day and are employed to manage obesity. With appropriate medical supervision, obese individuals prescribed these diets usually lose a significant amount of weight in a short period of time (Anderson *et al.*, 1992). Different formats of VLCD products are available including soups, bars and flavoured beverages. Milk-derived proteins such as caseinates, skim milk powders and whey protein isolates make up the majority of the protein complement of VLCD products. The proteins and other components in the VLCD products are often supplied as powders in single serve sachets, designed to be mixed with water and consumed at regular intervals throughout the day. Examples of VLCD products include Optifast®, Medifast and Kicstart™.

Sports performance beverages

Milk proteins are well positioned in the sport nutritional market and are employed in body building, exercise enhancing and exercise recovery products. Many of these products are supplied as a powdered base, which is mixed with water or fresh milk to form a beverage for consumption. There is support for the use of milk-derived proteins to improve muscle growth and repair over other proteins. For example, Wilkinson *et al.* (2007) has shown that milk-derived proteins promote muscle protein accretion to

a greater extent than soy-based proteins. Owing to the high content of essential amino acids, whey proteins have a strong position in the sports performance market. There have been studies showing that whey proteins improve muscle protein synthesis post exercise (Tang *et al*., 2007a); however, there seems to be little difference between whey proteins and other milk proteins (caseins) ability to stimulate muscle protein synthesis (Tang *et al*., 2007b). Milk beverages have also been reported to improve the ability to undertake strenuous exercise (Karp *et al*., 2006) and improve hydration after exercise (Shirreffs *et al*., 2007).

There are many commercial products that contain milk proteins and are reported to enhance sports performance and muscle growth. Dairy Farmers of America Incorporated produce a range of flavoured canned shelf-stable milk drinks marketed under the name 'Sport Shake'. These products contain skim milk, sugar, flavours and stabilisers and compared with other leading sports beverages they are reported to have a much greater protein, calcium, carbohydrate and potassium content. Murray Goulburn Cooperative (Australia) has recently released a range of milk-derived protein products that are reported to enhance muscle strength and muscle recovery (Farnfield *et al*., 2005). There are many other products on the market containing dairy-derived proteins that are also reported to increase muscle growth and endurance.

Hyperimmune milk and products

In spite of the low relative abundance of immunoglobulins in milk, hyperimmune milk products are in the marketplace. These products are produced with elevated levels of immunglobulins by challenging mucosal surfaces (e.g. nasal tract) of animals with human enteropathogenic microorganisms. Milk products produced using this method are generally regarded as safe (Gapper *et al*., 2007) and benefits are reported for treatment of protozoal gastrointestinal disorders (Beck and Kotler, 1992), immune suppression (Beck and Stolle, 1999), rheumatoid arthritis (Stolle and Beck, 1991), elevated cholesterol (Sharpe *et al*., 1994), inflammation (Zenk *et al*., 2002), elevated blood pressure (Sharpe *et al*., 1994), prevention of suppression of T-lymphocyte function (Beck and Stolle, 1999) and for passive immunisation (Humanetics Corporation). However, while the claims for these products have been clinically substantiated, it is not clear whether their bioactivities are related to the immunoglobulins per se or that the hyperimmune milk represents a patent-protected form of typical milk. The reported survival of orally ingested bovine colostrum concentrate through the stomach to the lower bowel and associated protective benefits against infection have elicited speculation regarding the application of this product as a functional food ingredient for at-risk populations, including developing nations (Solomons, 2002).

There are a number of companies throughout the world producing products based on milk and colostrum from hyperimmunised cows. MucoVax

BV (Netherlands) produces a spray dried whey powder derived from the milk of hyperimmunised cows which is reported to prevent relapse of *Clostridium difficile* infection (Van Dissel *et al*., 2005). This product maybe incorporated into infant formula products or other nutritional products in the future. In addition, Stolle Milk Biologics Incorporated (USA) has developed and patented the production of hyperimmune milk products that are reported to have anti-inflammatory and antihypertensive properties. Gastrogard-R® is an example of a hyperimmune colostrum product manufactured by Anadis (Australia) which is reported to reduce the risk of rotavirus infection in young children.

Probiotic beverages

Modern consumers are increasingly focused on their personal health and well-being, and increasingly demanding that the foods they consume are prophylactic, and potentially therapeutic, in terms of prevention (and cure) of illness and lifestyle diseases. Regarding specific health outcomes, gut health represents a key sector for functional foods in Europe and Australia, and increasingly in the United States and Canada. The probiotic yoghurt market is established but a key growth sector recently has been in the area of probiotic drinks (Mattila-Sandholm *et al.*, 2002).

Stability and long-term viability of probiotics, notably in harsh environments (e.g. acid, ambient storage), represent technical challenges for industrial producers of probiotic drinks. Probiotic foods contain one or more specific probiotic strains and must maintain an appropriate content of viable cells during the product's shelf-life. In modern foods, the technological demands placed on probiotic strains are severe and as such, new manufacturing processes, formulation technologies and protective delivery systems may often be required for probiotic bacteria primarily selected for their functional health properties (Crittenden *et al*., 2006). Before probiotic strains can be delivered to consumers, they must first be commercially manufactured in a cost-effective manner, and then survive and retain their functionality during storage as frozen or freeze-dried cultures, and also in the food products into which they are finally formulated, in this case beverages often at low pH. The probiotic bacteria should also ideally survive the gastrointestinal stress factors and maintain their functionality within the host at the targeted delivery point. Packaging materials used and the conditions under which the products are stored are also important for the quality of probiotic drink products, and the stability and viability of the probiotics. Finally, they must be able to be incorporated into foods without producing any undesirable organoleptic effects.

Future technological advances in probiotic drinks will likely be in the areas of organism stability and long-term viability in harsh food and beverage environments. Such advances will likely focus on (i) development of new or modifed strains that reflect the same health promoting properties of the parent strain but are more hardy under the application conditions of

interest (e.g. Ganeden Biotech recently announced a number of new hardy probiotic strains, based on the sporeformer *Bacillus coagulans*, that are claimed to have a long shelf-life, and to survive harsh food and gastric environments); and (ii) approaches to the protection and delivery (including targeted gastronintestinal tract delivery) of probiotic organisms using protective mechanisms and barrier materials (e.g. microencapsulation) (Crittenden *et al*., 2006). In addition, keeping production costs low will remain the challenge for future probiotic process and formulation technologies.

Perhaps the most well known and established probiotic drink in the marketplace is Yakult, now manufactured and sold in more than 30 countries around the world. Yakult contains live, beneficial bacteria – the proprietary *Lactobacillus casei* Shirota strain. This probiotic strain in Yakult has been tested and shown to survive transit through the stomach and reach the intestine in a viable form (Kado *et al*., 2001). Yakult is now also manufactured in a 'light' version containing 30% less sugar.

The established position and popularity of Yakult has led to other similar products appearing in the market. For example, DanActive is a new generation probiotic drink containing a proprietary strain of *L. casei* Immunitas™, and is being aggressively marketed in the United States. Regular consumption of this product is claimed by the manufacturer to help strengthen the body's defence against disease (Guérin-Danan *et al*., 2001; Turchet *et al*., 2003; Hickson *et al*., 2007). Similarly, Natrel Pro, a probiotic milk beverage containing two active cultures (Lb IMMUNI T and BB-12), has recently launched in Canada. The manufacturer claims that daily consumption of Natrel Pro will assist in protecting the immune system from viral infections such as gastroenteritis and influenza, stimulate the immune system to fight harmful bacteria and reduce autoimmune diseases, enhance the body's recovery following antibiotic treatment, and promote digestion and overall health. Unlike some other fermented probiotic drinks, Natrel Pro is claimed to taste just like fresh milk.

A recent novel entrant into the probiotic drinks category in north Asia has been Interbalance L-92, a liquid probiotic yoghurt designed to fend off allergies. This product features *L. acidophilus* L-92, one of the more than 2000 strains owned by the manufacturer (Calpis). Seasonal allergies are a major problem in Japan where more than 1.5 million people suffer the symptoms in spring and autumn. According to the manufacturer of Interbalance L-92, a regimen of daily consumption of the product for a period of 6 weeks led to reduced or eliminated allergy symptoms in nearly 70% of subjects.

An increasing trend in the probiotic drinks category is the development of so-called combination products where a probiotic bacteria is included in the beverage together with another bioactive component, either dairy or non-dairy derived. An example of this type of product is a milk drink

manufactured and sold by Kyodu Milk in Japan that has been fermented with *L. casei* and also includes supplementation with added lactoferrin.

Bioactive beverages

In the midst of the functional foods revolution, a major growth area for functional (bioactive) beverages has been in the area of heart health, notably the use of natural components to lower blood pressure.

The first such product in this category, launched in 1997 in Japan, was a cultured milk beverage called Ameal-S, manufactured by Calpis and distributed successfully through the extensive vending machine and on-line shopping networks found in Japan. Ameal-S is a fermented milk-based product that relies upon the generation of tripeptides by a lactobacillus strain used in the fermentation. These tripeptides have a clinically proven effect on lowering both diastolic and systolic blood pressure (Hata *et al*., 1996). Ameal-S has been a market leader in Japan for 10 years and is the flagship product for the manufacturer. Recently, the product was relaunched in a convenient plastic bottle and with a new taste profile. The active ingredients in the product have also recently been developed into a tablet format for convenient over the counter (OTC) applications.

Success of the Ameal-S product in Japan, and burgeoning interest from health-conscious consumers, has stimulated the development and marketing of similar heart-health functional beverages in countries outside Asia. Evolus is a yoghurt beverage fortified with bioactive peptides and minerals, developed in Scandanavia, but now available in various European countries. The product is related to Ameal-S in that it is fermented to generate tripeptides with clinically proven effects on lowering hypertension. Evolus contains two tripeptides – isoleucine–proline–proline (IPP) and valine–proline–proline (VPP) – that are generated from casein protein in the milk by the *L. helveticus* strain used in the fermentation. The IPP and VPP peptides have several biochemical and physiological effects, including inhibition of angiotensin-converting enzyme (ACE), leading to reduced hypertension. The blood pressure-lowering effect of Evolus drinks has been demonstrated in clinical trials with subjects showing slightly elevated blood pressure, and the mechanism of action has been studied in empirical trials (Tuomilehto *et al*., 2004; Jauhiainen *et al*., 2005).

Bioactive beverages based on milk systems will continue to be developed, commercially stimulated, at least in part, by the lucrative market for such products. Taste profile, convenience, price and strong evidence of health benefit will be critical factors in product success.

Other beverages

There are many other beverages on the market that utilise milk or ingredients derived from milk in combination with ingredients from cereals and other sources. Sanitarium's Up-and-Go™ (Australia) is a UHT-treated

beverage that contains a mixture of proteins and carbohydrates from cereal, soy and milk sources with fruit extracts, minerals, flavours and colours. The product is available in a range of flavours and is marketed as a quick and nutritious liquid breakfast.

8.6 Future trends

For several decades, beverage manufacturers attracted consumers with their tempting range of sugar-filled soft drinks, and the resulting expansion in sales occurred to some extent at the expense of milk consumption. Today, both dairy and beverage sectors have more to gain by combining their strengths to address new market opportunities. This comes at a time when consumers are having second thoughts about the relatively high carbohydrate loading of such soft drinks and their negative effects on children's diets. The middle ground is now being increasingly occupied by a rapidly growing market for functional beverages – drinks that contain biologically active ingredients which when consumed confer specific physiological benefits in the body. This is the dairy industry's strength as milk is the source of many bioactive components which can be transformed into functional ingredients that may be subsequently used by beverage manufacturers. Beverage companies, in turn, offer market access via their own brands and as suppliers to multiple supermarket chains. However, processing of these new functional beverages is more complex and demanding than traditional soft drinks and this is where dairy technologists have much expertise to offer. Furthermore, food labelling laws governing putative health benefits are becoming increasingly stricter and it will be necessary to have established scientific and clinical supporting evidence to support such claims.

Meanwhile, new markets for milk and milk products are opening up around the world especially in geographical regions where there has been little or no history of milk consumption. Consequently, these new consumers are not 'programmed' by the traditional taste of milk, but seem to be more attracted to the diversity of milk-based beverages offering a variety of fortifying and functional ingredients.

Milk is likely to remain a valuable source of ingredients that have nutritional, biological and functional properties. Future developments are likely to see the identification of novel components in milk, and a greater understanding of their role in human health and disease prevention. With the development of commercial-scale concentration and isolation techniques, these novel components will be manufactured and incorporated into a range of foods and beverages. Gene technology is developed to the extent that valuable milk components can be produced in non-animal sources. One company (http://www.ventriabio.com/) is manufacturing recombinant human lactoferrin and lysozyme in rice, and marketing the products as ingredients for food and pharmaceutical use. There is continuing debate

about the safety and ethics of ingredients produced using transgenic procedures, and the use of such products in the food chain. However, as the amount of information available on the safety of transgenic foods and ingredients increases, we may see a greater number of products in the market.

Future milk beverage developments are likely to include extensions of well-known brands (for example, chocolates), flavoured milks with artificial sweeteners, milk beverages with natural fruit purees, milk with bioactive components such as phytosterols, peptides and antioxidants. As traditional beverage companies strive to differentiate their products, milk and milk ingredients may be blended with a range of traditional beverages, enabling beverage companies to capitalise on the nutritional and functional benefits of milk and milk-derived ingredients.

8.7 Sources of further information and advice

The following websites provide useful information on milk processing and the production of milk-derived ingredients:

http://www.glanbianutritionals.com
http://www.dmv-international.com
http://www.mgc.com.au
http://www.mgc.com.au/business/mgnutritionals.asp
http://www.tatua.com
http://www.arlafoodsingredients.com
http://www.morinagamilk.co.jp
http://www.fonterra.com
http://www.domo.nl/nutrition/
http://www.euroserum.com
http://www.daviscofoods.com
http://www.eriefoods.com
http://www.armor-proteines.com

The following websites provide useful information on beverages that utilise milk or components from milk:

http://www.amealbp.com
http://www.calpis.net/
http://www.natrelpro.ca
http://www.ascendsport.com.au
http://www.dairyfarmers.com.au/df/ourproductsandrecipes/milk/farmersbest/
http://www.devondale.com/products/milk_reduce.asp
http://www.pura.com.au/pura_brands_heartactive.aspx
http://www.upandgo.com.au/
http:// www.humaneticscorp.com/microlactin/index.html

8.8 References

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9

Milk-based functional beverages*

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Abstract: This chapter discusses the rheology and defects of protein-based drinks. It also discusses the properties of milk components (casein, whey, lactose, minerals, milk fat), and the effects that processing (emulsification, acidification, heating) or additives (hydrocolloids, low molecular-weight emulsifiers) have. The effect of current trends (sugar and fat reduction, better sources of protein or fat, fortification) on formulation of dairy drinks is also discussed.

Key words: milk, sedimentation, viscosity.

9.1 Introduction

Cow's milk-based drinks are among the beverages with the longest history of consumption. For a long time, regular full-fat milk was consumed only by infants. Regular consumption of milk by adults occurred only after our ancestors started herding cattle. The ensuing possible excess milk production made microbiological and physical stability of dairy products relevant, triggering inventions such as cheese and yogurt production, as liquid milk itself was not easy to keep. Only in the past century or so have inventions such as milk powder production, pasteurisation and sterilisation changed this situation. Optimal treatment of the milk, however, required a deeper understanding of the chemical and physical properties of milk, giving rise to the field of dairy science. The importance of this field has increased even further in the past decade, as the trend of healthy fortified drinks (such as pre- and probiotics, sterol esters, fish oil, calcium, soy protein, light variants) has reached the mass market, introducing ever new forms of product instability. Issues arising from this development are for instance rancid and bitter

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off-taste, and textural defects such as sedimentation and creaming upon storage.

Solving these issues in protein-based drinks requires considerable knowledge of composition, microstructure and interactions of proteins and polysaccharides in these beverages. Great care has to be taken in combining certain ingredients, or specific processing steps. This chapter will review the most important microstructural aspects related to the above, with reference to industrial practice.

9.2 Basic properties of milk

9.2.1 Introduction

The main constituent of milk-based beverages is milk, or powders derived therefrom. For an overview of the composition of milk, see Table 9.1. The production of cow's milk is schematically described in Fig. 9.1. Note that during the process almost all fat is removed by centrifugation ('skimmed milk' contains approx. 0.4% fat), and later added under agitation (homogenisation). One of the last steps is pasteurisation. After this the milk can usually be kept at 5 °C for 2 weeks.

In order to make (skimmed) milk powder, which can be used to enrich drinks with protein, milk is first concentrated by film evaporation and next spray/roll dried. The heat treatment of the product during spray drying is important for its functional properties, because of denaturation of whey upon heating (see Section 9.4.3). Heat treatment is more severe for high-heat skimmed milk powder (SMP) than for low-heat SMP. The heat-history during powder manufacture is measured by the whey protein nitrogen index (WPNI). To measure the WPNI the powder is dissolved and acidified, which flocculates the casein and denatured whey. The supernatant is heated, which denatures and flocculates the remainder of the whey. The amount of flocculated whey is determined by measuring the nitrogen (e.g. by a Kjeldahl or Dumas test). The result is expressed as the quantity of undenatured whey/serum protein per gram of skimmed milk powder. The WPNI values for the various types of powder are: ≥ 6 (low heat), 1.5 to 6 (medium heat) and ≤ 1.5 (high heat). The main constituents in milk will be discussed next.

9.2.2 Casein

Caseins represent four-fifths of the total protein content of milk. They are proteins with very little secondary structure, i.e. they are heat stable. This is because the primary structure contains many proline amino acid residues, which prevents the chain from folding. The iso-electric point (IEP = pH at which the polyelectrolyte has zero net charge) is approximately 4.6. Below the IEP molecules are positively charged. At the IEP the caseins are

Table 9.1 Detailed composition of cow's milk. Numbers taken from Walstra *et al.* (1999).

Compound	Approximate average quantities in 1 kg of milk			
	Milk serum	Casein micelles	Fat globules	Globule membrane
Water	790 g	80 g	60 mg	+
Total protein				700 mg
casein	+	25 g		
β-lactoglobulin	3.3 g			
α-lactalbtunin	1.0 g			
serum albumin	0.3 g			
immiunoglobulins	0.7 g			
peptides	+	+		
Triglycerides			40 g	
Diglycerides			0.1 g	
Fatty acids	20 mg		60 mg	15 mg
Phospholipids	100 mg			250 mg
(Chole)sterols	70 mg		90 mg	15 mg
Ca, bound	300 mg	850 mg		
Ca, ions	90 mg			
Ph	1100 tng	1000 mg		
Mg	70 mg	+		
K	1500 mg	+		
Na	450 mg	+		
Cu	20 µg			4 µg
Fe	120 µg			100 µg
Vitamins A, D, E, K			+	
Vitamin B	200 mg			
Vitamin C	20 mg			
Urea	250 mg			
Citrate	1600 mg			

Note: $^{+}$ indicates that the component is present at low levels.

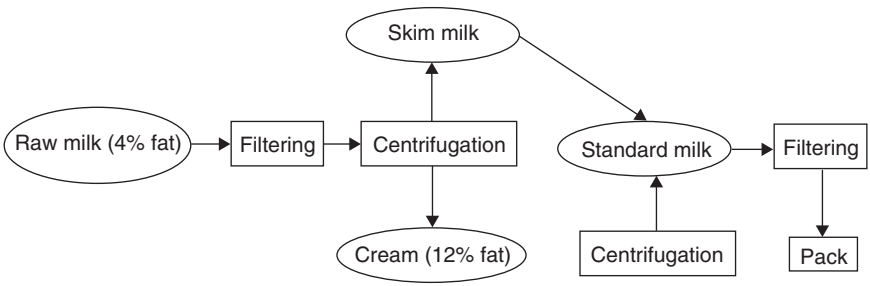

Fig. 9.1 Typical processing scheme for milk.

uncharged, because of the widely varying IEP of each of the amino acid residues (Walstra *et al.*, 1999).

Caseins are organised in so-called *casein micelles*, which are more or less spherical agglomerates of approximately 20–300 nm in diameter. One micelle contains thousands of (hydrophobic) casein molecules of different types (α_{S1}, α_{S2}, β and κ), colloidal calcium phosphate (CCP or CaPh) crystal particles, and approx. 60% water (Walstra *et al.*, 1999).

Inside the micelles there are at least three mechanisms keeping the casein molecules together (Horne, 1998; Mellema *et al.*, 1999): (a) casein bridges between hydrophobic domains on proteins, (b) casein bridges between domains with a high amount of (hydrophobic) colloidal CaPh, and (c) CaPh bridges between caseins. Clearly calcium plays a central role in the stability of the micelles. The α_{S1}-casein, because of its triblock configuration (hydrophobic–hydrophilic–hydrophobic), is excellent to play the role of bridging agent between hydrophobic entities according to mechanisms (a) and (b). The β-casein has a diblock configuration (hydrophilic–hydrophobic) but also contains a lot of phosphate (serine) groups, which are sensitive to calcium and contribute to mechanism (c). The κ-casein resides mainly at the surface of the micelles because it has a distinct diblock configuration but without many phosphate groups. This 'hairy layer' of κ-casein tails is approx. 7 nm thick and is responsible for the colloidal stability of the casein micelles in milk. The repulsion between the micelles is mainly of steric origin, although electrostatic aspects are important in an indirect way (to keep the hairs extruding into the solution and not collapsed) (de Kruif and Zhulina, 1996). An overview of the main industrial processing steps leading to changes in the micelles and aggregation of the micelles is given in Table 9.2.

Non-aggregated casein is available only in the form of caseinate salts. To prepare caseinate, casein micelles are isolated by decreasing pH, disrupted by increasing pH, while cations (e.g. sodium) are added. Because of their lack of secondary structure, and their amphiphilic nature, they are good oil-in-water emulsifiers. Unfortunately, caseinate can have some off-taste.

9.2.3 Whey proteins

The second most important group of proteins in milk are the whey or serum proteins, of which β-lactoglobulin is the most important. The protein β-lactoglobulin has a similar hydrophobicity as the caseins, but it has a somewhat higher IEP (5.3) and considerable secondary structure (Boye *et al.*, 1997). The latter means that upon heating, the molecule will denature. It is important to note that β-lactoglobulin has one reactive sulphur group, which contributes to the stability of the secondary structure or (after denaturation) of a whey gel structure. The sulphur group can initiate a polymerisation that can lead to the formation of a covalent network (Roefs and de Kruif, 1994; Vasbinder *et al.*, 2003).

Table 9.2 Various causes of aggregation of casein micelles (partly taken from Walstra *et al.*, 1999)

Origin of instability	Micelles changed?	Aggregation reversible?	Aggregation also at low storage temperature?
Acidification	Calcium dissociates, κ-casein hairs collapse	Yes*	At lower pH at high temperature
Heating	Chemical changes interaction with whey	No	–
Calcium addition	Calcium bridging less electrostatic repulsion	Yes	Less than at high temperature
Hydrocolloid addition	No	Mostly yes	Yes
Age gelation	Fibre-like extensions	No	Less
Rennet	Hairs cut	No	No
Air inclusion	Spread at air surface	Mostly not*	No
Freeze–thaw	Probably	Mostly yes	–
Ethanol addition**	Lowering solvent quality, collapse of κ-casein hairy layer	No	?

* Depends on timescale.
** See also de Kruif (1999).

Whey proteins are relatively hydrophobic, like most milk proteins. They are soluble in water because their hydrophobicity is 'masked' by the secondary structure. If the secondary structure is destroyed, e.g. by heating, hydrophobic patches on the proteins are liberated. These patches will have a strong tendency to complex with calcium (Bryant and McClements, 2000), or adsorb to casein micelles, each other, oil/water interfaces or to the surface of the vessel ('fouling'; Jeurnink *et al.*, 1996) (see also Section 9.4.3). On top of this, thiol groups may attack disulphide bonds, which leads to irreversible aggregation. Note that hydrophobic interactions are strongest at 60 °C.

The behaviour of α-lactalbumin (the second most important whey protein in terms of concentration) is similar to that of β-lactoglobulin. The denaturation of α-lactalbumin is somewhat slower and has a tendency to be reversible (in the absence of disulphide bond forming proteins like β-lactoglobulin) (Chen *et al.*, 1998).

9.2.4 Lactose

Lactose (density 1780 kg m^{-3}) is the main carbohydrate in milk. It is a disaccharide of glucose and galactose. Lactose is important for the taste of milk, and is considered to be of minor importance for the physical properties. Lactose is digested by the action of lactase (= β-D-galactosidase).

Lactose-intolerant people lack this enzyme. The milk sugar is 0.3 times as sweet as sucrose. The sweet taste is somewhat masked by the caseins. Glucose and galactose, which are formed as a result of lactose hydrolysis, are much sweeter than lactose.

In dairy products more sugar is often added than is naturally present in milk, usually in the form of sucrose. This is usually done for taste reasons (sometimes for masking reasons), but can also have an effect on other product properties. There are some indications that the presence of sugars can alter the solvent quality for the proteins, and thereby indirectly affect processes such as denaturation and occurrence of protein/hydrocolloid incompatibility. For instance it has also been shown that sucrose increases the temperature at which protein denaturation occurs (Spiegel, 1999; Kulmyrzaev *et al*., 2000). In the case of milk-based beverages, there are indications that addition of sugar decreases acid protein sedimentation by acting as a co-solvent for the proteins.

9.2.5 Salts and minerals

The most abundant salts in milk are sodium and potassium chloride. These are the main salts responsible for the ionic strength of milk, which is 0.075 mol/l. Note that the solubility of many charged macromolecules, such as casein, depends on the ionic strength. A minimal amount of salt is needed to solubilise proteins (salting-in), but too much salt leads to screening of excess charge, leading to agglomeration (salting-out).

The main mineral in milk is calcium. About 8 wt% of the casein micelles is calcium phosphate (about 1.25% of skimmed milk powder is pure calcium). Intake of calcium is considered to be of importance for reduced risk of osteoporosis. Surprisingly, deficiencies remain, even in the Western world. Casein micelles are an ideal carrier of cations such as calcium (but also magnesium and all other insoluble salts), which is mainly present in the micelles in the form of phosphate crystals. These crystals are insoluble in water at neutral pH, but because they reside in hydrophobic pockets at the interior of the casein micelles, they are kept 'in solution'. Acidification leads to dissolution of the crystals. If milk is further enriched in calcium, considerable taste (soluble calcium causes bitterness and in-line fouling) and texture (insoluble calcium causes sandiness, sedimentation and wearing of homogenisers) issues can arise.

Some new insights on the interaction between proteins and calcium have been gained over the past years, especially as a function of (reversible or cycled) pH (Canabady-Rochelle *et al*., 2007; Raouche *et al.*, 2007, 2008).

9.2.6 Milk fat

Normal fresh cow's milk contains only about 3.5% milk fat, depending on the cow breed. Milk fat, also known as butterfat, is composed of triacylglycerols (TAGs) containing a wide range of fatty acid moieties. The most

abundant fatty acids have both odd and even chain lengths between C_4 (butyric acid) and C_{18} (stearic acid). Milk fat typically contains about 65% saturated fatty acids (SAFA), as well as 30% monounsaturated fatty acids (MUFA) and 4% polyunsaturated fatty acids (PUFA). The amount of *trans* fatty acids, unsaturated fatty acids containing a trans double bond, naturally occurring in milk fat varies with feed of the cow, but usually exceeds 3% on fat phase. This is much higher than in unhardened vegetable oils, in which they are essentially not present (van Duijn *et al.*, 2006).

Milk fat (density 918 kg m^{-3}) has a texture, taste and flavour which are commonly perceived as positive, but the presence of cholesterol and high amounts of saturated fatty acids has given it a negative health reputation. In nature, milk fat resides in milk in the form of globules (Walstra and Jennes, 1984). The interface of these globules has a special composition and consists of various components (25% phosphatides, 70% proteins – mainly whey proteins, and cholesterol). Note that because consumption milk is usually heated and/or homogenised or even reconstituted, this interfacial composition is somewhat altered and some of these components have left the interface.

At low temperature, agglutination can occur, caused by partial coalescence of the lipid droplets (Walstra *et al.*, 1999). This can lead to an increase in viscosity or creaming of the milk fat. By applying homogenisation, creaming can be delayed considerably, especially if additional healthy oils such as sterol esters or fish oil are added to the milk.

9.3 Milk-based beverages

9.3.1 Introduction

Milk-based beverages are liquid, processed milk products. They are mixtures of (skim) milk or (skim) milk powder with water, with for example colorants, flavours, acids, functional ingredients, fruit mixes/juices, sugar and preservatives. The milk-based beverages market is still a niche market compared with the sales of yoghurt and milk. However, there have been many innovations and currently it is one of the fastest growing dairy segments.

Buttermilk could be considered one of the oldest acid milk-based beverages. Buttermilk used to be the by-product of butter manufacture but nowadays it is made by processing skimmed milk. The milk is homogenised, pasteurised (this increases the viscosity of the end product), acidificied to pH ~4.5 with a buttermilk culture, stirred until smooth, and packed. Although buttermilk can be a zero-fat product, usually at least 0.4% fat is included to prevent excessive acid impression.

Yogurt does not fall under the category of drinks, because it is too firm and texturised. It can be processed (diluted with fruit juice, stirred), however, in order to achieve a consistency suitable for pouring and drinking. Many

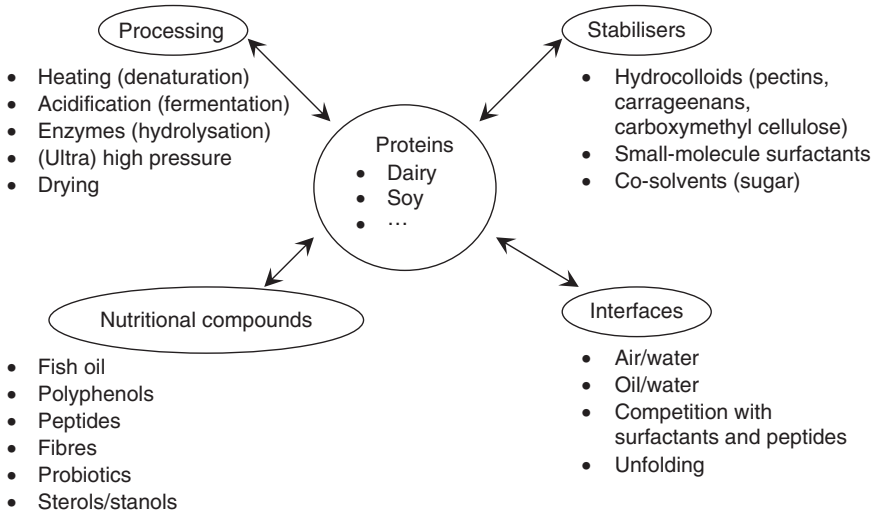

Fig. 9.2 Schematic overview of interactions of proteins typically found in modern milk-based beverages, with consequences on the microstructure and hence on texture and stability.

of the current milk-based beverages are mixes of yogurt and fruit juice. The viscosity of milk-based beverages is usually higher than of milk (which has twice the viscosity of water), and the pH is usually lower (3.8–4.4 vs. 6.7 for normal milk).

In the next sections we will discuss the effects of processing, the use of stabilising hydrocolloids and health trends of milk-based beverages. An overview of the main microstructural interactions that play a role in product quality of modern milk-based beverages is given in Fig. 9.2.

9.4 Effects of processing on milk-based beverages

9.4.1 Homogenisation

The most common processing step applied to improve textural stability of dairy drinks is homogenisation. The technique basically relies on reducing fat droplet or protein particle size by high shear, achieved by pushing the beverage through a narrow hole by high pressure. The technique is commonly used to emulsify fats, e.g. to achieve standard levels of fat. However, it can also be used to reduce protein particle size (Anema *et al*., 2005). This is particularly relevant for acid milk-based drinks. It is even more relevant if the protein is replaced by soy protein, or if chemical acidification is used instead of fermentation. In both situations the starting point is relatively large, compact, particles, which need to be made smaller to prevent such textural issues as powderiness or sedimentation.

Common issues with homogenisers are wearing (e.g. by inclusion of minerals) and blockage (e.g. by air inclusion). Also note that high homogenisation pressures or several homogenisation steps are cost- and time consuming.

9.4.2 Acidification

The pH of native cow's milk is between 6.6 and 6.8. Acidification is a highly important processing step in the production of many dairy products. Traditionally acidification is used to prolong shelf-life. Nowadays it is also used to reach a pleasant fresh taste and creamy texture. Although (e.g. chocolate or vanilla flavoured) neutral milk-based beverages are a big market, the main growth is in the acid milk-based beverages.

The target pH of acidification is usually around 3.6–4.6. Passing the IEP of the kappa-casein hairs (around a pH of 5) induces a collapse of the hairy layer (de Kruif and Zhulina, 1996; Horne, 1998). At the IEP the net charge of a macromolecule is zero, reducing the repulsion between the molecules and promoting mutual attraction. In the case of casein micelles, the charge neutralisation leads to a collapse of the hairy layer on the surface of the micelles. This strongly reduces the colloidal stability of the protein dispersion and leads to aggregation and can lead (if left undisturbed) to the formation of a gel. The attractive forces between the micelles are probably a combination of van der Waals, hydrophobic interactions and α_{S1}-casein bridging (Mellema *et al.*, 1999). Acidification can be achieved by bacterial and chemical acidification. The former is a slow form of acidification leading to the formation of large, tenuous casein aggregates which boost the viscosity.

Lactic acid bacteria (which are usually Gram-positive bacteria such as *Streptococcus* and *Lactobacillus*) can be used to produce a soured product. These bacteria convert lactose to lactic acid. At the same time they produce flavours that are typically associated with soured milk products (thermophilic bacteria used for yogurt mainly produce acetaldehydes and diacetyles, mesophilic bacteria produce a wider spectrum of flavours). Note that the choice of fermentation bacteria is crucial for yogurt drink properties, even beyond flavour (Folkenberg and Martens, 2003).

Typical yogurt fermentation takes 3 hours at 44 °C with *Streptococcus thermophilus* or *Lactobacillus bulgaricus.* Buttermilk fermentation conditions differ from those for yogurt because mesophilic bacteria are used: typical fermentation takes 18 h at 22 °C with *Streptococcus cremoris*/*diacetylactis* or *Leuconostoc cremoris*. Note that bacterial acidification also results in CO_2 formation. Dissolved CO_2 can lead to deformation of packaging (panelling) on chilling, if the product is hot-filled.

The inclusion of probiotics has had a major impact on the market of yogurt (drinks) over the past decade. Probiotics are specific lactic acid

bacteria that can survive the acid conditions in the stomach and reach the lower intestines, where they are assumed to improve resistance to bacterial infections. Only a few specific *Lactobacillus* and *Bifidobacterium* strains allow claims on immune health and natural defence, or gut health and diarrhoea. It must be said that these claims are often disputed.

The application of probiotics is limited to products that can guarantee the delivery of the right amount of live probiotic cells to the consumer, i.e. the interaction with other food components and the processing are also important.

Note that in order for sufficient amounts of bacteria to survive until they reach the gut, probiotic bacteria are added in large quantities. This imparts a slimy and astringent texture, which is commonly overcome by increasing sweetness.

To obtain gradual acidification without using live organisms, gluconadelta-lactone (GDL) can be used. GDL is often used for research purposes in model systems (Banon and Hardy, 1992). Acidification can also be achieved by pressurised CO_2 (Gevaudan *et al.*, 1996; Raouche *et al.*, 2007, 2008), though industrial applications are limited.

Chemical acidification is a quick form of acidification, leading to the formation of more compact aggregates which have to be torn up by a subsequent homogenisation. The most commonly used acids for chemical acidification are lactic, citric, malic and phosphoric acid. The choice of acid depends mostly on taste requirements. For instance, citric acid gives a more astringent and (citric) fruity impression and lactic acid a more yogurt and slimy impression. Note also that some acids give a more pronounced decrease in pH than others (at a given concentration). Also note that carbonates, phosphates and proteins have a strong buffering capacity.

Acidification is often followed by mixing with fruit juices (drinks) or pulp (smoothies) and/or intensive stirring of any form to retain the liquid character of the dairy drink. By doing so the tenuous structures are partly maintained, with positive effects on mouthfeel and stability. However, tenuous aggregates will slowly rearrange upon storage of the drink. Rearrangements are facilitated by the loss of the colloidal calcium phosphate from the micelles and the increase in hydrophobicity of the proteins upon acidification. The dissolution of the CaPh renders the aggregated casein material to be deformable at a molecular (or sub-micellar) scale. The increase in hydrophobicity increases the attraction between the proteins. Hence the aggregates rearrange into compact structures and eventually the (whey) liquid will be expelled (i.e. syneresis will occur) (Mellema *et al.,* 2002). This phenomenon is most widely studied in textured products such as yogurt, quark and dairy spreads (Bot *et al.*, 2007b), but can also occur in drinks and can be distinguished from acid-induced sedimentation by the early formation of a more transparant layer on top of the product, instead of a turbid layer at the bottom.

9.4.3 Heating

Introduction

The main purpose of heating drinks is to inactivate bacteria and enzymes. This is important for shelf-life stability of drinks. Pasteurisation (typically 15–20 s at 72–75 °C) kills vegetative microorganisms. Ultra-high-temperature (UHT) sterilisation (typically 4–15 s at 135–150 °C) kills all sporeforming microorganisms too. Novel processing methods to improve microbiological product stability such as *ultra*-high-pressure treatments (Cruz *et al*., 2007; Perada *et al*., 2007), are not common at present.

A very intensive heating is required to inactivate all enzymes, which is often not even attained upon sterilisation. Note that most enzymes reside at the interface of the milk fat globules, which is why skimmed milk contains fewer enzymes. Some enzymes (plasmins) are known to contribute to a defect commonly found in UHT milks: age gelation (Datta and Deeth, 2001).

There are many more effects of heating, of which the most important physical effects will be discussed in the following sections (whey protein denaturation, heat coagulation). The rate of breakdown of vitamins and oxidation is also enhanced upon heating, but this is not discussed here.

Denaturation of whey

Upon heating at temperatures above 70 °C, whey proteins denature and bind to each other and to casein micelles via hydrophobic (which are particularly strong at high temperatures) and disulphide interactions. This is the main reason why UHT milks with added whey-based ingredients (which are usually cheaper) show increased particle size and are hence less stable upon storage (Williams *et al*., 2000). Vasbinder and de Kruif (2003) reported that heating skimmed milk at 90 °C for 10 minutes leads to 40% coating of casein micelles by whey proteins. However, details of the interaction depend strongly on temperature and pH. At lower pH (at or below 6.5), the coating of micelles with denatured whey proteins is more pronounced (coating in the form of aggregates).

During heating, the formation of a whey protein 'coating' on micelles can prevent disaggregation of the micelles and release of casein molecules in the serum phase. During acidification, denatured whey proteins act as bridging agents between aggregating casein micelles. Because of this, the presence of denatured whey at the micelle surface has been shown to increase gel firmness (Lucey *et al*., 2000; Vasbinder and de Kruif, 2003), which is indicative of increased interactions. With respect to the kinetics of this aggregation process it is important to know that the rate at which the whey proteins unfold can be delayed by the presence of sugars (Spiegel, 1999; Kulmyrzaev *et al*., 2000), and that α-lactalbumin generally unfolds slower than β-lactoglobulin. Recently new insights into the interaction between whey proteins and casein micelles have been gained in the context

of acidification (Vasbinder and de Kruif, 2003) and renneting (Dinkov and Dushkova, 2007).

The above clearly illustrates the difference between heated and unheated milk, but also between low- and high-heat skimmed milk powder. Use of low-heat milk powder (or soy protein) with added calcium can lead to unwanted precipitation during the pasteurisation stage.

Denatured whey also adsorbs at the oil interface. Because of this, one of the positive effects of the whey denaturation is a decrease in creaming tendency. Adsorption to walls of the processing vessels or tubes can also occur, particularly at the heat exchanger. This is called fouling (Jeurnink *et al*., 1996), and poses the risk of contamination of the drink with pieces of aggregated whey proteins. If homogenisation is applied as a last step in the process homogenisation is applied, the aggregates can be torn apart again. However, fouling remains undesired because it can influence the hygiene of the process. In industry, such deposits are usually removed by swelling the deposit with a high-pH fluid that flows though pipes at relatively high flow rates (Mercade-Prieto and Chen, 2006)

Denatured whey proteins are good emulsion stabilisers because they are capable of forming an elastic film (Mellema and Isenbart, 2004). Denatured whey can also be used to improve texture, provided that aggregation is well controlled (Gustaw *et al*., 2006).

Heat coagulation

Even though casein micelles themselves are considered surprisingly stable upon heating, some physical changes may occur upon extreme changes in temperature. At fridge temperature, casein micelles swell because of lower hydrophobic interactions and dissolution of calcium phosphate while releasing some of the casein in the serum as well. At very high temperatures (>100 °C), particularly at or around neutral pH, part of the κ-casein goes into the serum. Because of this, the micelles may aggregate (this is called 'heat coagulation'). Fortunately the dissolution of κ-casein is partly reversible. Note that transglutaminase could decrease the dissolution of κ-casein, by cross-linking the proteins together (O'Sullivan *et al*., 2002).

Particularly at low pH, heating increases the chances of permanent instability. In fact, the temperature of heat coagulation of the casein micelles decreases with decreasing pH: it is approximately 150 °C at pH 6.7, and 50 °C at pH 5.4. The pH sensitivity of the heat stability is very critical: large differences in storage stability were reported for milk sterilised at pH 6.6 and pH 6.8. However, if pH is not too low and if the milk is homogenised after heating, coagulation of casein micelles should hardly affect product properties. One clear exception is condensed milk. If the condensed milk is also homogenised, the micelles will migrate to the interface of the fat globules, which will then behave as giant casein micelles and aggregate. Because of this, heat coagulation can increase viscosity. The issue is easily resolved by heating the milk before it is concentrated. This is because casein micelles

with coated denatured whey proteins are less sensitive to heat coagulation. Another way of solving the issue is by adding sequestering agents such as citrate, or compounds such as urea, formaldehyde, polyphenols or lecithin.

Other changes
Removal of oxygen can occur during heating if the oxygen is free to escape. This is a positive effect of heating with respect to oxidation processes (see Section 9.7.4). A gel-like behaviour does not seem to be a common attribute for drinks. However, sometimes a weak gel is formed upon long storage of sterilised milks, either resulting from insufficient inactivation of enzymes (Datta and Deeth, 2001) or carrageenan gelation (see next sections).

Besides physical changes, there are many chemical consequences of heating leading to changes in taste, e.g. cooked milk flavour formation. Two different types of cooked milk flavour are developed during heat treatment of milk: heated flavour and sterilised flavour. Heated flavour is a characteristic and volatile smell originating from sulphydryls and other sulphur compounds. Denaturation accelerates the formation of this flavour by liberating sulphur groups on proteins. This type of flavour decreases upon storage if sufficient oxygen is present. Sterilised flavour only develops above 90 °C and probably originates from Maillard reactions (reactions between proteins and sugars). Sterilised flavour is considered part of the typical flavour of a UHT milk (Burton, 1985), and is accompanied by a browning reaction. Many factors affect the formation of sterilised flavour of a UHT milk, for instance milk quality, severity and type of heating, packaging and storage (Andersson and Oste, 1992).

In general most heat-induced changes occur quicker at high pH (except hydrolysis, protein aggregation/coagulation, which is much stronger at low pH, and lactulose formation). Note that heating can also influence the pH: at high temperature the pH will decrease (especially for skimmed milk and buttermilk). Even though the actual pH decrease is reversible, it can contribute to irreversible processed-like heat-induced aggregation.

9.4.4 Enzymes as processing aid

The application of enzymes as a processing aid has recently gained attention. The use of rennet (chymosin) enzyme to produce cheese is well known, but not applied in beverages. More generally hydrolysis of proteins by enzymes can be used to improve texture and stability of digestibility.

Protein hydrolysis is well known to improve of protein stability upon acidification (especially for soy proteins). The behaviour of the peptide itself in stabilising air/water (Davis *et al*., 2005) or oil/water (Girardet *et al*., 2000) interfaces and the interaction between proteins and peptides (which show similarities with emulsifiers in this respect; Haque, 1993) are areas still open to further exploration. Note that extensive hydrolysis can lead to reduced emulsion stability and increased bitterness.

The use of hydrolysis for whey proteins is common in the development of whey-based sports drinks (Sinha *et al*., 2007). Such hydrolysates are often used to enhance protein availability to the body, to maintain muscles (e.g. quicker recovery/body building).

In addition to enzymes that cleave proteins, there are also those that generate cross-links. The use of the cross-linking enzyme transglutaminase in dairy products is of growing interest. Best known for its usage in the manufacture of textured fish products such as surumi, it is an enzyme which catalyses the formation of covalent cross-links between glutamine and lysine residues in proteins. It is suggested that transglutaminase can render protein structures more resistant, and is hence a useful tool for the production of dairy products (Ozrenk, 2006).

9.5 Effects of stabilising hydrocolloids

9.5.1 Introduction

Hydrocolloids are stabilising polysaccharides from vegetable sources (plants, fruits, seeds, seaweed), and sometimes originate from micro-organisms (exopolysaccharides). Hydrocolloids (or 'thickeners') are typically added to drinks to reduce sedimentation or creaming upon storage. Of course homogenisation and proper hydration of proteins also contribute to stabilisation, but this is often not sufficient for shelf-life of several months or longer. A concise overview of the implications of mixing hydrocolloids with protein-based systems has been given by Benichou *et al*. (2002).

In drinks, hydrocolloids have three basic possible beneficial mechanisms of action:

1. Viscosifying (rate of sedimentation of particles is reduced by high–medium viscosity).
2. Gelling (particles are trapped in a reversible gel with low yield stress).
3. Complexing (colloidal repulsion between particles is increased).

The viscosifying and gelling action of hydrocolloids can be probed by viscometry (see, e.g., Hemar *et al.*, 2000). The complexing action of hydrocolloids can be probed by measuring particle size upon ageing by light scattering techniques.

Viscosity is important for various product properties. Different product properties can be probed by different deformation rates. Each deformation rate relates to the viscosity relevant to a different product property. Typical deformation rates are

- pumping/stirring/mixing: 10–1000 s^{-1};
- chewing: 10–100 s^{-1};
- pouring: 1–10 s^{-1};
- sedimentation: 0.001–0.1 s^{-1}.

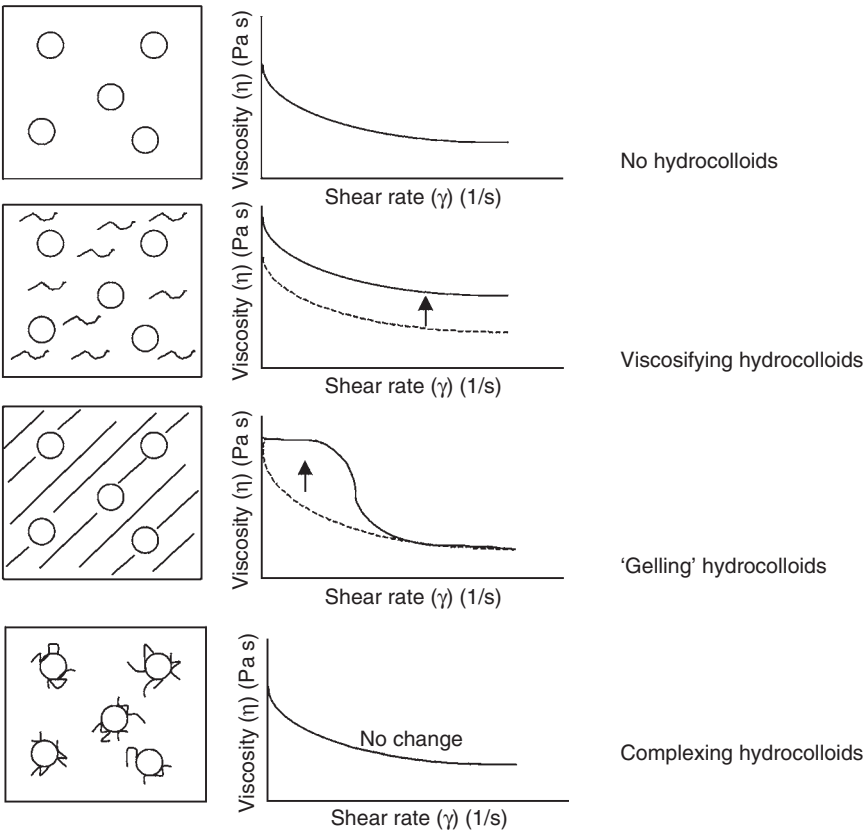

Fig. 9.3 Schematic representation of the microstructure of drinks with protein particles with various types of hydrocolloids, with accompanying viscosity profiles. The dashed lines indicate the profile for a drink without thickeners.

Examples of typical viscosity profiles for protein-based drinks are given in Fig. 9.3.

The above approach of measuring the viscosity profile allows a quick screening of the effectiveness of a range of thickeners. The combination of rheological data with centrifugation and particle size measurements, provides a very powerful tool for screening thickeners and understanding sedimentation (Bjerrum *et al.*, 2000).

The gelling and complexation route is preferred because ideally there should be no major changes in viscosity at high deformation rate. However, in practice all complexing and gelling hydrocolloids also show some viscosifying effect.

Viscosifying hydrocolloids

Most hydrocolloids simply act by increasing viscosity. This is particularly true for those that are uncharged, like starch (incl. maltodextrin), and

galactomannans such as locust bean gum (LBG) and guar gum. Because of the lack of charge these molecules are relatively difficult to dissolve, and usually require prolonged heating and stirring to be effective. Unfortunately this also increases the chances of breaking the large molecules, which results in a loss in viscosifying action.

Note that the lack of attractive electrostatic interactions increases the likelihood of phase separation (see Section 9.5.2). This is why the concentrations of guar and LBG recommended are usually relatively low when compared with charged polysaccharides.

Gelling hydrocolloids

Whether hydrocolloids in a dairy drink really have a gelling capability is a point of ongoing debate. Of course the existence of a weak, reversible gel would in theory be ideal for stopping sedimentation, and some hydrocolloids (xanthan, agar, low-methoxy (LM) pectin, alginate) are indeed capable of giving firm gels in model solutions. However, it is unlikely to expect the formation of a true gel at the thickener concentrations and storage timescales commonly relevant to drinks. In fact, often such gelling would lead to unwanted aggregation or lumpiness because the product has to be stirred after formation of the gel. At the same time, it should be kept in mind that the desired 'weak gelling behaviour' in milk-based beverages is very difficult to measure. The end of the plateau in the viscosity curve of Fig. 9.3 (gelling) could be seen as indicative of an 'apparent' yielding behaviour.

A well-known example of a 'gelling' hydrocolloid is xanthan. Traditionally the stabilisation of salad dressing by xanthan is explained by the yield stress of its solutions. However, Parker *et al*. (1995) proposed an alternative mechanism for the stabilisation given by xanthan, which assumes the occurrence of depletion flocculation inducing the formation of a weakly flocculated network of oil droplets. We speculate that the same might be true for complexing hydrocolloids: at sufficiently high concentrations they could act as weak and reversible bridges between particles (provided the particle concentration is high enough to establish a three-dimensional network throughout the volume of the system).

Complexing hydrocolloids

One type of hydrocolloids with a distinct complexing action at low pH, is high-methoxy (HM) pectin. HM pectin is less charged and hence less calcium sensitive than the low methoxy variant. The good functionality of HM pectins at low pH renders it widely applied in acid protein-based drinks (Pedersen and Jorgensen, 1991; Amice-Quemeneur *et al*., 1995; Lucey *et al*., 1999; Laurent and Boulenguer, 2003). Pectin stabilises acid drinks by contributing to the colloidal stability of the protein aggregates (Dickinson *et al*., 1998; Maroziene and de Kruif, 2000). The molecular mechanism probably involves the adsorption of pectin onto the surface of the protein particle. The interaction between the protein and the pectin is assumed to be electrostatic (Dickinson, 1998; Tuinier *et al*., 2002). To obtain full

effectiveness of HM pectin, it is commonly added in the process as a hydrated slurry (incl. part of the water and sugars).

There are not too many alternatives for HM pectin in acid protein drinks. Because of the chemical diversity of different types of carboxymethyl cellulose, some (short chain) variants could potentially mimic the behaviour of pectin (Du *et al*., 2007). Also propyleneglycol alginate (PGA) and soybean polysaccharides have been reported to have similar stabilising capacity as pectin, though the mechanism may be different (Nakamura *et al*., 2003, 2006). Unfortunately PGA is not always allowed in drinks and soy polysaccharides are currently (even) more expensive than pectins.

The hydrocolloid κ-carrageenan can act in a similar way as pectin, but in this case at neutral pH. Though many details are still unknown, there are indications that the κ-carrageenan makes a complex with the κ-casein in the hairy layer (Mora-Gutierrez *et al*., 1998). In such a way, colloidal stability is improved by the formation of a hairy layer.

9.5.2 Issues related to the use of hydrocolloids

Besides increasing costs, there are various risks connected to the use of thickeners, like unwanted changes in texture or stability. Usually these defects occur through use of too high concentrations, but sometimes they are due to unexpected interactions with other hydrocolloids, proteins or calcium (especially at high temperatures). The most common origins of defects are shown in Fig. 9.4. Phase separation and bridging flocculation are more elaborately explained next.

Phase separation

Phase separation generally results from an incompatibility between two species of molecules (Tolstoguzov, 2000). This means that increasing differences in size, charge, flexibility, etc., will lead to increasing tendency to phase separate. In drinks the phase separation due to incompatibility between proteins and thickeners is likely to be most relevant. Depletion flocculation as the underlying mechanism is also a possible explanation (Maroziene and de Kruif, 2000). However, because large differences in size between the phase separating species is needed to get depletion flocculation, it is a more likely mechanism for the destabilisation of protein-stabilised emulsions. In those cases where depletion flocculation occurs, it can possibly be suppressed by adding a compound that interacts with the smallest species, e.g. Blijdenstein *et al*. (2003) have shown that depletion flocculation of an oil/water emulsion induced by dextran can be suppressed by addition of β-lactoglobulin.

Phase separation between proteins and thickeners by any mechanism can be avoided by choosing a sufficiently low concentration of hydrocolloid, or by choosing a hydrocolloid which exhibits some interaction with the proteins. An alternative way to prevent phase separation could be to

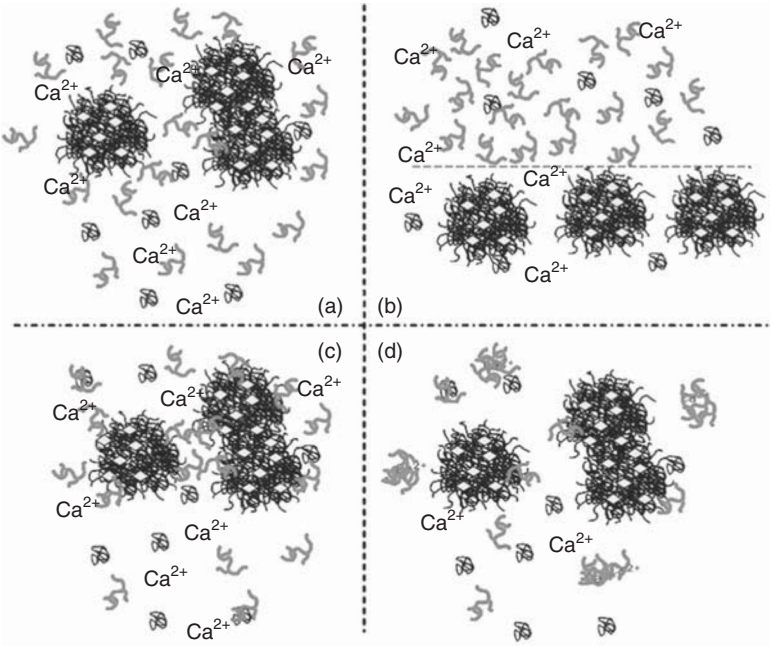

Fig. 9.4 Schematic representation of the occurrence of product defects resulting from the addition of hydrocolloids to skimmed dairy milk: (a) excess of hydrocolloids resulting in too high viscosity; (b) phase separation; (c) bridging flocculation; and (d) improper order of addition in the premix, in this case leading to complexation between ionic calcium and the hydrocolloids.

cross-link proteins to hydrocolloids, e.g. by making Maillard-type protein–polysaccharide conjugates. In that way, the repulsion between the two species will never lead to a separation into two distinct phases. The industrial application of Maillard complexes has been discussed by Kato (2002), even though the clearance for use in foods is still under discussion.

Bridging flocculation

Charged hydrocolloids added to form a stabilising complex with the protein should preferably have one block or segment associating with the protein, and a separate block dangling free in solution. In that way a stabilising hairy layer is obtained. However, hardly any commercial mix of hydrocolloids contains only such molecules. Usually the mix contains molecules that have an affinity with the protein along the whole chain (or at both ends). In that case a bridge can be formed between two protein particles. This will particularly occur at high concentrations of complexing hydrocolloids, and will lead to *more* sedimentation. A general overview of bridging flocculation is given by Dickinson and Eriksson (1991). The occurrence of bridging flocculation is difficult to assess. Bridging between casein micelles has been

reported to occur for λ- and ι-carrageenan (Langendorff *et al*., 2000). There are indications that carrageenans and pectins can show this behaviour in milk systems also. In the case of κ-carrageenan, process conditions may play a role in the development of (weak) gels (Sedlemeyer and Kulozik, 2006). If such products are stored over long times at relatively high temperatures (e.g. UHT milks), this could evolve to a weakly aggregated situation with elevated viscosity. This gel-like structure could become apparent by the consumer and hence become a quality issue (Tijssen *et al*., 2007). Note that some minor degree of bridging flocculation can also lead to improved stability by the formation of a weak gel.

Finally we shortly discuss synergism between hydrocolloids. Synergism has been shown between xanthan or carrageenan, with guar gum or LBG: model solutions of mixtures of these hydrocolloids exhibit higher firmness/viscosity than model solutions of each separate hydrocolloid (Schorsch *et al*., 1997, Cases *et al*., 2000).

9.6 Effects of small-molecule surfactants

In industry, most small-molecule surfactants used are emulsifiers, which are added to aid the emulsification of oils and fats. It is beyond the scope of this chapter to deal with the subject of small-molecule surfactants and oils in isolation. We will shortly touch upon the interaction with proteins.

Most research in this area has been devoted to such subjects as competitive adsorption (some recent examples are Mackie *et al*., 2000, and van der Meeren, 2005). Considering these small-molecule surfactants and proteins are often found in combination in foods the amount of papers on their *direct* interactions is relatively limited. There have been some recent papers dealing with this subject in more detail, e.g. in relation to its consequences on adsorption behaviour of the proteins (Dickinson *et al*., 2003), or the behaviour of the proteins upon heating (Giroux and Britten, 2003). Antipova *et al*. (2001) have shown that surfactants can influence inter- and intramolecular repulsion forces with considerable effects in stability.

9.7 Health trends

9.7.1 Introduction

In this section the effect of changed product formulations with respect to consumer health is addressed. In terms of optimising the product formulation there are five major directions.

9.7.2 Sugar reduction

In milk-based beverages, reduction of added sugar is usually achieved by increasing milk sugar or fruit sugars. Total sugar reduction is usually achieved

Table 9.3 Relative sweetness of sweeteners

Nutritive sweeteners		Non-nutritive sweeteners	
Sugars		Artificial high-intensity sweeteners	
Fructose	1.3	Acesulfame-K	200
Glucose	0.7	Alitame	2000
Galactose	0.5	Aspartame	200
Lactose	0.4	Cyclamate	30
Maltose	0.4	Neotame	8000
Trehalose	0.45	Saccharine	250–300
Tagatose	0.9	Sucralose	600
Isomaltulose	0.5	Twinsweet	350
Sugar alcohols		Natural high-intensity sweeteners	
Erythritol	0.7	Brazzein	500
Lactitol	0.4	Glycyrrhizin	170
Mannitol	0.7	Neohesperidine	1800
Sorbitol	0.6	Stevioside	300
Xylitol	1	Thaumatin	2000–3000

by replacing part of the added sugar by high-intensity artificial sweeteners. If this is done, the relative sweetness of all sweet compounds has to be taken into account. The fact that not all sweeteners are allowed in drinks, for instance because they cause flatulence at high intake, must also be taken into account. An overview of sweeteners is given in Table 9.3. Note that some sweeteners are less stable upon heating (e.g. aspartame). The most commonly used artificial sweeteners are mixtures of aspartame and Acesulfame K. Recently sucralose has gained popularity, partly because it does not have a negative image with consumers, (unlike aspartame, which consumers dislike) and the name resembles sucrose.

Lactase is the enzyme that breaks down lactose into glucose and galactose. It can be (post-)added to milk-based beverages/products to obtain a product suitable for people who are lactose intolerant.

9.7.3 Fat reduction

Most milk-based beverages are based on milk, which is relatively low in fat (3.5–5%). However, since daily dairy consumption is typically high, a reduced fat level can still have a significant effect on fat intake. This brought forward what is probably one of the most successful low-fat products: semi-skimmed milk. In many countries, sales of semi-skimmed milk is similar to or even exceeds that of full-fat milk. Although the taste profile of full fat and semi-skimmed milk do differ, the mouthfeel of the product is still quite acceptable. This is not completely true for skimmed milk. Skimmed or (a more recent trend) zero fat milk has a more watery consistency and

transparent appearance than regular milk. The industry has tried to compensate for this by means of the addition of thickeners such as modified starches, despite the fact that the resulting product usually can no longer be designated (skimmed) milk.

Recently attention has been given to the fundamental understanding of the sensory attribute 'creaminess' (Frost and Janhoj, 2007; Silletti, 2008). Silletti suggested the general role that reversibly flocculated emulsions are mostly perceived as creamy, while irreversibly flocculated emulsions are mostly perceived as dry and rough. There is probably a large role of the particle size under shear; smaller protein particles probably relate to higher creaminess.

9.7.4 Modifying the fat composition

The general consensus concerning the nutritional quality of saturated fatty acids and *trans* fatty acids suggests that it is desirable to reduce the amount of these compounds in the diet. The most obvious route to achieve this is to produce low-fat products, but an alternative is to replace the milk fat by more healthy options. Replacing dairy fat by TAGs rich in unsaturated fatty acids is not trivial; this makes the product more sensitive to fat oxidation, resulting in rancid off-taste. Two obvious fat sources to achieve this goal are liquid vegetable oils and marine oils.

The first products on the market to apply this principle were so-called filled milks which consisted of skimmed milk to which a liquid vegetable oil like canola oil was added. Since these oils are rich in MUFA and PUFA, the main concern is their stability against oil oxidation as PUFA are most sensitive to oxidation, MUFA less, and SAFA least. Thus, unfortunately, healthy fat blends tend to be most sensitive to the formation of off-taste (Flöter and Bot, 2006). Such problems are solved by using high quality oils (low level of polar compounds, as measured by the per oxide value, POV), minimal headspace in the pack, low transparency of the packaging to light, and sometimes the addition of specific anti-oxidants, emulsifiers or sequestrants. The textural and mouthfeel effects of using liquid oil instead of milk fat are relatively minor (cf. Bot *et al*., 2007a).

Recently, interest has shifted to drinks containing fats with a more articulated benefit, more specifically those containing long-chain polyunsaturated fatty acids (LC-PUFA) such as α-linolenic acid (C18:3 *n*-3), eicosapentaenoic acid (C20:5 *n*-3, EPA) and docosahexaenoic acid (C22:6 *n*-3, DHA). Although many benefits of these LC-PUFAs are claimed, those involving a reduced risk for cardiovascular disease seem best established.

Modification of the fatty acid profile of the TAGs in milk fat has been attempted by modifying the feed of the cows (Singh *et al*., 2004; Givens and Shingfield, 2006). Although this interesting approach does result in increased LC-PUFA levels, this is usually accompanied by undesirable increases in *trans* fatty acids as well as a consequence of complex interactions in the

ruminant digestion and metabolism. Since there is no evidence that ruminant *trans* fatty acids have different health implications than *trans* fatty acids from other sources (Weggemans *et al*., 2004), this implies that this route is not as attractive as adding vegetable or marine sources of LC-PUFA fats.

Most traditional vegetable oil sources such as canola oil provide insufficiently high concentrations of LC-PUFAs to allow any health claims in the present low-fat drinks. Therefore, non-traditional fat sources such as linseed oil or marine oils were introduced in milk-based beverages. It is needless to say that these oils are even more sensitive to oxidation than the traditional PUFA-rich oils, and that the precautions against oxidation need to be pursued even more vigorously in LC-PUFA enriched products.

Specific tailoring of the fatty acid profile in infant foods to mimic human breast milk is gaining more and more popularity as the technologies to do so progress. The effect of proteins on oxidative problems with oils is probably relatively limited (storage conditions and anti-oxidants play a larger role here). Iron catalyses lipid oxidation and has a large impact on development of rancidity. Note that iron is also known to accelerate the oxidative breakdown of vitamins (especially vitamin C).

9.7.5 Modifying the protein composition

The basic dairy proteins may be modified for functional or nutritional reasons. For example, non-micellar casein (especially caseinate) is often used to improve emulsification functionality. We have already discussed protein hydrolysis as a way of altering protein composition.

Other protein sources besides those of dairy origin have been used as well. Besides rice and oat milks which are still a small niche market, a wide range of drinks based on soy protein can be found in the market. Based on the availability we can expect more vegetable protein drinks to be developed in the near future (e.g. from potato, lupin and canola).

All vegetable protein-based drinks typically suffer from a green or cardboard off-taste resulting from the enzymatic reaction of the plant to processing. This is the main characteristic of these drinks, blocking their reaching high sales, in spite of potential health benefits. Soy drinks are one of the oldest vegetable alternatives to milk-based beverages and at the moment one of the most quickly growing areas of protein-based drinks.

9.7.6 Fortification

Instead of changing the source/composition of the fat or protein in a dairy drink, one can also introduce a new ingredient on top of the existing composition (fortification). In recent years, many such fortified products were launched. This area is considered the most important oppontunity for future growth of the market of milk-based beverages. In previous sections we have

mentioned and/or discussed the addition of fish oil and probiotics. Next we will touch upon addition of vitamins, minerals, polyphenols, fibres and sterols.

The oldest form of fortification is probably the addition of vitamins. The addition of water-soluble vitamins usually has little impact, although it should be realised that some vitamins are oxidation-sensitive. Mostly, this results in a lowering of the amount, and sometimes this can lead to taste or colour changes in the drink (e.g. riboflavin promotes oxidation during storage in the presence of light). Vitamin fortification is usually done in fruit/milk mixes and is not allowed in all countries if there is no deficiency of the vitamin.

The addition of minerals such as calcium or iron is also a relatively common. An important choice to be made concerns the source of mineral source. In many cases both soluble and insoluble mineral salts are available. The soluble forms are usually associated with good bioavailability but sometimes undesirable taste impact (and sometimes promoting protein aggregation). The insoluble forms are usually associated with good taste but sometimes sedimentation. Furthermore, specific cations, such as iron, may promote fat oxidation (Mehansho, 2006).

Polyphenols from, for example, tea, chocolate, grape or wine have gained considerable attention over the past decade. Although their nutritional effects are subject of ongoing debate, there seem to be some indications in the area of heart health. It is well known that polyphenols can make (ionic) complexes with proteins, which can influence product stability. In clear drinks this can give rise to a haze (Siebert, 1999). In milky drinks it can give rise to increased protein sedimentation. Suggested changes in bioavailability resulting from the complexation are under investigation.

For fibres it is important to quantify the textural consequences, either because they are added for nutritional purposes (prebiotics) and textural changes need to be prevented, or because textural benefits are desired (citrus fibres).

Prebiotics are non-digestible (usually carbohydrate) food ingredients that selectively stimulate the growth and/or activity of beneficial micro-organisms in the colon. Examples are inulin, raw oats, or unrefined wheat or barley. The addition of such fibres, often in conjunction with fruit pulp in so-called smoothies, imposes thicker mouthfeel and sedimentation.

In recent years, more often products with ‘hard’ claims found their way to the market. Recent successful examples are drinks fortified with stanol- or sterolesters for reducing blood cholesterol levels. Phytosterols in this form tend to be nearly tasteless and impact little stability problems besides some minor lipid oxidation and creaming.

More generally, however, functional ingredients often have pronounced taste (e.g. bitter) or give rise to instability, and extensive precautions should be taken to ensure product quality. A generic example of such a precaution is encapsulation. However, encapsulation of ingredients in aqueous envi-

ronment is (much) less effective than encapsulation of ingredients for dry use (Mellema *et al*., 2006).

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10

Whey-based functional beverages

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Abstract: This chapter focuses on three types of beverages based on sweet or acid whey – the fruit juice type, the unfermented or fermented dairy type, and the carbonated thirst-quenching type. Specialised drinks and formulas based on whey protein are discussed only briefly. Main technological steps used in the manufacturing of these traditional whey beverages are highlighted, including membrane processes, fermentation, lactose hydrolysis and lactose removal. Technological and physiological functionalities are considered as related mainly to whey protein and lactose and their effect on the processing behaviour and sensory and nutritional qualities. Formulation examples of existing products are given.

Key words: whey beverage components, whey beverage formulation, fruit juice–whey mixtures, dairy-type whey beverages, thirst-quenching whey beverages, whey protein.

10.1 Introduction: definition of whey beverages

The subject of whey-based beverages has been of ongoing interest for a long time, and numerous technical sources can be found in the literature, including research and technical reports as well as substantial reviews of the subject (e.g. Holsinger *et al*., 1974; Kravchenko, 1988; Jelen, 1992; etc.). As described in these works, the term 'whey beverage' tends to focus mainly on a traditional drinkable product based on liquid whey as the main, or at least a significant, component. Specialised products that also may be based on liquid or dried whey and/or main whey components (lactose, whey protein) are usually not included in the 'whey beverage' group, although advertisements and web page promotions of 'whey drinks', 'whey shakes', 'miracle protein formulas' based on whey abound. Such products may include geriatric nutritional products containing easily digestible whey proteins or their hydrolysates; highly formulated functional liquid foods targeted to high-performance sportsmen and bodybuilders; some food product

resembling drinkable yogurts marketed in small containers as sources of healthful bacteria (e.g. similar to the Japanese product Yakult, the Finnish Benecol, Evolus or Gefilus, Activia from Danone, etc.), even infant formulas based on whey protein and lactose. These products, although often containing significant amounts of whey-based components, are going to be mentioned here only briefly. For purposes of this writing, whey beverages will be discussed as 'drinkable non-alcoholic products based primarily on whey and/or containing a significant amount of liquid whey ingredients'.

Such 'traditional whey beverages' are often viewed as a convenient vehicle for disposal of the frequently bothersome surplus whey resulting from small cheese-making operations. Although industrial examples of the use of whey for drinking purposes keep appearing on (as well as disappearing from) the food shelves, the ongoing publication activity in this regard seems to be driven primarily by academic curiosity. With few exceptions, there is little evidence in the marketplace that the whey-based beverages have made any lasting and significant inroads into the very competitive beverage market. Even in the context of dairy-based beverages, the whey drinks – if found on a dairy shelf at all – constitute a very small segment of the worldwide market in comparison with milk (white or flavoured) and fermented fluid milk products such as buttermilk or drinkable yogurt. This is somewhat surprising, as the dairy whey appears to be an ideal raw material for production of nutritious dairy beverages. Although whey is now being considered a very valuable source of many highly prized nutraceutical products and is being used by technically advanced companies specialising in whey processing for manufacture of these high-value ingredients (e.g. lactoferrin, lactoperoxidase, immunoglobulins, growth factors) , the use of minimally processed whey for production of beverages could still be advantageous for smaller cheese processors to alleviate their ever-present problem of the cheese whey disposal.

Whey, the residue left over after manufacture of cheese or industrial casein, contains about 93% water and thus appears to be a logical raw material for development of nutritious or thirst-quenching beverages. After removal of the caseins and milk fat from milk in the cheese- or casein-manufacturing processes, the remaining water-soluble milk components (whey proteins, lactose, minerals, water-soluble vitamins, etc., constituting about 50% of the original milk components) form the approximately 7% dry matter of whey. When the whey proteins are removed by ultrafiltration (UF) of either the whey or the original milk, the 'protein-free whey' (referred to usually as UF permeate) can be considered an even better potential raw material for the production of beverages. Table 10.1 presents general compositional information on the two principal types of whey and a UF permeate.

Although both whey and UF permeates have presented a major disposal problem for the cheese and casein manufacturers, the seemingly logical

Table 10.1 Compositional characteristics of sweet and acid whey and UF permeates*; average data compiled from various sources

Component	Sweet whey (g/l)	Acid whey (g/l)	UF permeates (g/l)
Total solids	63.0–70.0	60.0–68.0	52.0–58.0
Lactose	46.0–52.0	43.0–50.0	45.0–51.0
Protein	6.0–10.0	6.0–8.0	~1.2
Ash	5.0–5.7	4.8–7.0	~6.0
Calcium	0.4–0.6	1.2–1.6	**
Phosphate	1.0–3.0	2.0–4.5	**
Lactate	2.0	6.4	**
Chloride	1.1	1.1	**

* Average values for both milk and whey UF permeates.
** Similar to values for whey.

utilisation of these materials in beverage production has been rather sporadic. The only significant example of a whey beverage that has been consistently successful commercially is the Swiss product Rivella – manufactured not by a dairy company but a specialised beverage manufacturing firm. The product itself is not a typical whey beverage, as the highly processed whey (with the protein and riboflavin removed) constitutes only one-third of the product, the remainder being added water. However, the product is very successful on the Swiss national beverage market scene. The history of the firm and the marketing success of the product (Barth, 2001) illustrate some of the difficulties in conversion of whey or UF permeates into commercially viable beverages.

10.2 History and current market status of whey beverages

Documented use of milk whey as a nutritious or curative beverage dates to ancient history times, with the Greek physician Hippocrates being known to prescribe whey for various therapeutic uses (Holsinger *et al.*, 1974). In this and several other significant reviews of the subject of whey beverages (e.g. Kravchenko, 1988; Sienkiewicz and Riedel, 1990) the historical as well as many more recent experimental developments have been reviewed in detail and these will not be repeated here. Despite the ongoing research and product development efforts, the whey beverages remain a relatively marginal segment of the worldwide beverage market, although in some local conditions these products may be important. In a brief and very incomplete market survey conducted in the mid-1980s, eight different product brands were sampled in Germany, three in Switzerland, four in the Netherlands, and seven more in other European countries (Jelen, 1992); in

FIG. 10.1 Example of a new multiple-flavour whey drink product launch, with advertising stubs attached to the simple plastic bottles as found on a Swiss market.

a later search (Jelen, personal observations) several such products were discovered in Australia. A much more complete survey published by Riedel (1994) identified some of these products as enjoying limited international success, while many others from the earlier listing were not included and thus presumably were discontinued. In this latter compilation, eight producers of whey beverages were identified in Germany, four in the Netherlands, two in Switzerland, and one each in Finland, Austria and Norway. Germany appears to be one of the most important markets for these often obscure products, most of which are mixtures of whey and fruit juice preparations. Detailed compositional data and description of such products and their properties can be found from time to time in trade journals. Table 10.2 presents some examples in a summary form; the original reference (Piendl *et al*., 1995) contains detailed descriptions of 25 products mainly from Germany, some differing only in the fruit flavours used.

As a rule, whey beverages containing unprocessed or minimally processed whey are not carbonated and are most often marketed in the liquid, ready-to-drink form. The typical package used is the juice box, most often 250 ml or 0.5 l volumes, occasionally also yogurt-type cups, glass or various types of plastic bottles (Fig. 10.1), rarely pull-tab aluminium cans (in addition to some of the specialised high-protein formulas, the only whey drink known to be sold in such a package is the Rivella). Some products may exist as concentrates (one such product included in the compilation summarised in Table 10.2 calls for 1:5 dilution before home use), while unflavoured powdered whey products marketed as a powdered beverage for reconstitution by the consumer have recently appeared in the Czech Republic (Jelen, personal observation).

Table 10.2 Examples of typical product descriptions as given on labels, properties and compositional data for ready to drink whey beverages (*) and a whey beverage concentrate (+). Generalised from Piendl *et al.* (1995)

A: Product description:

* Mixed beverage from whey and fruit preparation . . . heat treated. Refreshing and thirst quenching, with exotic flavour of tropical fruits.

* Acid whey with multi-fruit nectar, prepared with special cultures producing lactic acid with over 90% of the total as L(+). Whey contains valuable minerals.

* Dietetic whey-based product with orange-passion fruit preparation, heat treated. With artificial sweeteners, especially suitable for inclusion as a component of nutritional programs concerning diabetes mellitus.

\+ Alcohol-free table beverage; concentrate for preparation of a drink with 30% milk serum. Contains nutritionally valuable minerals from milk as well as L+ lactic acid. The aroma comes from extracts of herbs and fruits. To prepare a drink, mix one part of the concentrate with five parts of cold water or lightly carbonated mineral water.

B: Ingredients:

* Acid whey, fruit juices (oranges, apple, passion fruit, pineapple, lemon and grapefruit), fruit pulp (apricots, peaches, mango and banana), water, invert sugar syrup. Fruit content minimum 25%.

* Sweet whey, apple juice, sugar, pectin, acidulant, aroma, vanillin. Fruit content 20% minimum.

* Whey, tropical fruit juice concentrate mix (orange, pineapple, mandarin, passion fruit, apple), with artificial sweeteners aspartame and acesulfame. Fruit content 20%, contains no added sugar.

\+ Whey concentrate, fruit sugar, pear concentrate, extracts from herbs and fruits, L+ lactic acid, caramel syrup.

C: Measured physico-chemical data

	Ready-to-drink product (*)	Concentrate (+)
Density (20°/20°C)	1.053	1.193
Water (g/1000 g)	868.3	571.1
Energy (kJ)	2192	7214
pH	3.3	3.17
Colour	'reddish'	EBC code # 25
Osmolality (mmol/kg)	729	not measured

D. Typical compositional data for ready-to drink products (sample data of a manufacturer):

Component	Amount (g)
Protein (g/l)	5.0
Carbohydrates (g/l)	42.0
Total lipids (g/l)	0.2
Minerals	1360
calcium (mg/l)	
potassium (mg/l)	1680
Vitamins (mg/l):	
Vitamin C	466.0
Vitamin E	37.0
Pantothenic acid	20.0
Organic acids (mg/l)	

10.3 Whey beverage types and their ingredients

In a scheme published in an earlier treatise on the subject (Jelen, 1992), four basic types of whey beverages were identified:

1. mixtures of whey (processed or unprocessed, including UF permeates) with fruit or (rarely) vegetable juices;
2. dairy-type, 'thick' beverages (fermented or unfermented);
3. thirst-quenching carbonated beverages (the 'Rivella-type'); and
4. alcoholic beverages (beer, wine or liqueurs).

With the advances of nutraceutical products and products for special nutritional needs (infant and geriatric nutrition, meal replacement liquid products, high-energy drinks for those active in sports, bodybuilders, etc.), several new categories could be added to this basic scheme. Some of these may contain peptides obtained by whey protein hydrolysis, as well as added micronutrients of non-dairy origin and other ingredients. To follow the focus adopted for this review, only the first three categories of the above scheme will be considered in some detail.

10.3.1 Fruit juice-type whey beverages

Mixtures of fruit juices and unprocessed or deproteinated whey or UF permeates are the most common types of whey drinks found on local markets today. These products usually fulfil a role similar to typical fruit juices, including breakfast-type beverage, healthful fruit juice snack-type drink, or drinks with a healthful image as a source of vitamins. The main two basic ingredients are typically liquid whey and liquid fruit juice or, more likely, fruit juice concentrate. The flavours used in these beverages most often include citrus fruits (mainly orange, followed by lemon, rarely grapefruit), as well as mango, passion fruit, pear, apple, strawberry, raspberry or fruit juice combinations with exotic descriptive terms ('tropic mix', 'multifruit', 'fruit nectar', etc.). Acid whey from the manufacture of quark or cottage cheese is typically used for these highly acidic drinks, for obvious reasons of flavour compatibility. These products are often fortified with vitamins, sometimes also minerals, especially in the case of isotonic sport drinks. The extensive review of Riedel (1994) identified no less than 29 such whey-fruit juice-type drinks (some differing only in the particular fruit flavour used) existing on the German market in 1991–1993.

Products containing vegetable juices (e.g. carrot) also can be found sporadically on some local markets. Based on our extensive research (Devkota, 1991; Salinas, 2007) significant opportunity seems to exist in developing a product based on mixing tomato juice with whey. The regular tomato juice seems to be ideally compatible with the acid whey and the resulting product could be marketed as a source of several nutraceutically important components including calcium, lycopene, whey protein and, if desired, probiotic bacteria.

Production details for manufacture of industrial products are usually not available. A typical production sequence includes blending of the two main liquid ingredients, followed by proper heat treatment and packaging. In most cases, especially when these products are conceived as a vehicle for utilisation of the surplus whey, the tendency is to use the equipment available in the factory, minimising additional capital outlay for specialised machinery as well as for proper marketing. However, this approach often leads to unexpected quality defects (including poorly designed package presentation, as shown in Fig. 10.1), thus tarnishing the reputation of the whey drinks as a beverage category. One such defect tends to be the development of a sediment due to the insolubilisation of whey proteins upon the heat treatment. Contrary to some earlier publications suggesting that the heat-denatured whey proteins could provide a sensation typical of a fruit pulp, the character of the sediment is different and thus of little benefit to the enhancement of the product quality. One possible measure to minimise the sedimentation of the heated whey protein is to adjust the final pH of the product before the heating to levels below pH 3.8–3.6, as below this critical pH range the whey proteins become resistant to heat-induced coagulation (Patocka *et al*., 1986). The pH of some of the products found on the market can be as low as pH 3.1 (Jelen *et al*., 1987b; Piendl *et al*., 1995), presumably to counteract this sedimentation problem. However, another sedimentation problem can be encountered in this very low pH range due to interactions of whey proteins and pectins from the juice (Devkota, 1991). As confirmed experimentally, the treatment of the juice with an enzyme pectin esterase could be used to minimise this problem especially in cases where the juice is known to contain significant quantities of pectin (e.g. apple or tomato).

10.3.2 Dairy-type beverages

In contrast to whey beverages resembling fruit juices, the use of whey or whey components in a drinkable yogurt or a similar dairy-type beverage is less straightforward. Even the concepts of a dairy product containing some whey vs. a true whey beverage can be blurred and these products can be discussed under either heading.

There are two basic types of dairy beverages: (i) unfermented milk and milk derivatives represented by market milk, milk shakes, flavoured milk and similar products (based on skim, partially skimmed, full-fat or even fat-enriched products); and (ii) fermented products such as sour milk, buttermilk, kefir and other similar cultured dairy beverages. Both product types can be made using whey. The main difference in the quality characteristics of these two product types is the pH; in the former case the pH is close to the neutral range (pH 6.2–6.5) typical of cow's milk or sweet whey, while most fermented dairy products and acid whey are quite acidic, with the pH in the vicinity of the 4.8–4.5 range, in some cases even considerably less. In

investigations with an experimental kefir-based beverage, some of the samples were fermented to a pH as low as 3.0 (Athanasiadis *et al*., 2004). Correspondingly, the main sensory impact of the cultured products is their high acidity due to the high content of lactic acid, produced by conversion of lactose found in the milk of any of the main milking species to the lactic acid by the lactic acid bacteria (LAB) cultures. This causes a major functional complication in comparison with the non-fermented dairy beverage, as the main milk protein casein, becomes insoluble and forms a coagulum at about pH 4.8 or less. Thus, cultured dairy beverages based on milk typically contain non-dairy ingredients (pectin, carrageenan, various gums and similar hydrocolloids) to ensure that the coagulated casein is appropriately stabilised and does not form a sediment. In this regard, dairy-type whey beverages based on fermentation of liquid whey protein concentrates (WPC) could offer a major opportunity for production of non-sedimenting, 'thin' fermented dairy products (Jelen *et al*., 1987a). The problem of heat-induced insolubilisation of whey protein upon thermal processing – the likely traditional final step in any industrial process ensuring the microbiological safety of such products – would probably need to be solved, possibly by using alternative non-thermal processing methods.

The problem of insolubilised protein sedimentation is much less important in the case of unfermented, market-milk-type dairy beverages. Even in the case of products based solely on liquid WPCs, the heating could be much less damaging due to the tendency of the whey proteins to form 'non-sedimenting aggregates' (Britten and Giroux, 2001) when heated at neutral pH. Clearly, for drinkable dairy-based beverages containing liquid WPCs, the sweet whey would be the preferred raw material. Use of dry WPC and WPI products for protein enrichment of milk and milk-based beverages appears feasible, although documented information is scarce (Peter *et al.*, 1996). Similarly, although using whey protein materials in yogurts is widespread, much less information is available on yogurt drinks produced by incorporation of whey into the drinkable yogurt formula and/or by fermentation of liquid UF whey protein retentates. Such developments appear to be feasible (Burton-Trapp, 1991; Johnson *et al*., 1996) but literature on commercial whey-based drinkable yogurt products is lacking.

10.3.3 Thirst-quenching carbonated beverages

The most typical product representing this type of whey beverage is the Swiss Rivella. To illustrate its current popularity, it is worthwhile to cite from the presentation of the creator of the legend, the Swiss industrialist Robert Barth at the 3rd International Whey Conference in Munich (Barth 2001):

> Rivella is the strongest original Swiss soft drink brand and definitely No. 2 in the Swiss soft drink market with a market share of 14.2% in quantity and 16% in value. Per capita, Swiss drink more than 11 litres of Rivella per

> year. . . . Since the market introduction of Rivella 50 years ago, over 400 000 tons of whey have been purchased from the cheese industry in Switzerland . . . (it is) the leading national brand in Switzerland, ranking No. 6 on the list of Swiss power brands in the year 2000. . . .

The product is an archetype of a thirst-quenching, carbonated drink, resembling the most typical 'pop-type beverages' like Coca-Cola, Fanta or Sprite with the main ingredient being water. Since the liquid whey component is highly clarified, containing no whey protein, the carbonation of the product is not complicated by the strong foaming characteristic of the whey protein (Jelen, 1973). This is a major disadvantage of the traditional whey-based beverages and an advantage for a product whose main role is thirst-quenching rather than enhanced nutrition. However, one of the latest variants of the Rivella, the 'Rivella-green' has been developed using herbal extracts from green tea as a move towards enhanced nutritional benefits; paraphrasing Barth (2001) '. . . to alleviate the stress situations of the modern world . . .' – clearly a move towards the functional beverage concept.

Several attempts to mimic the success of Rivella have been recorded in the literature. At least one competing Swiss product named Surelli is currently available on the Swiss market (Jelen, personal observation, 2008). A similar product was developed by the Swiss food retailing giant Migros, indicating the importance of Rivella in the local food market conditions. The original Rivella enjoys a limited international success, with documented earlier marketing efforts in the Netherlands and Japan, while a similar attempt in Canada was completely unsuccessful. Current company web page indicates availability in six European countries (France, Austria, the Netherlands, Luxembourg, Germany and France). Information about other such similar whey-based products is not easy to find.

Carbonation is one of the critical aspects for success of the thirst-quenching whey beverages. This seemingly simple technological step is, in reality, quite complex and extremely difficult to accomplish with regular dairy whey containing the highly foamable whey proteins. From the viewpoint of nutritional/functional beverages, the carbonation is an epitomy of the 'empty calorie' drinks and thus unlikely to be compatible with the idea of healthful beverages based on whey.

10.3.4 Other beverages based on whey and whey components

Technical literature contains many marketing-type articles on whey protein-based nutritional drinks, as well as on dietary supplements and specialised high protein-containing formulas. Whey protein is one of the more easily digestible proteins and thus may be ideally suited as the basis for a high-protein 'liquid meal drink' intended for persons under stress or in high-anxiety situations, such as those faced by the top competitive sportspeople during the high anxiety pre-competition period.

Products resembling flavoured drinkable yogurts but formulated from reconstituted WPC or WPI keep appearing with increasing regularity at food product exhibitions and in the literature, often promoted as sources of high-quality protein or as nutritional specialities for targeted consumer groups (lactating mothers, adolescents, senior citizens, etc.).

Conversely, UF permeates from milk or whey processing are essentially solutions of a relatively easily digestible carbohydrate (lactose, especially if it has been pre-hydrolysed) and some important electrolytes in almost isotonic concentrations. Thus these permeates, essentially devoid of protein, should be ideal for development of sport drinks offering high energy and proper mineral replenishment. Indeed, several such whey beverages referred to as 'isotonic fruit drink' or 'multi-mineral sport drink' were described in the review by Riedel (1994).

Products resembling sparkling wine, based on fermentation of whey lactose and/or other carbohydrates added to whey or UF permeates have been described in the literature and are reported to be popular in certain national markets (e.g. Poland). Ideas for many other drinkable foods containing whey and/or whey components have been published, and undoubtedly many more tried in the industrial product development laboratories. In many cases, whey has served as an inexpensive component in fruit juice drinks to gain marketing advantage. One such notorious example is the case of the product named Frusighurt, an 'official drink' of the 1984 Winter Olympic Games in Sarajevo, produced by an orange juice manufacturer. The product was essentially a fruit juice-type whey drink (see Section 10.3.1) based on orange juice with a very small addition of yogurt (thus the 'catchy name'). The product life after the games was relatively short, but most likely the venture was still worth the effort.

10.4 Technological aspects of whey beverage production

There are several technological operations that play an important role in the production of whey beverages or of dairy ingredients used in these. Apart from the routine operations used in pre-processing of the liquid cheese whey (centrifugation or filtration to remove residual milk fat and casein fines and possibly pre-pasteurisation), the production steps in most cases typically include formulation and mixing of the main product ingredients, pasteurisation or other thermal processing steps, cooling and packaging. If a long shelf-life of the products is desired, ultra-high temperature (UHT) processing may be applicable in some cases where the sensitivity of the whey protein to heat could be overcome. Production of frozen whey beverage concentrates (similar to the frozen orange juice concentrates) should be feasible as freezing of whey protein concentrates does not seem to cause major insolubilisation or other problems (Bhargava and Jelen, 1995); however, whether any such products have been commercialised has not been ascertained. Similarly, drying of complex whey beverages for mar-

Table 10.3 Product information and compositional data for a dry whey-based beverage powder, as found on the package label of a commercial product found on the Czech market

A: Product description (front of package): with. . . .
. . . Increased protein content
. . . Low glycaemic index
. . . Added L-carnitin, lecithin, barley fibre, vitamins, minerals

B: Compositional information (side of package): contains . . .
. . . Mixture of milk proteins
. . . Dried whey (30% w/w)
. . . Barley fibre
. . . Vitamin–mineral premix
. . . L-carnitin

C: Nutritional values in 250 ml reconstituted drink (recommended reconstitution 25 g powder in 225 ml water):

Component	Amount (g)
Protein	5.7
Carbohydrates	13.0
Total lipids	1.5 (incl. lecithin 1.3)
Total fibre	1.1 (incl. insoluble 0.6)
L-Carnitin	0.25
Calcium	0.45

keting in powdered form is rare, although modified dried whey preparations in retail consumer packages for reconstitution into a healthful beverage are presently being marketed in the Czech Republic with great success; composition of one such product as per the consumer information on the package is summarised in Table 10.3.

Several rather specialised steps also may be included in the modification of the whey base materials or in the processing of the final products. These may include (i) membrane processes to remove and/or concentrate the whey protein; (ii) partial or full demineralisation of the whey base using electrodialysis, ion exchange or nanofiltration; (iii) fermentation of the mix if the case of cultured dairy product types; (iv) lactose hydrolysis for reasons related to nutrition (to avoid problems of lactose intolerance), sensory desirability (to increase sweetness) or technology (if using specific micro-organisms such as yeasts which do not ferment lactose); or (v) partial lactose removal by crystallisation or by the chromatographic process patented by Valio (Jelen and Tossavainen, 2003). Detailed technical information about these operations is available in several books and many review articles; thus only the most important aspects having possible impact on the whey beverage production will be briefly considered here.

10.4.1 Membrane processing and demineralisation

Of the four major membrane processing operations (microfiltration – MF; ultrafiltration – UF; nanofiltration – NF; and reverse osmosis – RO), UF is probably the most important one by far for production of traditional whey beverages and/or the main ingredients for use in some of the specialised high-protein products. The effectiveness of the UF is in separating and concentrating the soluble whey proteins in the retentate, which can serve directly as a base material for some of the whey beverages, or can be dried for later use as an ingredient or after reconstitution as the primary liquid component. The liquid by-product of the UF processing of whey into the protein-rich retentate is the UF permeate, containing the low molecular milk components, primarily lactose and water-soluble minerals; as indicated above, this 'whey without protein' could be used for production of thirst-quenching drinks such as Rivella or the isotonic and energy-rich sport drinks. The processing of skim milk by UF technology, used often in production of soft cheeses, results in a UF permeate of a chemical composition which is very similar (not identical in the minor details) to that of the whey UF permeate, and thus obviously also suitable as a potential raw material for thirst-quenching drinks that can be included as whey drinks.

Processing by NF or MF could also be important in the production of some whey beverages. The MF of skim milk is suitable for separation of the casein, thus producing 'ideal whey' containing all the whey proteins but not contaminated by casein fines from the cheesemaking or by the starter cultures used in the process. The NF is suitable for partial whey demineralisation and, in the case of acid whey, also partial deacidification, thus removing two components that may cause sensory defects in some of the beverages, as the mineral content of whey is about 10% of the total whey solids. When a more complete demineralisation is desired (e.g. in the manufacture of the WPC or WPI, but especially in the production of infant formulae), processing the whey by ion exchange or by electrodialysis can achieve up to 90% demineralisation (Burling, 2003).

10.4.2 Fermentation

Since whey and whey-like UF permeates contain lactose as the main solute, it is not surprising that fermentation of these liquids by lactic acid bacteria is not only feasible but constitutes a potentially attractive option for production of dairy-like beverages based on sweet whey or containing whey as a significant component. The conduct of the fermentation step should not cause any unusual problems, although plain whey has been shown to be a nutritionally incomplete medium for the lactobacilli, causing their growth to be slower than when additional nutrients were added (Vasiljevic and Jelen, 2001).

An added benefit of the fermented whey beverages could be the possibility to produce bioactive peptides during the fermentation step, when proper

strains of the LAB are used in the fermentation process (Korhonen and Pihlanto, 2006). Utilisation of whey in production of drinkable yogurts (either adding the sweet whey before the fermentation of the final mix or adding acid whey as a diluent of the previously fermented yogurt) appears logical and was shown to be feasible (Burton-Trapp, 1991), but little information is available on whether this practice is being utilised by the industry. Development of acidity in the fermentation of high-protein UF retentates by regular yogurt cultures was shown to proceed at much slower rates than in the case of simple whey substrates (Johnson *et al*., 1996), due not only to the buffering capacity of the whey protein but especially to the co-concentration of calcium and phosphorus in the UF process.

Fermentation of whey or whey permeates by kefir grains has been studied (Athanasiadis *et al*., 2004) and found to be feasible, with the fermentation carried to pH 4.1 shown to be optimal for flavour development. However, many yeasts and bacteria do not have the ability to ferment lactose and thus, if these cultures were to be used in the fermentation process for specific nutraceutical purposes, the lactose would have to be pre-hydrolysed into its monosaccharide constituents glucose and galactose.

10.4.3 Lactose hydrolysis

The process of lactose hydrolysis has been reviewed thoroughly in many publications (e.g. Gänzle *et al*., 2008) and these can be consulted for detailed information. Briefly, there are several alternative avenues available to accomplish the task (Table 10.4). Industrial applications in production of whey beverages are known to exist, utilising either the immobilised enzyme route (the Finnish Valio process), or by using soluble enzymes.

In addition to the possible application before the fermentation by non-lactose fermenting microorganisms, the lactose hydrolysis in whey

Table 10.4 Alternative lactose hydrolysis technologies potentially applicable in industrial production of whey beverages

Process	Characteristics
Acid-catalysed hydrolysis	Aqueous lactose solution heated at pH < 1.5
–direct addition of acid	–temperature about 90 °C
–ion exchange	–temperature about 150 °C
Immobilised enzyme technology	Reactor containing lactase immobilised on suitable carier in a column or on a membrane
Free (soluble) enzymes	Purified enzyme preparation added to final product, single use, enzyme not recovered
Membrane reactors with soluble enzymes	Soluble (free) enzyme kept in the reactor by continuous UF separation
Crude cellular extracts	Homogenate of lactase-producing microbial culture used as the enzyme source

beverages may be advantageous for reduction of the high caloric content of these products. Especially in the case of the fruit juice-type beverages, where the whey component often exceeds 80% of the total volume, the high lactose content may contribute 'empty calories' in the best sense of this word. As lactose is not sweet enough to balance the high acidity of these products, additional sweeteners must be added, usually fructose, glucose or sucrose. By hydrolysing the lactose, its contribution to the product sweetness can be increased about 4-fold. Together with the use of non-nutritive sweeteners such as saccharin, cyclamates, aspartame or acesulfame-K, dietary whey beverages can be produced (Beukema and Jelen, 1990) and these sweeteners are indeed being used in some of the countries with high market acceptance of the whey beverages (Germany, Switzerland). Of course, the lactose hydrolysis also will be advantageous for making these healthful beverages accessible to consumers suffering from lactose intolerance. In Finland, a probiotic whey beverage Gefilus (containing the probiotic strain *Lactobacillus rhamnosus* GG) has been developed based on lactose-hydrolysed demineralised whey syrup (Mann, 1994).

10.4.4 Lactose removal

The chromatographic process for removal of lactose from milk and other dairy beverages is well suited for applications concerning whey. Currently the main industrial applications are the production of lactose-free milk, which has enjoyed a considerable success on the Finnish market (Jelen and Tossavainen, 2003). It is conceivable (although not documented in the literature) that flavoured dairy-type whey drinks (in fact whey protein-based or enriched milk drinks) could be produced from liquid WPC or by adding sweet whey to the milk before subjecting the mix to the chromatographic lactose removal process.

Another possibility for removing significant amounts of lactose from whey destined for beverage production could be the crystallisation from pre-concentrated whey. The lactose-reduced whey concentrate could then be used as a basis for production of concentrated versions of the various types of whey beverages.

10.5 Technological and physiological functionality, sensory quality and nutritional aspects

Protein and lactose, the two main whey components after water, present technological challenges as well as opportunities in the manufacture of whey-based beverages. The main problem with the whey protein is its heat sensitivity, while its physiological functionality presents one of the major opportunities for health-promoting dairy beverages. In the case of lactose, the problems are related to its limited sweetness and, in the case of

concentrated whey product, its limited solubility, in addition to the possible nutritional problem of lactose intolerance. Other, less well-documented technological problems can be caused by the vitamins present in – or added to – the whey drinks.

10.5.1 Whey protein

As is well known, the whey proteins are heat sensitive and will denature when exposed to temperatures above about 70 °C. In the absence of casein, the whey proteins will form insoluble complexes owing to their propensity to self-association. This could pose a problem in the final heat-treatment step which the whey drinks normally undergo to ensure the food safety requirements, resulting in undesirable sedimentation. The problem could become more severe in the case of high heat treatment (UHT or equivalent) if the final product is intended to have increased shelf stability without refrigeration. The relatively severe heat treatment equivalent to a full 'pressure cook' would be required especially for non-acidic beverages with the pH close to neutrality, in contrast to the acidic beverages where a much less severe 'atmospheric cook' or even a hot fill is satisfactory. However, the whey proteins will not form sedimentable aggregates when the heat treatment is carried out below approximately pH 3.8 (Patocka *et al.*, 1986), while heating close to the neutrality (at about pH 6.5) will produce non-sedimenting 'soluble polymers' (Britten and Giroux, 2001). In addition, the suppliers of the whey protein products have developed heat-stable whey protein isolate (WPI) ingredients specifically for these applications. Thus, with judicial thermoprocessing and product formulation, the negative technological aspects of the heat sensitivity of whey proteins can be overcome, in addition to the stabilisation of the drinks by using the hydrocolloids as is traditional in the dairy industry in similar cases. A clear whey protein beverage has been developed by using iced green tea fortified with a WPI (Gerdes, 2007). As green tea is less astringent than black tea, the protein coagulation by tannins in this product was less of a problem, especially at the 3.0–3.4 pH range which was found to be the best for both clarity and heat stability.

In the context of the innumerable 'whey protein drinks' being marketed by the various purveyors of functional food products (typically in the dry form), the heat sensitivity of the whey protein fraction is also an important consideration in the drying process producing the WPC and WPI ingredients. In this regard the problem has two dimensions, concerning both the solubility of the powder and also possibly affecting the known (or alleged) physiological functionality of the whey protein components.

An additional technological problem related to the functionality of whey proteins could be caused by their tendency to foam, especially in highly heated whey (Jelen, 1973). As indicated above, this could be particularly bothersome in the case of producing carbonated whey beverages containing whey proteins, as well as pop-type beverages fortified with the WPI.

10.5.2 Lactose

As mentioned above, lactose is more a problem than an advantage in whey-based beverages. Its limited sweetness does not contribute positively to the sensory profile of the products, while its presence in relatively high quantities and its high energy content could be viewed as a nutritional disadvantage, in addition to its impact for lactose-intolerant consumers. Furthermore, in production of concentrated or dried whey-based beverages, the limited solubility of lactose could pose a problem owing to its propensity to crystallisation. If whey concentrates containing high amounts of lactose are spray dried, the resulting powders will be hygroscopic as the non-crystalline lactose will form the hygroscopic lactose glass. Such products must be packaged properly in water vapour-impermeable package to avoid caking. To produce a non-hygroscopic, non-caking lactose-containing powder, the lactose needs to be precrystallised before the drying step. However, in the production of the high-protein powders with generally low lactose content, the lactose crystallisation effects will be minimal.

10.5.3 Other components

The presence of lactic and other organic acids in the fruit juice-type beverages provides the main general sensory characteristics of these products. Since the lactic acid in the L+ isomeric form is generally considered nutritionally preferable owing to its alleged faster metabolic breakdown (Renner, 1992), the selection of appropriate dairy cultures in this regard could be important. From the technological viewpoint, producing these drinks as dry powders would be very difficult as drying materials that contain such high amounts of acids is exceedingly complex.

The presence of vitamins, particularly the riboflavin of the milk, can also cause problems owing to its sensitivity to photooxidation if light-permeable packaging is used. As illustrated by the case of Rivella (Jelen, personal communications), this oxidative instability was a problem in the early development stages, leading to sensory defects noticeable to consumers. Vitamin-caused sensory problems in whey beverages can be caused by indiscriminate fortification without careful product development trials – a case of off-flavour development due to the vitamin E added to an acidic whey drink has been recorded in the literature (Jelen, 1992). When using acid whey, its higher mineral content compared with the sweet whey may lead to saltiness perceptible in many juice-type whey beverages.

10.5.4 Sensory and nutritional aspects

The most important aspect of whey beverages – as is true for most foods – is their flavour. In the case of the fruit juice types, the compatibility of the juice with the peculiar flavour of the whey (sometimes referred to as ‘wet

dog') cannot be taken for granted. Acid whey can be used for these products advantageously, blending well with the acidic character of the fruit juices, especially orange and other citrus fruit juices used most often. As documented in the various surveys mentioned earlier, the 'traditional' whey beverages are marketed mostly as healthful sources of vitamins, minerals and other fruit-based constituents. Products with other typical 'dairy-related' flavours (such as chocolate, vanilla, and coconut) can be found on the market shelves more sporadically, primarily in the 'dairy-type' form. These flavours are much more typical for the high-protein products that, although marketed as 'whey beverages', are much less representative of the classical whey drinks. In fact in most of these cases the word 'whey' is a misnomer, being used incorrectly as denoting 'whey protein'.

The textural differences between the two principally different kinds of whey beverages, the juice-type vs. high-protein beverages are also significant. The juice-type drinks have usually low viscosity and 'thin', weak body, while the high-protein products are thick, with relatively high viscosity, often being served in a form of a milk-shake. The viscosity of the fermented milk-type products and drinkable yogurts could be quite low if a liquid whey protein concentrate would be used without casein (Jelen *et al*., 1987a).

Nutritionally, the whey protein has become the 'selling feature' of the whey beverages with high protein content. Reports abound on the physiological functionality of the whey protein fraction as a whole, as well as of the individual whey protein species; several recent literature reviews collected in a special volume of the *International Dairy Journal* provide an excellent source of literature references – as well as detailed summary of the current knowledge concerning the individual whey proteins (Boots and Floris, 2006; Chatterton *et al*., 2006; Mehra *et al*., 2006; Pan *et al*., 2006; Wakabayashi *et al*., 2006). Some of the whey proteins are now considered to have important nutraceutical and health-protecting properties *per se* (e.g. lactoferrin, immunoglobulins, lactoperoxidase); in addition, several peptides produced from α-la and β-lg, the main whey proteins, by enzymatic hydrolysis have been shown to possess a potential physiological functionality, e.g. immunomodulatory (Gauthier *et al*., 2006) or ACE-inhibitory activities (Otte *et al*., 2007). A whole new category of specialised whey-based beverages seems to be emerging, based on the protein hydrolysates, as well as hydrolysates of other dairy and non-dairy proteins. In 2007, a new product called 'WheyUP' was developed for the rapidly expanding energy drink market. The product is said to contain, in addition to the WPI, some other ingredients typical for the high-energy drinks, such as caffeine, taurine, B-vitamins, etc. (Wright, 2007).

The various whey drinks can be also a rich source of other nutritionally important minor milk components as well as added micronutrients and probiotic bacteria, assuming similar importance as the probiotic yogurts. The traditional juice-like whey drinks have been usually formulated with

overabundance of vitamins and minerals and marketed as rich sources of these. Such products, especially when made from acid whey with its high calcium content and fortified with vitamin D and additional whey protein, could be justifiably promoted as being equivalent to market milk. These milk-like beverages would be eminently suitable for consumers who limit their consumption of fluid milk owing to the inability to properly digest the acid-clotted casein, complaining about a condition referred to as 'heavy stomach'. If also lactose hydrolysis would be incorporated in the process sequence, a truly physiologically functional milk-like beverage based on whey could be developed.

10.6 Future trends

In the present fiercely competitive beverage market, the classical whey drinks may be facing tough times. Long gone is the era of the whey houses where 'Little Miss Muffet sat on a tuffet, eating her curds and drinking whey'. For a beverage to be accepted by the modern consumers, it has to satisfy at least some of the main determinants of success – desirable sensory quality, thirst-quenching effectiveness, favourable price and positive 'health image'. With the peculiarities of the whey flavour interfering with many flavouring ingredients and the processing costs adding to the rapidly rising value of the formerly bothersome waste, the future of the whey beverages might lie mainly in the last attribute, the special nutraceutical qualities of some of the whey components.

Future efforts in using whole whey for the production of functional beverages may follow the path of other functional foods, building on the perceived healthfulness of the whey and whey protein. Likely approaches will include increasing the physiological functionality of these products by targeted processing or by incorporation of specific nutraceutical components of dairy or even non-dairy origin. Adding probiotics seem to be one such obvious possibility, while fortification by isolated whey proteins, proteins from other sources such as soy, rapeseed, egg white, etc., or by protein hydrolysates could also be predicted to continue as a trend in expanding the offerings of speciality whey protein drinks. The desired final beverage characteristics will play an important role in these developments, with more drinkable yogurt-type and similar lactic beverage products likely to appear as these seem to show continued market appeal worldwide (Mann, 2003).

Another avenue likely to be pursued in future will include enhanced nutrient delivery of specific components including probiotics. Innovative methods presently under investigation include microencapsulation, nanoparticulation, double emulsification and similar techniques producing microparticulate matter compatible with the beverage characteristics. From this angle the heat-coagulable whey proteins could be considered as a

particularly suitable encapsulation material (Picot and Lacroix, 2004), especially for inclusion in the more viscous whey protein-containing products.

To increase the acceptability of the traditional juice-like whey beverages, carbonation will have to become more widely applied. In 2003, an Austrian company claimed to be the first to have produced a carbonated whey beverage, with a significant increase in its export share to Germany reported due to the carbonation (Anon, 2003). Production of carbonated thirst-quenching beverages based on UF permeates should be relatively simple; alternatively, as the yeast-containing kefir grains can ferment lactose, it might be possible to produce lightly carbonated whey beverages using fermentation of whey by kefir cultures.

The consumer appeal of the whey beverages can be increased by attractive packaging, including the use of clear polyethylene terephthalate (PET) and other plastic bottles as presently found on the Czech market (Jelen, personal observations). The presently popular 'juice box' or 'gable-top' cartons obviously would not be suitable for the carbonated beverages. Production of whey drink concentrates in the liquid or perhaps frozen form, as well as dried products for food service or for at-home reconstitution could also be viewed as a commercial opportunity.

As scarcity of water will increase in many parts of the world, it is not inconceivable that the water from whey or UF permeates may become the 'ultimate whey drink'. With the inclusion of hydrolysed lactose and undenatured whey proteins, both of which are fully soluble in water, a crystal clear carbonated whey beverage could be produced that would be both thirst quenching and nutritionally satisfying. The long quest for protein fortification of common 'empty calorie' beverages could thus be successfully accomplished.

10.7 Sources of further information and advice

The general subject of whey utilisation including the incorporation into beverages has been reviewed may times in technical articles and book chapters. In addition to the reviews and book chapters cited in the text above, the following sources of specialised information can be recommended.

10.7.1 Whey utilisation

Sienkiewicz, T. and Riedel, K. H. 1990. *Whey and Whey Utilization*, Verlag Th. Mann, Gelsenkirchen Buer, Germany, 378 pp.

This is currently the most significant source of information on all aspects of whey processing and utilisation. The sub-chapter 4.5.1 discusses all main forms of beverages in which whey is used, in fluid as well as dried form; alcoholic beverages based on whey are also included.

Anon. 1998. *Whey. Proceedings of the 2nd Int. Whey Conference*, Chicago, IL, USA, 27–29 October 1997. Publication SI 9804, Int. Dairy Fed., Brussels, Belgium.

A collection of current research reports and other contributions on many aspects of whey utilisation technology, as well as new health-related applications of lactose, whey protein and derivatives.

10.7.2 Lactose and lactose hydrolysis

Fox, P. F. (ed.). 1997. *Advanced dairy chemistry* – volume 3: *Lactose, water, salts and vitamins*. Chapman and Hall, London, 535 pp.

Chemistry, reactions, nutritional significance and enzymatic modifications of lactose, including hydrolysis, are covered in the first five chapters of this 2nd edition of the 'Advanced dairy chemistry' series presently being republished as 3rd edition.

IDF, 1993. *Monograph on Lactose Hydrolysis*. Bulletin 289, Int. Dairy Federation, Brussels, Belgium, 71 pp.

Various aspects of the process, its technological alternatives and its effects on alleviating the lactose intolerance symptoms are covered.

10.7.3 Whey protein

Fox, P. F. and P. L. H. Mcsweeney (ed.). 2003. *Advanced Dairy Chemistry*, Volume 1: *Proteins*, 3rd. ed., part A. Kluwer Academic/Plenum Publishers, New York, 603 pp.

Several chapters are devoted to all main individual whey proteins, describing their molecular structure, functionality and, in some cases, also their specific biological functions.

Roginski, H., Fuquay, J. W. and Fox, P. F. 2003. *Encyclopedia of Dairy Sciences*. Academic Press, London, Volume 3, pp. 1924–1966.

Each of the main whey proteins is discussed in separate sections; in addition, one section describes briefly the main processing methods used for production of whey protein concentrates and isolates, and one section is devoted to the subject of bioactive peptides.

10.7.4 Bioactive components of milk

International Dairy Journal, Volume 16, Issue 11: Special Issue on technological and health aspects of bioactive components in milk. Korhonen, H., Guest Ed. (2006).

This special issue contains 22 review articles on all the main bioactive milk components, including the main whey component lactose and its deriva-

tives, as well as whey protein and bioactive peptides from the main whey proteins.

10.8 References

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11

Beverages based on milk fat globule membrane (MFGM) and other novel concepts for dairy-based functional beverages

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Abstract: Industrialization of butter and subsequent development of commodity dairy products combine for the current status of buttermilk as an industrial by-product. In the United States authentic buttermilk from sweet cream is most commonly found as a dry powder with applications in processed foods and bakery goods. In addition, fermented skim milk took the place of traditional drinkable buttermilk. This chapter highlights the chemical, biochemical and nutritional advantages that the authentic buttermilk has over the current beverages. It focuses on the current status of the research linking human health to components within buttermilk and butter serum.

Key words: buttermilk, milk fat globule membrane, phospholipids, sphingomyelin and ceramide, glyco-proteins and glyco-lipids, lipids and human health.

11.1 Introduction

Scientific and commercial interest on milk fat globule membrane (MFGM) components is growing from a scientific and commercial perspective because of its unique composition which is illustrated in Table 11.1 (Corredig and Dalgleish, 1997; Sachdeva and Buchheim, 1997; Astaire *et al.*, 2003; Corredig *et al.*, 2003, Morin *et al.*, 2004). When milk fat globules are disrupted during the churning of cream, the membrane covering the lipid core is excluded from the lipid matrix and is recovered in buttermilk along with most of the proteins, lactose and minerals contained in the aqueous phase. The MFGM

Table 11.1 Phospholipids and protein content in different dairy products as reference for beverages containing milk fat globule membrane (adapted from Fong *et al*., 2007; Jiménez-Flores and Brisson, 2008; Dewettinck *et al*., 2008)

Product	Phospholipids wet basis (g/100 g)	Phospholipids dry basis (g/100 g)	Protein in product Average†	Protein in dry product Average†
Raw milk	0.035	0.28	3.3	–
Skim milk	0.02	0.28	3.41	7.55
Cream	0.19	0.40	3.16	–
Isolated MFGM	0.65	6.5*	1.8	60*
Butter	0.19	0.22	0.85	-
Buttermilk	0.16	2.03	3.31	32.95*
Butterserum	1.25	11.54	–	–
Fresh acid buttermilk	0.31	1.86	3.31	–
Acid buttermilk whey	0.10	1.84	–	84.7*
Cheddar cheese	0.15	0.25	–	24.9
Cheddar cheese whey	0.02	0.26	3.49	35.2

† Average from several sources and laboratory data.
* Numbers from the author's laboratory data.

is particularly rich in various proteins and phospholipids which have outstanding potential for functional and nutraceutical applications related to the prevention or amelioration of widespread chronic diseases such as cancer, obesity, diabetes and cardiovascular disorders. For example, it has been shown that sphingomyelin (SM) could help in the prevention of colon cancer (Schmelz *et al.*, 2000) and that phosphatidylcholine (PC) could interfere with the development of hepatic diseases (Niederau *et al.*, 1998). Although the biochemical and physiological mechanisms leading to such benefits are not yet fully understood, there is a growing need to produce fractions enriched in these components to further study their individual effects on metabolism under controlled conditions and to manufacture enriched foods and beverages for the benefit of he consumer at large. Interestingly, no patents have yet been granted on applications of sphingolipids found in the MFGM. However, Nieuwenhuizen (2006) has applied for protection of sphingolipid use to reduce plasma cholesterol and triacylglycerol levels as well as the method of usage in humans. Also, Takada *et al.* (2001) have developed a therapeutic agent based on ceramide, SM, sphingoglycolipids or gangliosides useful in the prevention of osteoporosis, lumbago and rheumatism.

Various attempts have been made to fractionate buttermilk in order to create an enriched fraction in MFGM components. Surel and Famelart (1995) reported the use of microfiltration (MF) to fractionate buttermilk. One important finding from their study was the similarity in size of the casein micelles and the MFGM components present in buttermilk. The number average diameter of casein micelles has been reported as 25 nm up

to 140 nm (Schmidt *et al.*, 1973). In order to overcome this problem, Sachdeva and Buchheim (1997) used renneting and acid coagulation of buttermilk to remove caseins prior to fractionation by combining MF and ultrafiltration (UF). The authors reported a recovery of 70–77% of the total phospholipids of buttermilk using this process. These results were highly dependent on various coagulation factors. Corredig *et al.* (2003) reported the use of citrate to disrupt the casein micelles followed by either UF or MF on polysulfone membranes. This approach is effective to concentrate MFGM proteins in the retentate. However, data from this study did not show the efficiency of this process to recover other important components such as phospholipids. The high levels of citrate in the permeate limit the potential use of this process for the recovery of MFGM from buttermilk. More recently, Rombaut and co-workers (Rombaut and Dewettinck, 2007; Rombaut *et al.*, 2007a) successfully experimented with tangential filtration techniques to purify MFGM fragments from acid buttermilk cheese whey. This by-product is rich in MFGM and devoid of casein micelles.

11.2 Milk fat globule membrane lipids

MFGM phospholipids represent less than 1% of total lipids and mainly comprise three different types (expressed as a percentage of total phospholipid content): SM (26%), PC (29%) and phosphotidylethanolamine (PE) (31%). Corredig *et al.* (2003) could only find the PC and PE fractions in commercially sourced buttermilk powder and in the retentates subsequently prepared.

Complex biological lipids including sphingolipids (SL) and phospholipids (PL) are of interest because they define the structural properties of membranes and lipoproteins and function as intracellular signaling molecules in a variety of biological processes, including regulation of cell growth, development, adhesion, and cross-membrane trafficking. Complex lipids are also associated with metabolic and age-related diseases, stress responses and apoptosis (Bouhours and Bouhours, 1981; Conklin, 2002; Parodi, 2003, 2004; German and Dillard, 2004, 2006; Spitsberg, 2005; Singh, 2006). Certain sphingolipids also influence cellular apoptotic pathways (Schmelz *et al.*, 1996; Parodi, 2003; Sawai *et al.*, 2005; Spitsberg, 2005; German and Dillard, 2006), and their beneficial effects may lead to potential constituents of an anti-cancer regimen or as health-favoring ingredients. Two reports on the phospho and sphingolipid content of the main dairy products found in the Belgian market have been published (Rombaut *et al.*, 2005; Rombaut *et al.*, 2007b). Sour and sweet buttermilk and quark-skimmed cheese are the highest polar lipid-containing products. Ahn and Schroeder (2002) analyzed sphingolipid content in dairy products frequently consumed in the United States. They found the concentration of total sphingolipids in the fermented dairy products to be similar to that of non-fat dry milk, suggesting that

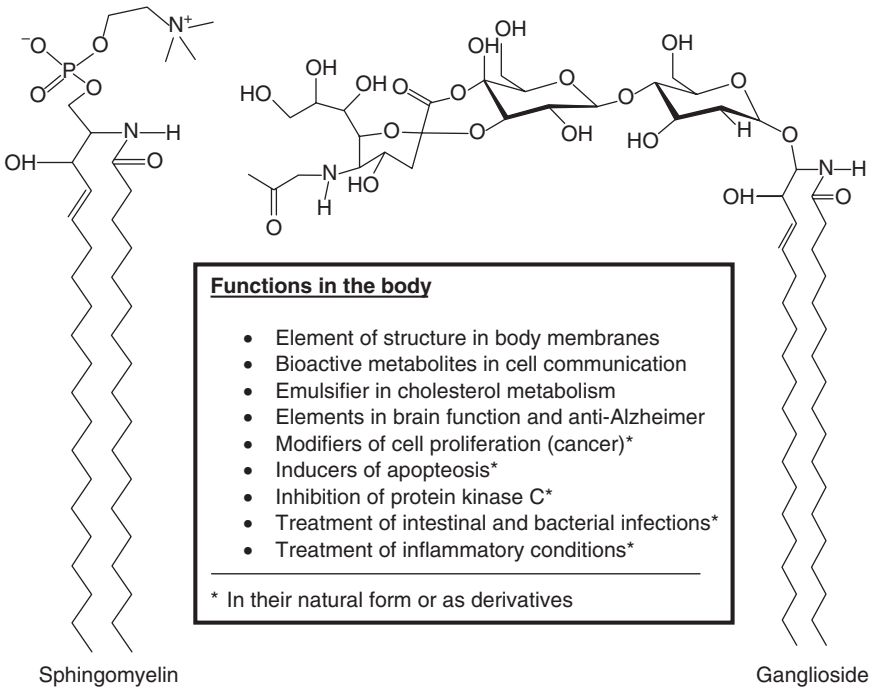


Fig. 11.1 Illustration of the structure of sphingomyelin and gangliosides present in milk.

starter cultures containing sphingomyelin do not significantly contribute to the total sphingolipid concentration of the fermented foods.

Recent consideration of the organization of the lipids is being sought as a potential explanation of the particular properties of the MFGM. Figure 11.1 is a good representation of the surface behavior of the lipids from bovine milk. A challenge to the technologist is to, first understand the biological mechanism of nutrition delivery, and then to elaborate foods and beverages that stabilize an optimal structure of these lipids to deliver nutrition.

11.3 Milk fat globule membrane proteins

The protein composition of MFGM is highly complex with over 40 different polypeptides ranging in molecular weight (MW) from 15 000 to 240 000 Da. The major classes of polypeptides including their respective MWs include xanthine oxidoreductase (170 kDa); butyrophilin (67 000); PAS 6 (50 000) and PAS 7 (49 000). Ye *et al.* (2002) confirmed that protein content of MFGM varies with lactation – butyrophilin and xanthine oxidase are higher during the early and late stages compared with mid-lactation. They found

that the molar ratio between these two proteins was practically the same throughout the season and speculated they may be cross-linked in some form. They also contended that the same proteins did not influence fat globule size.

11.3.1 Butyrophilin

Butyrophilin (BPH) is a protein with a molecular weight of 66 kDa. It is the major component of the MFGM, making up approximately 40% by weight. BPH is thought to be involved in milk fat secretion. In addition, it belongs to the immunoglobulin family of proteins and may be involved in immune recognition of foreign substances entering the body. Gorewit and Spitsberg (1996) suggest that BPH can act as an enzyme that transfers phosphorus groups to tyrosine residues in proteins. Most phosphorylated proteins exert their actions after binding to phosphorus. BPH would then play a critical role in controlling the physiological activity of all proteins within the mammary cell and the MFGM.

A controversial aspect relates to the role of BPH in inducing multiple sclerosis or experimental autoimmune encephalomyelitis (Dewettinck *et al.*, 2008). Yet, Mather and Linington (1999) have developed methods of modifying BPH in order to suppress an autoimmune response. This could be done using modified dairy foods. A further application of this development is the diagnostic procedures to screen for susceptibility to multiple sclerosis. The BPH gene promoter has also been used to produce an heterologous protein in the milk of transgenic animals and for cancer detection (Mather *et al.*, 1998).

11.3.2 Xanthine oxidoreductase

Bovine milk xanthine oxidoreductase (XOR) is a complex molybdoflavoenzyme comprising an homodimer of 147 kDa associated with phospholipid membranes that occurs as a major protein component of MFGM. The physiological roles of XOR have been described recently (Harrison, 2004). It has two basic forms, xanthine oxidase (XO) and xanthine dehidrogenase (XD). Their distribution has been studied by Silanikove and Shapiro (2007). About a third of XO is bound to the outer surface of the MFGM. This enzyme has broad substrate specificity and is capable of reducing oxygen to generate the reactive species, nitric oxide and peroxynitrite by reduction of NO_2 and is, therefore, thought to play a role in antimicrobial defense in the neonatal gut as freely available extra-membrane XO.

11.3.3 Mammary-derived growth inhibitor/fatty acid binding protein (MDGI/FABP)

Long-chain non-esterified free fatty acids (FFA) are the building blocks from which triglycerides are synthesized. The FFAs are important energy

substrates in various tissues, essential building blocks for lipid components of cell membrane and precursors for the synthesis of imported biological mediators such as prostaglandins. They are also increasingly being recognized as intracellular mediators of gene expression. Fatty acid binding proteins (FABP) appear closely involved in fat metabolism and are present in the MFGM. They also influence cellular proliferation and differentiation and inhibit the growth of various cell types including bovine mammary and human breast cancer cells.

Preliminary data show that tyrosine is phosphorylated within FABP upon incubation of MFGM with [^{32}P]ATP and that FABP is phosphorylated in mammary gland cells on a tyrosine residue (Gorewit and Spitsberg, 1996). Gorewit and co-workers also hypothesize that insulin receptor kinase-mediated phosphorylation of FABP controls the pleiotropic affects of FABP and lipid metabolism, growth and differentiation of the mammary cell. Ingredients with different composition and content of MFGM components would be helpful in this type of research. Spitsberg and Gorewit (1997a, b) have developed a method to detect treatments with bovine somatotrophin based on the evidence that the FABP of the MFGM of cows treated with the hormone display significantly reduced levels of phosphorylation.

11.3.4 Breast-ovarian cancer susceptibility protein (BRCA1)

The *BRCA1* breast-ovarian cancer susceptibility gene was identified in the recent past (Miki *et al.*, 1994). Mutations in the *BRCA1* gene may be responsible for a significant number of breast and ovarian cancers. The gene produces *BRCA1* protein which acts as a negative regulator of tumor growth (tumor suppressor). The proteins expressed by the *BRAC1* gene have been identified in the MFGM of human and bovine milk by western blot and immunoprecipitation (Spitsberg and Gorewit, 1999; Vissac *et al.*, 2002). Spitsberg and Gorewit (1999) obtained a patent for a method to detect expression of the *BRCA1* gene and isolate the encoded protein. The method also claims to provide a measurement of the probability that a woman will develop breast cancer by quantifying the level of the protein expression during lactation and comparing it with normal values.

11.4 Technical aspects of the milk fat globule membrane

It seems that if we take milk as a food that has evolved to deliver nutrition (German and Dillard, 2006) it is interesting to see that the interface of the fat with the environment is a membrane composed of specific PL and amphiphilic proteins. Therefore, we want to advance that the idea of a dairy beverage rich in these components and judiciously processed may bring forth multiple health benefits as previously described.

MacGibbon and his group (Fong *et al.*, 2007), have described the technological, compositional and nutritional properties of MFGM. In this section we want to emphasize the potential advantages that a beverage with these components may represent. Caution has to be taken in the processing and concentration of the MFGM and its components to maintain bioactivity and to exercise an economically feasible process. Jiménez-Flores and Brisson (2008) have reported on the challenges posed by the processes to concentrate MFGM elements while maintaining their biological value. The use of cross-flow membrane filtration, particularly microfiltration of buttermilk, has been explored but success has been limited by the presence of casein fractions. Other fractionation methods include the use of biosilicates to remove proteins and non-polar lipids from buttermilk as well as use of supercritical CO_2 extraction on microfiltered buttermilk powder to separate polar and non-polar lipids (Astaire *et al.*, 2003). These authors also report on the use of novel techniques, namely, atomic force microscopy and laser tweezers to study the fundamental properties of the MFGM components. Gorewit (2002a, b) has obtained two patents covering the isolation of the MFGM with simple physical procedures and the preparation of functional beverages, tablets or capsules. The method includes pasteurization of un-homogenized milk, whipping to produce two distinct phases (cream and aqueous phases), washing the cream phase to create an additional water phase containing the MFGM solids, which is later filtered and dried. The MFGM solids thus obtained can then be used as an ingredient for the manufacture of functional dairy products.

11.5 Beverages based on buttermilk

Although buttermilk has been virtually displaced from the dairy cabinet shelf in many countries, an increasing interest in using it as a supplement or ingredient in dairy beverages is evident from the number of patent applications being filed in recent years. For instance, an application for a formulation of a nutritious and functional dairy beverage includes buttermilk as an ingredient (Konkoli *et al.*, 2006). Similarly, but using a combination of soy isolate and yogurt-forming microorganisms, Miskovsky (2006) developed a variety of products, some of which may contain buttermilk. Shukla *et al.* (2004) developed a functional dairy beverage based on buttermilk and fruit juices and pulps that contained similar levels of protein to those found in milk and have acceptable organoleptic characteristics. The major advantages of using buttermilk solids in functional food systems pertain to their significant antioxidant activity and buffering capacity (Wong and Kitts, 2003) and to its high phospholipid content which may have a suppressing effect on certain pathogens. The use of condensed sweet cream buttermilk at low levels of supplementation improved the yield of pizza

cheese without affecting compositional, rheological or sensory properties (Govindasamy-Lucey *et al.*, 2007).

11.6 Beverages including prebiotic oligosaccharides

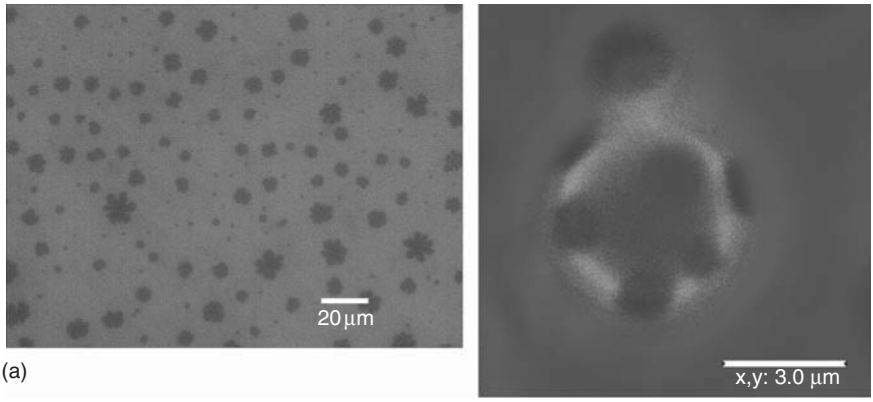
A significant body of research has accumulated on the beneficial effects of prebiotics, particularly fructo-oligosaccharides and galacto-oligosaccharides when included as food and infant formula ingredients. Anticancer effects, improvement in mineral absorption and anti-inflammatory response as well as beneficial immunological effects are among such favorable responses (Macfarlane *et al.*, 2008). Human milk oligosaccharides (HMO) may function as analogs of pathogen receptors in a manner which inhibits their adhesion to epithelial surfaces. The structural complexity of HMO has, so far, limited their availability as functional ingredients (Boehm and Stahl, 2007). Yet, a recent review by Espinosa *et al.* (2007) highlights the significant limitations of traditional approaches to emulate the biological functions of HMOs and propose a more focused approach including the synthesis of an *in vivo* assessment of fucosyl-lactoses which have been shown in pediatric practice to offer significant protection.

Recently, the use of traditional prebiotics is finding alternative applications in dairy foods and beverages, particularly for the manufacture of synbiotics. Mäyra-Mäniken *et al.* (2005) have protected an innovative combination of probiotics and prebiotics (galacto-oligosaccharides) providing marked advantages on immune response and diminishing carcinogenic substance formation in the colon. Most oligosaccharides found in domesticated animal milks have common structural features with human milk oligosaccharides.

Although the production of prebiotics from the MFGM components has yet to be explored, empirical evidence has shown significant adhesion of bacteria to some of its components. Figures 11.2 and 11.3 demonstrate the high content of natural sugars, and saccharide-linked proteins and phospholipids available for bacterial binding on the surface of the MFGM. Current research demonstrates the importance of these components in probiotics and human health.

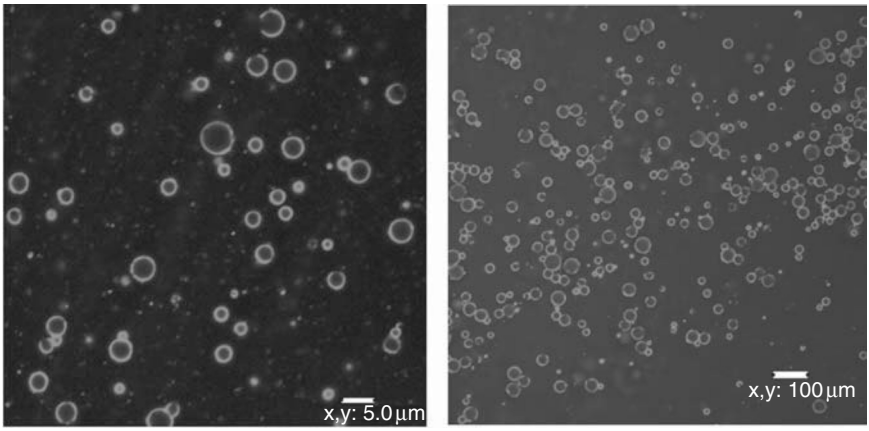
11.7 Colostrum and colostrum ingredients for functional dairy beverages

In this section we explore the connection between the MFGM and colostrums. There are several interesting factors associating colostrum and early lactation milk with small MFGM and composition. The MFGM gangliosides, for example, change remarkably from colostrums and early lactation to mature milk (Martin *et al.*, 2001). Additionally, several studies measuring



(a) (b)

Fig. 11.2 (a) Epifluorescent picture of milk phospholipids layered on a Langmuir trough. Fluorescently labeled Texas red-phosphatidylcholine indicates that the lipids from milk form specific domains, change phase at given temperature pressure conditions, and in this image the domains are shown as dark spots. (b) Confocal microscopic image of a native milk fat globule stained with rhodamine-phsopatidyl ethanolamine. The dark spots indicate that the fluorescent probe, is excluded. Both pictures illustrate the fact that the surfaces of fat globules represent localized areas where different component can be found at different concentrations. This is a particular characteristic of the MFGM currently under study by various scientific groups ((a) courtesy of Dr Derek Gragson; (b) courtesy of Dr Cristelle Lopez).



(a) (b)

Fig. 11.3 Confocal microscopic view of native milk fat globules from bovine milk, showing milk fat globules stained with a fluorescently labeled Concanavalin A marker: (a) fluorescent only picture; (b) overlaying of the optical and fluorescent images. Both pictures indicate that the surface of the bovine milk fat globules are rich in oligosaccharides (pictures contributed by Dr Cristelle Lopez).

the size of the fat globule in early lactation as compared with those of mature milk, have found that small globules are the norm in mammals during the production of colostrums and early lactation milk. Technologically, the work of Michalsky and co-workers, make it very interesting and within the realm of possibility the more general use of this valuable dairy product (Michalski *et al.*, 2006a, b).

In spite of its numerous health-related benefits, colostrum is an underutilized food at the dairy farm as well as in the dairy industry. Various studies have described the high content of immunoglobulins, growth factors and other bioactive molecules such as lactoferrin contained in colostrum (Pakkanen and Aalto, 1997). The stimulation of the immunological system by colostrum administration has been shown by several authors and reviewed by Kelly (2003). In spite of its multiple benefits, the use of colostrum or any of its ingredients in functional dairy beverages is still very limited owing to consumer resistance and its high perishability. In the extensive product review provided by Sharma (2005) on functional dairy beverages currently marketed worldwide, one product sold in Japan is reported to be fortified with IgG. Gohlke and Cockrum (2001, 2002, 2004) have obtained several US patents claiming title to a dietary supplement containing colostrum, lactoferrin and citrus-derived pectin and its method of use. Citric acid is a further optional component of the formulation which can be absorbed through the oral mucosal membranes thus maintaining the bioactivity of the individual components.

The use of lactoferrin, a major protein in colostrum by itself, has been associated with a number of beneficial immunological, antimicrobial and anticarcinogenic properties. This protein has been reported to maintain its biological activity when administered by mouth (Knowles and Gill, 2004). Various patent applications for nutraceutical formulations have been submitted recently utilizing lactoferrin as an essential component for the prevention or treatment of cardiovascular disease (Mao *et al.*, 2007) or for the treatment of a variety of ailments.

Also, relevant efforts have been developed for the processing of colostrum in order to obtain a stable and readily available product. Scammell (2001) has used centrifugation and thermal treatments to diminish the microbial load in colostrum, thus providing a product which may be added to a variety of dairy food products or beverages to improve their functionality and potential to stimulate the immune system.

Ogasa *et al.* (1980) disclosed a method based on the use of mono- and disaccharides as well as other protective substances to preserve bovine or human colostrum in powder form. Playford *et al.* (2000) have reviewed the use of bovine colostrum for the treatment of gastrointestinal disorders. Among its many benefits, colostrum has been shown to prevent gut damage derived from the use of non-steroidal anti-inflammatory drugs. Other applications include colostrum preparations to be used as immunomodulators

in the treatment of allergic diseases, ulcerative colitis and similar disorders (Yasunobu and Yasuko, 2006).

11.8 Other products and ingredients

Emulsion-based functional foods are a broad class of products finding new applications in the marketplace. The amphiphilic nature of the components in MFGM, phospholipids and glycoproteins, gives them excellent emulsifying properties (Kanno *et al.*, 1991; Corredig and Dalgleish, 1997). Thus, Yoshiaki-Yano *et al.* (1994) have taken advantage of such characteristics and developed a method to protect and deliver numerous bioactive compounds using it as their basic substrate. It has also been shown that in some instances, that phospholipids (PL) derived from milk yield more stable emulsions and liposomes than PL derived from plant sources (Singh, 2006). Lysophospholipids released from the MFGM by treating milk with phospholipase provide better emulsification of water and fat during the processing of mozzarella cheese (Lilbæk, 2006). Mora-Gutierrez and Gurin (2007) have developed a very complex set of combinations for various nutraceuticals in emulsion-based systems. The emulsification properties of the MFGM have been used to prepare stable compositions of bioactive compounds with an hydrophobic nature, based on specific grain size fractions containing a high density of lipid microspheres with high emulsifying stability.

Also, the use of nanotechnological procedures will provide a novel means of incorporating nutraceuticals into functional foods. Nanoparticles made with biopolymers can, for instance, become an effective way to deliver bioactive compounds to specific sites where they will exert their beneficial properties. Shefer and Shefer (2005) have developed a novel controlled-release system based on solid nanospheres of hydrophobic nature encapsulated in moisture-sensitive microspheres with enhanced stability and delivery control.

All the above developments point to a bright future for the incorporation of MFGM components into functional dairy beverages but various obstacles will have to be resolved before the benefits reach the final consumer. Among these obstacles are the complex regulatory aspects surrounding functional foods in general, as well as the multiple technological limitations posed by the physical and chemical environment where the desirable bioactive ingredients have to maintain their properties. Few studies have been performed on this specific topic, but recently Uzzan *et al.*, (2007) analyzed the processing and storage stability of five candidate ingredients for milk fortification: isoflavones and chondrotin sulphate were stable during the processing and storage conditions used in the experiments, whereas glucosamine, lactoferrin and creatine exhibited very unstable

behavior, thus losing a significant part of their desirable properties. Studies of this type will have to be performed with MFGM components in potential functional dairy products in order to determine conditions for maintaining optimum organoleptic properties as well as full assurance to consumers that the health benefits of the added compounds will still be present when the food/beverage vehicles are finally consumed.

In summary, based on the number of patents and scientific publications on the subject, it seems very likely that beverages containing high concentrations of phospholipids, glycolipids, glycoproteins, or other specific components from the MFGM, which provide significant health and functional advantages, will find their way into the market in the near future. There are two very good reasons to anticipate the development of such beverages (preferentially, but other dairy products are also very likely). One is the general consumer's expectation of health-oriented and nutritious foods that fit their ideas and lifestyles. The second is that there are valuable components in buttermilk, cream, and whey, mostly in the category of phospholipids and gangliosides that are not being utilized commercially. We hope that in the near future the detailed nutritional studies that further expand our understanding of the biological fundamentals for optimal nutrition will make it possible to increase the availability of such beverages or products for the benefit of the consumer.

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Part III

Plant-based beverages

12

New directions in fruit juice processing

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Abstract: This chapter considers trends in the consumption of fruit juices and provides a brief overview of their nutritional components and health benefits. Processing technologies are considered, beginning with traditional methods and then examining the potential for newer processes including the use of freeze concentration and membrane filtration. A section on final product processing follows and includes pasteurisation technologies as well as newer methods of processing such as high-pressure processing. The chapter concludes with a review of fruit juice-based products and then considers future trends and opportunities in this field.

Key words: fruit juices, fruit processing, fruit-based products, future trends for fruit-based products.

12.1 Introduction: trends in the consumption of fruit juices

Packaged fruit juices are now to be found in retail markets in most countries of the world. The availability of these products has been facilitated by the production of sufficient raw materials and processing capacity as well as the development of suitable packaging to give end products with a long shelf-life. Long-life juices are typically packaged either in glass or, increasingly, in laminated cartons that are formed and filled in an environment that allows for aseptic filling. There are significant advantages in the use of laminated packages as the quality of the product is often improved by the use of less heat treatment than would be required for packaging in glass. Other benefits include the more efficient use of space during transit and storage as well as much lower weight of packaging material. Laminated packs permit more colourful and all-over labelling and offer many opportunities for improved marketing of the product. Other sectors of the market that rely on cold distribution channels have encouraged the development of short shelf-life juices with minimal processing.

These developments have facilitated a steady growth in the consumption of fruit juices in the years since the Second World War with the market

being dominated by the production of concentrated fruit juices which are reconstituted by the addition of water just prior to the final packing. The re-addition of concentrated fruit juice volatile substances removed during the process of evaporation is also encouraged and in some countries now obligatory. For products to be designated as pure fruit juices no other additions are normally allowed, although ascorbic acid levels are sometimes fortified to replace what is lost during processing and to provide improved anti-oxidant protection for the product.

In the last two decades, markets in the United States and Europe in particular have seen the introduction and dramatic growth of freshly squeezed and direct fruit juices (the latter being designated 'not from concentrate' or NFC in some countries). Freshly squeezed orange juice refers to juice that is obtained by pressing fruit as close to the end market as possible, removing the waste and some of the pulp, and then packaging the resulting product in single serving packages without any processing other than reduction in temperature. By processing in clean conditions and storing freshly squeezed juice at around 2–3 °C, a shelf-life of several days can be achieved. In markets with well-developed cold distribution chains this is sufficient to get the product to the end consumer in good condition.

Direct or NFC product is produced in a similar way to freshly squeezed juices by processing the fruit in the end-use country removing fruit waste and then subjecting the bulk product to what is often described as a 'light pasteurisation' before packing in clean (but not usually aseptic) conditions into appropriate containers. Such products when stored at around 2–3 °C usually demonstrate a shelf-life of around 2–3 months. The term 'light pasteurisation' is usually a flash pasteurisation process of 2 or 3 seconds at around 90–92 °C.

These direct juices are popular with manufacturers, retailers and consumers as they attract premium prices but offer a high-quality juice in a convenient form. Growth in this premium sector will undoubtedly continue, mainly at the expense of the traditional long-life market. In countries where the cold distribution sector is non-existent or less well developed there is an obvious limitation to such products. In less developed countries (where citrus is often grown) there is the opportunity for individual and very small processing units to supply freshly squeezed juices to individual hotels or other small markets where a premium price can be sustained.

The levels of fruit juice consumption vary from country to country and there are a number of specialist companies that provide such data on a subscription basis.

12.2 Brief overview of the health benefits of fruit juices

12.2.1 Fruit composition

Fruits contain a wide variety of different compounds and they can be grouped into the following principal categories: water; carbohydrates;

proteins; fats; minerals and vitamins; other components such as organic acids, polyphenols, anthocyanins and carotenes. Most of these components are essential to humans although the amounts required by the body vary depending on age, body mass, gender and physical activity.

12.2.2 Water

Water is the most abundant component in all fruits and the level varies from about 82% in grapes to around 90–92% in strawberries. The physical structure of fruit types is an important determinant for selecting the appropriate processing method to obtain the best yield of juice which, at its simplest, involves releasing water and the dissolved constituents from the different cellular structures.

12.2.3 Carbohydrates

Carbohydrates form the next most important group of components in most fruits. The typical carbohydrate spectrum in fruits includes polysaccharides such as starch, cellulose, hemicelluloses and pectic material. Saccharides including sucrose, fructose and dextrose are present in most fruits. The amounts of these components vary widely and at maturity the simpler sugars are usually abundant. In fruit such as peaches, nectarines and apricots, sucrose is the main sugar whereas apples and pears are rich in fructose. Other mono- and disaccharide sugars such as xylose, mannose arabinose, galactose and maltose may also be found in trace quantities in some fruits. Sorbitol, a polyol related to sugars and with laxative effects, is found in significant quantities in pears and plums. Cellulose, hemicellulose and pectic substances form the cellular structure of fruits. Pectin is commercially important to the food industry as it is widely used as a gelling agent. Total carbohydrate levels in fruits at maturity range between about 15% in grapes to 3% in lemons and tomatoes.

Fruits can be an important source of dietary fibre which, by definition, includes the cell wall components that are resistant to digestion by stomach enzymes. The dietary fibre content of fruits vary between about 0.7% and 4.7%.

12.2.4 Proteins

The protein content of most fruit is below 1% and for the most part does not contribute significantly to the human diet. The protein level of fruit is calculated by determining the level of nitrogen by digestion and multiplying the resulting figure by a factor of 6.25. The value so obtained will be quoted as protein but will also include any individual amino acids that are the individual building blocks of proteins as well as any other nitrogenous compounds. Of this latter group, substances such as asparagine and glutamine, and their related compounds aspartic and glutamic acids, are

abundant in citrus fruit, strawberries and tomatoes. Asparagine occurs in relatively large quantities in apples and pears while oranges are rich in proline.

12.2.5 Lipids

Lipids rarely exceed 1% of the weight of most fruits and apart from the fact that they contribute to the overall energy value of a food, the body requires small quantities of unsaturated fatty acids such as linoleic acid. Fruits in general are not considered to be significant sources of fat and the process of extracting fruit juices effectively removes any fat from the final juice product.

12.2.6 Minerals

Minerals are present in fruits although vegetables are generally a more important source. Potassium is the most abundant element in fruit and usually occurs in combination with organic acids. In strawberries and tomatoes, calcium, magnesium, phosphorus and chlorine are the only elements that occur in a quantity exceeding 10 mg/100 g of fruit. Because of their solubility in water fruit juices will normally contain any minerals present in the fruit. Some elements are bound into complex organic molecules and may not be extracted into juice or if extracted may have limited bioavailability. For example, calcium plays a role in the pectic structure of cell walls and magnesium in the chlorophyll molecule.

12.2.7 Vitamins

Fruits in general are an especially good source of vitamin C, ascorbic acid, although the amounts vary considerably from variety to variety. Ascorbic acid is present in fruit juice although a significant proportion may be lost during the process. Citrus fruits in particular contain carotenoids, which are known as provitamin A, and can be converted in the body to vitamin A. Fruit is a moderate to poor source of members of the B vitamin group. Vitamin E occurs in very small quantities in some fruits.

12.2.8 Other components

The remaining constituents of fruits include pigments, organic acids, which are important for delivering an organoleptic balance to the sweetness of most juices, and a number of minor components. The significance of polyphenolic components and other minor ingredients with anti-oxidant properties are currently being emphasised as positive dietary factors that may assist in protecting the body against various diseases. Because fruit and fruit juices contain some of these essential nutrients in higher concentrations

than other foods, they play an important role in balancing the human diet. Fruit juices are often rich in iron, carotene and ascorbic acid. Evidence has been found that indicates ascorbic acid of natural origin is apparently superior in nutritional benefits to that made synthetically.

12.3 Processing technologies

12.3.1 Traditional methods

The most basic method of processing fruit involves a sequence that incorporates the removal of foreign material and diseased fruit, washing and then the presentation of fruit to the pressing facility. This basic process is modified in many ways to suit the different botanical structure of main fruit types. For example, apples, pears and berry fruits such as blackcurrant and raspberry are invariably milled and subjected to the addition of pectolytic enzymes to supplement the natural content. The milled mash is often held at temperatures of 30–40 °C for about an hour to enhance the breakdown of cell walls and the release of juice.

Citrus fruit processing usually involves a process to release essential oils from the flavedo layer before the fruit is pressed to obtain juice. Fruit that contain pits (fruit stones) are often heated to soften the flesh in a way that will facilitate removal of the stone as well as releasing juice and pulp.

12.3.2 Soft fruit processing

Fruit used for juicing must be sound, free from gross damage or contamination, especially mold or rot which can lead to tainted juices or, especially in apples, the presence of toxic mould metabolites such as Patulin. Mechanical harvesting can cause bruising and skin penetration which may cause off-flavours or the growth of pathogenic organisms. Mechanical harvesting is used extensively on apples and blackcurrants, whereas berries are mainly picked by hand. Most temperate deciduous fruits, including apples, pears, grapes, berries, blackcurrants and redcurrants, are processed by milling, i.e. comminuting the whole fruit and then pressing the juice from the resulting mash. Processing equipment for stone fruits such a peaches or apricots requires an additional stage such as a heat treatment, to facilitate removal of the stones prior to pressing. Many tropical soft fruits require specialised processing stages (Fig. 12.1).

Milling

All fruit used for juicing must be thoroughly washed to both clean it from field dirt and reduce the microbial load. A variety of methods are used to break the fruit to release juice. These methods depend on the structure of raw material, the clarity desired in the final juice, enzymatic discoloration, and destruction of pectin. The most common disintegrator used in North

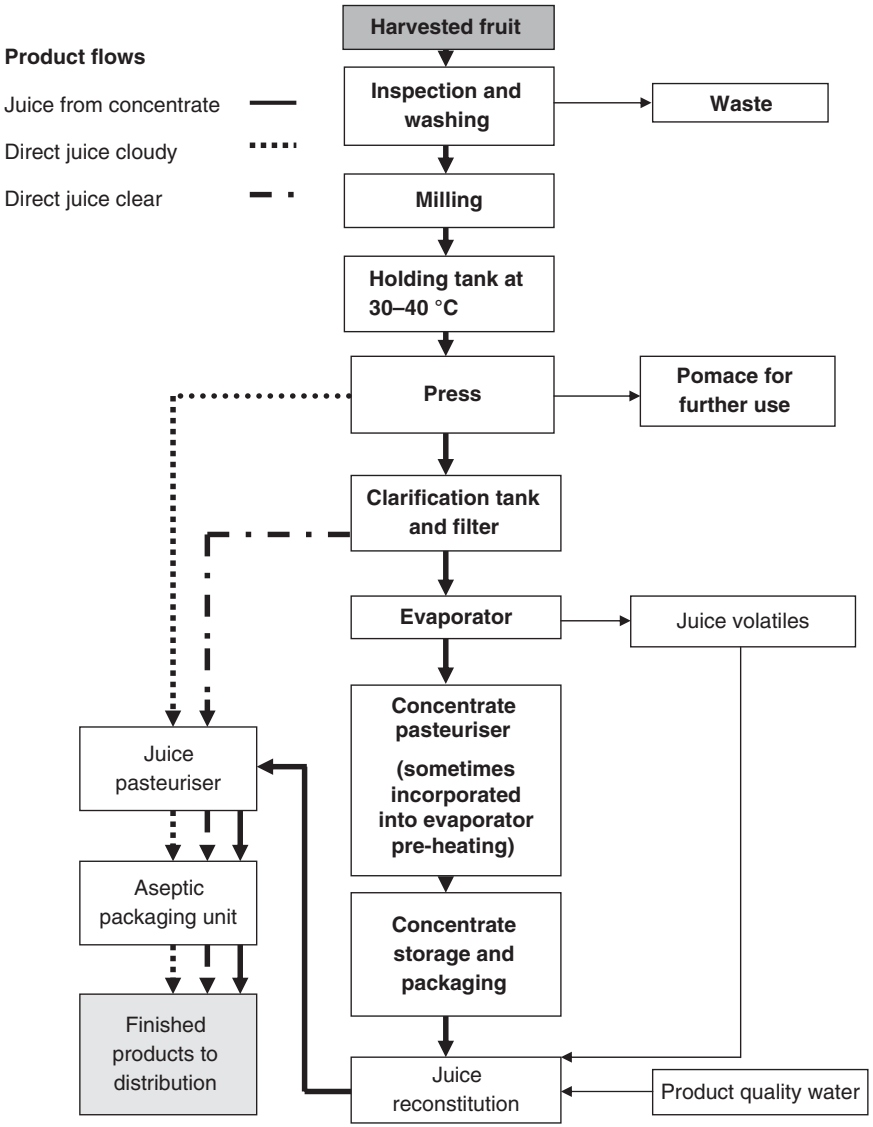

Fig. 12.1 Outline typical process for soft fruit processing.

America is the hammermill, or a variation of it, where fixed or free-swinging hammers force the fruit particles through a screen. The degree of disintegration is varied by type of hammer used, i.e. blunt hammers for impact disintegration or sharp hammers for chopping; diameters of the screen holes beneath the hammers; and hammer speed. Some processors believe this type of breakdown gives higher juice yields than other methods.

Another common system is the fixed knife mill. This consists of a chamber containing fixed knives and a rotating three-armed spider that shreds the fruit by forcing it against the fixed knives. Fruits are gravity fed into the chamber from an opening in the top. Small holes under the knives permit the mash to fall through to a conveyor.

Milling is essential for relatively hard fruit such as apples, whereas grapes and berries need only light crushing. Some soft fruit, such as raspberries and strawberries, are received frozen and must be defrosted prior to processing. Grapes and other fruit harvested in clusters are mechanically destemmed prior to crushing. For crushing, a series of intermeshed arms mounted on two cylinders shred the grapes as they pass through to produce a mash.

The pulp from stone fruit, such as apricots and peaches, must be removed in a way that prevents damage to the stone; the stone often contains components which can affect juice flavour and storage stability. A mill for stoned fruit typically contains hard rubber-lobed wheels that rotate together to force the fruit down and strip most of the pulp from the intact stone. An alternative process for peaches involves halving and pitting the fruit in a twist pitter, pulping the fruit halves, and heating the pulp to 82 °C in a steam cooker. A fruit disintegrator is then used to produce a purée which is put through a finisher. Such pulpy fruit purées can be treated with sugar syrup to produce nectar drinks containing 25–50% juice.

Enzyme treatment of pulp

Because of their cell structure, some fruits are not easily juiced by milling and pressing. Commercial pectolytic enzymes are widely used to break down cell structure and degrade pectins in the juice to improve extraction efficiency. The principal disadvantage to enzyme use is the cost relative to the additional juice yield obtained. For fresh apples and pears, efficient juicing can occur without the use of enzymes, but after a period of storage of the fruit, the cell structure changes and enzyme treatment is of greater benefit. Many other fruits benefit from enzyme treatment prior to juicing as well, including strawberries, cherries, plums, blackcurrants and raspberries. Fruit for processing is received either frozen or at ambient temperature. It usually must be mixed with the pectolytic enzyme and heated for 1–2 h for optimum pressing efficiency, e.g. 1 h at 15–30 °C for apples. By use of an appropriate cocktail of enzymes it is now possible to obtain almost complete liquefaction of fruit such as apples although the legal status of juice so obtained must be in question. This has been an increasing concern in recent years because the authenticity of fruit juices has become a major issue in many countries.

12.3.3 Citrus processing

In major producing countries, citrus fruits generally are transported from growing areas by truck for processing (Fig. 12.2). The truck is unloaded by

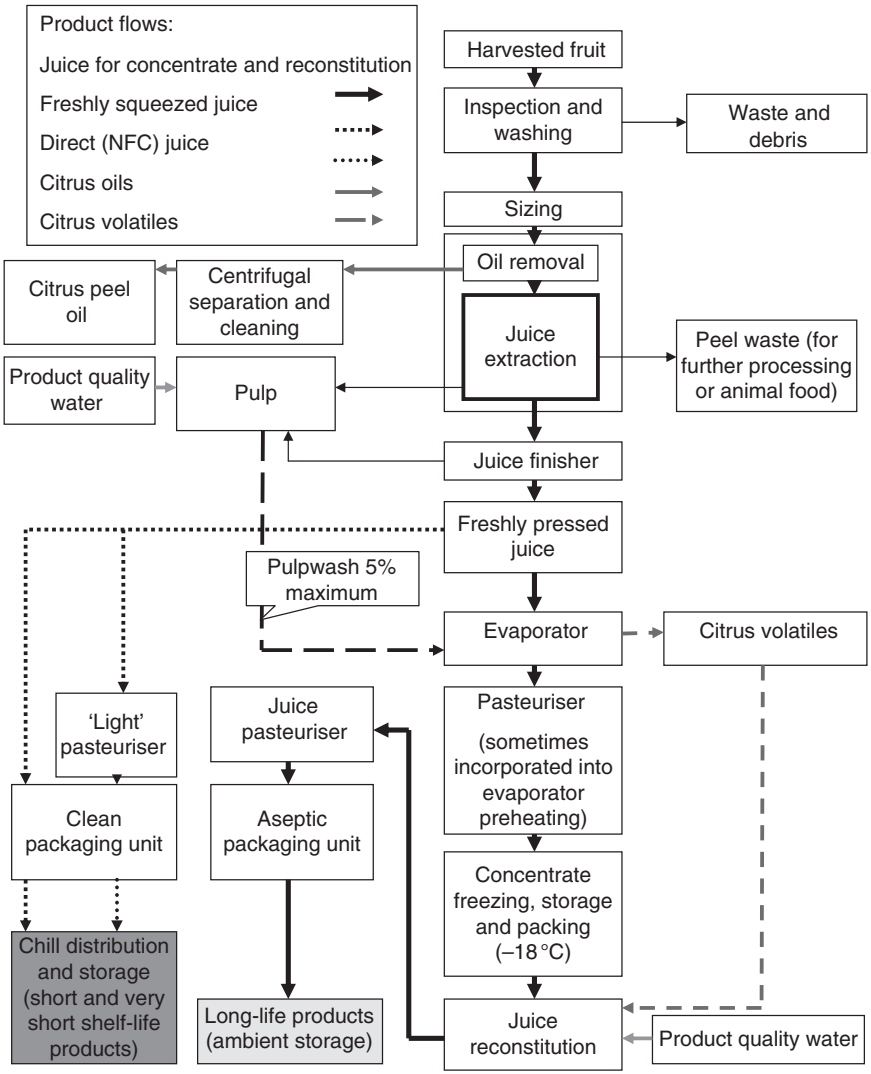

Fig. 12.2 Outline typical process for citrus fruit processing.

gravity feed to a conveyor belt that transfers the fruit to a storage bin. In the United States, an official government inspector checks an 18 kg sample of the fruit for minimum standards of dissolved solids (°Brix) and acidity before it is certified as meeting maturity requirements for processing. The fruit is washed, sometimes with a detergent, as it passes over roller brushes, then rinsed and dried. Washing removes debris and dirt, and reduces the number of microorganisms on the fruit thereby making subsequent juice pasteurisation processes more effective. Graders remove unwholesome

fruit as the fruit passes over roller conveyors and is segregated automatically into several sizes prior to juice extraction by one of several types of extractors. Use of computer-controlled sizing and grading of each load of fruit, based on fruit colour, size, shape and weight, is increasing. There is potential for total electronic grading and thus total automated quality control.

In one extractor (FMC Inc.), the fruit is located between two cups having sharp-edged metal tubes at their base and metal fingers that intermesh. The upper cup descends and the many fingers on each cup mesh to express the juice as the tubes cut holes in the top and bottom of the fruit. On further compression, the rag, seeds and juice sacs are compressed into the bottom tube between the two plugs of peel. A piston moves up inside the bottom tube forcing the juice through perforations in the tube wall. A simultaneous water spray washes the peel oil expressed during extraction away from the peel as an oil–water emulsion; the peel oil is recovered separately from the emulsion usually by a centrifugal process. Each extractor unit has several cups of a single size and in a typical factory installation banks of extractors process different fruit sizes.

In another type of extractor, the individual fruits are cut in half as they pass a stationary knife. The halves are oriented in a vertical plane, picked up by synthetic rubber cups, and positioned across plastic serrated reamers revolving in a synchronised carrier in a vertical plane. As the fruit halves progress around the extractor turntable, the rotating reamers exert increasing pressure and express the juice. The oil and pulp contents in the juice increase with greater reaming pressure. The recoverable oil is removed in a separate step prior to juice extraction. Needle-sharp spikes prick the peel of the whole fruit, releasing oil that is washed away with water and recovered from the oil–water emulsion. Most citrus juice extraction equipment is based on these two types of extractor although plant similar to that originating in the United States is now made in Europe.

In the extraction of citrus juices it is desirable to have as gentle an extraction pressure as possible. There should be minimal contact time between juice and pulp to reduce the amount of bitter substances expressed from the peel into the juice. The amount of suspended solids and pulp in citrus juice is controlled in a subsequent separation in a finisher. A screw action is used to force juice through a rotating perforated screen and separate the larger pulp particles from the juice. The oil level in the juice may, if required, be adjusted by vaporising under a vacuum. Separated pulp is washed and finished several times to produce a solution which is then either added back to the juice to increase juice yield, or concentrated to produce pulp wash solids, also called water extract of orange solids, which can be used as a base for cloudy beverages. The use of so-called pulp wash has been the subject of much debate in the juice authentication issue although it is now generally regarded as acceptable if added back in an ‘in-line’ process in small (<5%) quantities. Its use off line is not generally now acceptable for authentic juice.

Empirical methods for measuring pulp wash content have been developed using UV spectrophotometry.

Navel orange juice containing Limonin, grapefruit juice and pulp wash solids can be excessively bitter. A commercial process developed in about 1992 for debittering these liquids involves separating the pulp by centrifugation or ultrafiltration, passing the resulting juice through a column packed with a neutral resin to selectively remove the bitter juice components, and recombining the pulp with the debittered juice prior to concentration in an evaporator. The debittered juice or pulp wash solids are often used in blended orange or grapefruit juice products. Again, the issue of the authenticity of debittered citrus juice has been raised and differing views are taken in different producing countries on this point.

12.3.4 Membrane filtration

The use of membrane filtration in fruit processing is generally limited to the production of clarified juices. It is of particular significance where the final product is required to be as light as possible in colour and this is especially significant in the production of clarified lemon and lime juices. Traditional lime juice processing relies on the presence of the pectolytic enzymes that occurred naturally in the fruit which is washed, milled and allowed to stand in tropical conditions for periods of several weeks. During this time the natural clarification process takes place and the level of browning that occurred is limited by the presence of sulphur dioxide which is added after milling and maintained at a level of around 1500 to 2000 mg per litre. Following the period of standing and natural clarification the clear layer of juice is separated, polished by filtration and then concentrated to the required level. Traditional lime juice concentrates were shipped at ambient temperature, again relying on the presence of sulphur dioxide at around 1500 ppm to maintain the colour. Consumer demands for even lighter coloured juice coupled with legislative pressure to remove preservatives in general and sulphur dioxide in particular from juice-based products have become the driver for the introduction of alternative technology. Today most clarified lime juice and some lemon juice is produced by membrane technology and the resulting juices are a pale straw colour even after concentration. Where traditionally lemon and lime juices were stored at ambient temperature in the presence of sulphur dioxide, they are today mainly supplied as frozen and unpreserved concentrates.

Membrane technology has found little wider use partly because of its significant capital and running costs, the product losses that are inevitable and the limited range of juices that are extremely colour sensitive. Enzyme technology has now enabled clarified juices from soft fruit to be produced in higher yields that can be obtained by membrane filtration and at significantly lower cost. The colour of most soft fruit juices is often not as critical as that in the production of lemon and lime juices.

12.3.5 Concentration and aroma recovery

Most of the orange and grapefruit juice sold and transported internationally is as frozen concentrate, usually 60–66 °Brix. Because single-strength citrus juices generally are 8–15 °Brix solutions, a fivefold or greater concentration has occurred, making the concentrate a more economical product to freeze, store and transport. Apple juice is typically concentrated to around 70 °Brix and provided it is stored at a constant cool ambient temperature of around 10 °C does not need freezing to remain in a satisfactory stable condition.

Citrus juices are almost exclusively concentrated in a thermally accelerated short-time evaporator (TASTE) which has optimised efficiency for the amount of water removed per kg of steam used; e.g. in a seven-effect evaporator, 2.77 kg (6.1 lbs) of water are removed for each kg of steam used. Each effect consists of a bundle of tubes, with falling films of juice flowing inside, mounted inside a cylinder so that steam flowing into the first-effect cylinder can condense on the outside walls of the tube bundle as it transfers heat to vaporise a portion of the water contained in the juice. The hot water vapour thus produced is used to heat juice in the next effect and so on. This results in successively lower temperatures with each consecutive effect, starting at 98 °C in the hottest effect and reaching 45 °C in the last effect. Concentrated juice is discharged from the last effect to a vacuum flash cooler producing a product temperature of about 13 °C. The system is under vacuum with a progressively higher vacuum in each successive effect sufficient to cause water evaporation.

During water removal, much of the desirable flavour characteristic of the citrus juice is carried off with the vapour, especially in the early effects. Essence recovery units are used routinely to condense the volatile flavour substances as a water-phase essence, industrially termed aroma, and an oil-phase essence oil. These are valuable commercial flavour fractions saved separately to be added back later to concentrated juice. The concentrate is cooled quickly and stored at minus 10 °C in bulk vessels up to 500 000 litres or greater, refrigerated stainless steel tanks, i.e. bulk storage or tank farms. At the time a processor is ready to prepare a commercial juice pack for retail sale, concentrate from several batches in bulk storage is blended to achieve the desired sugar-to-acid ratio and colour. Water-phase essence and oil fractions are added to restore flavour lost during concentration. Most of the single-strength orange juice marketed worldwide is prepared by reconstitution of such blended concentrates to between 11.2 and 11.8 °Brix juice, followed by pasteurisation and chilling prior to retail packaging, usually in aseptic packs of 1.0 litre. In European markets there is a code of practice requirement to reconstitute orange juice to a minimum of 11.2 °Brix while in countries that follow the Codex guideline the minimum must be 11.5 °Brix.

A newer juice concentration process, requiring minimal heat treatment, has been applied commercially in Japan to citrus juice concentration. The pulp is separated from the juice by ultrafiltration and pasteurised.

The separated juice containing the volatile flavourings is concentrated at 10 °C by reverse osmosis and the concentrate and pulp are recombined to produce a 42–51 °Brix citrus juice concentrate. The flavour of this concentrate has been judged superior to that of commercially available concentrate, and close to that of fresh juice. Clarified citrus juices (other than lemon or lime) are not regarded as acceptable to most consumers in Europe or the United States.

A further process using freeze concentration has also been developed and has been used on a very limited commercial scale. Fresh juice is cooled to below 0 °C, and the water crystals that form are removed and washed by more juice. This process is repeated four or five times until the residual juice has a concentration of around 45 °Brix. The juice is said to have flavour characteristics that resemble those of the fresh juice. The economics of the process are said not to be favourable for most markets and applications.

Pasteurised single-strength orange juice, NFC or direct juice, often called premium orange juice in the United States, is increasing in sales worldwide with a corresponding reduction in the retail market share of frozen concentrated orange juice in the United States. NFC juice requires special extraction and storage conditions to ensure high quality. Juices are expressed with extractors designed to produce juice having low oil content (<0.035%). Juice must be chilled and stored aseptically or in frozen blocks to provide a year-round supply to packers.

Unpasteurised orange and grapefruit juices also are increasingly popular products in the United States and Europe. Fruit must be extracted under strict conditions of hygiene and the juice kept just above 0 °C during its approximately 17-day shelf-life. Year-round juice supply relies on the importation of fresh fruit from different growing countries according to the harvest season and it is often difficult to maintain consistency of flavour and Brix–acid ratio.

Apple and soft fruit juices are concentrated in a similar way to citrus juices with the volatile fractions being collected for later use. The efficiency of concentration processes can mean that it is possible to remove most if not all of the varietal flavour characteristics during the process. One consequence of this is that when juices are reconstituted, the aroma that is added back can be used to change the apparent variety of the original juice or more commonly to produce a standard blend for consumption that can be identified as a particular branded product yet can be described as a pure fruit juice, albeit reconstituted from a concentrate.

12.4 Final product processing

Leaving aside the relatively small proportion of the market for freshly squeezed (i.e. unprocessed) juices for rapid consumption, juice products must be processed to ensure protection from microbial spoilage. Enzymic

spoilage can also occur and processing technologies may need to deal with both potential problems. Processing varies depending on the packaging to be used and the shelf-life required.

12.4.1 Flash pasteurisation

Juices for which a long shelf-life are required (typically 12 months) are normally processed by flash pasteurisation and packaging in an integral aseptic packaging system. The largest market for such juices is in laminated board cartons such as those produced by Tetra Pak or Combibloc although systems are now available that will permit aseptic packaging in poly(ethylene terephthalate) (PET) and other plastic containers. For long-life products, typical flash pasteurisation conditions will involve heating the product in a recirculating plate pasteuriser to 85–90 °C for 15–30 seconds. The level of pasteurisation is calculated in pasteurisation units (PUs) and will depend on the assumed microbial load.

Pasteurisation units are calculated from the following:

$$\text{Number of PUs per minute} = 1.389^{(t \times 60)}$$

where t is the pasteurisation temperature in °C. A level of between 600 and 2000 PUs is normally satisfactory to ensure microbial stability in an aseptic system although this is dependent on the microbial load at the start of processing.

The current market trend towards consumption of so-called 'lightly pasteurised' products which are mostly NFC juices packed in clean but not aseptic conditions usually employs a very short pasteurisation time of a few seconds at a temperature of around 90–92 °C. By such a process a satisfactory microbial stability can be achieved for the shelf-life required (10–12 weeks) provided that products so treated are stored in cold conditions (2–5 °C). A benefit of such minimal pasteurisation conditions is that thermal damage to the flavour of the product is kept to a minimum and the juice is maintained in a stable state with a taste close to that of freshly pressed juice.

12.4.2 In-pack pasteurisation

The process of in-pack pasteurisation (often called tunnel pasteurisation because of the nature of the equipment used) involves packaging the product in its container and closure and then subjecting product and packaging to a process of preheating, to minimise thermal shock to the container, and then to a period of heating at pasteurisation temperature before cooling to ambient. Typical conditions for the pasteurisation are 20 minutes at 70 °C with shorter periods in the preheating and cooling sections of the operation. The process is used for juices and juice products packed in glass and can;

it has also been employed for some types of plastic container. The flavour of products so processed is usually affected to a greater or lesser extent by the prolonged period of heating although this is minimised by rapid and effective cooling by water sprays. If containers are not cooled to below about 25 °C and are then stacked in close proximity, the heat of the mass of products will cause significant damage to flavour and colour and reduce the effective organoleptic shelf-life.

Containers must also be dried effectively following the cooling process to avoid corrosion in canned products and to permit labelling of bottled products.

12.4.3 Hot packing

This is something of a hybrid process where modified plate pasteurisers similar to the equipment used for flash pasteurisation are employed to bring the temperature of product rapidly to around 85–90 °C. The hot product is immediately filled into its containers and closed allowing residual heat to pasteurise product, package and closure. Mechanical rotation of containers is necessary to ensure hot liquid comes into contact with all internal parts of package and closure. The process is very demanding in terms of energy and adequate cooling facilities must be used with similar needs for drying containers.

12.4.4 Refrigeration

In the United States in particular, there is a market for frozen freshly squeezed juices which, without any pasteurisation and when maintained at below –10 °C, have a long shelf-life with little flavour degradation. In many other markets freshly squeezed juices, with no other processing, are held at chill temperatures (2–5 °C) for very limited periods. As already indicated, the use of light pasteurisation coupled with chilled distribution and storage can be employed to give NFC fruit juices a shelf-life of many weeks.

Freezing is widely used for the transport and storage of citrus and concentrated soft fruit juices, other than apple, when products at around 60–66 °Brix may be held for considerable periods without significant deterioration of flavour or colour. In juices where natural pectolytic enzyme activity has not been suppressed some residual effect on the physical stability of the reconstituted juice may sometimes occur. Pectolytic activity can normally be destroyed by flash pasteurising juice for a very short time at around 95 °C before concentration.

12.4.5 Non-thermal processing

Although thermal effects on juices can be minimised by the use of very short heating periods, the organoleptic profile of pasteurised juices is slightly

different from juices that have not been heated. Various alternative processes that can be used to produce juices that have microbial stability are now available although the commercial exploitation of such processes is so far limited. The most promising technologies use high-pressure processing or irradiation.

High-pressure processing
Batches of product, which need to be packed in flexible containers, are placed in a pressure vessel and subjected to pressures of around 600 MPa. Current processes are labour and capital intensive and are limited by the necessity of batch operation, so the process is limited to premium products. Even then the benefits that can be achieved for freshly squeezed product only allow for an extension of shelf-life by a factor of two or three times that of unprocessed juice. The consumer benefit is thus limited with a significant increase in processing costs and consequent selling price. This technology is still under development in both academia and the commercial world but scope for its widespread adoption for relatively low-value products such as fruit juices is probably limited.

Irradiation
Although the use of gamma irradiation from a source such as cobalt 60 or caesium 137 is a very effective way of ensuring microbial stability of food and beverage products, the technique is permitted by the US Food and Drug Administration (FDA) for only a limited range of foods which does not include fruit juices or other beverages. In most other countries the use of gamma irradiation for foods is even more limited. However, the use of X-rays or high-energy electron beams is also very effective and may be more acceptable to consumers although consumer acceptance of any irradiated foods remains a major obstacle. Developments in this technology are progressing widely but are likely, in the short to medium term, to be limited to foods where pathogens constitute a significant microbial hazard. With few exceptions this hazard is not present in fruit juices.

12.5 Novel fruit juice-based products

At the time of writing, there are several principal areas of development interest for fruit juice-based products. These include the market for products that are perceived as pure and fresh; products that show developments in texture, principally exemplified by ‘smoothies’; products that are based on red fruits which can support claims for high levels of anti-oxidant activity; fortified juices and blends of juices with other products such as soy milk or whey. The development of fresh juice products in the widest sense is supported by a move by the UK Government to encourage individuals to consume ‘five-a-day’; i.e. five portions of fresh fruit and vegetables daily.

As indicated earlier, the need to ensure the authenticity of fruit juices is now regarded as a standing requirement for all juices. This requirement is in the light of the widespread sale of adulterated juices in the recent past. Although product safety was not at issue, consumers were widely misled over the quality of various products.

12.5.1 Fresh juices

Mention has already been made of freshly pressed juices that are described as direct or not from concentrate juice. However, the extremely short shelf-life of such products limits their market potential. Products that are made from direct juice but with minimal heat treatment supported by chilled storage and distribution are nevertheless growing in popularity, with a wide range of different types of citrus juices, tropical juice blends and products such as pomegranate in evidence. The marketing of organic and fair trade juices that have an appeal to the consumer with ethical concerns is an important and rapid growth sector of the NFC market.

12.5.2 Textured products – smoothies

This product area is based on the concept of pure juice products that show more texture than a simple fruit juice or blend of juices. The texture is achieved by including relatively high level of juice pulp and by incorporating into most smoothie products a base of banana purée. The range of fruits that can then be included within the '100% fruit' label is much wider than is possible with single juices because banana purée can be obtained with up to 20% sugars which can offset the use of much more acidic fruit juices such as raspberry and cranberry. Some interesting fruit blends are available as smoothies and imaginative marketing has helped to promote these products particularly for consumption by children. In addition to the presence of higher levels of natural minor ingredients such as ascorbic acid, smoothies are of nutritional interest as good sources of fibre.

12.5.3 Citrus comminutes

Citrus comminutes (Fig. 12.3) are a unique group of fruit materials that were initially developed in the 1950s. At its simplest, a citrus comminute is produced by taking a whole citrus fruit and comminuting it in a suitable mill. The resulting product would then contain all the juice, albedo, flavedo and peel oils that were present in the whole fruit. It was soon apparent that such a product was of limited value and was generally unacceptable for consumption as such. However, using the same principle, comminutes have been developed by taking the components of citrus from a normal juice processing operation and recombining them in different proportions according to the product required. Thus it is possible to enhance the flavour

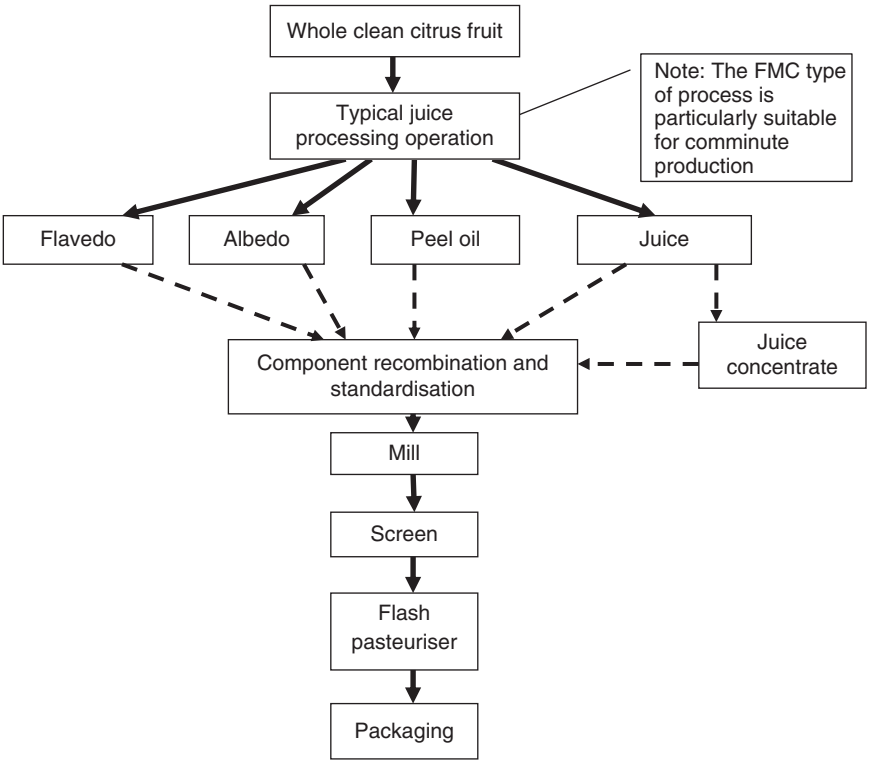

Fig. 12.3 Typical production outline for citrus comminutes.

intensity by ensuring that peel oils are recombined, the texture and cloudiness by addition of albedo and colour by addition of more flavedo components. It is also possible to produce concentrated comminutes by the use of concentrated rather than direct juice.

The components of a comminute are combined in the required proportions, milled to remove particulate material, screened and then subjected to pasteurisation. Citrus comminutes are not offered as single products for direct consumption but are used as ingredients in fruit-based drinks. Indeed, they originally gave rise to a group of products that were legally defined in the United Kingdom as 'whole fruit drinks' if they were in a dilute-to-taste formulation and 'citrus crushes' in a ready-to-drink formulation. Today citrus comminutes are probably used to a lesser degree than before but still offer a range of interesting and valuable raw materials.

12.5.4 Anti-oxidant products

In recent years much interest has been focused on products that have significant anti-oxidant levels. It is known that damaging free radicals, such

as singlet oxygen, can be produced in the human body by various means, such as from the effects of smoking. The presence of anti-oxidants which can absorb such free radicals is then an important protection that can reduce some of the free radical damage. A new quality parameter, 'oxygen radical absorbance capacity' (ORAC), is now being applied to fruit juices to indicate comparative levels of this activity. Much work is being carried out in this important area of the minor constituents of fruit juices but at present the quantitative significance of the ORAC measure and any claims that may possibly arise from the use of juices with a high ORAC value remain to be determined. ORAC values can be determined by measuring the amount of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) that reacts with a given quantity of a juice. The higher the amount of Trolox the higher the ORAC value. Another measure used to determine anti-oxidant effectiveness is the FRAP value (ferric reducing power of plasma) although this can only be determined indirectly following consumption of a particular product.

Of particular interest is that some red fruits have significantly higher ORAC values than juices such as orange, and that fruit such as prunes, which have been dried, appear to have the highest values although this may, to a greater or lesser extent, reflect the remaining moisture content.

12.5.5 Blended juice products

There is considerable interest in the opportunities presented by blending fruit juices with other natural products such as fruit, cream, soya milk, whey and similar dairy materials. There are a number of specific problems that must be overcome in individual formulations, such as the avoidance of curdling, and close control of pH is thus vital. Some products rely on the use of emulsifiers and stabilisers which can be used to great effect but may not be acceptable if the end product is to be designated as free from additives. Pasteurisation of such blended products may present particular difficulties because of the different materials used and the viscosity of the mixture. It may be necessary to employ a tubular pasteuriser which is usually a modified flash pasteuriser with holding tube that will allow the free passage of viscous products. Plant hygiene can also present difficulties when blends of juices and dairy products are put through the same machinery and many juice packers will not tolerate the presence of dairy materials on the same production site.

12.6 Future trends and opportunities

Developments in beverage formulations are often cyclical. Almost all products demonstrate a life cycle which today is often masked by the use of branding to develop range extensions to support the original product.

However, because of the commodity nature of pure single fruit juices the market for them appears very stable with little growth in individual countries. When considering fruit juices alone, the changes that occur in a market tend to be between the amounts consumed as NFC, which are increasing rapidly, and that consumed as reconstituted which for most markets is at best static or even showing slight decline. However, because the proportion consumed as NFC is generally small the overall effect of these changes on markets tend to be small. As disposable incomes rise and ethical considerations taken more into account by consumers, the consumption of premium juices such as freshly squeezed and other NFC products appears set to continue to grow. The sectors of that market which can be designated as organic or fair trade can be expected to show proportionately even greater growth. Another consideration that is beginning to be widely debated is the so-called 'carbon footprint' of products and the overall sustainability of their production. The effect on the market of these factors is as yet unquantified.

Beverage manufacturers will, however, continue to develop new products that can appeal to specific market sectors and juice blends with other natural products will continue be a focus of interest as many can be designated 'all natural' and in some cases organic.

There is too considerable interest in fortifying juices and blends with nutritional substances such as vitamins and minerals. Of these, vitamins A and C present the simplest fortification possibilities as they are naturally present in many juices. Vitamin C has the double benefit of protecting the product against oxidation damage. Vitamins from the B complex are more difficult to incorporate, as in many cases they have a significant organoleptic effect that is often out of character with the product.

Calcium is often selected as the preferred mineral for fortification but particular care must be taken because where the pectin components of a juice have been degraded, calcium is likely to bind to residual pectic acid to form a gel.

12.7 Sources of further information and advice

ARTHEY, D., and ASHURST, P.R., (Eds) 1996 *Fruit Processing*, Blackie, Glasgow.
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13

Isolated soy protein usage in beverages

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Abstract: Isolated soy proteins are an important category of plant-derived ingredients, which are used in a variety of healthy beverages today. The high nutritional quality and health benefits of these plant proteins are discussed. Beverage application recommendations are provided to optimize usage of isolated soy proteins in dry blended, ready-to-drink acid and ready-to-drink neutral beverages. Recent developments in soy protein processing and mixed protein beverages are highlighted as opportunities for future growth.

Key words: isolated soy protein, healthy beverage, food processing, soy protein nutrition.

13.1 Introduction

In today's food markets, beverages are more than refreshment or rehydration. They have become a frontier for the delivery of portable health and nutrition. Over the past 20 years, we have seen an explosion of beverages sold to virtually every consumer niche that marketers can discover. Protein, and specifically protein via isolated soy protein (ISP), has been formulated into many of these beverages. Consumers recognize the basic need for high-quality protein in their diets and also appreciate the health benefits that ride alongside.

Isolated soy proteins can be seen on the labels of many beverages today. Figure 13.1 (Lu, 2007) displays the number of beverages launched with protein ingredients and soy protein ingredients between January 2001 and October 2007. Depending on the region, soy protein was included in 9–53% of the beverages. These beverages span the nutritional market categories including infant nutrition, medical nutrition, sports performance, weight management, milk alternative, and senior adult nutrition (Paulsen *et al*., 2006). Some of these beverages contain multiple protein sources and isolated soy proteins are primarily economical alternatives to other

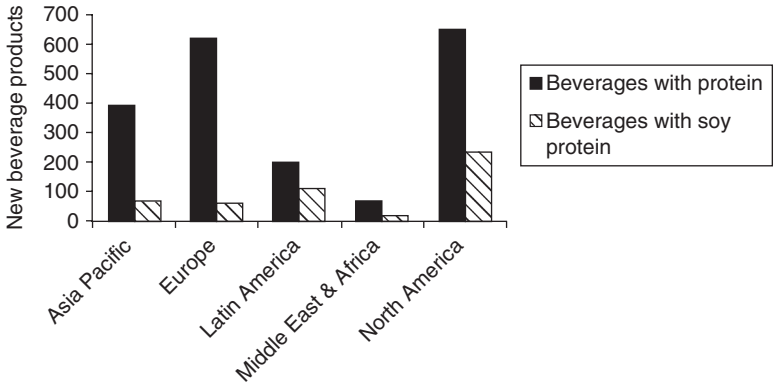

Fig. 13.1 Plot of new beverage launches with any 'protein' or 'soy protein' on the ingredient label for different world regions. The figure was created by Solae, LLC from data tracked by Mintel GNPD, Chicago between January 2001 and October 2007.

high-quality proteins. Certain of the products highlight the inclusion of soy protein and its health-promoting benefits on their labels.

Consumer research has shown that healthy consumers are looking for balance in their lives. Analyses of eastern and western diets suggest that balancing protein contribution, between plant and animal, may have a positive impact on overall health (Campbell and Campbell, 2006). Nutritional beverages with isolated soy protein can offer a convenient and pleasing option for the consumer to balance their protein intake. Another trend is snack drinks. People in Western society are on the move and often do not stop to eat. In order to satisfy healthy eating and well-being, the 'snack drink' market has developed (Smerz, 2006). Many of these recently launched 'snack drink' beverages contain fruit juice and soy, thus also offering many new and diverse tastes. Today beverages containing soy are positioned to appeal to all health-conscious consumers and not just those who are keen to avoid dairy products (Post, 2005). Modern technology has developed bland-tasting isolated soy proteins with low viscosity, so expanding the development potential for new beverages.

13.2 What is isolated soy protein?

ISPs are the primary protein components obtained from soybeans after the removal of oil, fiber and soluble carbohydrates. ISP is defined in most standards by a minimum protein content of 90%, dry basis. This is a simplistic definition primarily used for regulatory purposes. Throughout most of this text, I will refer to ISPs as a single entity; however in reality ISPs consist of various associated soy proteins which can vary in composition depending

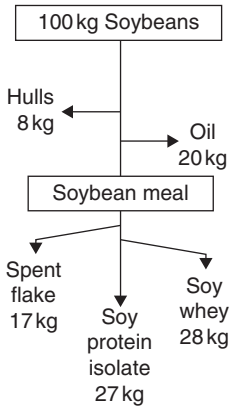

Fig. 13.2 Diagram of soy protein isolation process.

on the source beans and manufacturing process. Thus in practice, there are multiple ISPs from suppliers designed to deliver specific health and functional attributes.

Liu (1999) provides an extensive description of the protein isolation process, but as an introduction, a typical process is shown in Fig. 13.2. The hulls and oils are removed from the soybean creating an intermediary product, called 'soybean meal'. The soybean meal should contain less than 1% residual oil and will be characterized by having a high protein dispersibility index (PDI). At this point, the process may begin to deviate depending on the manufacturer, but in all cases the objective is to cause separation of the fiber (primarily cell wall) and carbohydrate (sucrose and various oligosaccharides) from the protein. The more common approach is to extract the soybean meal with alkaline water and separate the protein and carbohydrate from fibrous materials, called 'spent flake'. The protein is then separated from the carbohydrates (soy whey) by isoelectric precipitation at pH 4.5. The resulting isolated protein is washed with additional water, processed further to optimize functionality, and dried (Markley, 1950; Smith and Circle, 1978; Liu, 1999).

A new approach to obtain ISP utilizes combinations of centrifuges and membrane systems. The soybean meal is dispersed into warm water to solubilize the protein and carbohydrate; a similar separation of spent flake is done. The extract is next passed over membranes with selected pore sizes that retain the major proteins, but allow the smaller carbohydrates to pass. The proteins concentrate in the retentate; soy whey collects as permeate. As before, the isolated protein will be further processed to optimize functionality and dried.

The native soybean protein is a mixture of many different types of proteins. Some proteins are structural elements, some are enzymes, but

the majority is storage protein. Most of the structural elements and the enzymes are captured in the spent flake or soy whey during traditional processing. Isolated soy protein is primarily composed of storage proteins that have globular quaternary structures and it should be considered as an associated polymer of these. It is convenient to classify the protein mixtures by their sedimentation behavior upon dissociation. Commercially, ISP is primarily composed of denatured forms of 7S and 11S storage protein fractions.

The 11S fraction, primarily glycinin proteins, is quite different in amino acid composition and structure from the 7S, which are primarily conglycinin (beta and gamma) proteins. Owing to the differences in composition and structure, these protein fractions exhibit differences in functional properties. For example, 7S proteins show higher emulsifying capacity and emulsion stability, which would be an advantage in certain lipid-containing beverages. Soy protein researchers take advantage of these differences to optimize ISP performance via protein engineering and genetic improvements.

The manufacturer modifies the functionality of the ISP by four primary means. These are reduction/oxidation additives, ionic effects, enzymes, and thermal treatments. Reductions/oxidation additives are primarily sulfiting agents. Ionic effects are selective use of alkaline earths such as sodium hydroxide or calcium oxide. Enzymes are primarily proteolytic in specificity. Thermal treatments may range from vat milk pasteurization time/temperature to conditions of ultra-high temperature and short-time sterilization. These steps may be used singly or in combination to achieve the target functional property. The correct drying system operation is critical to retain protein functionality from the previous process steps and impart user friendliness (non-dusting, dispersibility, density control) to the ISP powder. The drying systems may include post-drying blending, oil or lecithin spray-on systems, and agglomeration capabilities. The specific treatments for a defined product are closely held trade secrets by manufacturers.

ISPs are the most purified form of soy protein ingredients available in the market. In general, ISPs are very low in flavor, highly digestible, and easy to use in food, beverage, and baking formulations (Golbitz and Jordan, 2006). For applications with discriminating needs for flavor and functionality, ISPs provide advantage over whole bean extracts, soy flour or soy concentrates.

13.3 Soy protein nutrition and health benefits

The soybean is a rich source of beneficial nutrients (Golbitz and Jordan, 2006). The oil is a quite healthful fat containing approximately 50% linoleic acid, an essential polyunsaturated fatty acid, and as much as

8% alpha-linolenic acid, which is an omega-3 fatty acid. Lecithin contains choline, which is required for normal liver function and nerve development (Miller, 2002).

Certain materials that were rejected as anti-nutritional factors in years past are now being reconsidered. Phytic acid, trypsin inhibitors, and Bowman Birk inhibitor were associated with inadequate mineral and protein absorption, but today they are potential agents for cancer risk reduction. Raffinose and stachyose were derided for their flatulence effect, but today they are considered fermentable carbohydrates for promotion of intestinal health (Golbitz and Jordan, 2006).

Soybeans also contain essential minerals such as iron, magnesium, and copper which enable certain metabolic enzymes. Soluble and insoluble fibers benefit digestion, increase transit time, and help slow delivery of carbohydrates and energy to the body. Phenolic compounds and vitamin E are potent anti-oxidants. The protein derived from soybeans also has nutrition and health benefits (Liu, 1999; Golbitz and Jordan, 2006).

13.3.1 Nutrition

ISP is a high-quality, plant-based protein that supports healthy growth and development. Soy protein is a complete protein in that it meets all the essential amino acid requirements to support normal growth and development of infants and children. Soy protein is also low in fat and free of saturated fat and cholesterol. It is an ideal protein source to boost the nutrient density of foods. Accordingly, ISP fits within the current recommendations of nutrition experts to focus on lean protein sources and nutritionally dense foods. When corrected for digestibility, soy protein, like dairy and eggs, has a protein digestibility corrected amino acid score (PDCAAS) of 1.0 (Mai and Lo, 2004), which is the highest attainable. Soy protein is the only vegetable protein that is a complete protein.

Beyond sustaining humans, research has recently focused on the ability of ISP to provide for muscle development in athletes or compromised individuals. Wilkinson *et al*. (2007) noted both soy-protein-based beverages and milk resulted in positive net protein balance after exercise. The consumption of either milk or soy protein with resistance training should promote muscle mass maintenance and gains. However, milk protein promoted a greater net positive protein balance than did soy protein. This effect was attributed to the rapid digestions and transit time of amino acids from soy protein; the response (for soy protein) was considered similar to milk whey protein. In a clinical study of kidney patients, Chen *et al*. (2005) served hemodialysis patients 30 g milk protein or ISP in a breakfast or post-dialysis beverage. He found no significant differences in nutritional parameters between the groups at the end of the study, indicating that when substituting soy protein (30 g/day) for animal proteins, the nutritional status of the hemodialysis patients could be maintained.

13.3.2 Health benefits

ISP is associated with several health benefits including heart health, cardiovascular health, weight management, menopausal symptom relief, and reduction of cancer risk. The processing used in preparation of ISP will have an impact on the delivery of health benefits, owing to alterations in the protein structure and to the removal or modification of beneficial compounds such as isoflavones. If health function is important for a beverage, it is very important to discuss the expected outcomes with your ISP supplier. Not all products will deliver similar health benefits.

Cholesterol lowering, and thus, the reduction of heart disease risk has been an area of research where ISP has been extensively studied since the 1970s. ISP has been shown to reduce total and low-density lipoprotein (LDL) cholesterol (the 'bad' type), while maintaining or slightly raising high-density lipoprotein (HDL) cholesterol (the 'good' type) in multiple studies. It was initially believed that the isoflavones were required along with the protein to lower cholesterol (Crouse *et al*., 1999); yet many other studies failed to show cholesterol lowering effects of isoflavones alone (Sitori *et al*., 2005), providing support for the cholesterol-lowering effect of soy protein itself. A very recent study, however, may shed some light on this controversy (Clerici *et al.*, 2007). It appears that isoflavones may play a role in cholesterol lowering for individuals who are 'equol producers' (their gut flora can convert the isoflavone daidzein into a more potent isoflavone, equol). Equol producers had a 4% decrease in serum cholesterol and 9% decrease in LDL cholesterol compared with non-equol producers (Clerici *et al*., 2007) when fed a pasta enriched with isoflavones but without soy protein. Soy isoflavones have been implicated for some of the improvements in artery health observed when soy protein is consumed (Steinberg, 2003).

ISP when used in combination with other heart healthy ingredients, 'a portfolio diet', can have an additive effect in reducing risk factors of cardiovascular disease (Jenkins *et al*., 2003). Indeed, when a low-saturated fat, low-cholesterol diet containing soy protein, plant sterols, oat fiber and nuts was fed to volunteers (see Fig. 13.3), LDL cholesterol decreased by nearly 30% and the ratio of LDL:HDL cholesterol, another indicator of heart disease risk, decreased by 25%. These changes were similar to the decrease observed in volunteers receiving drug therapy for cholesterol reduction. This additive effect has also been observed for blood pressure improvement in diets containing ISP and dietary fiber from *Psyllium* (Burke, 2001).

Some research suggests the protein health benefits may be derived from the peptides created upon digestion of ISP. Research by Rho *et al*. (2007) and Ishihara *et al*. (2003) report hydrolysate peptides from yellow soybeans and black soybean can inhibit the absorption of dietary lipids in rodent models. Black soybean peptides (BSP) caused significant prevention of body, liver, and epididymal adipose tissue weight gains over a caseinate diet. BSP showed lower total cholesterol and LDL/HDL ratios in serum. Also

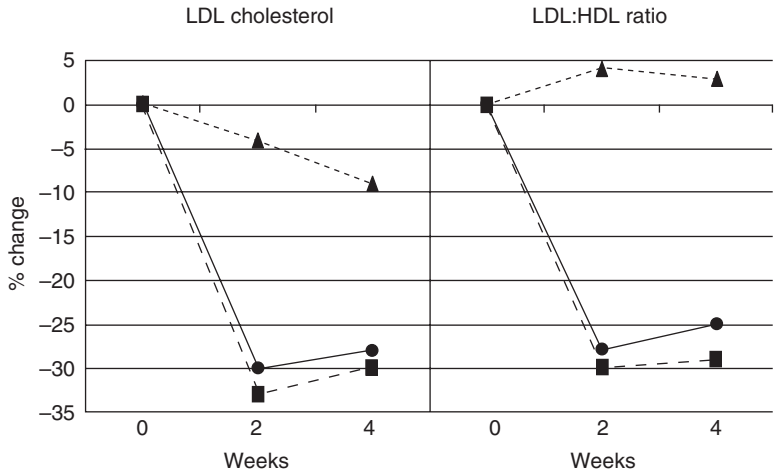

Fig. 13.3 Impact of low saturated fat, low cholesterol (portfolio) diet containing ISP, plant sterols, oat fiber, and nuts verses drug therapy on serum cholesterol: ▲ control low-fat, low-cholesterol diet; ● control plus portfolio ingredients; ■ control plus statins. Adapted from Jenkins *et al*. (2003).

there was a lower level of hepatic triglycerides and higher excretion of triglycerides in feces versus casein control diets. The authors suggest the peptide products might impact insulin and glucagon secretion to alter triglyceride metabolism. Vegetable proteins may enhance glucagon levels, which would down-regulate certain lipogenic enzymes and cholesterol synthesis.

Certain countries allow the declaration of health benefits associated with ingredients on labels for products. The first country to authorize a health claim for soy protein and heart disease was Japan in 1996. Three years later the health claim was authorized in the United States. Currently, nine countries have authorized health claim declarations for soy protein either for cholesterol reduction or reduction in risk of heart disease.

Protein is the most satiating macronutrient and studies show that if you feel full longer, you will eat fewer calories and thus lose weight. Weigle *et al*. (2005) showed how satiety can be related to weight loss. When subjects were moved to a diet with higher protein as a percentage of calories, the subjects felt fuller. When the subjects were allowed to consume a diet with higher protein *ad libitum*, they reduced their total calorie intake.

Soy protein helps reduce hunger in part by lowering a food's glycemic index. The glycemic index is a value given to a food based on its ability to raise blood glucose. The value actually represents the change that occurs in blood glucose for 2 hours after consumption of a test food containing 50 g of carbohydrate compared with a glucose solution providing the same carbohydrate load. Consuming lower-glycemic index foods throughout the day can help modulate blood sugar swings, which may help you feel more

satiated and energetic throughout the day. Thus, including high-quality protein such as soy protein at every meal can help control blood glucose swings, so keeping you alert and feeling full longer. Studies have indicated commercial beverages containing soy protein deliver low to medium glycemic index depending on the presence of fiber and type of carbohydrate (Blair *et al*., 2006; Torres y Torres *et al*., 2006).

Soy protein will provide effective nutrition in weight management programs. When obese and genetically disposed obese rodents were fed ISP or hydrolysate of ISP verses a casein control, the absorbability of dietary energy and fat was lower with both soy diets (Aoyama *et al*., 2000). Total body fat was also lower with soy protein or hydrolysate diets. Liao *et al*. (2007) investigated the relative performance of a traditional low-calorie (1200 kcal/day) diet and one based on soy foods for 8 weeks. Two-thirds of protein in the traditional meal replacement protocol was animal protein. The soy-based diet resulted in greater effect on reducing body fat percentage than the traditional diet. Also serum total cholesterol and LDL cholesterol were significantly reduced ($P < 0.05$). In another study, an ISP-containing commercial, meal replacer provided slight, but not significantly greater, weight loss over a 12 week period than milk-containing, meal replacer used in low-calorie diet programs (Anderson and Hoie, 2005). Weight loss achieved in a combined analysis was approximately 8.5%. Very low-energy diets have enabled many obese individuals to lose substantial amounts of weight and maintain these losses. Soy protein has been a part of such diets for over 20 years.

A negative physiological effect of menopause are hot flushes. The prevalence of hot flushes varies among women in different countries. Hot flushes are reported by 70–85% of menopausal US and European women. This is in contrast to Asian countries where less than 20% of menopausal women report hot flushes. Hormone replacement therapy (HRT) is a very effective treatment for relief of hot flushes; however, 80–85% of women in the United States refuse to take HRT. Soy is a staple in traditional Asian diets and the question was raised as to whether soy consumption may impact the lower incidence of hot flushes in women living in Asian countries. A study published by Nagata *et al*. (2001) examined whether soy food consumption was associated with the onset of hot flushes in 1100 Japanese women. Women were premenopausal at study entry and were tracked for 6 years. The group with moderate soyfood consumption (75 g of food/day) had approximately a 20% reduction in hot flushes, while the group consuming the greatest amount of soyfood had greater than a 50% reduction in hot flushes. This study suggested an inverse relationship between soyfood consumption and hot flush incidence.

Many clinical studies examining isolated soy protein have resulted in reductions in hot flushes of between 40 and 55% compared with reductions from placebo products of 20–35% (Albertazzi *et al*., 1998; St. Germain *et al*., 2001; Knight *et al*., 2001). These effects were related to isoflavone

quantity and composition (Willamson-Hughes, 2006). Reduction in the number of hot flashes seems to be dependent on the frequency of hot flushes in women (Messina and Hughes, 2003) and the isoflavone level contained in the soy protein.

Cancers of the breast, prostate, and colon are reduced in populations and subpopulations that consume higher levels of soy foods. For example, death rates from breast and prostate cancer are about four times lower in Japan than in the United States. Linking food consumption and cancer promotion or prevention is difficult since cancer is a multi-stage process of initiation, promotion, and proliferation. In spite of this, studies have been completed in simple systems which suggest an inhibitory role for ISP or its components on various cancers. Badger *et al*. (2005) reported that the incidence of induced mammary and colon prostate tumors could be significantly reduced (versus a casein-based diet) in rats by ISP. ISP may protect one from cancer by altering mammary gland differentiation, decreasing activation of pro-carcinogens to carcinogens, or regulating genes that signal transduction pathways.

A meta-analysis was completed in 2004 by Solae LLC of published cancer epidemiological studies and results published in Badger *et al*. (2005). The intent of the meta-analysis was to use a statistical approach to examine the relationship between soy protein consumption and cancer risk reduction from a set of published studies. Only published studies with reported odds ratios or relative risks (risk estimates) of cancer development and 95% confidence intervals were included in the analysis. A pooled analysis was calculated using a random-effects model in which the weighted effect measures were the log of the odds ratio or relative risk. Figure 13.4 presents the pooled risk estimates from three meta-analyses that subjects would develop the specified type of cancer. A value of 1.00 would indicate the treatment had no effect.

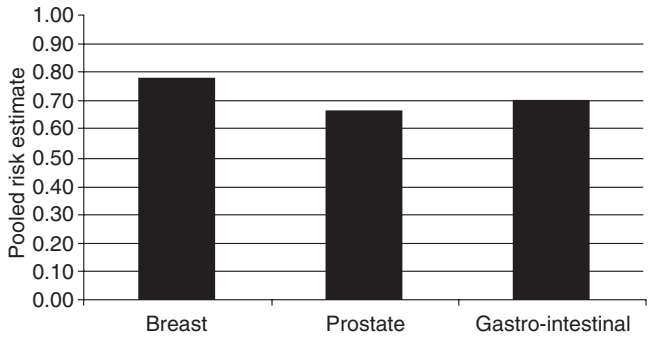

Fig. 13.4 Pooled risk assessment from three meta-analyses of published clinical studies on soy protein consumption and cancer prevention. Analyses considered 14 breast cancer studies on women (all ages), 6 prostate cancer studies on men, and 15 gastrointestinal studies on men and women. Adapted from Badger *et al*. (2005).

For breast cancer, soy protein-containing foods are associated with a 22% reduction (95% confidence interval 9–32%) in the risk of developing breast cancer compared to subjects consuming no or low amounts of soy foods. For prostate cancer, soy protein-containing foods are associated with a 34% reduction (95% confidence interval 19–46%) in the risk of developing prostate cancer. Finally, consumption of soy protein-containing foods is associated with a 30% reduction in the risk (95% confidence interval 20–39%) of developing cancers of the gastrointestinal tract. The estimated protein consumption required to deliver this impact was 5 g of ISP. The data further suggested that subjects consuming the greatest amount of soy protein had the lowest risk of these cancers.

Soy proteins will also provide advantages for nutritional beverages targeted to individuals with health problems. For example, patients on hemodialysis are at elevated risk for hyperlipidemia and atherosclerosis. Managing this is an important goal for hemodialysis patients. In the previously mentioned study by Chen *et al.* (2005), 80 hypercholesterolemic subjects received soy protein or casein (placebo) daily over a 4-week period. Hyperlipidemic subjects with the soy beverage had significantly decreased total cholesterol (–18.6%), triglycerides (–43%), LDL (–25%), and insulin levels (–49%). HDL was increased (17%). In a separate study, Teixeira *et al.* (2004) observed significant decrease in serum total and LDL cholesterol in type 2 diabetes patients with nephropathy consuming only soy versus milk protein. Moreover, subjects consuming soy protein had signs of reduced urinary albumin excretion compared with the milk protein. Thus, incorporating ISP into diets of renal patients maintains an adequate nutritional status, delivers blood lipid improvements and can positively influence factors associated with kidney disease progression.

13.3.3 Allergenicity

Food allergies are relatively rare, but some individuals experience violent reaction of the immune system to food proteins. The Food and Agriculture Organization of the United Nations includes soy proteins in its list of the eight most significant food allergens. Food processors must use good manufacturing processes whenever new allergens are introduced into their facility to manage cross-contamination. However, soy protein requires no greater diligence than other allergens. In fact, *in vitro* and blinded food challenge studies with susceptible subjects 'show lower allergenic reactivity for soy protein verses other food allergens' (Cordle, 2004). Investigations in animal models confirm these clinical studies of diminished immunological reactivity for soy proteins (Cordle, 2004).

Food allergy reactions to soy proteins do occur and so correct labeling of products must be enforced to assure susceptible consumers can avoid ingestion. There has been shown to be certain cross-reactivity for persons allergic to other plants such as birch pollen (Suss *et al.*, 2005). Birch pollen

cross-reactivity has also been observed for a range of plant materials including apples, pears, hazelnuts, potato, kiwi, carrots and celery. Processors can assure the non-presence of soy proteins by various analytical techniques such as polyacrylamide gel electrophoresis, capillary electrophoresis, high-pressure liquid chromatography, immunoblotting, enzyme-linked immunosorbent assay (ELISA), and other immunoassays. The development of monoclonal antibodies to detect 7S protein fractions may further improve the speed and accuracy of detecting specific soy epitopes with allergenic potential (Bittencourt *et al.*, 2005).

13.4 Formulating and processing of primary beverages

Proteins have physical roles in beverages that enable a satisfying consumer experience. The ISP must provide correct functionality for a selected beverage type. A common classification of beverages is by delivery form and by pH of final product (Paulsen *et al.*, 2006). In delivery form there are simply liquid ready-to-drink (RTD) products and dry blended beverages (DBB). In the first case the consumer takes home a bottle of processed food and the second the consumer purchases a powder in package that must be mixed into some fluid for consumption. For pH, it is useful to consider products that are used at approximately pH 7 (RTD-N or DBB-N) and approximately pH < 5 (RTD-A or DBB-A).

DBB products require ISP with high dispersibility and wettability, controlled density, controlled particle size, moderate solubility, and good flowability. RTD products require ISP with high solubility, good emulsification, low dusting (for processor), and controlled viscosity. The impact of pH on these relevant functionalities must then be considered. ISPs have by nature low solubility in the acid (pH 4–5) range and so they must be functionally enhanced by processing to deliver good RTD or DBB performance. Minerals added to a beverage can carry along soluble ions which can impact protein rehydration, also. The principal culprits are the divalent cations calcium and magnesium. Some ISP have been designed for less sensitivity to pH and minerals.

For the purposes of this text, I will define dry blended beverages as dry powders that are blended with liquid (milk, water, juice) by stirring, shaking, or whisking before consuming. These products are usually consumed immediately after preparation. ISP is included in the formulations for nutritional or health benefit reasons. Protein can impact the flavor, mouthfeel, density, and dispersibility of dry blended beverages, significantly.

Dry beverage powders can be prepared by combining the necessary ingredients in a wet state, homogenizing, heat processing, and spray drying. When lipid content is a criterion, the wet process method is preferred. This system generally creates the highest-quality product, but it is also most expensive. Dry blended products are prepared in much simpler operations

– usually only requiring a dry mixing system. Dry blended operations will be inexpensive, easily maintained, and requires minimally trained personnel. However, generally the product quality is not as high and the formula will require spray-on fat or a fat powder for any lipid requirements.

Formulating a dry blended beverage requires the developer to consider the overall nutrient delivery per serving, the manner of rehydration, the process mixing available, and the drinking experience desired. When considering the nutrient delivery and ISP, the expected protein concentration is a critical calculation. As added protein increases beyond 2% (hydrated basis), there will be a significant impact on viscosity (increase), which will in turn affect mouthfeel, flavor, and overall liking. Depending on the eating experience desired, refreshment or indulgent pleasure, the viscosity effect can be good or bad for a given ISP. Recognizing the expected impact and desired outcome will enable the developer to accurately communicate needs to the ISP supplier.

Additionally, a high-protein DBB (>25% protein in powder) will cause the powder density to be highly dependent on the density of the ISP. Density is an important powder attribute when considering packaging size. The ISP supplier must closely control the density within narrow limits for high-protein DBBs to prevent issues during filling (low density, cannot fit into package) or at the consumer (high density, package looks under-filled).

Flavor is always an important attribute for consumer acceptance. It will be affected by the proteins selected, rehydration media (common examples would be water, cow's milk, or fruit juice), color of the system and certainly the other ingredients. Flavor can also be negatively impacted by mouthfeel. Mouthfeel is a common problem in DBB and is defined as the physical sensation of the beverage in the mouth; this is characterized primarily by smoothness, grittiness, and viscosity. Mouthfeel is impacted by particle size of powder, shear during make-up, time allowed to make-up, and the reconstitution medium. Water make-up generally provides the best environment for good mouthfeel with ISP, whereas juice will be more difficult to deliver good mouthfeel due to its low pH negatively impacting ISP solubility. Milk will have an intermediate effect; available calcium ions can be a difficult environment for ISP rehydration.

Dispersibility is the ease with which the powder can be mixed into the recommended make-up liquid, resulting in a homogeneous dispersion. This property is often incorrectly assumed to be directly correlated with protein solubility. Highest solubility often results in poor dispersibility and highest dispersibility often results in lowest solubility. In most cases for ISP, the developer must accept a compromise between good dispersibility and good solubility, rather than reach for the extreme in either case. Dispersibility is influenced by other ingredients (especially available lipid), the temperature of the make-up liquid, the ISP percent inclusion in formula, and process (lipid incorporation, agglomeration, and surfactant application).

Table 13.1 Beverage dispersion and hydration conditions for isolated soy protein

Disperse ISP into soft water at 25–55 °C (77–130 °F) depending on shear of dispersing equipment
Hydrocolloid stabilizers may be added with ISP
Heat slurry to 77 °C (170 °F) and hold for 15 minutes

There are four primary steps in the processing of ready to drink nutritional beverages with ISP. These are dispersion/hydration, homogenization/stabilization, thermal processing, and packaging. ISP must be rehydrated from powder to a similar degree of solubility of that before drying. ISP powder, being a hydrocolloidal material, will repel water and prefer to adhere to itself. Thus early process steps must provide sufficient shearing to enable water to penetrate to each spray dried particle. Following this, the particle must be given sufficient time for water to penetrate. This penetration process, also called hydration, can be accelerated by heat and additional shearing energy. This process can be slowed or prevented by the environment into which the protein is dispersed. Low pH or high ionic environments are to be avoided during dispersion. It is normally better to hydrate under conditions which enable protein hydration; then adjusting the pH or ionic strength of the beverage will have reduced impact. Some recommended conditions for dispersing and hydrating ISP are provided in Table 13.1.

As mentioned earlier, minerals added to a beverage can impact protein stability. It is normal behavior to add ingredients that can tie up (chelate) divalent cations, to prevent negative reactions with protein. Monovalent ions such as sodium or potassium are rarely a significant issue, but the divalent ions calcium and magnesium are issues. As water hardness exceeds 100 ppm (as calcium carbonate), the use of chelating agents becomes very important for ISP performance. Phosphates and citrates are preferred ingredients for this. In addition to binding divalent ions, these ingredients aid in protein hydration and enhance suspendability in storage.

Homogenization helps the beverage achieve uniform fat distribution. It also improves protein and hydrocolloid hydration. The primary objective is to achieve particle size reduction. The outcome is a smoother, creamier body and, where required a more white color. Floury *et al*. (2002) examined homogenization (20–350 MPa) of emulsions stabilized by 11S soy proteins and found the droplet sizes of emulsions and coalescence stability were greatly reduced with homogenization. Pressures below 100 MPa appear sufficient for beverages. Very high pressures (above 150 MPa) will disrupt the tertiary and quaternary structure of globular proteins, leading to aggregation and very stable emulsions through the formation of a gel-like particulate network. This aggregation may be desirable for some foods, but would likely not be desirable in beverages. Similar protein denaturing

Table 13.2 Typical isolated soy protein beverage heat processing conditions

High-temperature short-time pasteurization:
–Temperature/time: 85–90 °C/15 seconds
Ultra-high-temperature process:
–Temperature/time: 129–143 °C/3–5 seconds (ESL)
–Temperature/time: 137–142 °C/4–17 seconds (shelf stable)
Retorted in-container process:
–Temperature/time: 121 °C/10 minutes

effects were observed for 7S and 11S proteins treated with high-pressure (100–600 MPa) environments (Zhang *et al*., 2005).

Thermal treatments kill the pathogenic and food spoilage organisms. The severity of heat processing determines the shelf-life of a product. In certain cases the point is to reach a commercially sterile product. Heat processing can significantly impact the final product characteristics – such as flavor, color, nutritional values, and stability. Proteins must be selected for heat stability. Examples of heat processing conditions are presented in Table 13.2.

The effect of heat treatment on the delivery of the desired health benefit in a beverage must also be considered. This can range from minimizing non-enzymatic browning and its impact on amino acids such as lysine or changes that might occur in bioactives. Hoie *et al*. (2006) reported loss of cholesterol-lowering effect from the ultra-high temperature (UHT) treatment of an ISP plus milk RTD-N versus a similar product delivered in a DBB. In another ISP containing, chocolate RTD-N, significant changes in isoflavone profiles were reported pre- and post-UHT processing (Hayes *et al*., 2004). These changes did not reduce the total isoflavone content, but isoflavone conjugates within a family did change in ratio to each other. These results suggest further investigation of the link between heat processing and delivery of soy health benefits would be desirable.

The product then is filled into packaging which must be correctly matched with the heat processing and expected delivery system. The types of packaging systems can be classified into non-aseptic, aseptic, and in-container sterilization. Combining pasteurization conditions for heat processing and non-aseptic package normally results in a 14 day, refrigerated shelf-life. Combining UHT sterilization conditions with non-aseptic packaging produces a 60-day, refrigerated shelf-life. Aseptic processing is normally combined with UHT sterilization to create products with a 6 month ambient temperature shelf-life. In-container sterilization is for products where the heat processing is done within the packaging and results in a shelf-stable beverage.

When the nutritional profile of a product is established, the developer must consider the product category and the country regulations for that category. For example, in the EU, there are three primary legislations that

Table 13.3 Three examples of different isolated soy protein beverage formulae

Ingredients	Percent of formula		
	DBB	RTD-A	RTD-N
Isolated soy protein	34.0	3.3	3.4
Water	–	68.0	90.1
Juice	–	20.0	–
Vegetable oil	–	–	1.0
Fat powder, SD	10.0	–	–
Fructose	21.9	–	–
Sucrose	–	8.0	4.8
Maltodextrin	17.5	–	–
Fructo-oligosaccharides	4.0	–	–
Soy fiber	5.0	–	–
Sodium citrate	–	0.2	0.3
Citric acid	–	to pH 3.8*	–
Flavor	3.4	+	+
Color	–	+	–
High methoxy pectin	–	0.5	–
Hydrocolloid stabilizer	2.0	–	0.4
Vitamin & mineral premix	2.2	–	+
Total	100.0	100.0	100.0

* Getting the product to the correct pH is more important than the exact amount of citric acid. Sufficient citric acid must be added to reach this pH.
+ Indicates ingredient is added, but addition level is typically below 0.1% of formula.

direct nutritional compositions as follows: PARNUTS – Foods for PARticular NUTritional purposeS Directive (1989 – modified 1996 and 1999), Weight Loss Directive (1996), and FSMP – Foods for Special Medical Purposes Directive (1999). Under the Weight Loss Directive (Commission Directive 96/8/EC on foods for use in energy-restricted diets for weight reduction) energy for a meal replacer should be 200–400 kcal. Then protein should be a 25–50% of calories, fat should be a maximum 30% of calories with a minimum 1 g/meal of linoleic acid, and fiber should be present in sufficient quantity to achieve 10–30 g/day. According to the directive, there are also requirements on protein quality which can be met by most ISP.

Table 13.3 provides example formulae for three types of beverages – an RTD-A, an RTD-N, and a DBB product. These formulae illustrate some of the formulation differences a developer would expect for protein beverages containing ISP. The DBB could be a generic weight-loss formula for dispersion into water; it would provide 210 kcal per 60 g with 30% of energy from protein and 24% from fat. The product would also contain 5.6 g dietary fiber per serving. In the cased of the DBB, all ingredients have been selected for dry addition. The need for lipid has been supplied by a spray dried fat powder of soybean oil. A mixture of carbohydrates which supply calories, sweetness and dietary fiber was selected. These materials must be of

compatible particle size with the ISP to prevent later segregation in the packaging, particularly if not packed by serving size. The stabilizer will be selected from hydrocolloids that may hydrate quickly and evenly in the desired medium.

Generally, there are three main types of soy protein containing RTD-A as follows:

1. Fruit-based products, containing low protein levels (0.5–2.0%). They are wellness and refreshment drinks in which soy is a 'plus'.
2. Fruit-based drinks containing higher protein levels (3–5%) where soy is a predominant ingredient. This could be a heart health claim beverage or a meal replacer smoothie. A higher viscosity than juice is normal. The RTD-A formula provided in Table 13.3 is an example.
3. Fortified soy fruit beverage containing valuable secondary soy plant extracts is the third category. Today the secondary ingredients are more prominent in the consumer focus. This mixed concept of native protein and secondary plant extracts is chiefly targeted toward special wellness themes, such as heart health, weight management, or energy (Smerz, 2006).

The RTD-A product in Table 13.3 contains both water and juice as liquid medium. Ideally, the soy protein and pectin will be dispersed and hydrated into the water, then have the juice added later after pH adjustment with the citric acid. Sodium citrate and citric acid are added to control final product pH. Frequently colors and flavors must be added to create the appropriate consumer appeal. ISPs selected for this application should have good acid pH solubility. High-methoxy pectin is selected for its ability to stabilize protein suspensions. Lam *et al*. (2007) examined the stabilization of simple soy protein systems and found high-methoxy pectin was better to prevent protein aggregation and sedimentation at pH 3.8. Pectins interact with soy proteins via electrostatic interactions at pH 3.8. Pectins, with higher charge (low-methoxy types), associate less with the protein particles than those with lower charge (high-methoxy types). Another stabilizer that has shown good acid stabilization effect and found use in some Asian beverages is soy soluble polysaccharides (SSPS) (Roudsari *et al*., 2006).

Some fruits contain high amounts of native pectin compounds. In these cases, the native pectin may negatively impact product quality and increase soy protein aggregation at low pH. For example, developments to add 1% isolated soy protein to tomato juice were unsuccessful owing to increased viscosity and increased dynamic modulus values. The authors proposed 'interractions occurred between the protein and pectins as well as all other particles dissolved in the colloidal tomato juice system, resulting in aggregate formation. The higher viscosity observed at higher shear rates in tomato juice with soy may be due to a further aggregation of the soy protein with pectins' (Tiziani and Vodovotz, 2005a). Later work (Tiziani and Vodovotz, 2005b) indicated that 1.5% soy germ did not show this effect. This may be

due to all the different components, including soy polysaccharides, in the soy germ material and their ability to alter the system interactions.

The RTD-A area has been very active for new product introductions in the past few years. There have also been several patent introductions to deliver improved product quality. Shen (2007) describes a process that maximizes high-methoxy pectin stabilization by separate hydration from soy protein in a US patent application. Patel *et al*. (2004) discloses the composition of an enteral formula delivering the best of soy and other plant nutrients from fruits in a US patent. A stable alcoholic, acidic soy beverage is presented in the World Intellectual Property Organization (WIPO) patent application from Van Dijk and Smulders (2005). Other patents on processing soy proteins for this application will be presented in Section 13.6.

The RTD-N is an all soy protein-based nutritional beverage, which could be formulated as a milk alternative, a soy milk, or a disease-specific adult beverage depending on the vitamin and mineral package. Water is the only hydrating medium, and sodium citrate is added to control pH and bind-up free calcium ions. Liquid vegetable oil can be emulsified into this beverage to align with a low-saturated fat position. The stabilizers recommended are iota and lambda carrageenans; sometimes mixtures of these with other viscosifying stabilizers such as microcrystalline cellulose or xanthan gum have been found effective. The soy protein should exhibit high neutral pH solubility, good emulsifying properties, and have heat stability. This type of beverage is normally UHT processed and aseptically packaged.

13.5 Optimizing beverage sensory qualities

The key to product success is all about good taste. Regardless of the health benefits touted by a new beverage, good taste is a requirement to obtain continued consumer purchase. In order to achieve good taste, one must first start with (and later end with) the consumer. Figure 13.5 contains a process chart for developing beverages with optimized hedonic performance. This process relies heavily on good sensory science linked to market research to provide the roadmap necessary for the beverage developer. Similar processes are described by Moskowitz (2007).

The process starts with a marketing brief. Although, not shown, this brief could have been developed by full-scale market research including focus group testing of concepts, or it may be done by Internet surveys and expert groups. Regardless of the method, the beverage developer must obtain from the marketing brief the main expected claims and the intended target consumer group. It is also important to understand how often the product will be consumed and in what quantity. Any hedonic sensory testing should be with the target consumer; this enables consumer-directed development.

The developer then must identify commercial products and create a range of prototypes that could occupy the potential market space described

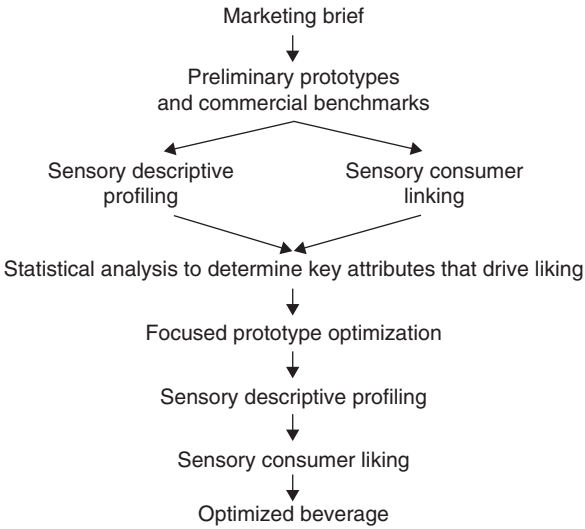

Fig. 13.5 Process flow for consumer directed development of beverage sensory qualities.

in the brief. Generally, it is important to obtain or create as wide a range as is feasible to represent diversity in sensory characteristics. In beverages, designed experiments that test the limits of viscosity, sweetness, saltiness, intensity of characterizing flavor, and color are needed at minimum. Whenever possible, it is best to use the minimum amount of active ingredients required to meet the fortification requirements for the product. The base should be neutral as possible, so always use the highest quality active ingredients. Also, select ingredients that are stable to oxidation. When requiring soy protein, ISP will meet these demands (Potter *et al*., 2007; Deshpande *et al*., 2005).

Next it is important to have a professional descriptive panel that has a developed lexicon that effectively describes the diversity found within the range of beverages that define the category. Russell *et al*. (2006) describes a lexicon for protein ingredients, Chambers *et al*. (2006) provides a lexicon to describe plain flavored soy milk beverages, and Childs *et al*. (2007) presents a lexicon for meal replacers that can serve as examples. This panel will be the measuring stick for beverage attributes. It is very useful to have a lexicon with identified standards for training and recalibration of the panel. Another panel of the target consumer, untrained, will be required to ascertain how much the beverage is liked. The size of the panel can vary depending on the demographic cross-section which must be sampled, but generally would need at least 100 members.

After sensory testing (both descriptive and hedonic) is completed, the data are analyzed for associations between attributes and liking. The output of this exercise is a correlation chart that indicates key attributes that drive

liking (or disliking) of the beverages. These key drivers then become focus points of further development – maximize the positive drivers and minimize the negative drivers. The developer then works in a loop with the sensory descriptive panel to address the attributes through formulation and process. When significant progress has been made to alter the attribute, as measured by the descriptive panels, the improved product is taken back to the target consumer panel for confirmation. Once products show achievement of market brief goals for consumer liking, the product optimization is completed and the product moves on to scale-up development.

The inherent flavors of a formula and the actual flavor that you add should never be separated in development; they need to work together in the finished product. The use of masking techniques may help bridge differences. An ideal masking flavor will tone down undesirable attributes in a beverage base without producing any characterizing aromas of its own. Another way to balance a characterizing ingredient is to use flavors that work well with an attribute. For example, cooked protein aromas go well with cooked brown flavors, caramel, chocolate, coffee latté, malt, and butterscotch. Generally these work well with soy protein RTD-N (Mai and Lo, 2004), although other flavors such as cream, almond, passion fruit, peach, apple, cereal, coconut, banana, and honeydew melon and cantaloupe have also produced good tastes. When addressing flavor, do not forget that appearance is the first thing that a customer notices.

The following are some examples of the links between liking and attributes for some soy protein beverages. Wang *et al*. (2001) described the difficulty of achieving a good tasting beverage with traditional soy milk. They reported an evaluation of 14 commercial soy milk samples and only 1 was considered moderately acceptable to panelists. In order to improve the acceptance of soy milks, they identified higher sweetness levels, addition of flavors (chocolate and almond were best), and gum addition (iota carrageenan) to mask some beany flavor in the soy-milk base.

Deshpande *et al*. (2005) wanted to obtain a best chocolate-flavored peanut–soy beverage formulation. They tested a three-component, constrained mixture design with peanut, soy (either ISP or soy flour), and chocolate syrup blend. Previous screening work to achieve lysine content, acceptable range of viscosity, and visual stability index (like suspension) were done to determine upper and lower boundary constraints in comparison to a commercial benchmark. Higher viscosity was not liked, but lower viscosity correlated with more beverage sedimentation. A compromise viscosity was selected to provide visual stability, but acceptable mouthfeel. Formulations prepared with ISP were better than those with soy flour, especially for the sensory attributes aftertaste, aroma, flavor, and overall acceptability. The mid-range of chocolate syrup was preferred. The best formulation (containing ISP) obtained overall ratings comparable to the target, and higher ratings for appearance, color, and sweetness.

Potter *et al*. (2007) described a 2×2 experimental design (with soy isolate versus soy milk, and juice concentrate versus brown rice syrup) to optimize product acceptance for a wild blueberry–soy beverage. Principal component analysis (PCA) was completed on the descriptive and liking sensory panels. Overall acceptability was strongly correlated with flavor. In PCA, there was strong positive correlations to sweet, sour, and blueberry flavor. Negative correlations were identified with painty, grassy, chalkiness, nutty, bitter, and creamy flavors. The ISP and juice concentrate rated highest with good blueberry flavor. Soy milk provided painty and nutty aroma with low blueberry attribute.

Childs *et al*. (2007) examined different vanilla meal replacement beverages made with soy protein, dairy whey protein, or combinations of soy and dairy whey proteins. Soy and whey had different contributing attributes. Prototype products with soy protein alone were characterized by cereal/grain flavors. Whey alone delivered higher sweet aromatic and vanillin, but also had metallic and bitter flavor. Soy beverages were mostly differentiated from whey beverages by texture and provided higher viscosity and more particles in mouth. Vanilla perception was decreased in the all soy beverages. Sensory scores were higher for whey protein beverages and combination soy and whey, than for soy protein alone beverages. These results identify beverage attributes that need further development to optimize soy protein usage for this meal replacement formula. The results are representative of the formulating challenges when optimizing beverage taste with soy and dairy proteins.

13.6 Processing soy protein for function

Progress has been made over the past 50 years to improve the functionality of ISP. There is a much wider variety of products targeted to different applications today; however, efforts to innovate have not stopped. Three key areas of development are very relevant to this chapter – enhanced health benefits, improved flavor, and increased acid pH suspension stability.

13.6.1 Enhanced health benefits

Kohno *et al*. (2003) claimed a composition of matter for ISP which has the ability to reduce fat accumulation and exhibits enhanced cholesterol-lowering benefits. The composition is dependent on a high level of 7S protein (>50%), a low level of residual phytic acid (<0.2%), and very low content of oil-body-associated proteins (<1%). Potential processes to achieve this composition are presented.

13.6.2 Improved flavor

Wong *et al*. (2007) has disclosed an ISP composition to deliver calcium along with good flavor (low levels of aroma compounds). The ISP is treated with a specific protease enzyme to a degree of hydrolysis (1.8–4%) and sufficient calcium (0.1–0.6%) to enable the improvement. This protein would have particular use in DBB due to its density (0.28–0.48 g/cm^3).

Astringency of protein beverages increases as pH is decreased; this attribute has been a negative driver of liking for RTD-A. Ishimoto *et al*. (2006) claimed a process to prepare protein with less astringency, by causing it to be coagulated and precipitated from solution. They believe astringency primarily occurs when soluble proteins interact with saliva proteins causing insoluble particles sensed as astringents. So by rendering the protein insoluble the astringent attribute is negated. The solution requires the addition of certain soluble soy polysaccharide, soluble basic salt, alkali metal salt of an organic acid, basic monosaccharide and basic oligosaccharide to a protein dissolved under acidic conditions.

Akashe and Melibach (2006) claimed a method to 'deflavour' soy protein for RTD-N soy milk or soy-milk smoothie beverages. The principles employed were alkaline (pH 9–12) extraction of flavor components, followed by removal with aqueous separations. Ultrafiltration and diafiltration are recommended to enable the aqueous separation of protein from undesirable flavor compounds. The analytical and sensory results indicate the resultant protein has fewer green, cardboard, nutty, grain, brown and astringent attributes.

One of the aromatic compounds from isolated soy proteins, methanethiol, can be traced to mixtures of manganese and sulfite reacting in the presence of oxygen with soy proteins (Lei and Boatright, 2007). Methanethiol results from the one-electron oxidation of methionine. The researchers have shown the reaction can be stopped by the addition of reducing process aids such as cystine or potassium iodate. Sulfite is sometimes used as a process aid for soy proteins, so this discovery may impact future practices.

Besides changing the protein, some companies are trying to alter the proteins perception (Gelski, 2007). Solae LLC has partnered with Senomyx to understand and control bitterness in soy applications and particularly in sports drinks, clear beverages, and acid beverages. The objective is to develop 'bitter blockers' that will prevent this undesirable taste. Senomyx will explore 25 receptors on the tongue and their reaction to components from soy proteins.

13.6.3 Increased acid suspension stability

A soybean beverage was patented by Kohno and Miyazaka (2007) that required an ISP with a defined 7S (<60%) and phytate (<0.2%) content. The product showed excellent stability in a weak acidic pH range (3.0–4.5). The protein would be prepared by fractionating and purifying

beta-conglycinin after phytase (or phosphatase) treatment. This process alters the solubility profile at different pH of the ISP. The pH range over which precipitation occurs is narrower and becomes slightly higher. The beverage made with this protein requires a hydrocolloid stabilizer such as pectin or soluble soy polysaccharides (preferably a 9:1 ratio of the two). The inventors reported excellent taste and mouthfeel for the beverage, as well.

A patent was also issued to Wong *et al*. (2006) for the use of phytase to alter the solubility characteristics of ISP. The ISP is described for use in RTD-A with specific hydrocolloid stabilizers and organic acid sources. The beverage will ideally have a pH of 3.0–4.5 pH for optimal performance. The claims of altered solubility with pH were reported beneficial for the beverage application.

Benitez *et al*. (2005) claimed to create an acid-soluble soy protein by the steps of heating, high shear and pH adjustments at specific times. Soy protein at neutral pH (6.8–7.4) is dispersed into water and then heated to high temperatures (65–140°C) with shearing force. The protein slurry is then reduced to pH 3.3 and again sheared; finally the pH is adjusted to pH 3.5–4.1 to make the beverage suitable for drinking.

In another disclosure, ISP was immersed in an aqueous environment with multi-anion salts (Wu *et al*., 2006). The protein was then heated and dried to retain functionality. The preferred operation required processing at pH 3.2–3.8. The multi-anion salts could be sodium citrate, sodium phosphate, or sodium sulfate. The resulting composition show improved solubility, suspendability, and stability in acidic (pH 3.0–3.8) environment, which would be useful for acidic beverages.

13.7 Combining different nutrient sources for advantage

There is much to be gained from combining ISP with other nutritional sources to create better tasting, more stable and greater health-enabling beverages. In Section 13.3, a 'portfolio diet' was discussed that could match cholesterol lowering performance of pharmaceutical statins (Jenkins *et al*., 2003). In Section 13.5, the sensory performance of soy proteins in a meal replacement beverage were improved in blends with whey protein (Childs *et al*., 2007). Soy and fruit each benefit from natural and healthy images, and if you put these together wellness themes take hold in the consumers' eyes (Smerz, 2006).

Future beverage development should investigate the complementary role of ISP with other additives. As an example, the combining of ISP with probiotics or with milk proteins could be fertile areas. The isoflavones in soy protein are typically present as glycosides. The more easily digested and biologically active isoflavone forms are aglycones. Pham and Shah (2007) tested the ability of two *Bifidobacterium animalis* strains to biotransform isoflavone glycosides in an ISP beverage. A conversion rate of 85% could

Table 13.4 Complementary health and nutrition benefits of isolated soy protein and whey protein in beverages

	Soy protein	Whey protein
High-quality protein	✓	✓
Benefits in weight management	✓	✓
Contain isoflavones, other botanical compounds associated with health benefits	✓	
Reduces cholesterol, can maintain healthy arteries	✓	
May reduce high blood pressure	✓	✓
Increases glutathione concentrations – may reduce the risk of cancer	✓	✓
Can increase muscle mass, when combined with exercise	✓	✓
Reduces inflammation in muscle following exercise	✓	

be achieved by combining the ISP with milk. In similar work, Tsangalis *et al*. (2005) attempted to enhance the level of aglycone form by feeding a ISP soy milk in combination with *Bifidobacteria animalis*. The fermentation again created more aglycone forms for consumption. Unfortunately, aglycone enhancement did not result in higher urinary levels of isoflavones with this study.

Whey protein and soy protein have complementary health attributes (see Table 13.4). Soy protein isolate and whey protein isolate are high-quality proteins (with a PDCAAS = 1.0). Soy protein significantly reduces cholesterol and may help maintain healthy arteries. Soy protein and whey protein may reduce high blood pressure. Both soy isoflavones in ISP and whey protein increase glutathione concentrations – a powerful antioxidant that may prevent cancer and retard the aging process. Soy and whey increase muscle mass and help muscles recover after exercise for improved athletic performance. Whey and soy also have complementary functional attributes. Whey protein provides good acid pH solubility. Soy proteins provide good emulsifying attributes and heat stability. The flavor of whey proteins alone has the negative attributes of metal and soap, whereas ISP produce cereal and roasted attributes (Russell *et al*., 2006); by combining the proteins the flavor negatives may be minimized, resulting in a more neutral base for certain beverage types. When formulating ISP into beverages, developers should be open to explore new combinations of functional ingredients for potential consumer advantages.

13.8 Future trends

Although soybean oil is a primary source of vegetable oil for human consumption, only a small portion of the residual soybean meal is processed

into human foods. As both environmental concerns and health concerns mount regarding consumption of animal proteins, the ability to transform soybean meal into healthy and tasty foods must develop. Steps are already underway to breed soybeans for better utilization in foods. Some of the key output traits being developed are flavor improvement (low lipoxygenase and low polyunsaturated oil), nutritional additives (omega-3 oils), protein increases, type of protein (7S:11S ratios), and carbohydrate changes (high sucrose). This genetic activity will impact the production and use of ISP; ISP manufacturers are working in close collaborations with seed producers to assess and prepare for handling these new crop varieties.

The investigation of how soybean materials and in particular ISP materials impact human health will continue, although at a reduced funding level than a decade ago. The rush to incorporate soy and its botanical extracts into clinical tests resulted occasionally in conflicting conclusions. Isoflavones alone are not the cause of health benefit effects; the complexity of the materials and the response of individuals must be closely understood and controlled for future studies. New techniques borrowed from the pharmaceutical industry will enable nutritional researchers to scan more components of ISP for bioactivity against biologically significant receptors, enzymes, and signal transfer mechanisms.

This work on genetics and nutrition will enable the development of new ISP products that provide excellent nutritional benefits at an economically viable cost. Processors will improve their ability to fractionate the parts of the soybean and will understand how to apply these parts into differing foods for benefit. In some cases, processors will do less in order to minimize impact on the environment; the use of alternative solvents such as liquid CO_2, lipase and carbohydrase enzymes, and membrane systems may change the current process of ISP. Today protease enzymes have a role in altering the physical characteristics of ISP and tomorrow this role will increase with more specificity obtained in the enzyme action and the expected outcomes.

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14

Sports beverages for optimising physical performance

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Abstract: Dehydration and substrate depletion are major factors in fatigue during prolonged exercise, and carbohydrate and fluid have independent and additive effects on performance. Sports drinks supply substrate in the form of carbohydrate as well as water to replace sweat losses, and have a clear role to play during any activity where fatigue is likely to influence the outcome. This applies as much to team games, where skill generally deteriorates in the later stages of the game, as to endurance running or cycling events. The optimum type and concentration of carbohydrate and the amount of sodium will depend on various factors including the intensity and duration of exercise, the ambient environmental conditions and on the individual physiology of the athlete. The basic formulation can be varied by the addition of other components such as proteins and amino acids as well as a wide range of other ingredients.

Key words: energy, hydration, fatigue, glucose, sodium.

14.1 Introduction: challenges of athletic performance

Many different factors contribute to success in athletic contests. Strength, speed, stamina and skill in varying combinations are the obvious physical requirements. Motivation, tactical awareness and strategy are equally important mental attributes. The successful athlete has a genetic endowment that confers an advantage and builds on this with a programme of training and rehearsal. At the highest levels of sport, all the competitors are genetically gifted and all are highly trained and highly motivated, with the consequence that the margin between victory and defeat is generally small. Nutritional interventions – in training as well as in competition – offer a low-risk strategy for performance improvement.

Nutrition support aims to allow the athlete to train intensively and consistently without succumbing to illness or injury, but is also intended to ensure that the adaptations to training occur as effectively as possible. In

competition, the aim is to address the factors – whether physical or mental – that limit performance and to minimise their negative impact. The limitations to exercise performance clearly depend on the exercise activity itself. In short-duration, high-intensity exercise, the limitations are quite different from those that apply during prolonged exercise. Where the exercise duration is less than about 30–40 minutes, there is little scope for nutritional intervention during the event itself, but post-exercise drinks consumed after training sessions lasting this long may help the recovery and adaptation processes. In exercise lasting longer than this, and certainly when the duration exceeds about 1 hour, there is scope for the ingestion of foods and fluids that can influence performance (Aragon-Vargas, 2001).

Dehydration and substrate depletion are major factors in fatigue during prolonged exercise, and Below *et al*. (1995) showed that provisions of carbohydrate and fluid have independent and additive effects on performance. Sports drinks, which can supply substrate in the form of carbohydrate as well as water to replace sweat losses, have a clear role to play during any activity where fatigue is likely to influence the outcome. This applies as much to team games, where skill generally deteriorates in the later stages of the game, as to endurance running or cycling events. The optimum type and concentration of energy substrates and the presence or otherwise of electrolytes and other components give an infinite number of possible formulations. More recent additions to the array of commercial formulations available have included other components such as proteins and amino acids as well as a wide range of other, sometimes exotic, ingredients.

14.2 Formulation of sports drinks

Sports drinks should be formulated to be effective in improving performance, but there is no universal agreement on the most effective formulation. This perhaps reflects the diversity of situations in which these drinks are used and the various factors that contribute to efficacy. Drinks must taste good if they are to be a commercial success, and the importance of taste in encouraging consumption among even the most serious athletes is an important consideration. The basic sports drink formulation, however, is extremely simple: the essential elements of a sports drink are water, sugar and salt. The major factors that can be manipulated to alter the functional characteristics of sports drinks are the carbohydrate (sugar) concentration and the type of carbohydrate used, the electrolyte content, the osmolality and the flavouring components.

14.3 Carbohydrate content: concentration and type

Carbohydrate ingested during exercise enters the blood glucose pool and serves as a fuel for the working muscles and for the brain, supplementing

the body's limited endogenous stores (Coyle, 1997). In situations where performance is limited by the availability of carbohydrate, either from the endogenous liver or muscle glycogen stores, exercise capacity should be improved when carbohydrate is consumed. When glucose or maltodextrin are used, there appears to be an upper limit of about 1 gram per minute to the rate at which ingested carbohydrate can be oxidised, even when much larger amounts are ingested (Wagenmakers *et al*., 1993). This may be a result of limitations in the rate at which carbohydrate-containing drinks can be emptied from the stomach during exercise or from a limitation to intestinal absorption, as more recent studies have shown that the rate of exogenous carbohydrate oxidation can be increased by using combinations of different carbohydrates (Jentjens *et al*., 2005; Wallis *et al*., 2005).

It is sometimes said that drinking water alone may be as effective as drinking carbohydrate-containing drinks, implying that hydration is the key factor. This, however, is not supported by the available evidence and there are several studies showing that carbohydrate drinks are more effective that water alone (Maughan *et al*., 1996). Work from Coyle's laboratory (Fritzsche *et al*., 2000) suggests that ingestion of a commercial sports drink was more effective in attenuating the progressive decline in peak power-generating capacity that occurred during a prolonged (122 minute) exercise session in the heat than was the ingestion of the same volume of plain water. Ingestion of the carbohydrate alone gave the same response as the placebo trial. This supports the idea that maintenance of hydration should be the first priority during prolonged exercise in the heat.

As well as providing an energy substrate for the working muscles, the addition of carbohydrate to ingested drinks will promote water absorption in the small intestine, provided the concentration is not too high. Because of the role of sugars and sodium in promoting water uptake in the small intestine, it is sometimes difficult to separate the effects of water replacement from those of substrate and electrolyte replacement when carbohydrate–electrolyte solutions are ingested. Below *et al*. (1995) showed that ingestion of carbohydrate and water had separate and additive effects on performance capacity, and concluded that ingestion of dilute carbohydrate solutions would optimise performance. Most reviews of the available literature have come to the same conclusion (Maughan, 1994; Shirreffs *et al*., 2004; Cheuvront *et al*., 2007).

In most of the early studies, the ingested carbohydrate was in the form of glucose, but the type of carbohydrate does not appear to be critical, and glucose, sucrose and oligosaccharides have all been shown to be effective in maintaining the blood glucose concentration when ingested during prolonged exercise, and in improving endurance capacity (Maughan, 1994). There are theoretical advantages in the use of sugars other than glucose. Substitution of glucose polymers for glucose will allow an increased carbohydrate content without an increased osmolality, but the available evidence suggests that the use of glucose polymers rather than free glucose does not

alter the blood glucose response or the effect on exercise performance (Ivy *et al*., 1979; Coyle *et al*., 1983; Maughan *et al*., 1987; Coggan and Coyle, 1988; Hargreaves and Briggs, 1988), and similar effects are seen with the feeding of sucrose (Sasaki *et al*., 1987) or mixtures of sugars (Murray *et al*., 1987; Mitchell *et al*., 1988; Carter and Gisolfi, 1989). Taste issues may be more important than other qualities, as high-glucose concentrations may be perceived as being too sweet.

Some studies have suggested that orally ingested long-chain glucose polymers are more readily used by the muscles during exercise than are glucose or fructose (Noakes, 1990), but others have found no difference in the oxidation rates of ingested glucose or glucose polymer (Massicote *et al*., 1989; Rehrer, 1990). Massicote *et al*. (1989) also found that ingested fructose was less readily oxidised than glucose or glucose polymers. Mixtures of glucose and fructose in equal amounts seem to have some advantages: when ingested in combination there is an increased total exogenous carbohydrate oxidation (Adopo *et al*., 1994). Fructose in high concentrations is generally best avoided on account of the risk of gastrointestinal upset. The arguments advanced in favour of the ingestion of fructose during exercise (namely that it empties rapidly from the stomach and provides a readily available energy source but does not stimulate insulin release and consequent inhibition of fatty acid mobilisation) is in any case not well founded: insulin secretion is suppressed during exercise. These studies were reviewed by Maughan (1994). There may be benefits in including a number of different carbohydrates, including free glucose, sucrose and maltodextrin: this has taste implications, which may influence the amount consumed, and, by limiting the osmolality and providing a number of transportable solutes, may maximise the rate of sugar and water absorption in the small intestine (Shi *et al*., 1995).

The optimum concentration of sugar to be added to drinks will depend on individual circumstances. High carbohydrate concentrations delay gastric emptying, thus reducing the amount of fluid that is available for absorption, but increase the rate of carbohydrate delivery. If the concentration is high enough to result in a markedly hypertonic solution, a transient net secretion of water into the intestine will result, and this will actually increase the danger of dehydration. High sugar concentrations (>10%) may also increase the risk of gastrointestinal disturbances (Davis *et al*., 1988). Where the primary need is to supply an energy source during exercise, increasing the sugar content of drinks will increase the delivery of carbohydrate to the site of absorption in the small intestine. Beyond a certain limit, however, simply increasing carbohydrate intake will not continue to increase the rate of oxidation of ingested carbohydrate, although it is not entirely clear where the limit lies (Wagenmakers *et al*., 1993). Dilute glucose–electrolyte solutions may also be as effective in improving performance as more concentrated solutions (Davis *et al*., 1988), and adding as little as 90 mmol/l glucose (1.6%) may improve endurance performance as effectively as more concentrated solutions (Maughan *et al*., 1996).

Much interest was generated by a study showing that just rinsing the mouth with carbohydrate-containing solutions may be effective in improving performance (Carter *et al*., 2004), suggesting the presence of carbohydrate receptors in the mouth. This was not confirmed, however, by a subsequent study in another laboratory (Whitham and McKinney, 2007), so further investigation is required to understand the mechanisms involved.

14.4 Osmolality

It has become common to refer to carbohydrate–electrolyte sports drinks as isotonic drinks, as though the tonicity was their most important characteristic. The osmolality of ingested fluids is important as this can influence both the rates of gastric emptying and of intestinal water flux: these processes together will determine the effectiveness of rehydration fluids at delivering water for rehydration. An increasing osmolality of the gastric contents will tend to delay emptying, and increasing the carbohydrate or electrolyte content of sports drinks will generally result in an increased osmolality. The composition of the drinks, and the nature of the solutes is, however, of greater importance than the osmolality itself (Maughan, 1994).

Although osmolality is identified as an important factor influencing the rate of gastric emptying of liquid meals, there seems to be rather little effect of variations in the concentration of sodium or potassium on the emptying rate, even when this substantially changes the test meal osmolality (Rehrer, 1990). The effect of increasing osmolality is most consistently observed when nutrient-containing solutions are examined, and the most significant factor influencing the rate of gastric emptying is the energy density (Brener *et al*., 1983; Vist and Maughan, 1994). Substitution of glucose polymers for free glucose will result in a decreased osmolality for the same carbohydrate content, and this may be one way of maximising availability of carbohydrate without compromising fluid uptake. This has led to the inclusion of glucose polymers of varying chain length in the formulation of sports drinks. Vist and Maughan (1995) have shown that there is an acceleration of gastric emptying when glucose polymer solutions are substituted for free glucose solutions with the same energy density: at low (about 40 g/l) concentrations, this effect is small, but it becomes appreciable at higher (180 g/l) concentrations; where the osmolality is the same (as in the 40 g/l glucose solution and 180 g/l polymer solution), the energy density is shown to be of far greater significance in determining the rate of gastric emptying. This effect may therefore be important when large amounts of energy must be replaced after exercise, but is unlikely to be a major factor during exercise where more dilute drinks are taken.

Water absorption occurs largely in the proximal segment of the small intestine, and; although water movement is itself a passive process driven

by local osmotic gradients, is closely linked to the active transport of solute. Osmolality plays a key role in the flux of water across the upper part of the small intestine. Net flux is determined largely by the osmotic gradient between the luminal contents and intracellular fluid of the cells lining the intestine. Absorption of glucose is an active, energy-consuming process linked to the transport of sodium. The rate of glucose uptake is dependent on the luminal concentrations of glucose and sodium, and dilute glucose–electrolyte solutions with an osmolality which is slightly hypotonic with respect to plasma will maximise the rate of water uptake (Wapnir and Lifshitz, 1985). Solutions with a very high glucose concentration will not necessarily promote an increased glucose uptake relative to more dilute solutions, but, because of their high osmolality, will cause a net movement of fluid into the intestinal lumen (Gisolfi *et al*., 1990). This results in an effective loss of body water and will exacerbate any pre-existing dehydration with potentially negative implications for exercise performance. Other sugars, such as sucrose (Spiller *et al*., 1982) or glucose polymers (Jones *et al*., 1983, 1987) can be substituted for glucose without impairing glucose or water uptake, and may help by increasing the total transportable substrate without increasing osmolality. In contrast, iso-energetic solutions of fructose and glucose are isosmotic, and the absorption of fructose is not an active process in humans: it is absorbed less rapidly than glucose and promotes less water uptake (Fordtran, 1975). The use of different sugars which are absorbed by different mechanisms and which might thus promote increased water uptake is supported by recent evidence from an intestinal perfusion study (Shi *et al*., 1995).

Most of the popular sports drinks are formulated to have an osmolality close to that of body fluids (Murray and Stofan, 2001), and are promoted as isotonic drinks, but there is good evidence that hypotonic solutions may be more effective when rapid rehydration is desired (Wapnir and Lifshitz, 1985). Although it is argued that a higher osmolality is inevitable when adequate amounts of carbohydrate are to be included in sports drinks, the optimum amount of carbohydrate necessary to improve exercise performance has not been clearly established. Hypotonic sports drinks are now beginning to appear on the commercial market.

14.5 Electrolyte composition and concentration

The available evidence indicates that the only electrolyte that should be added to drinks consumed during exercise is sodium, which is usually added in the form of sodium chloride, though other sodium salts may have some advantages (Maughan, 1994). Sodium will stimulate sugar and water uptake in the small intestine and will help to maintain extracellular fluid volume. Most soft drinks of the cola or lemonade variety contain virtually no sodium (1–2 mmol/l); sports drinks commonly contain about 10–30 mmol/l; oral rehydration solutions intended for use in the treatment of diarrhoea-

induced dehydration, which may be fatal, have higher sodium concentrations, in the range 30–90 mmol/l. A high sodium content, although it may stimulate jejunal absorption of glucose and water, tends to make drinks unpalatable, and it is important that drinks intended for ingestion during or after exercise should have a pleasant taste in order to stimulate consumption.

When the exercise duration exceeds 3–4 h, sweat losses are likely to be high, especially in warm climates. If circumstances permit the ingestion of large volumes of fluid, there may be advantages in adding sodium to drinks to reduce the danger of dilutional hyponatraemia. Physicians dealing with individuals in distress at the end of long-distance races have become accustomed to dealing with hyperthermia and hypernatraemia associated with dehydration, but it has become clear that a small number of individuals at the end of very prolonged events may be suffering from hyponatraemia. The reported cases have been associated with ultramarathon or prolonged triathlon events, or with the slowest participants in standard marathon events; most of the cases have occurred in events lasting in excess of 5–6 h, and there are few reports of cases where the exercise duration is less than 4 h (Almond *et al*., 2005). Noakes *et al*. (1985) reported four cases of exercise-induced hyponatraemia; race times were between 7 and 10 h, and post-race serum sodium concentrations were between 115 and 125 mmol/l. Estimated fluid intakes were between 6 and 12 l, and consisted of water or drinks containing low levels of electrolytes; estimated total sodium chloride intake during the race was 20–40 mmol.

Frizell *et al*. (1986) reported even more astonishing fluid intakes of 20–24 l of fluids (an intake of almost 2.5 l/h sustained for a period of many hours, which is in excess of the maximum gastric emptying rate that has been reported) with a mean sodium content of only 5–10 mmol/l in two runners who collapsed after an ultramarathon run and who were found to be hyponatraemic (serum sodium concentration 118–123 mmol/l). It has been argued that there is no benefit to ingestion of drinks containing sodium in terms of a reduced risk of hyponatraemia (Hew-Butler *et al*., 2005), but there is evidence from laboratory studies that addition of salt to ingested drinks will slow the rate of decline of the plasma sodium concentration when large volumes of fluid are ingested (Vrijens and Rehrer, 1999). Some supplementation with sodium salts may be required in extremely prolonged events where large sweat losses can be expected and where it is possible to consume large volumes of fluid. Most carbohydrate–electrolyte sports drinks intended for consumption during prolonged exercise contain sodium at a concentration of about 10–30 mmol/l, which is rather lower than the normal sweat sodium concentration (Table 14.1). While the formulation of these drinks might represent a reasonable strategy for providing substrates and water (although it can be argued that a higher sodium concentration would enhance water uptake and that a higher carbohydrate content would increase substrate provision), these recommendations may not be appropri-

Table 14.1 Approximate concentration, in mmol/l, of the major electrolytes present in sweat, plasma and in intracellular (muscle) water in man. The values are collated from a variety of sources (see Maughan, 1994, for further details)

	Plasma	Sweat	Intracellular
Sodium	137–144	10–80	10
Potassium	3.5–4.9	4–8	148
Calcium	4.4–5.2	3–4	0–2
Magnesium	1.5–2.1	1–4	30–40
Chloride	100–108	20–70	2

ate in all circumstances. Palatability may be an issue at higher sodium concentrations, but it is possible, with a suitable choice of electrolytes, to formulate drinks with a high (up to 100 mmol/l) sodium concentration that are not unpalatable.

Restoration of fluid and electrolyte balance after exercise is an important part of the recovery process, especially if another training session or competition must follow after a short time interval. Several studies have investigated the effects of ingestion of water or of commercially available drinks on restoration of fluid balance after exercise-induced dehydration. Costill and Sparks (1973) showed that ingestion of a glucose–electrolyte solution after dehydration resulted in a greater restoration of plasma volume than did plain water: a higher urine output was observed on the water trial. Gonzalez-Alonso *et al*. (1992) confirmed that a dilute carbohydrate–electrolyte solution (60 g/l carbohydrate, 20 mmol/l Na^+, 3 mmol/l K^+) is more effective in promoting post-exercise rehydration than either plain water or a low-electrolyte diet cola: the difference between the drinks was primarily a result of differences in the volume of urine produced. Similar results were obtained by Nielsen *et al*. (1986). In none of these studies, however, could the mechanism of this action be identified, but they did establish that, because of the high urine flow that ensued, even drinking large volumes of electrolyte-free drinks did not allow subjects to remain in positive fluid balance for more than a very short time.

If large volumes of plain water are consumed rapidly after exercise-induced dehydration, a marked fall in plasma osmolality and in the plasma sodium concentration ensues, and both of these effects will stimulate urine output (Nose *et al*., 1988a,b). Ingestion of plain water also removes the drive to drink by causing plasma osmolality and sodium concentration to fall. This makes it difficult to achieve complete rehydration when fluid intake is on a volitional basis.

The importance of the addition of sodium to rehydration fluids was systematically evaluated by Maughan and Leiper (1995) who dehydrated subjects by the equivalent of 2% of body mass by intermittent exercise

in the heat: subjects then ingested a volume equal to 150% of the mass loss of one of the test drinks over a 60 min period and were followed for a further 6 h. The test drinks contained 0, 25, 50 or 100 mmol/l sodium. Urine output over the subsequent few hours was inversely proportional to the sodium content of the ingested fluid: only when the sodium content exceeded 50 mmol/l were the subjects in positive sodium balance, and only then did they remain in positive fluid balance throughout the recovery period.

These observations were confirmed in a further study that systematically varied the volume of fluid ingested as well as the sodium content of rehydration drinks administered after induced hypohydration (Shirreffs *et al*., 1996). Even drinking large volumes (twice the sweat loss) did not allow subjects to remain in positive fluid balance for more than 2 h when the sodium content of the drinks was low (20 mmol/l): increasing the sodium content to 60 mmol/l, however, allowed subjects to remain well hydrated when volumes equal to 1.5 times or twice the sweat loss were ingested.

It is clear from these studies that rehydration after exercise can be achieved only if the sodium lost in sweat is replaced as well as the water, and it might be suggested that rehydration drinks should have a sodium concentration similar to that of sweat. However, the sodium content of sweat varies widely (Table 14.1), and no single formulation will meet this requirement for all individuals in all situations. The upper end of the normal range for sodium concentration (80 mmol/l), however, is similar to the sodium concentration of many commercially produced oral rehydration solutions (ORS) intended for use in the treatment of diarrhoea-induced dehydration, and some of these are not unpalatable. The ORS recommended by the World Health Organization for rehydration in cases of severe diarrhoea has a sodium content of 90 mmol/l, reflecting the high sodium losses that may occur in this condition (Farthing, 1994). By contrast, the sodium content of most sports drinks is in the range of 10–30 mmol/l and is even lower in some cases; most commonly consumed soft drinks contain virtually no sodium and these drinks are therefore not ideal when the need for rehydration is paramount. The problem with high sodium concentrations is that this may exert a negative effect on taste, resulting in reduced consumption.

It has been speculated that inclusion of potassium, the major cation in the intracellular space, would enhance the replacement of intracellular water after exercise and thus promote rehydration (Nadel *et al*., 1990). Experimental investigation of this suggested that inclusion of potassium is as effective as sodium in retaining water ingested after exercise-induced dehydration (Maughan *et al*., 1994). Addition of either ion will significantly increase the fraction of the ingested fluid which is retained, but when the volume of fluid ingested is equal to that lost during the exercise period, there is no additive effect of including both ions as would be expected if they acted independently on different body fluid compartments.

Potassium is normally present in commercial sports drinks in concentrations similar to those in plasma and in sweat, but there is little evidence to support its inclusion. Although there is some loss of potassium in sweat (about 3–7 mmol/l; Shirreffs and Maughan, 1997), an increase in the circulating potassium concentration is the normal response to exercise: increasing this further by ingestion of potassium does not seem useful. The concentration of potassium in most sports drinks (about 3–6 mmol/l, or about 0.12–0.24 g/l) is close to that present in sweat, but the amounts are small compared with the total daily intake of potassium for normal sedentary adults in the United Kingdom (about 3.2 g; Gregory *et al*., 1990). Replacement of losses will normally be achieved after exercise from the potassium present in foods: the potassium content of milk is about 35 mmol/l, while tomato juice contains about 55 mmol/l.

Commercially available sports drinks intended for use by athletes in training and competition are generally rather similar in their electrolyte content, suggesting a consensus, at least among the manufacturers, as to the requirements for electrolyte replacement. Some drinks are now available in different formulations (e.g. 'endurance formulation') that often have increased sodium concentrations, and some companies produce sachets of electrolytes that can be added to existing product formulations to increase the electrolyte content. These modifications go some way to addressing the need for individualisation of rehydration strategies to cater for differences in sweat composition between individuals and also to allow for different drinks in different environmental conditions.

There is some debate as to the need to add magnesium: this is added in some countries (e.g. Germany) to products that are sold elsewhere without added magnesium. In spite of the commonly held belief that exercise-induced cramp is associated with a falling plasma magnesium concentration, there is little or no experimental evidence to substantiate this belief (Maughan, 1986). A slight decrease in the plasma magnesium concentration is generally observed during exercise, but this seems to be the result of a redistribution of the body magnesium stores, and there seems to be no good reason for its addition to drinks consumed during exercise (for review, see Maughan, 1994).

14.6 Flavouring components

Taste is an important factor influencing the consumption of fluids. The thirst mechanism is rather insensitive and will not stimulate drinking behaviour until some degree of dehydration has been incurred (Hubbard *et al*., 1990). This absence of a drive to drink is reflected in the rather small volumes of fluid that are typically consumed during exercise: in endurance running events, voluntary intake seldom exceeds about 0.5 l/h (Noakes, 1993). Sweat losses normally exceed this, even in cool conditions and a fluid deficit is

therefore almost inevitable. Several factors will influence palatability, and the addition of a variety of flavours has been shown to increase fluid intake relative to that ingested when only plain water is available. Hubbard *et al*. (1984) and Szlyk (1989) found that the addition of flavourings resulted in an increased consumption (by about 50%) of fluid during prolonged exercise. More recently, Bar-Or and Wilk (1996) have shown that the fluid intake during exercise of children presented with a variety of flavoured drinks is very much influenced by taste preference: under the conditions of this study, sufficient fluid to offset sweat losses was ingested only when a grape-flavoured beverage was available. In many of these studies, the addition of carbohydrates and/or electrolytes accompanied the flavouring agent, and the results must be interpreted with some degree of caution.

Given the need to add electrolytes to fluids intended to maximise the effectiveness of rehydration, there are clearly palatability issues that influence the formulation. Effective post-exercise rehydration requires replacement of electrolyte losses as well as the ingestion of a volume of fluid in excess of the volume of sweat loss (Shirreffs *et al*., 1996). When sweat electrolyte losses are high, replacement with drinks with a high sodium content can result in an unpalatable product. This can be alleviated to a large degree by substituting other anions for the chloride that is normally added. The addition of carbohydrate has a major impact on taste and mouthfeel, and a variety of different sugars with different taste characteristics can be added.

14.7 Future trends: other active ingredients

As our understanding of the factors that limit exercise performance is extended, so opportunities arise for novel nutritional interventions. It seems unlikely that the basic composition of the sports drink will change greatly, but some recognition of the need for different drinks in different situations and for different individuals may lead to alternative formulations.

There is already a growing trend for the formulation of sports drinks to be modified to include other components which might affect the functional characteristics of the drink. This raises many important issues, including not only efficacy, but also safety, stability and palatability (Horswill, 2000). Many of the drinks aimed at the active individual include a range of vitamins and minerals, but it is widely agreed that these are not generally necessary. There is also little convincing evidence for beneficial effects of the addition of purported ergogenic compounds such as taurine, ginseng or aspartate. There is good experimental evidence to support the use of caffeine, and some evidence that the addition of glycerol, protein and amino acids may confer benefits in some situations.

14.7.1 Glycerol

The difficulties of achieving an adequate fluid intake during many competitive sports events and the growing recognition of the negative effects of hypohydration have led to an increasing emphasis on ensuring an optimum hydration status before exercise begins. Given the small volumes that it is normally possible to consume during exercise, there would seem to be benefits from ingesting fluid before exercise begins. Ingestion of large volumes of fluid, however, are likely to invoke a diuretic response, which is undesirable in the pre-exercise period. Water balance is largely controlled by monitoring of the circulating osmolality: this in turn is determined largely by the plasma sodium concentration, as sodium is the major circulating osmotically active particle, and the osmoreceptor may be in the form of a sodium receptor. Changes in osmolality cause alterations in output of antidiuretic hormone (ADH) by the pituitary; ADH controls the reabsorption of water by the distal tubule of the nephron and by the collecting duct, thus determining the urine flow.

Some degree of temporary hypervolaemia and hyperhydration result when drinks with high sodium concentrations are ingested, and there may be benefits of this blood volume expansion for the endurance athlete (Luetkemeier *et al*., 1997). The acute effect of ingestion of saline solutions is, however, transient and is rapidly corrected by the appropriate renal response. Attempts have been made to induce a state of relative hyperhydration prior to exercise by administration of glycerol solutions: ingestion of glycerol results in a marked rise in the extracellular glycerol concentration, and thus osmolality (Gleeson *et al*., 1986). This has the effect of increasing total body water, but results in an increase in plasma osmolality, and so may be considered to result in a relative hypohydration, even though total body water is increased (Sawka and Pandolf, 1990). The elevation of the osmolality of the extracellular space may result in water movements from the intracellular space, and cell dehydration, resulting in tissue shrinkage, is a well-recognised consequence of the administration of large amounts of glycerol.

Several studies have recently reported that ingestion of glycerol together with water can elevate the plasma osmolality and increase the total body water content, and that there may be benefits for thermoregulation and exercise performance (Riedesel *et al*., 1987). Lyons *et al*. (1990) gave subjects glycerol plus water or water alone 2.5 h prior to a 90 min exercise test at 60% of $V_{O_{2max}}$ in a hot (42 °C) dry (25% relative humidity) environment. The addition of glycerol decreased urine output over the trial and resulted in an increased sweat rate and a smaller rise in rectal temperature during the exercise period: there was also a tendency, although not statistically significant, for heart rate to be lower on the glycerol trial. Freund *et al*. (1995) have also reported an enhanced fluid retention and reduced renal flow when glycerol was added to drinks ingested at rest, and proposed that this effect might be mediated by an effect on ADH output.

There have also been some reports of improvements in the ability to perform prolonged exercise after glycerol administration (Montner *et al*., 1996; Anderson *et al*., 2001). Montner *et al*. (1996) found that prior administration of glycerol (1.2 g/kg) plus water resulted in significant improvements in time to exhaustion compared with a water-alone trial in two exercise tests lasting about 90 min: the improved exercise performance was associated with a lower heart rate during exercise and a smaller rise in core temperature. Anderson *et al*. (2001) found a lower heart rate and core temperature during 90 minutes of exercise in the heat after ingestion of a glycerol solution compared with a water-only control: power output in a 15 minute cycle ride which followed was higher on the glycerol trial. Scheett *et al*. (2001) have also shown that addition of glycerol to fluids ingested after exercise-induced dehydration improves exercise performance in an exercise test carried out 3 hours later. More recent studies have generally not confirmed these observations, though in some cases this may be due to lack of sensitivity of the exercise test or lack of statistical power of the study (Wingo *et al*., 2004).

The recommendation that endurance athletes competing in the heat should ingest glycerol and water prior to exercise, or that glycerol should be added to drinks, might be premature at this stage, but the evidence of benefits is accumulating. Further developments in this area are awaited with interest.

14.7.2 Protein and amino acids

Protein is not a major fuel for oxidative energy supply during exercise, but there is an increased rate of protein oxidation during prolonged exercise. It has generally been assumed, however, that there is no reason to add protein to drinks intended for consumption before, during or immediately after exercise. In the recovery period, muscle glycogen synthesis is a priority, but synthesis of new proteins should perhaps be seen as being of equal or even greater importance. Because little attention has been paid to this area, it is not at present apparent what factors may be manipulated to influence these processes.

Protein synthesis is stimulated for some time after exercise, and this reduces the size of the free amino acid pool within the muscle cells. These amino acids provide the essential building blocks for incorporation into new proteins, and a reduced concentration in turn restricts the rate of synthesis of new proteins. Administration of as little as 6 grams of essential amino acids after a bout of strength training exercise can increase the rate of protein synthesis (Rasmussen *et al*., 2000). If this effect persists in the longer term, the net effect is an improvement in muscle protein balance.

It is increasingly being recognised that cell volume is an important regulator of metabolic processes (Waldegger and Lang, 1997; Lang *et al*., 1998), and there may be opportunities to manipulate this to promote synthesis of

both proteins and glycogen in the post-exercise period. During and after exercise there may be large changes in cell volume, secondary to osmotic pressure changes caused by metabolic activity, hydrostatic pressure changes, or by sweat loss. Alterations in cell volume induced by changes in osmolality are well known to alter the rate of glycogen synthesis in skeletal muscle (Low *et al*., 1996). Amino acid transport into muscles is also affected by changes in cell volume induced by manipulation of the trans-membrane osmotic gradient: skeletal muscle uptake of glutamine is stimulated by cell swelling and inhibited by cell shrinkage (Low *et al*., 1997), and the intracellular glutamine concentration appears to play an important role in a number of processes, including protein and glycogen synthesis (Rennie *et al*., 1998).

The full significance of these findings for the post-exercise recovery process and the roles they play in adaptation to a training programme remain to be established. Manipulation of fluid and electrolyte balance and the ingestion of a variety of osmotically active substances or their precursors offer potential for optimising the effectiveness of a training regimen. There are clearly implications for the formulation of sports drinks designed to promote recovery and enhance adaptations to training. The increasing availability of water-soluble protein hydrolysates and peptides has made it easier for manufacturers to add a protein source to drinks.

One option that seems likely to develop is the use of milk-based drinks: there is evidence that milk-based drinks can be effective in promoting recovery between successive exercise bouts (Karp *et al*., 2006). This effect is probably due in part to the effective restoration of muscle glycogen stores, but milk-based drinks are also effective in promoting recovery of fluid balance due to the relatively high electrolyte content (Shirreffs *et al*., 2007). Low-fat skim milk varieties have potential benefits, but the lactose that these drinks contain may be less effective in promoting water uptake in the small intestine, and may be less available for oxidation than other sugars such as glucose or sucrose (Burelle *et al*., 2006).

14.7.3 Branched chain amino acids

The subjective sensations of fatigue that accompany prolonged exercise are generally considered to be the result of events occurring in the muscles or the cardiovascular system, but there is growing evidence that the signals that arise in the periphery are modulated by events occurring within the central nervous system; this is generally referred to as the central fatigue hypothesis (Davis, 1996; Maughan *et al*., 2007). This hypothesis proposes that an increased brain serotonin (5-hydroxytryptamine, 5HT) concentration is associated with the onset of fatigue: increases in brain 5HT can result from an increase in the transport of the precursor tryptophan (Trp) from the plasma across the blood–brain barrier. Increasing the plasma concentration of the branched chain amino acids (BCAA), which are competitive

inhibitors of Trp uptake, can reduce brain 5HT accumulation, and these observations have led to suggestions that BCAA should be added to drinks intended for consumption during prolonged exercise (Davis, 1996).

Although the evidence supporting a role of 5HT in the fatigue process seems strong (Wilson and Maughan, 1992), attempts to improve performance by the administration of BCAA during exercise have not generally been successful. One study (Blomstrand *et al*., 1991) did show beneficial effects in a subgroup of marathon runners who ingested drinks containing BCAA during the race, but these results have not been reproduced under controlled laboratory conditions (Varnier *et al*., 1994; Verger *et al*., 1994; van Hall *et al*., 1995). In the study of Verger *et al*. (1994), which involved treadmill-running rats, BCAA feeding resulted in a shorter exercise time to fatigue. In spite of these rather unpromising findings, at least one sports drink which contains added BCAA is on sale.

14.7.4 Glutamine and antioxidants

Moderate exercise levels seem to be associated with a reduced risk of minor illness and infection, but there is some evidence that athletes in hard training may be at increased risk of opportunistic infections, especially those of the upper respiratory tract (Nieman, 1996). Although minor in themselves, these may be sufficient to interrupt training. Failure to recover fully between training sessions also leads to a condition of chronic fatigue, and although this condition is not well defined, it is well recognised and is characterised by underperformance (Budgett, 1999). Damage to tissues caused by an increased level of free radical generation during and after exercise (in part from increased rate of aerobic metabolism and in part from release by neutrophilic leucocytes during phagocytosis) has been proposed as one of the factors in incomplete recovery, leading to suggestions that an increased dietary intake of antioxidant nutrients may confer some protection.

A variety of nutritional interventions have been proposed to enhance immune function, to increase the antioxidant defence mechanisms and to improve the resistance of the athlete to illness. Glutamine is used by the cells of the immune system and hard exercise causes a fall in the plasma glutamine level: from here it is only a short step to propose that glutamine supplementation may enhance immune function (Walsh *et al*., 1998). Unfortunately, the available evidence does not support this suggestion at the present time (Pedersen, 1999), even though glutamine is currently being sold to athletes. A more effective dietary strategy to enhance immunity may be to ensure adequate dietary carbohydrate intake. This has the effect of minimising any rise in plasma levels of stress hormones (cortisol, catecholamines and growth hormone) known to have a negative effect on immunity, and is likely to be the most successful nutritional strategy (Nieman and Pedersen, 1999). More recent data suggest that ingestion of drinks containing glutamine or protein during and after exercise does abolish the

post-exercise fall in the plasma glutamine concentration but has no effect on markers of immune function (Kryzwkowski *et al*., 2001).

Although there is good evidence that the delayed onset muscle soreness and damage that are experienced after hard exercise may be mediated at least in part by the release of free radicals, it is less clear that supplementation of the endogenous defence mechanisms with an increased intake of antioxidant nutrients will have any effect on these processes (Powers *et al*., 2004). Supplementation with antioxidants may reduce some of the markers of free radical-mediated muscle damage, but this alone is not sufficient justification to recommend the use of dietary supplements, and there is no evidence of performance benefits for athletes (Packer, 1997). Again, a number of products, including sports drinks, are on sale with these ingredients added.

14.7.5 Caffeine

The beneficial effects of caffeine on exercise performance may arise from any one of several different mechanisms. Caffeine has metabolic effects by virtue of its ability to stimulate lipolysis and thus spare muscle glycogen stores (Costill *et al*., 1978), but it also has a variety of effects of the contractility of muscle (Spriet, 1997) and on the central nervous system (Plaskett and Cafarelli, 2001). The consensus view now is that the effects – at least at the low doses that are shown to improve both physical and cognitive performance – are primarily due to the effects of caffeine on adenosine receptors in the brain (Davis *et al*., 2003). The use of caffeine is not prohibited by the doping regulations that apply to elite sport: the restrictions that formerly applied were removed in 2004, and this has led to an increased interest in the addition of caffeine to commercially available sports drinks. In addition, it is now recognised that positive effects on performance can be achieved at doses far less than those formerly used by athletes. Kovacs *et al*. (1998) tested sports drink formulations containing varying does of caffeine and found that performance of a time trial lasting about 1 hour was improved when doses of 225 or 320 mg were included in the drinks, but that a dose of 150 mg did not result in a performance that was better than the sports drink itself. The 150 mg dose did result in a better performance than when water was drunk. An even smaller dose (about 1.5 mg/kg) was shown by Cox *et al*. (2002) to improve time trial performance in highly trained cyclists.

Caffeine has a diuretic action, promoting urine output, and this is clearly undesirable in a sports drink. At low doses, however, caffeine has little or no noticeable diuretic effect. Two comprehensive reviews of the published literature have concluded that caffeine doses of less than about 250–300 mg are unlikely to increase fluid loss in the urine, at least in those accustomed to caffeine intake (Armstrong, 2002; Maughan and Griffin, 2003). This is a far higher dose than is necessary to gain a performance benefit.

Table 14.2 Composition of selected mainstream commercial sports drinks. For comparison, the composition of typical fruit juices and a standard carbonated cola beverage are also shown. The composition of fruit juices can be highly variable, so these examples are for illustrative purposes only

	Carbohydrate (%)	Sodium (mmol/l)	Potassium (mmol/l)	Osmolality (mosmol/kg)
Gatorade	6	20	3	310
Isostar	7	30	5	290
Lucozade Sport	6.4	22	3	285
Powerade (UK)	6	23	2	280/290
Fruit juice				
Orange	10	4	45	660
Apple	13	1	26	–
Tomato	3	140	7	–
Cola	11	3	1	700

14.8 Commercially available formulations

Most of the mainstream commercial sports drinks have a rather similar formulation, suggesting some consensus among the manufacturers as to the key components of an effective sports drink (Table 14.2). In general, these drinks are approximately isotonic, with an osmolality that is typically between about 280 and 340 mosmol/kg. The carbohydrate content is usually about 6–7% and usually includes some combination of glucose, fructose, sucrose and maltodextrin. The sodium concentration in typically about 20–30 mmol/l and the potassium concentration about 5 mmol/l.

14.9 Sources of information and advice

A variety of paper and electronic resources provide information on sports drinks. Many have a commercial bias, but some of the commercially funded websites are nonetheless valuable resources. A recent publication devoted specifically to the properties of sports drinks and to the effects of ingesting them (Maughan and Murray, 2001) provides a broad introduction to the topic. Most textbooks on sports nutrition have one or more chapters devoted to issues relating to hydration and sports drinks, and recent texts include Burke and Deakin (2000) and Maughan (2000). There are also numerous reviews of the scientific evidence relating to specific aspects of the topic in the published literature and there are referred to throughout the text of this chapter as appropriate.

Of the available web-based sources of information, that published by the Gatorade Sports Science Institute (GSSI, www.gssiweb.com) is both extensive and reliable. Most of this information is also published in paper

format. Another site that provides a useful gateway to many resources is www.sportsoracle.com.

14.10 References and bibliography

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15

Coffee as a speciality and functional beverage

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Abstract: Coffee is the most consumed beverage in the world. This chapter presents the main coffee species in the world market, the production of coffee and coffee-based beverages as well as their chemical composition. The chapter also explores the health benefits and quality of coffee, looking at it as both functional and speciality beverage.

Key words: coffee, green coffee, roasted coffee, post-harvesting coffee processing, coffee roasting, decaffeinated coffee, monsooned coffee, coffee and health, health benefits of coffee, functional food.

15.1 Introduction

Coffee has been for decades the most commercialized food product and therefore the most widely consumed beverage in the world. Since the opening of the first coffee house in Meca at the end of the 15th century, coffee consumption has greatly increased all around the world. In 2006, coffee production reached 7.4 million tonnes worldwide (ICO, 2007), with the major consumer countries being the United States, Brazil, Italy and Germany (ABIC, 2007). However, the per capita consumption in the North European countries may reach more than two times that of the United States or Brazil (Farah, 2004). The reasons for this increase in coffee consumption include improvement in cup quality through selection of varieties and breeding; improvements in agricultural practices; creation of speciality shops; and change in coffee image through the dissemination of information on the health benefits of long-term coffee consumption. Today, coffee is considered by many as a functional food mainly owing to its high content of bioactive compounds with anti-oxidant and other beneficial biological properties. The characteristic flavour and richness of coffee aroma also make it a unique beverage, with almost a thousand volatile compounds being identified in roasted coffee (Yeretzian *et al.*, 2003). As a consequence,

a series of new coffee-based products are being created and massive research is been performed in the intent of combining the peculiar and appreciated flavour of coffee with its biological properties.

Although more than 80 coffee species have been identified in the world (Clarke, 2003), only two of them are economically important: *Coffea arabica,* also known as arabica coffee, which is responsible for about 60% of world coffee market, and *Coffea canephora* or robusta coffee, which after a recent increase in production accounts today for the remaining 40% (ICE, 2008). Arabica and robusta coffees are different in many forms, including ideal growing climate, physical aspects, chemical composition and characteristics of the beverage obtained from the roasting of their seeds. These aspects will be addressed later in this chapter. The chapter will also explore the health benefits and quality of coffee, looking at it as both functional and speciality beverage. The technology used to obtain different types of coffee products available in the market as well as their chemical composition will be addressed.

15.2 Production of coffee and coffee-based beverages

From the moment coffee fruits are harvested until they reach consumers in the form of beverage, a series of steps are involved. As it happens for wine or any speciality beverage, the more controlled is each step of the manufacturing process, the greater is the chance of production of a good-quality beverage. After harvesting, the fruits need to undergo a primary processing for separation of the seeds (also called beans). Special treatments such as decaffeination and steam treatment are performed prior to roasting. After roasting, coffee is ground and packed or further processed for instant coffee production.

15.2.1 Green coffee production

Harvesting is an important step in the production of a good-quality coffee. Although the awareness of quality is very important throughout the whole agricultural process, the degree of maturation of coffee fruit and the care to avoid undesirable fungus contamination and growth during harvesting, drying and storage of the seeds are critical points. The contamination with fungus may be prejudicial not only to the sensorial characteristics of the final beverage but also – in the case of mycotoxin production – to human health (Taniwaki *et al.*, 2003).

Harvesting of coffee fruits mainly occurs in three different ways: by picking, by manual stripping and mechanically. In the first method, only ripe fruits, also known as cherries, are picked one by one. Because usually coffee fruits do not reach ripeness at the same time, this is a time-consuming and therefore expensive method in places in which the workforce is not very

accessible. However, picking tends to produce better-quality coffee beans than other methods. The manual method consists of stripping the twigs, collecting immature, ripe and overripe seeds and leaves at the same time. Mechanical harvesting is done either by shaking the trees or by stripping the branches with an apparatus similar to a flexible comb. These two methods – manual and mechanical – yield a series of extrinsic and intrinsic defects derived from fruits in different degrees of maturation as well as fermented fruits. Extrinsic defective beans are stones, husks, twigs, etc., which may be incorporated to the fruits during harvesting. Intrinsic defective beans – considered to be the most relevant ones for cup quality – are immature beans, black beans, sour beans, black-immature beans, bored or insect-damaged beans, and broken beans.

After harvesting, coffee fruits need to be processed for the production of green coffee seeds. The most common methods for the pulp extraction are known as wet and dry methods. Dry processed seeds are dried by sun exposure or by air dryers until moisture content is about 10–12% to avoid bacterial and mould growth (Trugo, 2003). Unless air dryers are available, low rainfall during the harvesting period is very important to ensure a good-quality coffee. After drying, fruits are cleaned and de-hulled. Dried skin and pulp are removed, leaving the mucilaginous material which involves the seeds (silverskin) still adhering to their surface. In order to obtain a good-quality beverage, the seeds (two beans for each fruit) are mechanically and electronically sorted so that the defects are excluded from the healthy seeds. This method is commonly used in Brazil and Africa, where sun and space are abundant and where stripping is also more common.

Wet processing is more sophisticated and tends to generate a higher-quality beverage. In this type of processing, before de-hulling and separation of the seeds, the selection of cherries occur in flotation tanks, followed by soaking and fermentation. During fermentation, which may occur with addition of enzymes, the silverskin is removed, acidity increases and pH may go down to 4.5 (Flament, 2002). The seeds (parchment coffee) are then extensively washed, polished and dried by sun or hot air drying or a mixture of both methods. Frequently, wet processing is used in places where coffee is harvested by picking, such as Colombia, Asia and Central America.

The major difference between dry and wet processing is that in the wet processing all material that involves the seeds is removed before drying. Alternatively, a method called Natural has been created in Brazil, combining both dry and wet methods. The method consists of washing and selection of the seeds in flotation tanks without fermentation. Coffee seeds treated by the natural process are often used in espresso coffee blends as they tend to add more body to the beverage than the wet processed ones. This happens because the polysaccharides that are originally present in the silverskin are not fermented, remaining on the seeds.

After the beans are dried, coffee is sized, graded and mechanically, manually and/or electronically sorted to eliminate defective beans. This

process may be followed by an additional sorting with UV excitation to separate defects which may be produced during both dry and wet processing and which are very difficult to separate from healthy beans, except for the fact that they show special fluorescence (Flament, 2002; Bee *et al*., 2005). Green coffee beans are then ready to undergo roasting. Optionally, green coffee beans may be decaffeinated, steam-treated or stored prior to roasting.

15.2.2 Decaffeinated coffee production

Decaffeination is performed prior to roasting. The most common and least costly caffeine extraction methods in the coffee industry basically employ an organic solvent such as dichloromethane or ethyl acetate, associated with the use of water/vapour prior to and after extraction for washing and opening of the pores. Water alone has alternatively and increasingly been used to replace organic solvents in the process, especially in the United States and Europe. Beans are then dried until they reach the moisture similar to that prior to processing. At the end of the process, caffeine content is usually reduced from 1–2 g% to 0.02–0.3 g% (Balyaya and Clifford, 1995; Farah *et al*., 2006a; Toci *et al*., 2006). The caffeine extracted from the green beans may be recovered and commercialized for production of cola types of beverage, pharmaceutical drugs and other purposes. During the decaffeination process, losses of key flavour components generally occur (Silvarola *et al*., 2004), especially when using solvents that lack specificity, such as water. Different devices have been created in order to return the aromatic fraction lost during the caffeine extraction. Alternatively, coffee aroma may be added to the decaffeinated product.

There is a general concern regarding the consumption of residual methylene chloride in the coffee beverage after decaffeination process. Considering that the boiling point of this solvent is 40 °C and that coffee goes through temperatures around 70 °C for solvent volatilization, green coffee beans should not contain significant amounts of the solvent. In addition, roasting temperatures (commonly 210–230 °C) are high enough to allow volatilization of any remaining amount of dichloromethane in coffee. The US FDA (Food and Drug Administration) allows up to 10 ppm in roasted coffee, while the European Union allows up to 3 ppm. In most cases, according to industry reports, the residual concentration of dichloromethane is 100× lower (FDA, 2006). A method that is more efficient for maintaining the original flavour of regular coffee, although costly, is the supercritical carbon dioxide method, in which carbon dioxide is used at high pressure and temperature to replace the organic solvents (Farah *et al*., 2006a).

15.2.3 Steam-treated coffee production

Coffee may be steam-treated prior to roasting as a means to make coffee ‘less irritable’ to the stomach, and therefore acceptable to persons with

stomach problems (Steinhart and Luger, 1997). For this reason, this type of coffee may be called 'stomach friendly'. The decrease in stomach 'irritability' of steam-treated coffee has been attributed to the loss of chlorogenic acids during the process (Maier, 1994).

15.2.4 Monsooned coffee

Monsooned coffee is a speciality coffee of India, which undergoes a natural process of curing dry green arabica and robusta coffee beans by exposing them to moist monsoon winds prevailing in the west coast of southern India (Malabar coast), especially in the regions of Mangalore and Tellichery (Variyar *et al*., 2003). The history of monsooned coffee is quite curious. In the days of sailing ships, the cherry coffee sent to Europe used to take 6 months to arrive in their destination. The high relative humidity of the seas and the monsoon winds caused the beans to swell in size, change colour to light yellow and acquire an intensely mellow but aggressive, musty flavour. With the opening of the Suez Canal and speedy transport on steam ships, complaints were received from Europe that the coffee did not have the flavour it had earlier. It was only then that the coffee exporters in Mangalore devised a process known as 'monsooning coffee'. Today, monsooned coffee beans are exported from India to Europe, Asia, Africa and North America. Monsooned coffee is known to have good body, mild (reduced) acidity and pleasant aroma and flavour in the cup. For preparing monsooned coffees only dry processed arabica and robusta beans are used. This coffee is mainly used in blends to mellow and impart richness to rougher, more acidic coffees (Coffee Board of India, 2007).

15.2.5 Coffee roasting

The aroma of green coffee is very different from that of the roasted beans we know. It is only after roasting that coffee gains its characteristic aroma and flavour. During roasting, the temperature of the beans increases significantly and a series of physical and chemical changes take place. The roasting conditions will influence these changes considerably and, as a consequence, the results on the cup. The most common roasters available for domestic use and in coffee industries include different types of drum roasters (the beans are in contact with fire or hot surfaces) and fluidized/spouted bed roasters (the beans are in contact with hot air/gases), currently the most preferred by industries because they grant colour homogeneity and fastness in the process through the control of air/gas temperature and speed inside the roaster, allowing variation of heat input and management of bean temperature over time.

The temperatures used to roast the beans will depend on the type of roaster, but maximum temperatures commonly used in industrial fluidized bed roasters vary between 210 and 230 °C. In the initial phase of the roasting

process, evaporation of free and bound water occurs. When the bean temperature reaches 130 °C, caramelization of sucrose and browning take place, and the beans start to swell. The chemical changes in the initial phase of the process are relatively small compared with those at the end of the process. In temperatures higher than 160 °C, a series of exothermic and endothermic reactions take place and the colour of the beans changes to light brown; their volume increases considerably and the aroma formation starts. The chemical reactions responsible for generation of typical aroma and flavour of roasted coffee are triggered when the beans temperature reach about 190 °C. Maillard and Strecker reactions, in which carbohydrates, proteins and other classes of compounds participate, take place, with simultaneous degradation and production of low and high molecular weight compounds such as melanoidins. During this process, changes in the colour of the beans from light brown to almost black are observed (Farah, 2004). These reactions are interrupted at the desired point based on the colour of the bean or programmed time. The beans are rapidly cooled by cool air or water. Air is currently preferred because water increases humidity and chances of microorganism growth. After roasting, the beans are ground and marketed as ground roast coffee or used for instant coffee production.

The ground roast coffee may be available at the market in different roasting degrees, varying in colour from very light to very dark, according to the national preferences. In the United Kingdom and the United States, for example, light medium to medium roasts are preferred (Clarke, 2003), while in some parts of Europe dark coffee is preferred. In Brazil, dark medium to dark roasts are traditional. However, the Brazilian Association of Coffee Industry (ABIC) has been encouraging the manufacturers to lighten their coffees in order to take advantage of their potential as functional foods (see Section 15.4) and the results are already reflected in the market. The roasting degree standards for both commercial and scientific purposes are quite subjective, and may vary considerably. The mass loss during the roasting process may be considered as a good parameter on a small scale but on a large scale the mass may be difficult to control. The visual parameter continues to be the most accepted. In order to help build standards for colorimetric measurements, colour disks have been created by the Speciality Coffee Association of America (SCAA). This system, the AGTRON/SCAA Roast Classification Color Disc System, was obtained under controlled roasting conditions, in which a linear progression of colour development was determined, based on sugar caramelization and in the most commonly found roasting degrees in the market.

In addition to colour variations, roasting parameters such as amount of coffee in the roaster, temperature, time and speed of the hot air/gas circulation (in the case of fluidized and spouted bed roasters) may vary considerably to reach one single roasting degree. The speed with which the bean becomes dark will affect a series of physical-chemical parameters and therefore the flavour of the beverage. For example, two samples of the same

coffee roasted to the same degree (colour) may have distinct chemical compositions if they are roasted at different speeds. Coffees roasted at higher temperatures for a shorter period of time may present higher acidity, higher content of soluble solids and a different volatile profile then those roasted for a longer period of time and at lower temperatures, and this will be reflected in the cup.

15.2.6 Instant coffee production

In the typical process of instant coffee production, ground and roast coffee are treated with hot water and high pressure for extraction of the water-soluble material. The soluble material is then cooled, sometimes centrifuged, concentrated through heating and then dried through spray vaporizing, or freeze drying. The spray drying process uses high temperature under high pressure to volatilize the aqueous extract and the hot air dehydrates the small drops which are powdered. The freeze drying process uses very low temperatures for sublimation of a previously frozen aqueous extract and the direct change from the solid phase to the gas phase produces a higher-quality product compared with other methods.

Alternatively, steam/water and/or oil may be used to rewet the surface of instant coffee granules, followed by drying. This process is called agglomeration (ABICS, 2007). Additionally, different manufacturers use distinct techniques when preparing instant coffee in order to improve the appearance and taste of the final product.

As the soluble material is extracted, the insoluble material is separated from the extract and dehydrated to be discarded or reutilized as equipment fuel, or animal feeding or as a source for polysaccharides extraction (Adams and Dougan, 1987; Clarke and Macrae, 1987; ABICS, 2007). Although arabica coffee alone is often used for ground roast coffee production, in many places robusta coffee alone or as a high percentage of it compared with arabica coffee may be used in the blends designated for instant coffee production because arabica beans contain higher amount of soluble solids and therefore increase yield.

15.3 Coffee chemical composition and cup quality

The basic chemical composition of green coffee depends mainly on genetic aspects, such as species and variety, and on physiological aspects, such as degree of maturation. To a smaller degree, extrinsic aspects, for instance, soil composition, climate, agricultural practices and storage conditions will affect the seeds' physiology and consequently their chemical composition (Farah and Donangelo, 2006). But like wine, coffee is a very peculiar beverage and even the flavour of an excellent cup quality coffee may vary considerably among samples from the same species and variety but grown in

different regions. In this case, extrinsic aspects such as climate and soil composition (including the typical microbiota of the region) are quite relevant because of certain chemical compounds and perhaps minerals that even though present in very small amounts, may produce considerable changes in the sensory attributes of the beverage.

It is well known that *C. arabica* and *C. canephora* species have a very distinct chemical composition and that arabica coffees provide superior cup quality and aroma to robusta coffees. So why not use only arabica coffee in the production of coffee products? Starting from the agricultural point of view, robusta coffee trees, as the name says, are 'robust', stronger, more resistant and less demanding than arabica trees in various aspects, including the ideal growing climate and use of pesticides. All these aspects imply in lower prices in the market. For decades, there has been a scientific research effort in order to combine the resistance of robusta trees with the cup quality of arabica seeds through the production of hybrids, but it is likely that the chemistry responsible for a higher resistance is also responsible for a lower cup quality. As an example, one of the reasons for the differences between arabica and robusta coffees has been attributed to the content of chlorogenic acids, which are important for plant protection against pathogens such as microorganisms and insects and against UV radiation (Farah and Donangelo, 2006). Although low amounts of chlorogenic acids are important for flavour formation, there have been studies showing that high contents of chlorogenic acids in green coffee may generate low cup quality possibly because of the high formation of oxidation products prior to roasting (Farah *et al.*, 2006b). However, well-harvested and well-taken-care of robusta coffee seeds may give a better cup quality than inferior arabica coffees. Moreover, the use of good robusta coffees in industry and commercial blends offers the advantage of a higher content of soluble solids, compared with arabica coffees, which adds more body to the beverage and increases yield.

A description of the basic chemical composition of *C. arabica* and *C. canephora* seeds is now given.

15.3.1 Green coffee chemical composition

The non-volatile fraction of green coffee is mainly composed of water, carbohydrates and fibres, free amino acids, proteins, lipids, minerals, organic acids, chlorogenic acids, trigonelline and caffeine. From these components, caffeine, trigonelline and chlorogenic acids are bioactive compounds characteristic of coffee that contribute importantly to the flavour of the beverage after the roasting of the beans.

Caffeine, the main alkaloid found in coffee, is a xanthine derivative (Fig. 15.1) with bitter characteristics. However, caffeine is responsible for only up to 10% of the perceived bitterness of coffee beverage (Flament, 2002). The caffeine content in *C. canephora* is about two times that of

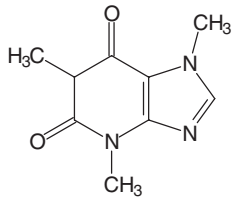

Fig. 15.1 Chemical structure of caffeine.

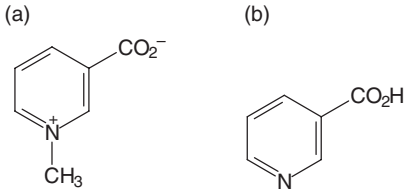

Fig. 15.2 Chemical structures of (a) trigonelline and (b) nicotinic acid.

C. arabica. Caffeine metabolites such as theophylline and theobromine have also been identified in coffee. Trigonelline is a methyl-betaine (Fig. 15.2) biologically derived from enzymatic methylation of nicotinic acid. It contributes to bitterness and as a precursor for the formation of different classes of volatile compounds during the roasting process (Farah *et al.*, 2006b). The trigonelline content in *C. canephora* corresponds to about two-thirds of the content in *C. arabica*. Although there has been an association between trigonelline content of green coffee and good cup quality (Farah *et al.,* 2006), one of the classes of volatile compounds derived from trigonelline may present an 'objectionable flavour', according to Flament (2002). Chlorogenic acids are a major class of phenolic compounds derived from the esterification of *trans*-cinnamic acids such as caffeic, ferulic and *p*-coumaric, with (–)-quinic acid. The main subclasses of chlorogenic acids in coffee are, therefore, in order of abundance, caffeoylquinic acids, dicaffeoylquinic acids, feruloylquinic acids and coumaroylquinic acids, with at least three positional isomers per subclass (Clifford, 2000; Farah *et al.*, 2005) (Fig. 15.3). These phenolic components confer astringency, bitterness and acidity to the coffee beverage. However, high amounts in green coffee, particularly the caffeoylquinic and feruloylquinic acids, may produce an undesirable flavour possibly because of negative notes of their products of oxidation and degradation prior to and during roasting. Chlorogenic acids are also precursors for the formation of (unpleasant) phenols and cathecols during roasting (Trugo, 2003). The content of chlorogenic acids in *C. canephora* is traditionally higher than in *C. arabica,* being quite variable in both species.

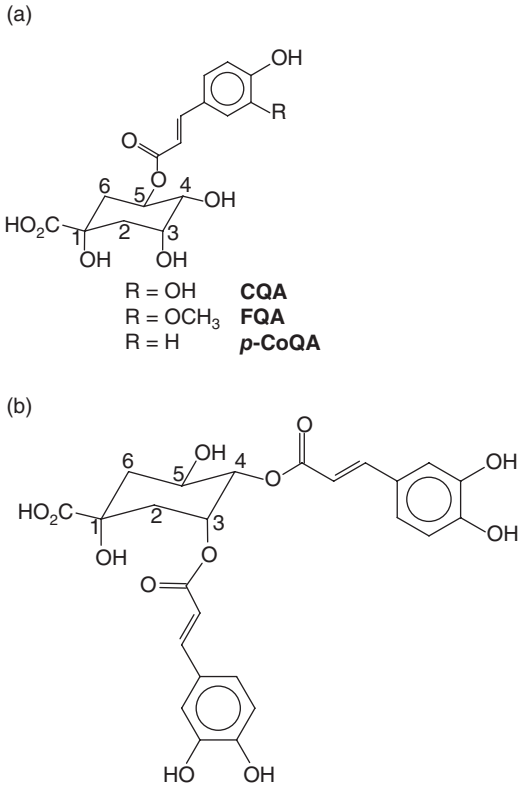

Fig. 15.3 Chemical structures of chlorogenic acids: (a) monoesters of *trans*-cinnamic acids with (–)-quinic acid (example of 5-isomers); (b) dicaffeoylquinic acids (example of 3,4-isomer).

Additionally, two bioactive terpenoids have been identified as characteristic of coffee, cafestol and kahweol (Fig. 15.4). These compounds and derivatives represent about 20% of the lipid fraction of coffee, mainly in the form of salts or esters of saturated (predominant) and unsaturated fatty acids (Wattenberg, 1983; Cavin *et al.,* 2002; Kölling-Speer and Speer, 2005). Cafestol is the main constituent of the unsaponifiable fraction of coffee oil, representing about 0.2–0.6% of coffee weight. Kahweol is more sensitive to heat, oxygen, light and acids, and therefore is less abundant (Flament, 2002). Higher contents of diterpens are found in *C. arabica* than in *C. canephora*.

The water content of the green seeds of *C. arabica* and *C. canephora* varies from about 8.5 to 10% (Farah, 2004). About 40% of the mineral content is composed of potassium, the remaining content being composed of 30 different elements. From these elements, only the magnesium content varies significantly with species (10–30 ppm for *C. canephora* and 25–60 ppm

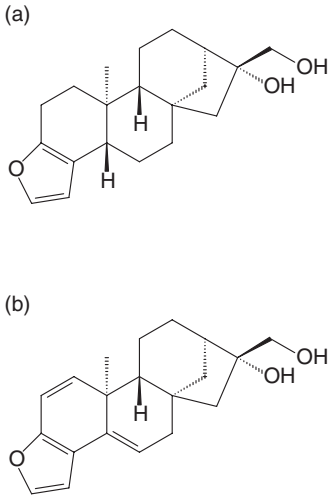

Fig. 15.4 Chemical structure of diterpens characteristic of coffee: (a) cafestol; (b) kahweol.

for *C. arabica*) (Clarke, 2003). While the variations observed in the protein content of both species seem to be only analytical, the lipid content in *C. arabica* (about 14%) is about twice that of *C. canephora* (Stephanucci *et al.*, 1979). Table 15.1 presents the typical non-volatile composition of green *C. arabica* and *C. canephora* seeds after primary processing (dry weight composition).

The volatile fraction of unroasted coffee beans is very poor, which gives them a weak, although characteristic, aroma. About 100 compounds have been identified in green coffee beans (Flament, 2002) of different types and origins. The most abundant classes of volatile compounds found in green coffee beans are esters, hydrocarbons, alcohols and aldehydes. Ketones, pyrazines, furans and sulphur compounds have also been identified (Flament, 2002; Toci and Farah, 2008).

15.3.2 Roast coffee chemical composition

During roasting, the beans' composition dramatically changes as a consequence of pyrolysis, caramelization and Maillard reactions, among others, with destruction of various compounds and formation of others, including bioactive compounds and substances of high and medium volatility, which play an important role in the formation of aroma and flavour of the beverage. The composition of roasted coffee will vary according to the raw material, roasting degree and roasting parameters such as time, temperature and speed in which the process occurs.

The moisture content of roasted coffee is very inferior when compared with green coffee beans, varying from about 1.7–5%, depending on the

Table 15.1 Chemical composition of the non-volatile fraction of green or unroasted coffee beans (Clarke and Macrae, 1985; Mazzafera, 1991; Viani, 1993; Debry, 1994; Clifford, 2000; Clarke, 2003; Farah *et al.*, 2005).

Component	Content[a] (g/100 g dry basis)	
	Coffea arabica	Coffea canephora
Carbohydrates and fibres		
Sucrose	6.0–8.0	4.0
Reducing sugars	0.1	0.4
Polysaccharides (arabinogalactan, mannan and glucan)	34–44	48–55
Lignin	3.0	3.0
Pectins	2.0	2.0
Nitrogenous compounds		
Protein	10.0–11.0	11.0–15.0
Free amino acids	0.5	0.8
Caffeine	0.9–1.2	1.5–2.5
Trigonelline	0.8–2.0	0.6–0.7
Lipids		
Coffee oil (triglycerides with unsaponifiables)	16.0	10.0
Diterpene esters	0.9	0.2
Minerals (41% K and 4% P)	3.0–4.2	4.4–4.5
Acids and esters		
Total chlorogenic acids	4.1–7.9	6.1–11.3
Aliphatic acids	1.0	1.0
Quinic acid	0.4	0.4

[a] Content varies with cultivars, cultivation climate, soil, methods of analysis, etc.

roasting degree (Trugo and Macrae, 1984a; Farah, 2004). Part of the protein is degraded and part reacts with other compounds such as carbohydrates, chlorogenic acids, etc. to form (via Maillard reaction) melanoidins, which are water-soluble polymers responsible for the brownish colour of roasted coffee and for about 25% of roasted coffee composition (Nicoli *et al.*, 1997). The free amino acids are consumed by Strecker reactions and sucrose is consumed by caramelization and (indirectly, after hydrolysis) Maillard reaction.

Caffeine is not significantly altered during coffee roasting, but small losses may occur owing to sublimation. In terms of percentage composition, an increase in the caffeine content may be observed owing to the loss of other compounds. Because of the chlorogenic acid's thermal instability, these compounds undergo many changes during roasting, including isomerization, epimerization, lactonization, degradation to low molecular weight compounds (including phenols and cathecols) and incorporation to melanoidins, which may alter their content, depending on the roasting degree, to less than 5% of their original content. Therefore, chlorogenic acids take

part in the generation of colour, flavour and aroma of coffee during roasting. The total chlorogenic acid content in commercial roasted coffee ranges from about 0.5 to 6 g%, depending on the type of processing, roasting degree, blend and analytical conditions (Farah *et al.,* 2005; Farah and Donangelo, 2006). The aliphatic acids (formic, acetic, glycolic and lactic) content, and therefore acidity, may increase as a result of degradation of sucrose, polysaccharides and other compounds (Clarke and Macrae, 1985; Ginz *et al*., 2000). Trigonelline is degraded, producing a great variety of compounds, including nicotinic acid, also called niacin, B_3 vitamin or PP vitamin (Fig. 15.2) (Trugo and Macrae, 1989) and volatile compounds such as pyrrols, pyridines, pyrazines and methyl nicotinate (Flament, 2002; Trugo, 2003).

Potential carcinogenic compounds such as benzo[*a*]pyrene and acrylamides may be also formed under certain conditions, especially at very high temperatures (Wattenberg *et al*., 1980; Wood *et al*., 1982; Huang *et al*., 1985). On the other hand, potentially harmful substances, such as mycotoxins (particularly Ocratoxin A), which may be present in green coffee beans as contaminants, are degraded during roasting (Taniwaki *et al*., 2003). Even though the diterpenes cafestol and kahweol are sensitive to heat, small amounts may still be found in roasted coffee, especially in *C. arabica*. The typical chemical composition of the non-volatile fraction of ground roast coffee beans is presented in Table 15.2.

Regarding the volatile fraction of roasted coffee, it is only after roasting that, by Strecker and Maillard reactions, the complex aroma of coffee is formed. The variety and amount of volatile compounds in roasted coffee will depend on the composition of the non-volatile fraction of the raw beans and on roasting conditions. Therefore, genetic aspects, soil, agricultural practices, climate and degree of maturation, among other factors, will influence the final composition of the volatile fraction of the roasted beans. Considering different types of coffee, origin, roasting degrees and analytical methods, more than 900 compounds have been identified in roasted coffee (Yeretzian *et al*., 2003). Typical classes of volatile compounds in roasted coffee are hydrocarbons: alcohols, aldehydes, ketones, acids and anhydrides, esters, lactones, phenols, furans and pyrans, thiophenes, pyrroles, oxazoles, thiazoles, pyridines, pyrazines, amines and different sulphur and nitrogen compounds (Flament, 2002; Toci and Farah, 2008).

15.3.3 Chemical composition of coffee brew

During the preparation of the filtered beverage from ground and roast coffee, basically water-soluble compounds are extracted, whereas most of the lipophilic fraction is left with the solid material. The brew composition and cup quality will vary according to several factors, which include the composition (blend) and the grid, the brewing method, the proportion of coffee to water, the water temperature and length of time coffee is in

Table 15.2 Chemical composition of the non-volatile fraction of roasted coffee beans (Debry, 1994; Clarke, 2003; Farah and Donangelo, 2006; Toci *et al.*, 2006; Perrone *et al.*, 2008).

Component	Content[a] (g/100 g dry basis)	
	Coffea arabica	Coffea canephora
Carbohydrates and fibres		
Sucrose	4tr–4.2	1tr–1.6
Reducing sugars	0.3	0.3
Polysaccharides (arabinogalactan, mannan and glucan)	31–33	37
Lignin	3.0	3.0
Pectins	2.0	2.0
Nitrogenous compounds		
Protein	7.5–10	7.5–10
Free amino acids	Not determined	Not determined
Caffeine	1.1–1.3	2.4–2.5
Trigoneline	0.2–1.2	0.3–0.7
Nicotinic acid	0.016–0.026	0.014–0.025
Lipids		
Coffee oil (triglycerides with unsaponifiables)	17.0	11.0
Diterpene esters	0.9	0.2
Minerals	4.5	4.7
Acids and esters		
Total chlorogenic acids	1.9–2.5	3.3–3.8
Aliphatic acids	1.6	1.6
Quinic acid	0.8	1.0
Melanoidins	25	25

[a] Contents vary for different cultivars, agricultural practices, climate, soil composition, methods of analysis and, critically, roasting degree.
tr, trace.

contact with water and the filter material. Espresso coffee is prepared by a special brewing technique in which a limited amount of hot water is percolated through ground coffee under high pressure in a short time. This process produces a concentrated brew of intense flavour covered with a foam layer. In this case, the composition and quality of the brew will also depend on the water pressure. Usually, the higher the temperature (under 100 °C for thermo-labile compounds) and the pressure, the greater the extraction of water-soluble components such as chlorogenic acids, caffeine, nicotinic acid (Trugo and Macrae, 1984b). Even though cafestol and kahweol are not water soluble, part of the remaining amount in the beans after roasting may be extracted owing to the high temperature of the water and, in the case of espresso coffee, high pressure. But it is likely that the oil particles will be retained in the paper filters or similar types of filters from domestic brewers. It appears that mostly unfiltered coffee and perhaps espresso coffee beverages will contain significant amount of these compounds.

For chlorogenic acid extraction in particular, the main components responsible for coffee functional properties, about 80–100% of the chlorogenic acids are extracted in domestic brewing (Clifford, 2000). Domestic extraction will result in 70–200 mg of chlorogenic acids per 200 ml cup, in the case of arabica coffee, and 70–350 mg in robusta coffee (Clifford, 1997). Keeping coffee brews at elevated temperature reduces the contents of chlorogenic acids and derivatives with anti-oxidant activity (Bennat *et al*., 1994; Schrader *et al*., 1996). Chlorogenic acids contents in light to dark-medium roasted coffees still stand out when compared with most food sources of these compounds (Farah *et al*., 2001; Clifford, 2000). While coffee abstainers may typically ingest less than 100 mg of chlorogenic acids per day, modest and heavy coffee drinkers' intake may range from 0.1 to 2 g (Clifford, 1997, 2000; Del Castillo *et al*., 2002).

15.3.4 Changes in coffee chemical composition during special coffee processing

Generally speaking, any processing coffee beans undergo produces chemical changes. For example, high temperatures will affect thermo-labile compounds while processing involving water/vapour may wash off water-soluble compounds such as oligosaccharides and increase water content of the beans. Furthermore, different solvents used during decaffeination process will present more affinity for specific compounds than others. For instance, dichloromethane decaffeination will tend to extract more trigonelline and chlorogenic acids than ethyl acetate or water alone (Farah, 2004). In addition, the overall aroma of decaffeinated coffee is extremely affected by decaffeination process, especially when water alone is used. The instant coffee chemical composition will reflect the blend composition, the roasting degree of the ground and roast coffee from which the brew is made, the brewing and the concentration methods used in the process. When the concentration method employs excessive heat, thermo-labile compounds are lost. In the case of caffeine, its content in instant coffee (2.5–5 g/100 g) will depend mostly on the blend composition and the extraction method, as this compound is quite resistant to heat. Once instant coffee is dissolved in water its composition should be similar to the ground roast coffee brew, and this has been the goal of instant coffee manufacturers. In the case of monsooned coffee and steam-treated coffee, in addition to partial hydrolysis of chlorogenic acids, and loss of low molecular weight compounds due to exposure to humidity, differences in the volatile composition profile are observed.

15.4 The health benefits of coffee drinking

15.4.1 Caffeine and health

Caffeine is an alkaloid known as a central nervous system stimulant through its adenosine antagonist action. Although caffeine is the most widely

consumed and studied psychoactive substance in history, research results seem to be inconclusive when trying to associate its intake with beneficial or detrimental effects on health (Shlonsky *et al*., 2003). While there has been an attempt to associate caffeine intake with high blood cholesterol, high risk of coronary diseases, and cancer, other studies suggested an association between its consumption and lower incidence of suicides and hepatic cirrhosis (Farah *et al*., 2006a); while low to moderate caffeine intake is generally associated with improvements in alertness, learning capacity, exercise performance and perhaps mood, high doses may produce negative effects in some sensitive individuals, including anxiety, tachycardia and insomnia, during its half-life, which varies from 2 to 6 hours after intake (Nehlig, 1999; Ogita *et al*., 2003; Farah *et al*., 2006a; Toci *et al*., 2006); while acute caffeine consumption showed to have negative effects on glucose tolerance and lower glucose disposal and insulin sensitivity in lean, obese and type 2 diabetic individuals, other compounds present in coffee are able to counteract this effect (Shearer *et al*., 2007). Acute caffeine intake also increases the urinary excretion of minerals such as calcium (Ribeiro-Alves *et al*., 2003). However, after long-term consumption, most of these acute effects tend to disappear due to metabolic adaptations in the body (Demirbag *et al*., 2006). In addition, caffeine metabolites, especially 1-methylxantine and 1-methylurate have exhibited antioxidant activity *in vitro* and regular coffee has shown a higher iron-reducing capacity *in vivo* compared with decaffeinated coffee (Lee, 2000).

With the availability of good-quality decaffeinated coffee in the market, caffeine consumption may be currently considered as a choice. Recently, Shlonsky *et al*. (2003) profiled decaffeinated coffee consumers. Their research data revealed that decaffeinated coffee is generally consumed owing to health disorders, or simply by people in search for a healthier lifestyle, with low incidence of smoking habit, low consumption of alcoholic beverages and high consumption of health supplements. These causes seem to be responsible for the growth and expansion of the decaffeinated coffee market. Currently, decaffeinated coffee is responsible for up to 10% of the coffee market (Ogita *et al*., 2003; Silvarola *et al*., 2004).

15.4.2 Phenolic compounds of coffee and health

In the last few years, a series of epidemiological and clinical studies have associated coffee consumption, independently of caffeine, with health benefits such as reduction of the relative risk of type 2 diabetes (Agardh *et al*., 2004; Salazar-Martinez *et al*., 2004; Rosengreen *et al*., 2004; Soriguer *et al*., 2004; van Dam *et al*., 2006; Bravi *et al*., 2007); Parkinson and Alzheimer diseases (Lindsay *et al*., 2002) and liver cancer (Ranheim and Halvorsen, 2005; Larsson and Wolk, 2007). *In vitro* and animal studies have mainly attributed such beneficial properties of coffee to the antioxidant and other mechanisms, involving chlorogenic acid compounds (Huang *et al*., 1985;

Arion *et al*., 1997; Hemmerle *et al*., 1997; Herling *et al*., 1998; Aruoma, 1999; Andrade-Cetto and Wiedenfeld, 2001; Natella and Scaccini, 2001; Natella *et al*., 2002; Gerin and van Schaftingen, 2002; Johnston *et al*., 2003; Pellegrini *et al*., 2003; Herrera-Arellano *et al*., 2004; Shearer *et al*., 2007). In fact, studies performed in Denmark and the United States, showed that, because of its high content of phenolic compounds, coffee is the most important contributor to antioxidant compounds in their diet (Svilaas *et al*., 2004; Vinson, 2005).

Long before the epidemiological studies involving coffee consumption, the antimutagenic property of chlorogenic acids and their metabolites had been demonstrated by a series of studies (Wattenberg *et al*., 1980; Wood *et al*., 1982; Stich *et al*., 1982; Wattenberg, 1983; Mori *et al.*, 1986; Namiki, 1990). Recent studies have confirmed this property and elucidated a few mechanisms involved among which are free radical scavenging, metal quelation, inactivation of reactive compounds and metabolic pathway changes (Mori *et al*., 1996; Pannala *et al*., 1998; Lo and Chung, 1999; Kasai *et al*., 2000; Cavin *et al*., 2002). Additional biopharmacological properties have been attributed to different caffeoylquinic and dicaffeoylquinic acids, for instance, antiviral activity against adenovirus and herpes virus (Chiang *et al*., 2002); hepatoprotective activity in injured liver experimental model (Basnet *et al*., 1996); immuno-stimulating activity (Tatefugi *et al*., 1996). Synthetic dicaffeoylquinic acids derivatives also inhibited the replication of HIV-1 in cells (Robinson *et al*., 1996a,b; McDougall *et al*., 1998; Kyng *et al*., 1999), which raises the possibility of production of new coffee-based anti-HIV drugs. Because only a few chlorogenic acid compounds are commercially available or synthesized in laboratories, studies on biological properties of feruloylquinic and coumaroylquinic acids are scarce.

Chlorogenic acid lactones or quinides, which are formed from up to 10% of chlorogenic acids of green coffee beans during roasting, have also exhibited hypoglycaemic activity in rats (Shearer *et al*., 2003) as well as the capacity to bind the brain μ-opioid receptors *in vitro* (De Paulis and Martin, 2004). This latter property raises the possibility of using coffee for certain types of drug addiction (Flores *et al*., 2000). However, it is most likely that once these lactones are consumed in the beverage, a major part of them returns to their chlorogenic acid form during digestion or metabolism (Farah, 2004; Farah *et al*., 2007), indirectly increasing, therefore, the chlorogenic acid intake.

15.4.3 Other relevant constituents of coffee and health

Cafestol and kahweol are two diterpenes with potential anti-carcinogenic and hepatoprotective properties that are present in coffee (Wattenberg, 1983; Cavin *et al*., 2002). On the other hand, a high consumption of these compounds has been associated with the increase in homocysteine and low-

density lipoprotein (LDL) levels in human plasma, which could result in higher risk of heart disease incidence (Olthof *et al.*, 2001). Nonetheless, considerable amounts of these compounds are mostly present in unfiltered coffee, because they are not water soluble and are mostly retained in paper filters.

Coffee is a peculiar beverage in the sense that, when submitted to very high temperatures, it produces a vitamin. A small part of the trigonelline contained in coffee is converted to nicotinic acid or niacin, a B vitamin (B_3) through demethylation. In humans, this vitamin participates as coenzyme in various metabolic processes and its deficiency causes Pellagra, a disease characterized by skin lesions. Even though the production of niacin increases in coffee as roasting progresses, a medium roasted 100 ml coffee cup is enough to supply about 20% of the daily DRI recommendations (DRI, 2000).

Another class of biologically active compounds of coffee is formed by the melanoidins, which are brown polymers of variable composition containing nitrogen (Ledl and Schleicher, 1990), that are formed mainly from proteins and carbohydrates during the roasting of the beans. Various other components of coffee such as the chlorogenic acids are incorporated in these water-soluble fractions. Different studies suggest that melanoidins are responsible for part of the anti-oxidant, antibacterial and metal chelating properties observed in coffee beverages (Daglia *et al.*, 1994, 2000; Homma and Murata, 1995; Nicoli *et al.*, 1997). However, their physiological relevance in humans is still unknown, partly because their chemical structures are varied and still unclear.

Recently, the soluble fibres of coffee brew, mainly galactomannans and type II arabinogalactans have received special attention. Because they are not digested by humans, they reach the colon and potentially serve as substrate for the beneficial colonic microbiota (Gntechwitz *et al.*, 2007). A high intake of dietary fibre is considered to be positively related to several physiological and metabolic effects, such as lowering of blood cholesterol and the moderation of blood glucose and insulin response. In addition, fermentable polysaccharides are degraded by the colonic microbiota to short-chain fatty acids (SCFA), mainly acetate, propionate and butyrate, which lowers the colonic pH, impeding the growth of certain pathogenic species and supporting the growth of bifidobacteria and other lactic acid bacteria that are considered to be beneficial for human health (Gntechwitz *et al.*, 2007).

As in many food products (french fries, smoked products, etc.) which are submitted to very high temperatures during processing, carcinogenic substances may be formed during coffee roasting, more specifically acrylamide and polycyclic aromatic hydrocarbons (PAH), of which benzo[*a*] pyrene is the most studied. On the other hand, chlorogenic acids have the capacity to inhibit both the formation of PAH as well as the mutagenicity of their carcinogenic metabolites (Wattenberg *et al.*, 1980; Huang *et al.*,

1985). Light to medium roasted coffees should contain less PAH than darker roasts. However, because acrylamide is formed in the beginning of the roasting cycle and destroyed by the end of the cycle, light to medium roasted coffees should contain more acrylamide than darker roasts. Recent studies have shown that roasting coffee in slow speed, i.e. at lower temperatures and for longer periods of time, helps prevent the formation of both PAH and acrylamide (Soares *et al.*, 2008).

Mycotoxins, more specifically Ocratoxin A, which has shown liver and kidney toxicity (Taniwaki *et al.*, 2003), may be present in low-quality green coffee due to poor agricultural practices, inadequate harvesting and/or storage conditions. This toxin is destroyed by the high temperatures to which coffee is submitted (Romani *et al.*, 2003) and therefore, unless the initial amounts in the beans are extremely high, medium to dark roasted coffees will not contain residual amounts.

In conclusion, epidemiological studies suggest that consumption of up to four cups of coffee a day is not harmful and may be beneficial to health. In order to obtain a functional or healthier coffee it is usually best to consider various aspects and, starting from good-quality beans, roast them from light to dark medium roasting degrees, preferentially at lower temperatures above 190 °C. Light to medium roasted coffees contain still high amounts of anti-oxidant compounds compared with other food products and a considerable amount of niacin, even though its content increases as roasting progress. For those individuals sensitive to caffeine effects and for those who wish to use coffee as an additional tool to prevent of type 2 diabetes development, decaffeinated coffee is indicated.

15.5 Future trends

As consumers are more demanding and as the speciality coffee public grows, the quality and differentiation among the various coffee brands will tend to increase, along with specific label information such as types of blends, roasting degree, sensorial attributes, etc. Additionally, a massive research effort is being made with the intent of combining the peculiar flavour of coffee with its biological properties and a series of new coffee-based 'functional' beverages and products are being created. The information on the potential benefits of coffee to human health, the content of anti-oxidant compounds or at least the indication of their presence will tend to appear in the product labels. As the need for practicality increases and the modern world market niches for semi-ready food products expand, the consumption of instant coffee and semi-ready coffee products tend to increase. Individual packages and sachets are already emerging in the market. Additionally, in view of the possible association of potentially beneficial compounds naturally present in coffee with nutrients for which inadequate intake is a risk factor for chronic degenerative diseases, coffee has been considered as a suitable vehicle for fortification.

15.6 Sources of further information and advice

15.6.1 Books

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15.6.2 Websites

Association for Science and Information on Coffee (ASIC): www.asic-cafe.org. Formerly known as the Association Scientifique Internationale pour le Café:

> 'ASIC was founded in 1966 following two colloquia on coffee organised in Paris by the Institut Français du Café et du Cacao (IFCC). It was created to fulfil the desire of the participants at these meetings to dispose of a permanent organisation, which would periodically bring together specialists from all over the world working in the different fields of coffee science and technology, giving them an opportunity of presenting and comparing their investigations'.

Since its creation, ASIC has organised a series of international scientific conferences in different coffee producing or consuming countries. 'Today, ASIC is the only completely independent organisation in the world whose scientific vocation is specifically devoted to the coffee tree, the coffee bean and the coffee drink'. In ASIC's site you will find the proceedings of the previous colloquia as well as information and links on the lastest publications on coffee agronomy, chemistry, technology, physiological effects of coffee and on coffee and health.

International coffee organization (ICO): www.ico.org. The International Coffee Organization (ICO) is the main intergovernmental organization for coffee, bringing together producing and consuming countries to tackle the challenges facing the world coffee sector through international cooperation. It makes a practical contribution to the world coffee economy and to improving standards of living in developing countries by: enabling government representatives to exchange views and coordinate coffee policies and priorities; enabling government representatives to exchange views and coordinate coffee policies and priorities at regular high level meetings.

In this site, in addition to information on world coffee production, exports and imports, you will find information and interviews on coffee and health.

National Coffee Association of the USA (NCA): www.ncausa.org. The National Coffee Association of USA was founded in 1911, one of the earliest trade associations formed in the United States and the first trade association for the US coffee industry. Its site supplies information for the public, coffee industry, and association members. There you will find information and recent research involving coffee.

Speciality Coffee Association of America (SCAA): www.scaa.org. The SCAA is the trade association for the speciality coffee industry. One of the SCAA's primary functions is to set the industry's standards for growing, roasting and brewing coffee. Members of the SCAA include coffee retailers, roasters, producers, exporters and importers, as well as manufacturers of coffee equipment and related products. In this site you will find hints on cupping, roasting, brewing, as well as other relevant speciality coffee consumer information.

15.7 Acknowledgements

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16

Teas and tea-based functional beverages

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Abstract: Teas and tea-based products have become some of the most important beverages around the world. Their consumption has grown in popularity due primarily to their claimed stimulant and health properties. Market expansion from their country of origin to other parts of the world has also led to new technological developments, not only to optimize their production but also to satisfy consumers, particularly regarding convenience and taste. This chapter describes health benefits and technological and sensory aspects of teas from different plant species. It includes the most popular teas from *Camellia sinensis* and herbal teas such as maté, hibiscus, peppermint, and chamomile. Teas and tea-based beverages will continue to grow as part of a healthy lifestyle of consumers.

Key words: tea chemistry and technology, *Camellia sinensis*, herbal teas, maté tea, tea health benefits.

16.1 Introduction: the range of teas and tea-based beverages and trends in their consumption

Tea is the most widely consumed beverage in the world, other than water; with nearly every culture consuming some variety of tea. The majority of teas consumed come from the C*amellia sinensis* plant and it is from this plant that green tea, black tea, oolong tea and white tea are produced. These types of teas are the most popular throughout the world and are known as non-herbal teas.[1] Teas from other plant species are known as herbal teas.

16.1.1 *Camellia sinensis* teas

Camellia sinensis is native to Southeast Asia but is produced throughout the world, from China to Kenya; it is one of the most important agricultural crops in these regions. China is currently the leader in tea production and

Table 16.1 World tea production

Country	Tonnes (×1000)
China	1050
India	893
Sri Lanka	311
Kenya	311
Turkey	205
Indonesia	171
Viet Nam	142
Japan	92
Argentina	68
Islamic Republic of Iran	59

Adapted from FAOSTAT, © FAO Statistics Division 2008, 9 January 2008.

has produced more than 200 different cultivars.[2] China produced over 1 million tonnes of tea in 2006 and India over 800 000 tonnes in 2006.[3] Table 16.1 lists the top ten producers of all teas in the world for 2006.

The *C. sinensis* plant was first cultivated over three thousand years ago and has traditionally been used in Chinese medicine. The difference between varieties of tea produced from *C. sinensis* is due to processing. An oxidative process known as fermentation, in which the tea leaves are exposed to air for different lengths of time, and the action of enzymes result in changes in flavor and chemical make-up of the tea. Black tea is fully fermented, while green and white teas are not fermented and oolong tea goes through only a moderate fermentation. To avoid the fermentation process both green and white teas are heated after harvesting to inactivate degradation enzymes, such as polyphenol oxidase. The difference between green tea and white tea lies in the time of harvest. White tea is produced from the youngest buds of the plant and even this type of tea has different grades; green tea on the other hand is produced from the leaves of the plant and the buds. From all of the tea produced, approximately 78% is black tea, primarily consumed in Western countries, 20% is green tea, primarily consumed in Asian countries, and 2% is oolong, mostly consumed in southern China.[3]

Two major classes of polyphenols are present in *C. sinensis*, the catechins and the theaflavins. The catechins are predominately found in green tea and include: epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC), (−)-epigallocatechin-3-gallate (EGCG), (+)-catechin, and (+)-gallocatechin (GC). EGCG is by far the most abundant, accounting for as much as 65% of all catechins, with one cup (250 ml) of tea containing between 100 and 200 mg.[4] The theaflavins are found in high levels in black tea and are produced through the fermentation process in which catechins are oxidized and polymerized, these include: theogallin, theaflavin 3-gallate; and thearubigins, which are found in high levels in black tea and contribute

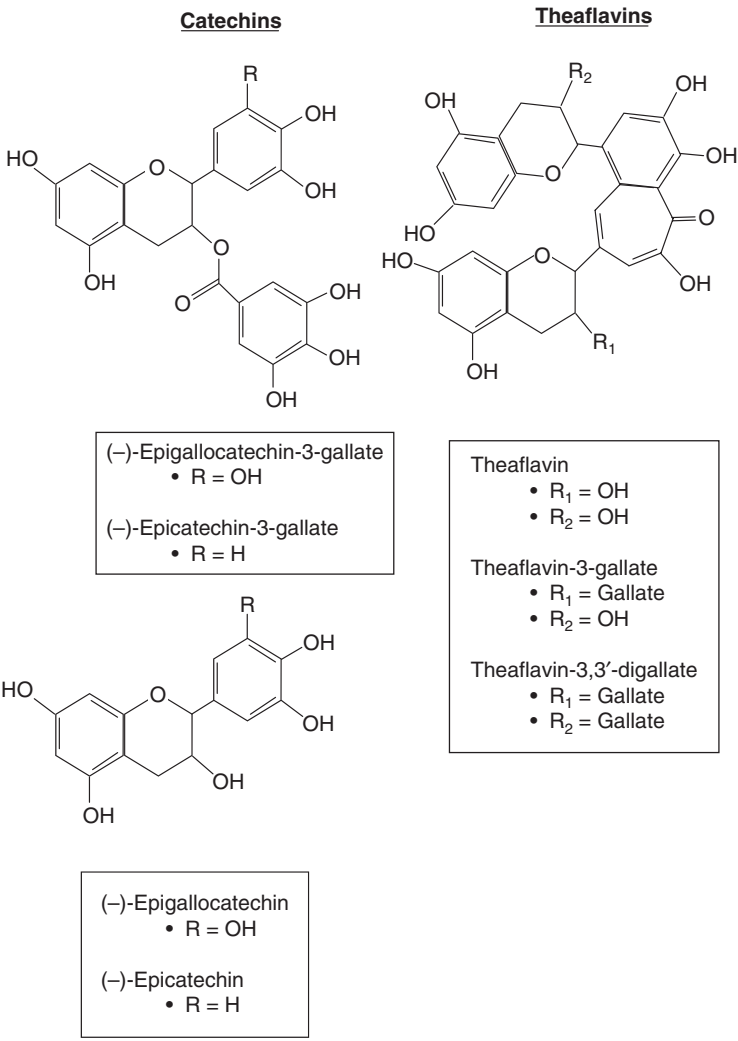

Fig. 16.1 Catechins and theaflavins present in *Camelia sinensis* teas (green tea, black tea, oolong tea and white tea).

to the color and flavor.[5–7] Figure 16.1 shows some of the chemical structures of catechins and theaflavins present in the *C. sinensis* teas. The polyphenols found in tea can account for as much as 30% of the weight of the leaf.[8] Oolong tea is only partially fermented, therefore; it contains high levels of catechins as well as theaflavins.[8] In all *C. sinensis* teas, alkaloids (caffeine and theobromine) as well as flavonols (quercetin, kaempferol and myricetin) are present and contribute to the stimulant effect and antioxidant activity, respectively.[7]

The relative proportion of catechins to theaflavins is what contributes to the teas having very different sensory properties. As the leaves ferment they lose their grassy aroma and become much more floral.[9] Not only do the polyphenols contribute to the sensory properties of the tea but they have become of interest to science for health purposes. Green tea has been associated with many health benefits, from boosting the immune system to possible cancer prevention.[4] For these reasons consumers are seeking out teas in an ever-increasing level.

16.1.2 Herbal teas

Tea produced from the *C. sinensis* plant is not the only tea consumed in the world. Herbal teas account for another category. While there are many different kinds of herbal teas, some of the most common varieties include: maté tea (*Ilex paraguariensis*), hibiscus tea (*Hibiscus sabdariffa)*, peppermint tea (*Mentha piperita*), and chamomile tea (*Matricaria recutita*). These teas are consumed by many different cultures for both medicinal purposes and for pleasure. Recently, they have begun to find their way into other cultures, outside their historical use, namely the United States and Europe, and because of this there is a growing interest in their study.

Maté tea originates from a region in southern South America, primarily in Argentina, Paraguay, Brazil and Uruguay. It is produced from the plant *I. paraguariensis* and is very different from the chemical and flavor standpoints of *C. sinensis* teas. Maté tea has been consumed for centuries by native people of South America and was adopted by the gauchos, becoming an important social aspect of life. Maté tea is prepared as an herbal infusion, often in a dried gourd, and drank through a metal straw called 'bombilla'. It is known for its earthy grassy aroma and taste as well as its astringent bitter taste. Maté tea is consumed largely for its stimulant properties, arising from its content of xanthine compounds.[10] It is consumed at very high levels, 1–3 liters per day, and with up to 50 g per infusion. Compared with traditional North American style, which is equivalent to 1–2 grams per cup.

Maté tea is made up of entirely different polyphenols from those in tea produced from *C. sinensis*. Instead of catechins, maté tea has a large concentration of chlorogenic acid, dicaffeoyl quinic acid and xanthines, i.e. caffeine and theobromine, (Fig. 16.2).[11] Nonetheless, it possesses a similar anti-oxidant capacity as green tea.[12] It is this high polyphenol concentration that has drawn so much attention for scientific study. Maté tea studies have steadily increased within the last 20 years. Maté tea is popular for its stimulant effect as well as for its associations to weight loss and possible effects on cancer.[13] This growing interest in maté tea has led to its introduction as an ingredient into other foods and concomitant increased interest in the consumer market.

Peppermint tea (*M. piperita*) is one of the most popular herbal teas throughout the world and, similar to maté tea, it has been used as an herbal

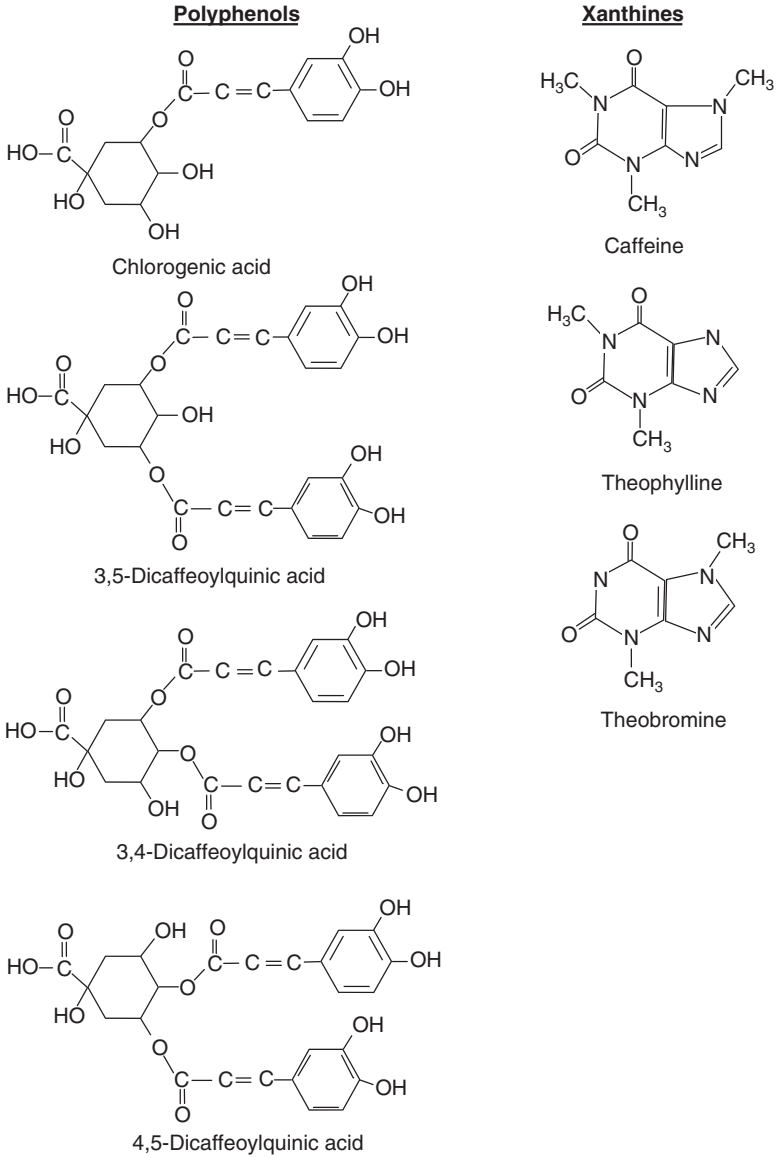

Fig. 16.2 Phenolic compounds in maté tea (*Ilex paraguariensis*).

remedy for centuries. Peppermint is cultivated around the world, is known for its distinctive flavor and aroma, and is used in everything from food to cosmetics. Polyphenols of peppermint leaves account for 19–23% (total flavonoids, 12%) of their weight; in an infusion, approximately 75% of the polyphenols are extracted. Making up this phenolic fraction are 59–67% eriocitrin and rosmarinic acid, 7–12% luteolin 7-*O*-rutinoside, 6–10%

hesperidin, and lower levels of 5,6-dihydroxy 7,8,3′,4′-tetramethoxyflavone, pebrellin, gardenin B, and apigenin.[14]

Hibiscus (*H. sabdariffa)* is an ancient medicinal herb used by many cultures to cure a number of diseases including hypertension, pyrexia and liver disorders. It can be consumed as an infusion but it is also found in other beverages, for instance as a popular soft drink in Taiwan. Hibiscus tea has shown to have potent antioxidant potential *in vitro*.[15]

Chamomile (*M. recutita*) is native to Europe and Western Asia and has been used as a medicinal plant for centuries due to its anti-inflammatory, spasmolytic, antipeptic, sedative, antibacterial and antifungal properties.[16] Chamomile has been shown to contain a number of polyphenols and to possess anti-oxidant potential.[17] Over 120 compounds have been identified in the flowers of chamomile, from which the infusions are prepared. Phenolic compounds include apigenin (16.8%), quercetin (9.9%), patuletin (6.5%), luteolin (1.9%), as well as cinnamic acid derivatives (39.1%), i.e. caffeic acid. However, its antioxidant capacity is relatively low compared with other herbs, as tested with the ferric-reducing ability of plasma (FRAP) assay. However, chamomile possesses one of the highest levels of apigenin which has shown potential as a chemopreventive and anti-inflammatory agent in *in vitro* and *in vivo* studies.[18] Thus, chamomile, while not the most potent herbal tea, is readily available and may be a good candidate for commercial extraction and introduction into other foods and beverages.

16.1.3 Tea-based beverage sales and production

The global tea market is one of the fastest growing industries in the consumer market. With increasing publicity from health magazines and commercial advertisement the number of products designed around tea is ever increasing at a rapid rate. This has caused consumers to desire more accessibility to tea products and has led the industry to look for better ways to get the tea to the consumer as well as improving its quality and safety.

With teas growing in popularity throughout the world it has become increasingly important for producers to find new ways to entice new consumers into the market. For instance, because maté tea has a very distinct grassy, hay-like and often smoky taste and aroma, producers have begun adding other herbs and ingredients, i.e. mint, citrus, milk, etc., to the teas and extracts to facilitate better taste for, primarily, American and European consumers. However, there are restrictions to these additions: Argentina admits up to 40% (by weight) addition of herbs, Paraguay admits up to 15%, while Brazil does not have maximum values.[19] Not only are producers developing new forms of dry tea products but also they have begun to fundamentally change the way in which people consume tea. By combining tea with other ingredients, i.e. soda and juice, as well as making it more accessible, by making instant products and ready-to-drink teas, producers are acquiring the next generation of tea consumers.

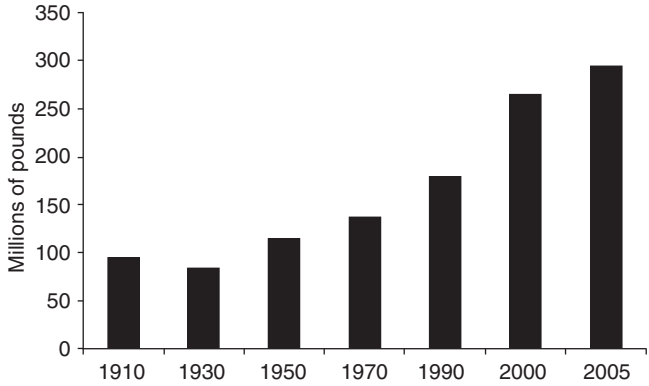

Fig. 16.3 Imports of all teas into the United Stated in millions of pounds from 1910 to 2005 (1 million pounds = 454 tonnes). Adapted from USDA/Economic Research Service 2007.

Overall tea imports have increased steadily from the early 1900s to the 1990s; particularly in the past 15 years these imports have drastically increased. As shown in Fig. 16.3, data collected by the USDA/Economic Research Service 2007 demonstrate the increased appetite of the American public for tea. This increased interest has led to a greater availability of tea in supermarkets as well as in coffee shops. Tea alone has not the only effect of this increased consumption. The development and production of tea-based beverages and ready-to-drink teas has also been on the rise.

16.2 Overview of the health benefits of tea

Teas have traditionally been consumed by cultures for particular health benefits. It was realized early on that these beverages were able to impart better health and in some instances used to treat specific diseases, such as inflammation, headache, and cancer. Peppermint tea purported benefits include liver disorders, dyspepsia, enteritis, flatulence, gastritis, intestinal colic, and spasms of the bile duct, gallbladder, and gastrointestinal tract.[14]

Although tea has been consumed for centuries it has only been within the past few decades that science has begun to fully examine its properties. Examinations of *in vitro* and *in vivo* mechanisms relating to its ability to affect certain diseases has shown many pertinent possibilities for its use. For instance, numerous anti-oxidant mechanisms have been examined in order to determine tea's ability to fight free radicals through the interactions of the free radicals with the potent polyphenols present in tea. Though the teas produced from *C. sinensis* and *I. paraguariensis* are substantially chemically different, they both possess a high anti-oxidant activity, which in recent years has become a chief marketing tool for the tea industry.

The many polyphenols in tea have not only been attributed to antioxidant activity but have shown to have a potent effect on other aspects of human health. There has recently been a breadth of studies published on the effects of non-herbal tea on inflammation, cardiac health, respiratory health, and oral health.[4,20,21] The major polyphenol found in green tea, EGCG, has been shown to have a substantial effect on inflammation. One suggested method is the inhibition of nitric oxide synthesis, which may damage cardiac tissue; some evidence has shown significant plasma antioxidant activity less than an hour after consumption of tea.[20]

Historical evidence has indicated that consumption of green tea can have an effect on weight loss. Recently, *in vitro* studies have shown that EGCG, found in green tea, can inhibit the proliferation and differentiation of adipocytes and fat cells as well as increasing lypolysis.[22] *In vivo* studies on mice and humans have shown that green tea and its catechins can reduce body weight.[23–25] Maté tea has shown substantial evidence supporting the effects it may have on weight loss and obesity. *In vivo* studies have shown that consuming maté tea resulted in a loss of weight for overweight individuals, possibly owing to the delay of gastric emptying.[26] Because of the increasing studies on weight loss, a number of over-the-counter diet pills have become available containing these teas or their extracts as well as other ingredients. Although studies have shown that some of these products indeed affect weight loss, it is still to be seen to what extent these products work, their mechanism of action, and their safety for human consumption.[27]

On the forefront of tea research for human health are the implications related to its ability to affect cancer. *In vitro* and *in vivo* studies of green tea polyphenols and maté tea polyphenols have both shown an affiliation to inhibit or prevent cancer growth. A number of different cancers have demonstrated to be affected by tea, including: breast, lung, liver, gastrointestinal, leukemia, pancreatic and prostate.[28–31] Numerous mechanisms have been suggested, including inhibition of topoisomerases,[32,33] proteasome inhibition, induction of apoptosis,[34] cell cycle arrest,[35] and inhibition of cancer cell proliferation.[36] However, research on the use of black tea in cancer prevention has thus far been inconclusive. It is then imperative that further research be conducted, especially on maté tea and other herbal teas, to determine their true effectiveness for cancer prevention and treatment.

Epidemiological evidence regarding the effect of different types of teas in humans has been mixed. However, recent in-depth studies have shown that *C. sinensis* tea consumption may have an effect on reducing cancer incidence.[28,37] An epidemiological study conducted in China reported, in 2007, that women who consume green tea had a lower incidence of breast cancer. As tea consumption increased, the rate of breast cancer decreased, and inversely when the rate of tea consumption decreased the rate of breast cancer increased.[38] Though the number of epidemiological studies is growing, it is still relatively inconclusive as to the effectiveness of tea and polyphenols on the risk of cancer in various populations. It is therefore necessary

to conduct controlled *in vivo* studies to understand green tea and maté tea's effectiveness on reducing the risk for developing cancer.

16.3 Improving the quality of leaf and bagged teas

16.3.1 Growing conditions

China and India are the leading producers of *Camellia sinensis*; however, many other countries also cultivate this plant, including Asian countries as well as African. In many of these areas the economy relies heavily on tea production. For this reason much emphasis in recent years has been placed on developing better cultivation practices to produce a healthier and more plentiful tea crop. This has become especially important with the increasing demand for tea.

Much of this emphasis has been focused on soil quality, i.e. fertilization and erosion. For instance, in Sri Lanka, a country dependent on tea cultivation for its economy, research has examined the use of fast-growing hedgerow crops planted between tea rows to help stop soil erosion, as the tea is grown on slopes in areas with high rainfall. It was determined that the plant *Euphatorium innulifolium* could be planted and effectively increased tea yields through the elimination of soil erosion, without being over-competitive to the tea plants. It was also noted that the use of the hedgerow clippings as mulch also increased tea yields.[39] These observations may be important in other parts of the world where cultivation on slopes takes place.

Utilizing state of the art methods, researchers are developing ways to analyze tea crops to further optimize their production. Some studies also focus on the density of the plants and the water use associated with it, and how this affects the growth and production of the tea plants.[40] Through the use of remote sensing, Rao *et al*. have been able to determine optimal areas for tea growth to produce the best crop. They, identified areas prone to waterlogging that could pose a detriment to tea crops.[41]

As with most any other crop, fertilization plays a key role in growing tea. Continuous application of nitrogen (N) containing fertilizers to Japanese fields has led to acidification of the soil. Excessive N not only can affect plant growth but also can induce nitrate contamination in local water and cause high nitrous oxide loss. Conventional high N fertilization is equivalent to 1100 kg N/ha. When utilizing calcium cyanamide (400 kg N/ha), a low N fertilizer, soil acidification was decreased and tea yields remained equal or higher than with previous high N treatment.[42] The studies signify the expanse to which cultivation research is being conducted and the importance to the countries that rely on tea production for economic stability.

Maté tea is primarily grown in the southeast regions of South America but despite this limited localization a range of cultivation and processing methods are utilized. In the wild, *I. paraguariensis* grows in the forest and

is covered by the canopy of the forest. However, to better facilitate an increase in harvest and reduce cost with mechanical harvesting much of the cultivation is done on large plantations. Nevertheless, some producers are also growing forest-grown maté tea, which is often marketed with an organic claim. To complicate matters even more, different countries use different cultivation methods. For instance, Argentina does not commercially produce forest-grown maté tea; while Paraguay produces both forest and plantation-grown products. With the global tea industry gaining an increasing interest in maté tea, it is becoming more evident to producers that developing growing characteristics that will produce the highest-quality product, i.e. higher caffeine and polyphenol levels, is in their best interest. There have been studies that have shown that utilizing different fertilization methods can influence the levels of the polyphenols in the tea extracts; sun-exposed plants, plantation grown, had higher extract yields.[43]

With maté tea's increasing rise in popularity outside South America it has become increasingly more desirable for producers to change their methods of production from the more ancient labor-intensive methods to newer, more cost-effective methods. Whether it is production on large plantations, with mechanical harvesting, or decreasing energy consumption, producers are looking for the best methods for producing larger quantities for export with improved quality and at cheaper cost.

16.3.2 Processing

Camellia sinensis processing involves many steps for the production of green tea, black tea, oolong tea, and white tea. White tea and green tea are the most minimally processed, being flash heated immediately after harvest to inhibit oxidation and not undergoing fermentation. This results in catechin content distinctly different from black tea and oolong. Firstly, harvested leaves go through a process called withering; this involves some loss of moisture and begins the changes in chemical make-up. This process is believed to give distinct flavor and aroma to black and oolong teas. Second is the process of maceration or rolling the leaves, which helps to further initiate the fermentation process where further changes in chemical make up take place and continues to change the flavor and aroma of the tea. This is where black and oolong teas diverge. Oolong is only minimally fermented and is quickly fired to dry the tea and inhibit further oxidation; black tea is allowed to progress through a longer fermentation before it is fired. It has become increasingly more evident that proper control over these processes greatly influences the quality of the tea. Not only do the processing methods affect the quality, but also even more important may be the handling of the tea from field to processing plant. It is believed that as much as 25% of the value of a tea can be lost owing to improper handling of the leaves in the field.[44]

Traditionally firing/drying of the tea leaves was accomplished through direct heat or pan-frying. Newer methods involve using steam or oven

drying. Recent studies however, have shown that utilizing microwave drying may produce a better tea. Microwave drying resulted in higher levels of polyphenols and catechins, improved color and gave a sweeter flavor. Parched and sun-dried teas had the lowest levels of compounds and a duller color; oven and steam drying were intermediate.[45]

The fermentation process that is utilized to produce the many wide-ranging varieties of teas from the *C. sinensis* plant plays many roles in developing the different aromas, tastes, and color of the tea. One chief and very measurable change is the oxidation and polymerization of the catechins. Oxidation is carried out by enzymes, polyphenol oxidase or, in the presence of oxygen, auto-oxidation. This oxidation of compounds like EGCG, ECG, EGC, or EC, among others, can result in the formation of polymers, forming theaflavins. Theaflavin is made of two catechins; others can then bind to form theaflavin-3-gallate and theaflavin-3,3′-digallate, for example (Fig. 16.1). This oxidation and polymerization of catechins to form theaflavins is one aspect for the distinct change in color, aroma and taste of fermented teas, black and oolong, compared with non-fermented teas, green and white.[9]

Maté tea production utilizes numerous processing methods. Traditionally, it is processed by flash heating through a drier where the leaves are in near direct contact with open flames. It is then dried over an open fire where it is in direct contact with smoke. However, over the past few years there has been growing concern that this method of open flame drying may be imparting impurities from combustion, such as polycyclic aromatic hydrocarbons. For this reason some producers have switched to drying with open fire but implementing filtration of the smoke/hot air to eliminate ash and contaminates. Furthermore, other producers have gone as far as to fully switch to hot air drying alone as well as the use of conveyor systems to facilitate faster and more even drying of the tea leaves. Recent literature has begun to examine what drying characteristics will allow for the most cost effective drying of maté tea products; for instance, the heat transfer required in packed bed and conveyor drying.[46,47] Finally, following the drying process the tea is then aged in cedar or concrete chambers. Again, this is another step that varies greatly. Some maté tea is aged in large sacks while others are aged loose. Not only this, but the temperature, humidity, and time are all variable. These characteristics vary by country: for instance, Argentina and Paraguay consume a moderately ground, aged maté tea with a high content of stems, while Brazil prefers a finely ground maté tea with fewer stems and a non-aged/greener product.[19]

There are many methods for analyzing the sensory properties of food matrices. These include a number of aroma analysis methods utilizing machine analysis as well as flavor and aroma analysis with trained panelists. These methods are generally time consuming and costly. They can also vary greatly between one another and be subject to bias. The mechanical aroma analysis methods, for example, generally require solvent extraction and are

not appropriate for rapid and extensive analysis of teas for quality analysis.[48,49] Recently, however, a method for rapid identification and quantification of aroma compounds in both green and maté teas has been applied.[50,51] This method is called solid phase microextraction (SPME) and involves the use of a polymer-coated needle to analyze the headspace of a prepared tea. This method has proven to be a reliable, rapid, and reproducible method for the identification of aroma compounds. From maté tea 70 aroma compounds were identified, 17 not previously described in maté tea, and showed a relative standard deviation below 10% between replicates.[51] Standard solvent extraction methods have been able to identify differences in maté tea aroma compounds between teas that were roasted from those that were not.[49] It may be possible to apply this same principle to SPME analysis for analyzing large number of samples for quality assurance or to identify aromas specific to production methods in large numbers of samples.

Similar solid phase microextraction methods have also been conducted on *C. sinensis* tea. Utilizing SPME, differing levels of many aroma compounds were identified in different green tea varieties.[50,52] Thirty-six compounds were described with differing levels in different teas, such as 1-penten-3-ol, linalool, (*E*)-2-pentenol, (*E*)-3-hexanol, (*E*,*Z*)-2–4-heptadienal and 3,7-dimethyl-1, 5,7-octatrien-3-ol; which contribute to the fruity and sweet aroma of green tea and were distinct between origins.[50]

Not only have aroma compounds been identified but also catechins. Furthermore, analysis of catechins with SPME analysis proved to be several times more sensitive than with liquid injection into a mass spectrometer.[53] SPME has been shown to be effective at detecting beneficial or desirable compounds as well as, with the use of different fibers, identifying pesticides in Chinese green teas.[54]

Through the use of novel aroma and component analysis rapid and accurate methods could be developed for the industry. Implementing these novel methods could ensure better quality and consumer acceptance; especially for determining the components that consumers will accept with consumer panels and correlation to these novel detection methods.

16.3.3 Storage

All teas are dried products and therefore are inherently a very shelf-stable product as long as water activity remains low. However, mold and bacterial growth are among the primary concerns in tea packaging, handling, and storage. There has been some evidence that shows both bacterial and fungal/aflatoxin presence in processed teas including *Aspergillus flavus* and *Aspergillus niger*.[55,56] Proper storage conditions of tea are critical as an increase in humidity levels above 90% allows for the growth of fungus. While proper storage of tea is the simplest method to ensure proper safety, new methods are being developed to eliminate bacteria and fungus from the product. One example of this is the use of irradiation to kill

Table 16.2 Change in tea beverage consumption (%)

Segment	2001–02	2002–03	2003–04	2004–05	2005–06
Ready to drink tea	–2.70	0.70	2.60	15.40	26.20
Instant tea	–4.00	–10.80	–1.40	–1.70	–6.00
Loose tea	0.20	0.80	1.00	2.10	3.00

Adapted from Beverage Marketing Corp. or New York, 2007, http://www.beveragemarketing.com.

microorganisms in processed teas. Through the use of gamma radiation it has been demonstrated that a dose of 5–10 kGy is sufficient to kill the microbes, while not affecting sensory or chemical attributes of the tea.[57,58]

16.4 Production of tea-based beverages

16.4.1 Consumer trends

As consumers seek out healthier food choices to improve their health and lifestyle, the market must continually advance to satisfy this need for the next great health food. Tea-based beverages and ready-to-drink tea (RTD) beverages are on the forefront of the consumer's health choices. They are becoming more readily available not only in health food stores but in the mainstream supermarket and convenience stores. Many of the largest beverage producers in the world have shifted their production to incorporate tea in their beverages. Within the tea industry, consumer interest in RTD beverages has dramatically increased over the past 5 years while interest in other convenient forms of tea, such as instant teas, has reduced, as shown in Table 16.2; perhaps due to consumers seeking even more convenience and higher-quality products.[59] Premium, super premium, and regularly priced RTD tea beverage grew by 22%, 35% and 10%, respectively, from 2004 to 2005.[60] This can be compared with soda sales, which were down 0.7%.[61] The increase in tea sales and decline in soda sales exemplifies the interest consumers have in consuming tea-based beverages and emphasizes the need for industry to continue to develop new and healthier products.

16.4.2 Instant tea

The first alternative form to traditional tea preparation was the development of instant dried tea. This involved the preparation of a tea infusion, followed by concentration and then drying with either spray drying or freeze drying. The process for instant tea production was first developed in 1940 in England and was produced from black tea.[62] Instant teas became popular because of the ease of use and longer shelf-life compared with fresh tea. However, they face the problems of generally being lower quality than

fresh tea, loss of aroma and flavor, as well as having a high production cost. New methods of production are thus currently being developed for extraction of a higher quality and less expensive product. One of these methods is the utilization of pressed juice of the tea leaves to prepare a solid extract which yields similar theaflavin levels to those in fresh tea, as well as good cold water solubility and good flavor and aroma.[62]

16.4.3 Concentrates

Tea concentrates are also another popular alternative to dried extract. They generally have the benefit of being better quality than dried tea, though usually with a lower shelf-life. They also have a reduced level of sanitation required in commercial applications compared with fresh tea. Concentrates can contain up to 35% solids.[63] They can also be made freeze–thaw stable through the addition of 2–4% water-soluble carbohydrates.[64] Enzymatic methods have also been employed to make tea concentrates chill stable and control the precipitation that forms when chilled and stored.[65] It is important to limit the precipitation of the polyphenols and catechins from the tea, as loss of these compounds would likely lead to loss of the heath protective action that tea provides. Numerous methods have been studied and patented by the industry for this purpose.[63,66–68] These extracts and concentrates can then be used to make tea-based beverages by diluting and dissolving the tea in water.

16.4.4 Ready-to-drink tea beverages

RTD tea beverages can range from tea alone to a combination of ingredients and may contain as much as 6% solids and possess a desirable flavor.[69,70] While tea beverages often contain varying levels of caffeine and are often consumed for the stimulation received from caffeine, some consumers are sensitive to caffeine. For these reasons, tea beverage producers often decaffeinate the tea, which commonly involves organic solvent extraction. These organic solvents not only pose an environmental hazard as waste but a potential health hazard from residues in the products. It has become increasingly necessary for producers to find new methods for extraction of caffeine, such as supercritical CO_2 extraction, which does not use organic solvents and can selectively extract caffeine; however, it is difficult to implement such methods commercially.[71,72] There has now been new research on the use of genetically modified tea plants that do not have caffeine. By genetically engineering the tea plants, the caffeine can be eliminated by overexpressing caffeine degradative pathway genes or silencing caffeine biosynthesis pathway genes, thus, eliminating caffeine in the raw product and eliminating extraction methods.[73]

Some teas are traditionally consumed with the addition of milk or sugar; most notably, black tea which often has one or the other or both added.

This may be of interest to beverage producers as a means for producing better tasting or more interesting tea-based beverages for the consumer. However, there is some evidence to suggest the addition of these ingredients can lower the total polyphenol levels and anti-oxidant capacity of the tea; there may be an interaction of the polyphenols and milk proteins, possibly casein.[74,75] Both the *in vivo* and *in vitro* studies have indicated the possibility of this effect of milk on the biological properties of tea; however, there is conflicting evidence that there is no effect from the addition of milk. Therefore, further studies are needed to determine the extent and validity of this effect to the human consumption of tea-based beverages.[76]

16.5 Improving the nutritional and sensory qualities of tea-based beverages

16.5.1 Detection of contaminants

The safety of consumer products is a top priority for many food companies. Tea production often utilizes tea from many different growers and, sometimes, different areas. It can be difficult to ensure that these products are not contaminated with harmful chemicals such as pesticides. Rapid and accurate analysis of tea-based beverages for pesticide residues is paramount for consumer safety. Recently a method has been developed for the extraction and quantification of minute levels of pesticides in RTD tea beverages. This method involves the use of dynamic hollow fiber-protected liquid phase microextraction (DHFP-LPME) and was able to detect organochlorine pesticides at levels below 1 μg/l. An important note on this method is that it only required 3 μl of organic solvent for the extraction.[77]

Though less critical to health is the issue of purity of tea-produced beverages. Tea is an agricultural product and in many areas of the world where it is cultivated there may not be stringent guidelines in place for its cultivation and harvest. It may be possible for growers to intentionally adulterate their product with other plant material that is cheaper or of lower quality. This is especially evident with the production of maté tea. There are many species of *Ilex*, e.g. *I. dumosa, I. brevicuspis*, and *I. theezans*, among others, that grow in the same region as *I. paraguariensis*, the plant used to make maté tea. These plants look similar to the maté plant; however, most possess no to little polyphenols and caffeine. They grow wild and make perfect adulterants, as they are very hard to detect. However, they can have a diluting effect on the polyphenol concentration in maté tea as well as changing the flavor and bitterness of the tea. New methods have been developed that could be used to rapidly and reliably detect these adulterating plants. *Ilex* plants, including maté, contain saponins, the glycoside compounds that often contribute bitterness to maté tea.[13] The interesting thing about these saponins is that each *Ilex* species possesses different saponins, with not one sharing a similar saponin with *I. paraguariensis*. Utilizing chromatographic

methods it is possible to identify saponins in maté and determine the presence of adulterating plant materials.[78] This method could prove a fast and reliable method for the identification of adulterants and may be applicable to other teas and foods in the industry.

16.5.2 Improving sensory properties of tea-based beverages

As the tea market reaches out to new consumers and begins integration of new teas, producers are looking for ways to improve the taste of the products to capture a wider audience. Maté tea, for example, is characterized by an astringent or bitter taste. This tea, with its high anti-oxidant capacity and caffeine concentration makes for a good candidate for energy and health drinks. However, the taste may turn off many consumers. Traditionally producers have used sugar to counteract the bitterness; however, this incorporates calories into what would be a healthy beverage. New methods, however, are being implemented to counteract bitterness without the addition of large quantities of sugar. For example, the addition of plant sterol esters may reduce bitterness, as well as incorporate the benefits associates with plant sterols, with cholesterol-lowering effects.[79]

When trying to reduce the calorie content of particular healthy beverages, such as sweetened tea beverages, replacing sugar with low-calorie sweeteners is commonly used. However, this can lead to a lower sensory quality through decreased mouthfeel. It may be possible to incorporate long-chain carbohydrates that could substantially improve mouthfeel. These carbohydrates, in combination with low-calorie sweeteners, could provide an appropriately sweetened beverage with good sensory properties.[80]

16.6 Future trends

The functional beverage market has experienced a 14% inflation/adjusted growth from 2002 to 2007 and has an estimated value of 9.8 billion in sales.[81] Consumers have made functional beverages part of their lifestyle according to their age and gender. This is because functional beverages address different lifestyles and needs such as excitement, energy boost, prevention against specific diseases, aging, fighting fatigue and stress, and making up for the lack of healthy eating.[82] Based on recent consumer research data, anti-oxidants and green tea/green tea extract have been ranked, after calcium, on the top three functional ingredients influencing consumer purchase decisions (60% and 52%, respectively). The health benefits of different types of teas as well as their high anti-oxidant capacity and flavor have been well documented.[83,84] In this context, teas are well positioned to grow in the future as a profitable part of the functional beverage segment. This trend will continue as long as science discovers novel attributes of

functional beverages and consumers become more conscious of the role of diet on their health and well-being. The functional tea market has registered a healthy inflation-adjusted growth of 59% from US$417 million in 2002 to US$756 million in 2007.[82]

Considering the composition, health attributes, and consumer perception of teas, the opportunities for their growth as functional beverages are the following:

- As ready-to-drink teas that offer convenience and taste.
- As functional teas promoting a holistic lifestyle that provide health benefits such as protection against common cold, digestion aid, and immunity boost.
- As premium-positioned functional teas that are marketed on the strength of inherent healthy attributes such as naturally occurring anti-oxidants.
- Since various teas have naturally occurring caffeine, they also represent an opportunity for their positioning as energy drinks, especially if added with other ingredients such as guarana or taurine.
- As ethnic teas from different countries having health/energy attributes. This may include yerba maté, hibiscus, chamomile, damiana, lemongrass and linden mint, among others.
- Finally, a more novel niche for teas is been developed as a functional beauty beverage. This will become a reality as more research is conducted to determine the role of anti-oxidants contained in the teas on skin health and beauty.

16.7 Sources of further information and advice

Sources of information on tea and marketing opportunities are presented in Table 16.3.

Table 16.3 Sources of information and marketing on teas

Source	Web address
Tea Association of the United States of America	http://www.teausa.com/general/teaassociation/
Specialty Tea Institute	http://www.teausa.com/general/star/
Tea Council of the United States of America	http://www.teausa.com/general/topline/pop_newsletter.cfm?nlnum=2 http://www.teausa.com/general/teacouncil/
United Kingdom Tea Council	http://www.tea.co.uk/index.php?pgId=24
Mintel International, Chicago, IL	www.mintel.com
Yerba Maté Association of the Americas	http://www.yerbamateassociation.org/

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Part IV

Beverage development and consumption

17

Consumer-oriented development of functional beverages

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Abstract: This chapter addresses the key consumer issues relevant to the technical development and strategic marketing of functional beverages. The chapter first gives an overview of the market dynamics and innovation trends in the functional beverages market. Following this, the key strategic marketing and product design challenges facing manufactures of functional beverages are explored, and the importance of integrating the consumer with the new product development (NPD) process is discussed. Finally, a case study is presented on the consumer-oriented development of functional cosmetic beverages, which can assist manufacturers manage consumer knowledge more effectively throughout the NPD process.

Key words: knowledge management, consumer orientation, new product development, functional beverages, cosmeceuticals.

17.1 Introduction

The development and marketing of functional beverages present considerable challenges to new product development (NPD) practitioners in terms of identifying and designing technological 'breakthrough' products on one hand, and the marketing of science and technology to consumers on the other. Although health and wellness have been the most significant drivers of NPD in recent years, failure rates for new functional products are reportedly high. This may suggest a failure by companies to manage consumer knowledge effectively throughout the NPD process. This chapter addresses the key consumer issues relevant to the functional beverages market, and illustrates how functional beverage manufacturers can manage consumer knowledge more effectively throughout the NPD process.

Initially, an overview of the market dynamics and NPD trends in the functional beverages market is presented. Following this, the key strategic

marketing and product design challenges facing manufacturers of functional beverages are explored, and the importance of integrating the consumer with the NPD process is discussed. Finally, a case study is introduced on the consumer-oriented development of functional cosmetic beverages. The consumer-oriented approach to NPD presented in this chapter can assist NPD practitioners manage consumer knowledge more effectively, and provide direction for the new product design and strategic marketing of consumer-oriented functional beverages.

17.2 Functional beverages: overview of market dynamics and new product development trends

Five general trends have influenced food and beverage innovations since 1985: convenience, pleasure, ethnic fusion, tradition and importantly, health and wellness (Datamonitor, 2007a; Longo, 2007; Foote, 2002). These trends have had a strong influence on the development of the functional beverages market. The continued decline in traditional carbonated soft drink sales in the maturing EU and US markets has been offset by consumers increasingly seeking beverage alternatives perceived as natural or healthy, such as flavoured and near-water drinks, premium chilled juices and non-carbonated fruit juice drinks, ready-to-drink ice tea, soy drinks, and functional beverages (Reynolds, 2007; *Beverage Industry*, 2006, 2003). The functional beverages market, valued at US$25bn in 2005, has indeed come to represent an important strategic and operational orientation for food and beverage, biotechnology and pharmaceutical companies (Datamonitor, 2006a).

The appeal of the functional beverages market to companies clearly lies in adding value, and potentially higher price premiums, to otherwise conventional drinks. More specifically, the functional beverages market has proved attractive to companies with average growth rates ranging from 15% to 20% per annum, in comparison to growth rates of 2–4% per annum for both the general foods market and lighter food and beverages market (*Retail Merchandiser*, 2007; Weststrate *et al*., 2002; Longman, 2001; Shah, 2001). A key question therefore arises: what factors are driving NPD activities in the functional beverages market? Overall, the market dynamics and NPD activities across functional beverage categories, such as sports and energy drinks, probiotic and nutraceutical beverages, are either influenced or driven by five key factor groupings: increased concentration in the global beverages market; diverging functional beverage trends worldwide; flavour innovations; product differentiation; and cross-category innovations.

17.2.1 Key market and new product development drivers in the functional beverages market

Consolidation within the global beverage industry, coupled with aggressive acquisition strategies, has accelerated the growth of many functional

beverage categories from specialist to mainstream market channels (Datamonitor, 2007b; Longman, 2001; Holway, 2000). Large functional food and beverage manufacturers are increasingly recognising the importance of adopting a corporate image that is congruent with their respective functional food and beverage brands in order to gain overall consumer acceptance. In that context, multinational firms such as Groupe Danone, Kelloggs and Nestlé have divested a number of non-core brands, while acquiring and investing organically in health-oriented brands, in order to reposition their corporate image as part of their long-term strategy for growth in the functional food and beverages market (*Dairy Industries International*, 2006; Datamonitor, 2005a; Mellentin, 2004). In addition, large multinational beverage companies continue to pursue aggressive joint venture, acquisition or merger strategies to offset declining soft drink sales, to broaden their brand portfolio, or to gain market share in growth markets such as the functional beverages market (Shearer, 2007; Hein, 2006; Hehn, 2001). For example, PepsiCo has become a global player in the functional beverages market through both its merger with the Quaker Oats Company, and its acquisition of South Beach Beverage Company in 2001 (Todd, 2003; Bruss, 2002). Similarly, Coca-Cola's acquisition of Odwalla, Mad River Traders and FUZE Beverage has helped increase its market share in selected non-carbonated beverage categories such as new age drinks and premium chilled juices and tea drinks (*Drug Store News*, 2007; Halleron, 2001; Leatherhead Food Research Association, 2001). Furthermore, with increased competition in the US market across beverage categories, Coca-Cola and PepsiCo have also looked to potential joint ventures with other multinational companies such as Starbucks, Groupe Danone, Nestlé and Novartis for market opportunities with functional and speciality beverages in international markets (*Beverage Industry*, 2007; Datamonitor, 2005b; Leatherhead Food Research Association, 2002a).

It would appear that functional beverage trends are more heterogeneous than homogeeous, evolving and growing at different rates both within and across countries, owing to socio-demographic and socio-cultural differences in consumers' perceptions and acceptance of functional products (Frewer *et al*., 2003; Bech-Larsen *et al*., 2001; Poulsen, 1999). For example, while US probiotic dairy categories have realised impressive growth rates in recent years, these categories still remain largely underdeveloped in volume and value terms in comparison to the French, German, Spanish and UK markets (*International Food Ingredients*, 2007, 2002; Heasman and Mellentin, 2001). Similarly, the sports drinks category remains largely underdeveloped outside the United States and Japan, where energy and stimulant drinks and ACE fortified drinks dominate a number of key European beverage markets (*International Food Ingredients*, 2005; Leatherhead Food Research Association, 2004; Cosgrove, 2003).

Market growth and NPD activities across many functional beverage categories are also driven by flavour innovations through the introduction of new flavour line extensions. In particular, functional beverages appear to

be clearly benefiting from increasing consumer demand for smoothies and premium juice drink blends that combine a range of exotic fruits (Dafoe, 2006). The introduction of premium juice and smoothie bars has coincided with greater consumer experimentation with innovative functional drinks with novel flavours and functional ingredients (Landi, 2007, 2006; Dafoe, 2006; Hunter, 2005; Kelleher, 2005). For example, in response to the continued growth of the European probiotic dairy drinks category, Groupe Danone has expanded its Actimel range of probiotic dairy drinks to include orange and multi-fruit flavoured variants, while Ocean Spray has entered the select European markets with Ocean Spray Cranberry probiotic drink (Ball, 2004; *Marketing Week UK*, 2003). Similarly, in the energy and stimulant drinks categories, product formulations appear to be moving away from citrus flavours towards exotic fruit-based flavours such as BooKoo Beverages' Jugo and Healthy Beverage's Steaz Energy, as well as cranberry and pomegranate flavours such as Atlantic Multipower's Crea Max Creatine Drink, Food Broker's Spiked Silver and Silver Arrow's Revitalise Silver (*Convenience Store News*, 2007; MarketWatch, 2007; Booth, 2002; *In-store Marketing*, 2002).

Although new flavour line extensions represent a successful strategic orientation across functional beverage categories, companies are increasingly attempting to further differentiate their brands through creative segmentation and product positioning strategies on the basis of superior functionality (Mellentin, 2007). For example, the global functional juice category is primarily dominated by calcium-fortified and other nutrient-enriched fruit juices. However, there is a growing awareness by companies that calcium fortification alone may no longer offer a distinct competitive advantage or unique selling point within the functional juice category. This is evident by the increasing number of new product introductions being launched by juice companies in the United States that include juices fortified with vitamin D and other nutrients associated with milk, as well as functional juices targeted at specific conditions ranging from heart health to immune and joint health such as Tropicana's Essentials and Minute Maid's Active and Heart Wise (Howell, 2006; *Brand Strategy*, 2005; Butler, 2002).

New market entrants and line extensions of existing stimulant drinks brands are replacing taurine with more natural stimulant ingredients to include both ginseng and guarana such as SoBe's Tsunami and Free Natural's Organic Energy, or guarana and ginkgo biloba such as Natural Beverage's Voodoo Rain (Hein, 2005; Datamonitor, 2004; Zenith International, 2002). There is also a growing NPD trend in established European markets towards multi-functional probiotic dairy drinks such as Yoplait's Everybody, Yofres's Puleva Omega 3 con Bifidus Activo, as well as synbiotic beverages such as Muller's Vitality and ProCult ranges, and Ganaderia Priegola's Priegola Simbiotic drink (Mellentin, 2007; Leatherhead Food Research Association, 2004; Rogers, 2004).

The increasing number of multi-functional probiotic foods and beverages on the European market, and juice-based nutraceutical drinks in the United States alludes to an emerging NPD trend in the functional beverages market, which Hehn (2001) describes as 'blurring between beverage categories'. Innovations transcending beverage categories not only refers to companies diversifying into new product categories, but also the movement of functional ingredients across and between beverage categories. For example, in the functional dairy products market, plant sterols appear to have made the successful transition from table spreads to dairy drinks such as Raisio's Benecol Yoghurt Drink and Danone's Danacol (Mellentin, 2005). Novel examples of such a trend in the stimulant drinks category would include Upstate Farm's Mocha Java Caffeine Kick, a stimulant milk-based drink positioned as a healthier alternative to traditional carbonated stimulant beverages or green tea and botanical-based stimulant drinks such as Energy69 (*Marketing*, 2007; Berry, 2002).

Fruit juice and juice drinks have come to represent important carriers or base products for a multitude of cross-category functional ingredients, ranging from probiotics, fibre and plant sterols to omega-3, collagen and glucosamine. For example, an increasing number of probiotic juices and juice drinks are being launched on Northern and Western Europe markets such as Valio's Gefilus, Skane's ProViva (one-litre carton) and ProViva Shots (250 ml bottle), Hero's Bienstar and, more recently, Pete & Johnny's It's Alive, a non-dairy fruit smoothie containing bifido cultures (Dairy Industries International, 2005; Dairy Foods, 2004; Leatherhead Food Research Association, 2004). Most recently, Tropicana launched a dual-branded heart benefit functional juice Essentials with Benecol, while Minute Maid launched Active, containing glucosamine for good joint health (Datamonitor, 2006b; Brand Strategy, 2005).

17.2.2 New product development strategies for functional beverages: technology or consumer-oriented?

It is argued that companies, rather than consumers, are the main drivers of innovation, and that 'science push' rather than 'consumer pull' strategic orientations characterise NPD activities in the functional food and beverages markets (Salavou and Lioukas, 2003; Wennström and Mellentin, 2003). For technology-oriented NPD companies, a differentiation strategy based solely on functionality, i.e. added functional benefits, offers a short-term competitive advantage only: 'often technology is used to create value for the producer and this can sometimes be a very different matter from creating customer value' (Wennström and Mellentin, 2003: 21). Indeed, the high reported failure rates for functional foods and beverages suggest that consumer acceptance issues are either ignored or poorly understood by companies (Sorenson and Bogue, 2005a; Verbeke, 2004; Wennström and Mellentin, 2003; Heasman and Mellentin, 2001). Future success in the

functional beverages market will clearly necessitate not only understanding consumer and market trends, but also greater involvement of the consumer throughout the NPD process than hitherto, in order to anticipate and meet consumers' changing tastes and needs. In the next section the major strategic marketing and product design challenges that companies must address before bringing new functional beverages to the marketplace are discussed.

17.3 Strategic marketing and new product development challenges in the functional beverages market

The technical development and strategic marketing of functional products present enormous challenges to companies. Specifically, although 'breakthrough' products potentially offer value to consumers over incumbent products, consumer acceptance of novel products such as functional foods and beverages are slower than for conventional products (Samli and Weber, 2000). Indeed, the high reported failure rates of 70–90% for new functional foods and beverages indicate that many of these products meet with poor consumer acceptance (Heasman and Mellentin, 2001; Wennström, 2000). From both a technological and marketing perspective, this would suggest a failure on the part of many companies to manage consumer knowledge effectively throughout the NPD process.

The key new product design and strategic marketing challenges to new product success in both the functional food and beverage markets can be grouped as follows: predicting where consumer demand can be expected in terms of functional benefits, and overcoming consumer acceptance issues from both a marketing and product design perspective (Sorenson and Bogue, 2005a; Luckow and Delahunty, 2004; Gray *et al*., 2003); identifying consumer groups to target with new and innovative functional products (Sorenson and Bogue, 2007, 2006; Wennström and Mellentin, 2003; Heasman and Mellentin, 2001); identifying optimal pricing strategies (Bogue and Sorenson, 2007; Sorenson and Bogue, 2007, 2006, 2005b; Wennström and Mellentin, 2003); and developing effective positioning and communication strategies (Wennström and Mellentin, 2003; Bistrom and Nordstrom, 2002; Hilliam and Young, 2000).

17.3.1 Consumer-oriented approach to concept ideation and concept development

Product development is a knowledge-intensive process where the generation of new ideas and concepts requires detailed knowledge of both products and consumers. Consumers have an extremely important role to play at the early stages of the NPD process in two respects: consumers as a resource and consumers as co-designers, since they can make an effective

contribution to product design and acceptability. Therefore, although analyses of emerging consumer and market trends is a very good starting point in terms of identifying potential new product opportunities at the concept ideation and concept generation stages of the NPD process; closer integration of the consumer with the NPD process is necessary to minimise consumer acceptance issues associated with more innovative functional beverage concepts. For example, multi-functionality is considered an important future functional beverage trend based on past product launches in Japan and the United States. Indeed, it is argued that calcium fortification no longer presents a distinct competitive advantage to US juice manufacturers. However, it would appear that Irish consumers are not receptive towards nutrient-enriched functional orange juice that offers the full nutritional benefits of milk (Sorenson and Bogue, 2005a). More so, in a follow-up study, young mothers who traditionally represent a key segment for nutrient-enriched beverages do not appear to perceive value from the addition of nutrients beyond calcium fortification (Sorenson and Bogue, 2007). This possibly represents a manifestation of high 'food risk' aversion and quality consciousness, as well as positive attitudes towards the provision of wholesome, nutritious products for their children (Sorenson and Bogue, 2007; Verbeke, 2004).

An exploratory consumer study on market opportunities for innovative probiotic beverages revealed scepticism among Irish consumers towards the concept of multi-functional probiotic drinks (Sorenson and Bogue, 2005a). A multi-functional strategy may very well be attractive to pursue for probiotic beverages in more competitive and mature markets, such as the Japanese market, where consumers are highly aware of the links between certain dietary components and a reduced risk from certain diseases. However, in the case of the Irish market, a probiotic drink offering a singular benefit would appear to present a lower risk in terms of new product failure than a multi-functional probiotic drink. This could be attributed to the relatively low but growing market share for probiotic products in Ireland, consumers' limited exposure to multi-functional products, and their limited understanding of the diverse health-enhancing properties of probiotics and synbiotic ingredients (Bogue *et al*., 2005a). Overall, the findings from these consumer insight studies highlight the importance of integrating consumers' views at the earliest stages of the NPD process in terms of pretesting emerging functional beverage trends prior to launching new functional beverages on the market.

The importance of integrating the consumer at the earliest stages of the NPD process is further evidenced by a growing body of consumer research, which supports the view that consumers' purchase intent towards functional products also depends upon the carrier or base product selected for enrichment (Ares and Gambaro, 2007; Sorenson and Bogue, 2007, 2006, 2005a,b; Bogue *et al*., 2006, 2005b; van Kleef *et al*., 2005; DeJong *et al*., 2003; Bech-Larsen *et al*., 2001; Bogue and Sorenson, 2001). This has implications

for a key NPD trend that is expected to drive future growth in the functional beverages market: functional ingredients transcending beverage categories. For example, it is predicted that juices and juice drinks will lead NPD activities for both gut-benefit and stimulant drinks. However, functional ingredients moving across product categories may not ultimately meet consumers' expectations and acceptance. Indeed, both purchasers and non-purchasers of stimulant drinks in Ireland appear extremely negative towards the concept of stimulant orange juice containing caffeine and taurine positioned as a healthier alternative to carbonated stimulant soft drinks (Sorenson and Bogue, 2005a). Similarly, Sorenson and Bogue's (2005a) exploratory study revealed low levels of acceptance among Irish consumers towards both fibre-enriched orange juice, and the concept of a probiotic orange juice designed to prevent diarrhoea.

Effective consumer knowledge management at the early stages of the NPD process can help minimise product design and consumer acceptance problems arising in the later stages of the NPD process, where development costs incurred are considerably high (Bogue and Sorenson, 2007). Identifying compatible functional ingredients and functional benefits for specific carriers or base products is therefore critical to achieving higher levels of consumer acceptance in the functional beverages market. However, the identification of consumer-oriented concepts alone will not guarantee success in the functional beverages market. Marketers and NPD practitioners must also identify those market segments that are functionality-driven and, importantly, those segments that have the potential to purchase functional beverages through differentiated product strategies.

17.3.2 Identifying and targeting consumer groups effectively through benefit segmentation

How can companies effectively segment the market when pursuing new product opportunities in the functional beverages market? The archetypical consumer group for health and wellness products is most frequently characterised as well educated, female, aged 35–55 years, and belonging to the ABC1 social class groupings. This is based upon their positive health beliefs, attitudes and behaviour towards diet and health, and relatively high disposable incomes (Bogue *et al*., 2006; Bogue and Ryan, 1999; International Food Information Council, 1999; Childs, 1997). Similarly, young adults aged between 18 and 34 years are considered the core target market for energy and stimulant drinks as evidenced by high market penetration and consumption rates among this demographic group of consumers (Mintel, 2004; Boyle and Emerton, 2002; Leatherhead Food Research Association, 2002a; Zenith International, 2002). However, this generalised approach to market segmentation presents potential pitfalls for companies, owing to socio-demographic, socio-cultural and attitudinal differences in consumer acceptance of functional products across categories (van Trijp and van der Lans,

2007; Frewer *et al*., 2003; Bech-Larsen *et al*., 2001; Poulsen, 1999). Identifying and targeting cognitively and attitudinally differentiated market segments therefore presents a major challenge for companies pursuing opportunities with functional beverages.

Given that functional products are primarily positioned and differentiated from conventional products on the basis of functionality, it would therefore seem most logical to segment a market through identifying and profiling those consumer groups that are lifestyle or needs driven, and perceive value from specific functional products. As Wennström and Mellentin (2003: 44) noted: 'the key to a winning strategy is to identify a single bridgehead of pragmatic consumers in a mainstream market and to accelerate the formation of 100 per cent of their whole product. The goal is to win a niche foothold in the mainstream as quickly as possible'. Bogue and Sorenson (2007) and Sorenson and Bogue's (2007, 2006, 2005b) benefit segmentation analyses across five functional beverage categories revealed four common groupings of consumers with similar values and preferences: a functionality-driven segment; a price-sensitive segment; a natural-driven segment; and a grouping of carrier attribute-driven segments.

The functionality-driven segment offers the greatest potential for functional beverages as it is least willing to make trade-offs between functionality and other intrinsic and extrinsic product design attributes, in order to obtain or achieve the desired benefits. In contrast, the price-sensitive segment is most willing to make trade-offs between functionality, carrier specific attributes and, price. The natural-driven segment does not perceive value from added functional ingredients. This segment represents those consumers that consider functional products less natural than conventional products, and instead, value wholesome foods and beverages (Sorenson and Bogue, 2005a; Frewer *et al*., 2003; Bogue and Sorenson, 2001). The final grouping characterises consumer segments that desire products associated with the maintenance of health and well-being, and therefore represents potential target markets for functional products. However, they are less willing or are unwilling to compromise on carrier-specific related attributes such as flavour, texture or convenience for reputed functional benefits (Bogue and Sorenson, 2007; Sorenson and Bogue, 2007, 2006, 2005b).

Although many companies have come to rely solely on functionality vis-à-vis the associated benefits to leverage competitive advantage in the functional products markets often there is only one consumer segment that is overtly functionality-driven in terms of purchase preferences. This is congruent with Wennström and Mellentin's (2003) strategic analysis of the functional food and beverages market where new functional product introductions are viewed as niche products at the early stage of the product life cycle. The niche market appeal of functional products suggests that many companies fail to appreciate that functional benefits may be secondary to taste and overall appeal for the vast majority of consumers (Urala and Lahteenmaki, 2003; Tuorila and Cardello, 2002; Zanoli and Naspetti, 2002;

Gilbert, 2000). As Milton (2003: 20) argues: 'new functional products must taste and look good, meet a consumer need, fit into consumers' lifestyles and then offer a functional and emotional benefit'. The key to achieving mainstream appeal for functional beverages therefore requires differentiated product strategies through identifying the optimal combination of product design attributes that drive consumer groups' choice motives (Sorenson and Bogue, 2007, 2006, 2005b).

Companies that adopt such a consumer-oriented approach to NPD are also well positioned to recognise consumers' emerging needs and rapidly assess consumers' responses to new functional beverages. This in turn can help identify underdeveloped market niches and segments, and potentially identify opportunities created by competitors' mistakes. For example, Sorenson and Bogue (2006) identified a niche group of consumers that perceived value from a stimulant juice drink enriched with ginseng and guarana. Importantly, this group represents a new target market beyond young adults aged 18–34 years who are traditionally targeted with stimulant drinks. Overall, identifying and targeting viable consumer segments with functional beverages can best be achieved through benefit segmentation whereby consumers are grouped according to the likely benefit they would seek or derive from specific functional beverages. Thereafter, secondary segmentation variables such as attitudes and behaviours, family lifestyle stage and socio-demographics become important in terms of differentiating between segment typologies.

17.3.3 Differentiated pricing strategies: identifying the optimal price premium for functional beverages

A premium pricing strategy remains a key objective for many companies that invest in NPD within the functional products market. Generally, functional foods and beverages have traditionally maintained a 10–20% premium above the price of non-functional comparable products, although premiums associated with radical innovations such as Raisio's Benecol have been reportedly higher (Maynard and Franklin, 2003; Heasman and Mellentin, 2001). However, a premium pricing strategy is one of the main reasons constraining the development of functional products categories, and specifically, the pursuance of a mass-marketed product through a premium pricing strategy (Heasman and Mellentin, 2001; Hilliam and Young, 2000). The sustainability of pricing strategies that seek premiums ranging from 100% to 500% above non-functional comparable products is therefore questionable given: the niche market appeal of premium priced functional products; poor product differentiation and positioning strategies; and differing levels of price sensitivity between product categories.

The optimal pricing strategy or premium that consumer segments are willing to pay for specific functional products depends upon whether consumers can make direct price comparisons with existing conventional

products, and whether premiums are already tolerated within the category (Sorenson and Bogue, 2006; Heasman and Mellentin, 2001). Importantly, benefit segmentation analysis studies suggest that a premium pricing strategy will only be tolerated by a niche market group of consumers (Bogue and Sorenson, 2007; Sorenson and Bogue, 2007, 2006, 2005b). However, the price sensitive and carrier attribute-driven segments do offer potential new product opportunities for functional products although they are less willing to compromise on price or carrier specific-related attributes for reputed functional benefits. For functional products to achieve more mainstream appeal among these consumer groups, NPD practitioners must first identify the product design attributes that these consumer groups value, and therefore, are willing to pay an optimal premium for in retail outlets. Thereafter, differentiated product design and pricing strategies can be used to leverage higher levels of acceptance and value, and potentially optimal premiums, among the functionality-driven, price sensitive and carrier attribute-driven segments.

17.3.4 Leveraging a distinct competitive advantage in the functional beverages market

A lack of understanding of a brand's position by consumers is a major cause of product failure in the marketplace, and remains a problematic area for companies attempting to leverage a distinct competitive advantage in the functional food and beverage markets (Wennström and Mellentin, 2003; Heasman and Mellentin, 2001). Therefore, how can companies ensure that their functional beverage brands' positioning and communication strategies are received, understood and accepted by consumers? Companies need to recognise that all elements of the marketing strategy, from product design and market segmentation to pricing and distribution, help define a functional beverage brand's position in the marketplace. Companies must therefore focus their consumer research efforts on identifying, and then measuring consumers' acceptance towards, potential positioning and communication strategies.

One of the most significant strategic marketing issues for a company is whether the positioning strategy adopted differentiates the functional beverage's brand from its competitors in the minds of the target consumer group. In that respect, it is argued that functionality cannot be relied upon solely to create value and leverage a distinct competitive advantage in the functional beverages market. This view is based upon the niche market appeal of functional products presently. For example, differentiating fruit juice-based stimulant beverages from traditional carbonated stimulant beverages on a functional platform that emphasises refreshment and naturalness, rather than healthiness, appears to offer a distinct competitive advantage from the consumers' perspective (Sorenson and Bogue, 2006, 2005a). Similarly, differentiating chilled probiotic and nutrient-enriched

orange juices on functional platforms that emphasise naturalness and general well-being also represent an important strategic orientation for functional beverage companies (Sorenson and Bogue, 2007, 2005a, b). Therefore, positioning strategies targeted at specific consumer segments, which marry functional benefits with other key intrinsic or extrinsic attributes, such as convenience, naturalness or sensory pleasure, would appear more likely to offer success than merely differentiating on the basis of functionality alone (Sorenson and Bogue, 2006, 2005b; Wennström and Mellentin, 2003). Once a distinct competitive advantage from the consumer's perspective is identified through consumer research, a company must then communicate the positioning proposition to the target consumer group.

Regulatory issues concerning the use of physiological and health claims are considered significant limiting factors constraining the development of functional food and beverage markets. However, a more fundamental issue is whether consumers possess the necessary nutrition knowledge to allow them to accept the science underpinning functional foods and beverages and, consequently, change their dietary and purchase behaviours. This is particularly relevant given that the use of both physiological and health claims as marketing tools is based on the premise that consumers possess the requisite knowledge to link these claims to improved health and well-being. Indeed, a growing body of consumer research suggests that consumers' negative perceptions of nutritional claims are directly linked to consumers' poor knowledge of the benefits associated with functional products (Bogue *et al*., 2005a; Frewer *et al*., 2003; Menrad, 2003). It is only when consumers link product attribute-related knowledge and consequence-related knowledge that they will then consider purchasing functional products (Wansink *et al*., 2005). Companies need to therefore focus their promotional activities on increasing consumer awareness and understanding the benefits associated with functional ingredients in order to achieve higher levels of new product success in the functional food and beverages markets (Bogue *et al*., 2006; Sorenson and Bogue, 2005a; Wennström and Mellentin, 2003). Such activities can range from traditional promotional tools such as advertising and promotional offers to product sampling, in-store education, and dissemination of health and nutritional information through targeted print media and websites.

From an NPD perspective, a pertinent question therefore arises: how can companies develop consumer-relevant communication strategies for functional beverages? Consumers' perceptions of a product's unique selling point can be considered a multidimensional choice factor, which can be perceived in many ways depending on the selected carrier or base product, and the benefits associated with the product. For example, functionality is linked to general well-being where the carrier is a product such as yoghurt or icecream, while functionality is linked to disease prevention in products such as table spreads (Urala and Lahteenmaki, 2003). The message content

through which a positioning strategy is communicated to consumers is therefore a critically important consideration for functional beverage manufacturers. In that context, consumer research tools such as the laddering technique, which link consumers' perceptions of a product's attributes with consumers' life values, can assist companies effectively communicate the benefits of functional beverages to consumers (McCarthy *et al.*, 2004). In effect, carefully defined positioning and communication strategies based on an understanding of consumers' choice motives, needs, perceived benefits and values can help broaden the appeal of functional beverages beyond the health-oriented consumer segments traditionally targeted by companies.

17.4 Consumer-oriented new product development and functional beverages

At this point a pertinent question that should be raised from both an organisational and operational perspective is: 'How can companies effectively integrate the consumer with the NPD process in order to increase new product success rates for functional beverages?' Companies must adopt the key success factor groupings in order to improve the success rates for functional beverages, and these include: a balanced NPD strategy; a systematic and well-defined NPD process that utilises a multidisciplinary NPD team; and a market or consumer-oriented approach to NPD (Bogue and Sorenson, 2007; Bogue, 2001; Lord, 2000; Cooper, 1994, 1993; Harmsen, 1994; Cooper and Kleinschmidt, 1987). Successful NPD activities therefore encompass the complete management and organisation of the NPD process and not just the development and design of new products.

A strong consumer orientation is particularly important throughout the NPD process, and especially at the early stages, to ensure the process is consumer-driven and market-focused (Bogue, 2001; Cooper, 1994, 1993). Contemporary consumer research techniques such as focus groups, quality functional deployment (QFD), sensory analysis and conjoint analysis (which can utilise both technical and marketing information), promote closer integration between the marketing and technical functions, and, importantly, a consumer-oriented approach to NPD (MacFie, 2007). Consumer research techniques that facilitate closer integration of consumers with the NPD process can assist companies adapt to changes in consumers' needs; overcome confusion and uncertainty concerning new functional product ideas; ascertain the feasibility and level of market acceptance of potential functional products; define target consumer groups; and guide strategic marketing decisions for new functional foods and beverages (Bogue and Sorenson, 2007; Sorenson and Bogue, 2007; van Kleef *et al.*, 2002). In the next section a consumer-oriented NPD case study is presented on the development of functional cosmetic beverages. The consumer-oriented approach to NPD presented in this case study provides a framework by which companies can

integrate 'voice of the consumer' information more effectively, and manage consumer knowledge more efficiently, at the early stage of the NPD process.

17.5 Consumer-oriented new product development case study: functional cosmetic beverages

The borders between the food and beverage, cosmetic and consumer healthcare industries are becoming increasingly blurred concurrent with the development of the functional food and beverages markets, and functional ingredients transcending product categories. In particular, the emergence of functional cosmetic beverages is taking beverage companies closer to domains where cosmetic and consumer healthcare companies are more comfortable, and therefore better positioned than beverage companies to exploit market opportunities for complementary cosmetic products in terms of brand recognition, and the marketing of 'cosmetic science' to consumers. This case study investigates Irish consumers' acceptance of functional cosmetic beverages and focuses on consumer research techniques that facilitate closer integration of the consumer as 'co-designer' with the NPD process. The case study is presented in three distinct sections, which reflect key pre-development stages in the NPD process: analyses of market and NPD trends; concept ideation and concept development through focus groups; and finally, concept optimisation and refinement through conjoint analysis.

17.5.1 Market and new product development activities for functional cosmetic beverages

The global market for cosmeceutical products has evolved and grown over the past decade as the boundaries between cosmetic, pharmaceutical and supplement categories have become increasingly blurred (Uctas and Baker, 2006; Matthews, 2005). The general industry-led term 'cosmeceutical' refers to a broad range of products containing active ingredients that provide anti-ageing or cosmetic-enhancing benefits (Uctas and Baker, 2006). While the market for supplements that target cosmetic benefits is well established, it remains to be seen whether functional ingredients that confer cosmetic-enhancing benefits can make the successful transition to functional foods and beverages. Indeed, outside Japan, functional cosmetic foods and beverages represent a niche opportunity within the functional food and beverages market presently (Food and Drink Europe, 2006). However, it is expected that future NPD activities in functional cosmetic food and beverages will continue to grow with: an increasingly ageing population and expectant increase in age-related disorders such as age-related macular degeneration (AMD); greater awareness of the role of lifestyle and dietary

factors in the ageing process; the increasing importance of grooming and appearance to consumers; and increasing competition and the need for further product differentiation across functional food and beverage categories (Cosun, 2007, 2003; Datamonitor, 2006c; Food and Drink Europe, 2006; Uctas and Baker, 2006).

In Japan, the largest market for functional cosmetic foods and beverages, NPD activities have primarily focused on functional cosmetic soft drinks and juice drinks enriched with collagen, vitamins C and E, aloe vera or royal jelly, such as Takara Shuzo's Life Line, Meidi-Ta's MY Aloe and Healthy Drink and Coca-Cola's Love Body (Heather, 2005; Mortimer, 2005; Leatherhead Food Research Association, 2002b). More recently, House Foods launched Moisturizing Beauty Proportion, a high fruit content juice drink enriched with collagen, elastin and hyaluronic acid in the same proportion as found in the human skin (McClain, 2006).

Similarly, there have been a number of fruit juice-based functional cosmetic beverages launched in key European markets such as Parmalat's Jeunesse containing coenzyme Q10, and Yagua's Beauty Juicer, a cocktail of pure grapefruit, guava, lemon and lychee juice with ginger, minerals, aloe vera, collagen, and added vitamins to cleanse the digestive system, and support the construction and generation of new cells while helping to keep hair and skin cells healthy (Britton, 2006). However, it would appear that dairy-based drinks still remain a favoured carrier for many functional ingredients in the EU based on past and recent product launches such as Danone's Bio Aloe Vera, Latteria Sociale Merano's Bionessere Henri Chenot Anti-Oxidant Yoghurt Drink, Central Lechera Asturiana's Natur Jalea con Aloe Vera and Emmi's Aloe Vera Yoghurt Drink (Emmi, 2007; Brinnehl, 2005; Mintel, 2005). Most recently, Danone has re-entered the functional cosmetic beverages market with Essensis, a multi-functional dairy product containing ProNutris, a complex of natural ingredients such as omegá 6 starflower oil, green tea polyphenols, vitamin E and an exclusive probiotic culture to improve skin health (Datamonitor, 2007c).

A key trend in the global functional cosmetic beverages market is the pursuance of joint venture strategies to gain a strong market presence in the marketplace. For example, Coca-Cola entered into a joint venture with the Japanese cosmetic company Shiseido to develop and launch Body Style Water and Aroma Works, while Nestlé and L'Oreal's joint venture led to the creation of Laboratoires Inneov (Kryhul, 2007; Mortimer, 2005; Heather, 2004). Similarly, the Lumae range of functional cosmetic beverages is a potential result of synergies between Coca-Cola and L'Oreal (Kryhul, 2007). On that basis it would appear that joint venture and co-branding strategies with either cosmetic or healthcare companies potentially represent viable strategic orientations for beverage companies to pursue when entering the functional cosmetic beverages market. This would be particularly true where beverage companies would lack experience in marketing 'cosmetic science' to consumers as well as poor brand recognition.

In addition, this strategic approach would naturally give rise to synergies in NPD by facilitating technical and marketing knowledge transfer between companies, as well as reducing the associated product development and marketing costs in launching functional cosmetic beverages in new markets (Mortimer, 2005).

Overall, a review of both the market and NPD activities would suggest that a viable platform on which to develop consumer-oriented functional cosmetic beverage concepts would be based on: a co-branded, individual nutrition, ready-to-drink (RTD) beverage; that would be either dairy or fruit juice-based carrier; and with the functional benefits oriented towards either eye, skin and/or hair health.

17.5.2 Concept generation and concept screening in new product development: focus group results

Focus groups represent an extremely important consumer research tool that can facilitate close integration of consumers' views and opinions at the earliest stages of the NPD process. In particular, focus groups are essential for concept ideation and concept screening as product developers can utilise potential users or purchasers of products as 'co-designers' at the early stages of the NPD process. Sixty-four respondents were recruited by means of intercept and convenience sampling to participate in eight focus groups held in two different centres in Ireland (Table 17.1). An experienced moderator conducted the focus groups, which were audiotape recorded and lasted approximately 1 hour and 30 minutes. The qualitative data was transcribed and then coded using the computer package N6™ (QSR International, 2002). The N6™ software package facilitated the process of identifying, coding and retrieving information for further analysis.

The level of nutrition-related knowledge concerning the benefits of certain foods and beverages to improved eye, skin and hair condition was limited, and varied widely across groups. In addition, the vast majority of respondents were generally unaware of consumer foods or beverages marketed in terms of their beauty or cosmetic benefits. Focus group participants were then introduced, by means of product prompts and information on a flipchart, to the concept of functional cosmetic beverages. The focus group discussions revealed that consumers were most interested in functional cosmetic beverages that would benefit either eye or skin health based upon their present purchase behaviour in terms of either supplements or cosmetic healthcare products. While most consumers were generally receptive towards functional cosmetic beverages that could offer multiple rather than singular benefits, a number of adults aged between 18 and 44 years were sceptical of multi-functional cosmetic beverages: 'It really is promising too much [multi-functional benefits]. If they promise too much who is going to believe it?' (FG 8).

Table 17.1 Socio-demographic profiles across focus groups

Socio-demographic variables	FG1	FG2	FG3	FG4	FG5	FG6	FG7	FG8
Group size	8	8	8	8	8	8	8	8
Age group (years)	18–34	45–54	35–44	55+	45–54	18–34	55+	35–44
Gender								
Male	8	0	8	0	8	0	8	0
Female	0	8	0	8	0	8	0	8
Marital status								
Single	4	0	2	0	1	6	1	0
Married	2	4	4	6	5	2	4	4
Separated	0	2	2	0	0	0	1	1
Cohabiting	2	0	0	0	0	0	0	2
Widowed	0	2	0	2	2	0	2	1
Education level								
Primary level	0	0	0	0	0	0	1	0
Junior cert.	0	0	1	0	0	0	1	0
Leaving cert.	0	0	1	2	2	0	2	4
Vocational	2	3	2	3	3	0	2	2
Third level	6	5	4	3	3	8	2	2
Social class	B, C1	B, C1	C1, C2, D	C1, C2	C2, D	C1, C2	C2, D	B, C1
Location	Cork	Cork	Dublin	Dublin	Dublin	Dublin	Cork	Cork

Exploratory discussions with focus group participants then identified a number of potential micronutrients that could be incorporated into functional cosmetic beverages. Specifically, participants across focus groups were aware from lifestyle magazines, and existing cosmetic products on the market, of the benefits of vitamins A, C and E to eye and skin health. Older female consumers were also aware of both supplements and cosmetic products containing collagen, royal jelly or aloe vera, although these consumers perceived potential off-flavours from the addition these ingredients to fruit juice-based beverages. However, consumers were neither aware of the functional ingredient lutein nor its potential benefit to good eye health. Similarly, consumers were not aware of the benefits of omega-3 oils to good eye health. In that context, participants were in agreement that more information would be needed to enhance the credibility of functional drinks targeting both skin and eye health. More so, from a product-positioning perspective, the type of functional proposition was also important to consumers, and appeared to differ markedly across age groups. For example, while younger adults were interested in messages that generally communicated skin and hair well-being, older adults desired more specific messages such as toning, anti-cellulite and intense moisturising.

Overall, berry flavoured fruit juice-based beverages that offered multi-functional benefits appeared to gain the highest levels of consumer acceptance for a range of functional cosmetic beverages. However, a central issue in the marketing of functional foods and beverages relates to identifying product concepts with the 'optimal' product design features, or more precisely, determining which consumer groups will make trade-offs between key product-related attributes and price for functional benefits.

17.5.3 Concept optimisation and concept refinement through conjoint analysis

The conjoint analysis technique was chosen to gain a deeper understanding of consumers' choice motivations, and to study the trade-offs consumers might be expected to make between key product attributes, in order to guide product design, branding, positioning and pricing strategies for functional cosmetic beverages. The product attributes, and associated attribute levels, used in this conjoint-based study were generated based on the focus group discussions (Table 17.2). Two well-known brands in the beverage and personal healthcare sectors were selected for purely illustrative purposes

Table 17.2 Attributes and attribute levels used in the conjoint-based study

Attribute	Attribute level
Branding strategy	Tropicana (beverage brand) Johnson & Johnson (healthcare brand) Tropicana and Johnson & Johnson (co-branding strategy)
Carrier	Juice drink (5% fruit juice) Juice drink (15% fruit juice) Pure fruit Juice
Flavour	Pomegranate and blueberry Raspberry and orange Grapefruit and peach
Functional benefits	None Contains aloe vera, collagen peptides, royal jelly and vitamins A, C & E to rejuvenate skin cells Contains omega-3 and lutein (marigold extract) to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells
Portion size	250 ml 330 ml 500 ml
Price	€2.00 €3.10 €4.20

to determine whether a co-branding strategy would add value from a consumer perspective. Tropicana was selected to represent a well-known beverage brand, while Johnson & Johnson was selected to represent a global brand in personal healthcare, although the latter is also associated with the functional food and beverages market vis-à-vis McNeill and the Benecol brand in the United States. Likewise, the carrier and functional benefit attribute levels were chosen to determine the extent of the trade-offs consumers would be willing to make between these attributes at various price points. A conjoint-based survey was then designed which presented 22 hypothetical beverages for consumer evaluation using a nine-point Likert scale. Additional questions, which related to consumers' beverage purchasing habits, lifestyles and their socio-demographic information, were also included in the conjoint-based survey. Four hundred and thirty-nine consumers were recruited to participate in the study. The questionnaires were analysed using SPSS v14 (SPSS, 2005).

The conjoint analysis revealed that the carrier, functional benefits, flavour and price attributes were most important in terms of choosing between alternative beverage concepts. Agglomerative hierarchical cluster analysis determined that three to five clusters existed based on consumers' attribute utility patterns. K-Means cluster analysis was then used to segment respondents into five clusters of consumers with similar preferences for juice-based beverages (Table 17.3). The socio-demographic profile of each cluster helped further distinguish between segments (Table 17.4). For example, Cluster 3 exhibited differing preferences for functional cosmetic beverages in comparison with Clusters 1, 4, and 5. Specifically, this segment preferred a singular benefit functional beverage (improve skin health only) to a multi-functional cosmetic beverage (improve both skin and eye health) (Table 17.3). In terms of its socio-demographic profile, Cluster 3 was primarily composed of females in the pre-family and early family lifestyle stages (Table 17.4). Importantly, Cluster 5 was functionality-driven in terms of its purchase preferences and represented a key consumer segment for functional cosmetic beverages. However, from a marketing perspective, although Cluster 5 appeared most receptive towards functional beverages, this segment was also more price sensitive relative to Clusters 3 and 4 (Table 17.3). Successfully targeting this important consumer segment with functional cosmetic beverages therefore presents considerable challenges to NPD practitioners in terms of leveraging a competitive advantage vis-à-vis functionality on one hand, and identifying the optimal product design and pricing strategy on the other.

Conjoint model predictions for functional cosmetic beverages

A Kendall's tau value of 1 for the four holdouts was obtained which suggested perfect agreement between the holdout ratings and the model predictions. It was therefore possible to analyse consumers' preferences for alternative functional cosmetic beverage concepts using choice simulators,

Table 17.3 Averaged utility values across clusters

Attribute	Attribute level	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5
Branding strategy	Tropicana	0.13	**0.10**	*–0.03*	0.11	0.04
	Johnson & Johnson	*–0.31*	*–0.05*	*–0.40*	*–0.28*	*–0.28*
	Tropicana and Johnson & Johnson	**0.18**	*–0.05*	**0.43**	**0.17**	**0.24**
Carrier	Juice drink (5% fruit juice)	*–0.51*	*–1.58*	*–0.58*	*–0.67*	*–0.36*
	Juice drink (15% fruit juice)	0.01	0.40	–0.20	0.11	–0.13
	Pure fruit juice	**0.50**	**1.18**	**0.78**	**0.56**	**0.49**
Flavour	Pomegranate and blueberry	**0.17**	0.23	**1.23**	**0.52**	**0.34**
	Raspberry and orange	0.08	**0.25**	*–1.39*	–0.23	0.10
	Grapefruit and peach	*–0.25*	*–0.48*	0.16	*–0.29*	*–0.44*
Functional benefits	None	*–0.28*	**0.14**	*–0.49*	*–0.21*	*–1.33*
	Contains aloe vera, collagen peptides, royal jelly and vitamins A, C & E to rejuvenate skin cells	–0.11	–0.02	**0.59**	–0.08	0.51
	Contains omega-3 and lutein (marigold extract) to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	**0.39**	*–0.12*	–0.10	**0.29**	**0.82**
Portion size	250 ml	*–0.14*	*–0.29*	*–0.35*	*–0.02*	*–0.09*
	330 ml	0.02	0.07	0.01	0.00	0.00
	500 ml	**0.12**	**0.22**	**0.34**	**0.02**	**0.09**
Price	€2.00	**1.82**	**0.31**	**0.45**	0.42	0.36
	€3.10	–0.18	0.10	0.11	0.18	–0.02
	€4.20	*–1.64*	*–0.41*	*–0.56*	*–0.60*	*–0.34*
Cluster size		130	59	86	42	122

The highest utility values are in bold and the lowest in italic.

Table 17.4 Socio-demographic profiles across clusters

Socio-demographic variables	Factor	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)	Cluster 4 (%)	Cluster 5 (%)
Gender	Male	49.6	44.3	31.0	12.8	53.9
	Female	50.4	55.7	69.0	87.2	46.1
Age group (years)	18–24	20.9	22.9	9.2	10.0	12.4
	25–34	30.7	26.6	24.4	14.7	27.2
	35–44	26.4	22.6	50.1	12.1	17.0
	45–54	11.0	18.5	5.3	11.3	21.5
	55–64	6.3	5.7	6.2	37.2	14.4
	65+	4.7	3.7	4.8	14.7	7.5
Marital status	Single	42.4	32.0	26.8	16.4	25.6
	Married	30.3	54.3	69.7	26.7	51.4
	Separated/divorced	12.8	4.1	3.5	8.7	10.9
	Cohabiting	10.1	9.6	0.0	0.0	3.8
	Widowed	4.4	0.0	0.0	48.2	8.3
Educational status	Completed primary level only	4.9	15.4	0.0	16.4	3.9
	Completed secondary level only	40.3	54.1	61.2	73.1	25.7
	Completed third level	54.8	30.5	38.8	10.5	70.4
Employment status	Employed full time	57.1	58.4	74.0	35.0	61.0
	Employed part time	16.0	12.5	5.0	15.0	9.0
	Student	23.0	21.4	10.9	0.0	12.9
	Retired	3.9	3.7	2.1	44.0	10.1
	Unemployed	0.0	4.0	8.0	6.0	7.0

both maximum and probability (BTL and Logit) modelling, across clusters. Eight hypothetical beverage concepts (COSBEV 1 to COSBEV 8) were generated for the group level simulation analysis across clusters (Table 17.5). COSBEV 1 and COSBEV 2 were included in the group level simulation analysis following analysis and interpretation of the cluster analysis data (Table 17.3). These products represented the ideal hypothetical functional cosmetic beverage concepts, which according to the cluster analysis results would yield high predicted preference scores across key consumer groups. However, new product concepts that combine the optimal product design attributes (e.g. COSBEV 1 and COSBEV 2) may not represent commercially feasible new products. Therefore, six further hypothetical beverage concepts (COSBEV 3 to COSBEV 8), which were variants of COSBEV 1 and COSBEV 2, were generated for inclusion in the simulation analysis (Table 17.5).

From a strategic marketing perspective, the simulation analysis procedure not only facilitates an evaluation of possible market entry strategies, it can also assist companies anticipate future changes to key elements of a brand's marketing plan. This is accomplished through modelling how consumer groups might react to potential competitor products in the later stages of the new product life-cycle. For example, according to product life cycle management theory, manufacturers of 'new-to-the-world' products could potentially pursue a premium product/pricing strategy (e.g. COSBEV 8) targeting innovator and early adopter consumer groups (e.g. Cluster 5) to maximise profits at the early stages of the product life cycle. However, the group level simulation analysis revealed that this form of market entry strategy would merely present a niche market opportunity for functional cosmetic beverages (Table 17.5). Specifically, it was revealed that Clusters 1, 3 and 4 would either trade-down to low fruit content functional cosmetic beverages (e.g. COSBEV 3 and COSBEV 4) or choose non-functional beverages (e.g. COSBEV 5), over super-premium priced functional cosmetic beverages (e.g. COSBEV 8) (Table 17.5).

Instead, analysis and interpretation of the group level simulation results would suggest that a penetration entry strategy with two medium priced functional cosmetic beverage concepts, COSBEV 6 and COSBEV 7, would probably represent a more commercially feasible strategy on which to build market share and brand loyalty, and ultimately garner mainstream appeal, in the embryonic functional cosmetic beverages market. This assessment was based on the high predicted levels of consumer acceptance (in bold) for both COSBEV 6 and COSBEV 7 relative to other alternative beverage concepts (Table 17.5). However, a company would need to consider changes to COSBEV 7's marketing mix over the product life cycle in order to retain Cluster 1's brand loyalty, owing to this segments price sensitivity. From a NPD perspective, Cluster 1 would clearly appeal to competitor-oriented companies that strongly pursue 'creative imitation' strategies when entering new markets.

Table 17.5 Simulation analysis for functional cosmetic beverage concepts across clusters

Attributes/ preference scores	COSBEV 1	COSBEV 2	COSBEV 3	COSBEV 4	COSBEV 5	COSBEV 6	COSBEV 7	COSBEV 8
Branding strategy	Tropicana and Johnson & Johnson	Tropicana and Johnson & Johnson	Tropicana and Johnson & Johnson	Tropicana and Johnson & Johnson	Tropicana	Tropicana and Johnson & Johnson	Tropicana and Johnson & Johnson	Tropicana and Johnson & Johnson
Carrier	Pure fruit juice	Pure fruit juice	Juice drink (5% fruit juice)	Juice drink (5% fruit juice)	Pure fruit juice	Juice drink (15% fruit juice)	Juice drink (15% fruit juice)	Pure fruit juice
Flavour	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry	Pomegranate and blueberry
Functional benefits	Omega-3 and lutein to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	Aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	Omega-3 and lutein to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	Aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	None	Omega-3 and lutein to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	Aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells	Omega-3 and lutein to promote healthy eyes, and aloe vera, collagen peptides and vitamins A, C & E to rejuvenate skin cells
Portion size	500 ml	500 ml	250 ml	330 ml	330 ml	250 ml	330 ml	250 ml
Price	€2.00	€2.00	€2.00	€2.00	€2.00	€3.10	€3.10	€4.20
Cluster 1 (pref. score)	8.7 out of 9	8.4 out of 9	7.8 out of 9	7.3 out of 9	7.5 out of 9	**7.0 out of 9**	6.7 out of 9	5.9 out of 9
Cluster 3 (pref. score)	8.3 out of 9	8.8 out of 9	6.6 out of 9	6.6 out of 9	6.6 out of 9	7.2 out of 9	**7.6 out of 9**	6.4 out of 9
Cluster 4 (pref. score)	8.7 out of 9	8.4 out of 9	6.9 out of 9	6.7 out of 9	7.0 out of 9	**7.8 out of 9**	7.2 out of 9	6.1 out of 9
Cluster 5 (pref. score)	8.9 out of 9	8.5 out of 9	7.2 out of 9	6.9 out of 9	6.7 out of 9	**7.8 out of 9**	7.3 out of 9	7.4 out of 9

Bold type indicates high predicted level of consumer acceptance.

17.5.4 Case study conclusion

What lessons can be learnt from this case study that can assist companies develop more consumer-oriented functional foods or beverages? First, consumers have an extremely significant contribution to make throughout the new product design process, and especially at the concept ideation and concept screening stages, in terms of identifying which carriers and functional ingredients meet with overall consumer acceptance. However, it is also important to acknowledge that consumer preferences elicited through focus groups alone may not necessarily reflect consumers' final purchase intentions given the complex nature of consumer choice. This is particularly true for functional food and beverages where consumers are inevitably forced to make trade-offs in terms of the desired sensory character, functional benefits and price. Therefore, consumer research techniques that facilitate an analysis of consumer trade-offs have an extremely central role to play in the product optimisation process for functional foods and beverages. Second, functionality cannot be relied upon solely to leverage a competitive advantage given that functionality is perceived and valued differently by different consumer groups.

Third, product design strategies that seek to add 'superior value' to functional foods or beverages must be viewed and assessed from the consumer's perspective. Indeed, this case study illustrates that adding 'superior value' from the company's perspective (e.g. COSBEV 8), by offering a premium quality carrier (pure fruit juice) with superior functionality (multi-functionality), does not necessarily add 'more value' from the consumer's perspective. This is based on the premise that different consumer groups can be expected to make trade-offs between functionality, price and other key product attributes to a greater or lesser degree. This trade-off approach to new product design goes some way to addressing a strategic marketing issue of concern to marketers and NPD practitioners: identifying the optimal pricing strategy for functional foods and beverages. Overall, the findings from this case study highlight the importance of integrating the consumer with the NPD process in order to make consumer-relevant product design and strategic marketing decisions when bringing innovative functional foods or beverages to the marketplace.

17.6 Summary

The functional beverages market offers new product opportunities to those companies that understand consumers' preferences and choice motives, and can develop and market functional beverages that meet consumers' expectations. Once companies implement an NPD strategy, and adopt a formal NPD process that is multidisciplinary in nature, advanced consumer research methodologies can then be used to manage knowledge more effectively and efficiently, leading to the development of consumer-oriented functional

beverages. The consumer-oriented approach to NPD discussed in this chapter illustrates how an understanding of consumers' choice motives and value systems can provide guidance to marketers in terms of segmentation, pricing, positioning and communication strategies, and to R&D personnel in terms of concept development and product design, when bringing new functional beverages to the marketplace. The utilisation of consumers' views throughout the NPD process therefore provides for a systematic means of managing consumer knowledge in product development, which can improve a company's competitiveness in the functional beverages market.

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18

The role of beverages in a healthy diet: key issues and guidelines

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Abstract: Caloric beverage intake is not associated with energy compensation and is linked with increased energy intake, particularly of refined carbohydrates. This is a review of the health effects of all beverages, caloric and non-caloric and includes a ranking. The rankings placed drinking water as the preferred beverage to fulfill daily water needs, followed by tea and coffee, low-fat (1.5% or 1%) and skim (non-fat) milk and soy beverages, non-calorically sweetened beverages, a set of beverages with some nutritional benefits (fruit/vegetable juices, whole milk, alcohol, and sports drinks), and ending with the lowest priority for calorically sweetened, nutrient-poor beverages.

Key words: caloric beverages, soft drinks, water, obesity, energy compensation.

18.1 Introduction: role of beverages in a healthy diet and the need for guidelines for beverage consumption

Over the past decade, major increases in obesity and overweight have occurred across the globe. One critical dimension has been the large increase in consumption of added caloric sweeteners in the diet, particularly from beverages (Popkin and Nielsen, 2003; Nielsen and Popkin, 2004). At the same time knowledge was growing that consumption of calorically sweetened beverages was not linked with any compensation related to reduction of food intake (Mattes, 1996; DiMeglio and Mattes, 2000). While the focus of the US Dietary Guidelines for Americans has been on food, energy intake from beverages currently represents 21% of the total energy intake for Americans aged >2 years (Nielsen and Popkin, 2004). In many other countries, we also experience similar excessive intakes of beverages and

caloric sweeteners (Somerset, 2003; Steyn, *et al*., 2003). This quantity of calories from fluids, which is predominately from calorically sweetened beverages, adds to energy of current foodstuffs in our diet and is a contributing factor to the energy excess needed to produce obesity (Ludwig *et al*., 2001; Raben *et al*., 2002; Schulze *et al*., 2004; Malik *et al*., 2006).

This chapter provides guidance on the relative health and nutritional benefits and risks of various beverage categories. A healthy diet does not rely on fluids to provide energy or nutrient needs. Therefore, potable water could be used to fulfill almost all the fluid needs of healthy individuals. However, to allow for variety and individual preferences, healthful diets may include several other types of beverage. Of course, many persons do not consume a healthy diet and beverages; in particular, cow and soy milk provide important nutrients for them.

There is evidence that beverages have weak satiety properties and elicit poor dietary compensation. Studies of appetitive sensations (e.g. hunger, fullness, prospective consumption) support the view that fluids are less satiating than solid foods (Hulshof *et al*., 1993; Raben *et al*., 1994; Haber *et al*., 1997). Dietary compensation (the adjustment in energy intake made by individuals in subsequent meals in response to earlier food intake) has been studied with solid, semi-solid, and fluid foods. For fluids, Mattes (1996) reported a complete lack of compensation, suggesting that fluid calories are not readily 'registered' for appetite regulation. Another study found that ingestion of 450 kcal of calorically sweetened fruit drink produced a significant increase in body weight that was not found when the same amount was consumed in solid form by the same individuals (DiMeglio and Mattes, 2000). The mechanisms for this weaker compensatory response to fluids are not known. Since fluid requirements vary widely among individuals and populations, there is no established requirement for water (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). That is, no EAR (estimated average requirement) has been set for water, and an AI (adequate intake) was defined instead. The AI, derived from the usual intake of total fluids in the general population, was set at 125 fluid ounces (3.7 l) per day for men and 91 fluid ounces (2.7 l) for women. About 80% of those daily needs is contributed by beverages, including water, and the rest from solid foods (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). Conversely, the contribution of fluids to meeting the RDA (recommended dietary allowance) for essential nutrients is minimal, except for milk and fruit juices. This balance between energy and nutrient content is a critical factor to define the role of beverages in a healthy diet.

18.2 The health benefits and costs of beverages

Different combinations of beverages can be used to fulfill the fluid needs of a healthy person. Potable water has the advantage that it is virtually

devoid of adverse effects when consumed within the allowable intake. Different beverages are discussed below from the most preferred (those that should be consumed as the major beverage, i.e. water) to the least preferred (beverages that should be consumed in limited quantities). It is not possible to define a set amount of water for each person, as water needs depend partially on overall diet and the water contained in the foods. Elsewhere the authors and also the Institute of Medicine (IOM) discuss total beverage needs (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004; Popkin *et al*., 2006).

18.2.1 Water

Water consumption is necessary for metabolism, normal physiological function, and may provide essential minerals such as calcium, magnesium, and fluoride. For a detailed review of the maintenance of water balance, see the IOM report on water and electrolytes (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). Despite the importance of water for human life, and because of our incomplete understanding of everyday water turnover, in recent years scientists have begun studies of human water requirements, the assessment of hydration, and the relationship between hydration status and human health (Armstrong, 2005; Manz and Wentz, 2005; Sawka *et al*., 2005).

Acute dehydration results in impaired cognition, moodiness, poor thermoregulation, reduced cardiovascular function, and impaired physical work capacity. These expenses can be charged to an overhead or trust account (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). The effects of dehydration on cognitive function have been studied in several randomized, controlled clinical trials, in which dehydration was achieved by fluid restriction, heat exposure, exercise, or combinations thereof (Ritz and Berrut, 2005). In healthy young adults, dehydration to 2.8% body weight loss by heat exposure or exercise significantly decreased alertness, concentration, tracking performance, and short-term memory; this also increased tiredness, headaches, and reaction time (Cian *et al*., 2001). In the only study performed in older subjects (healthy 50–82-year-olds), dehydration by overnight fluid restriction was related to slower psychomotor processing speed, poorer attention, and diminished memory (Suhr *et al*., 2004).

The adverse effects of acute dehydration on physical work capacity and exercise performance are well established (Maughan, 2003), especially when dehydration exceeds 1–2% of body weight (Cheuvront *et al*., 2003; Shirreffs *et al*., 2004). Chronic dehydration increases the risk of bladder cancer (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). However, some of the effects are not well established, in that few studies have focused on chronic disease outcomes. Between 2001 and 2004, 11 of 13 studies showed a significant association between improved hydra-

tion status and reduced kidney stone occurrence (Borghi *et al*., 1996; Siener and Hesse, 2003).

Excess water intake can occur; however, this is rare in healthy individuals with properly functioning kidneys because the kidneys can produce a large volume of urine in a relatively short period of time to correct the disturbance. Only in exceptional circumstances does hyperhydration occur (i.e. 1 out of 1000 ultra-endurance competitors), resulting in dilution of body fluids and a low serum sodium concentration (i.e. <136 (meq/l Na^+ or mEq $Na^+ l^{-1}$)) (Hew-Butler *et al*., 2005). For minerals, drinking water may contain different levels of Ca^{2+} and Mg^{2+} which contribute to meeting the recommended dietary intakes of these minerals (Azoulay *et al*., 2001). Calcium and magnesium from bottled water are well absorbed and utilized (Couzy *et al*., 1995; Aptel *et al*., 1999; Sabatier *et al*., 2002). The fluoride content of bottled water is usually much lower than fluorinated tap water, but on occasion it may exceed advisable levels (Lalumandier and Ayers, 2000).

18.2.2 Tea and coffee

Tea

Black, green, and oolong tea are the three main categories of tea consumed in the world. Tea provides a variety of flavonoids and antioxidants as well as a few micronutrients, in particular fluorine (Steele *et al*., 1999). While there is solid evidence that tea protects against chemically induced cancers in experimental animals, it remains unclear whether tea consumption lowers cancer risk in humans (Higdon and Frei, 2003). Tea also provides some amino acids, of which the majority is theanine. Recently, theanine has been shown to enhance innate immunity – the body's ability to resist infections – by stimulating gamma-delta T cells (Kamath *et al*., 2003), and this effect has been replicated with regular (5–6 cups/day) tea consumption in humans (Bukowski *et al*., 1999; Kamath *et al*., 2001, 2003). Tea consumption may also increase bone density (Chen *et al*., 2003), reduce tooth decay and cavities (Jones *et al*., 1999), and reduce kidney stones (Curhan *et al*., 1996, 1998).

Numerous epidemiological studies have examined the association between tea consumption and the risk of cardiovascular diseases. A meta-analysis that combined data of ten prospective cohort studies and seven case-control studies concluded that an increase in tea consumption of three large cups/day (24 fl oz or 710 ml) is associated with an 11% decrease in the risk of myocardial infarction (Peters *et al*., 2001). However, the results among prospective cohort studies are inconsistent. A 6 year study of Dutch men and women found that those who drank at least three cups/day (about 13 fl oz) had a significantly lower risk of myocardial infarction than non-drinkers (Geleijnse *et al*., 2002). A 7 year study of US women found that the risk of vascular events was significantly lower in a small number of women who drank at least four cups/day of black tea (Sesso *et al*., 2003b).

Finally, a 15 year study of US men found no association between tea consumption and cardiovascular disease risk, but tea consumption in this population was relatively low, averaging one cup/day (Sesso *et al*., 2003a). Overall, the current data suggest that consumption of at least three cups/day of black tea may modestly decrease the risk of myocardial infarction. Although green tea consumption may confer a similar benefit (Nakachi *et al*., 2000), there is currently not enough data to draw firm conclusions.

Recent evidence suggests that tea consumption improves endothelium-dependent vasodilation, which could explain – at least in part – a reduction in cardiovascular disease risk (Duffy *et al*., 2001). Two clinical studies found that daily consumption of four or five cups (30–40 fl oz) of black tea for 4 weeks significantly improved endothelium-dependent vasodilation in patients with coronary artery disease (Duffy *et al*., 2001) and in patients with mildly elevated serum cholesterol levels (Hodgson *et al*., 2002) compared with the equivalent amount of caffeine or hot water. In agreement with these studies, a recent double-blind crossover study found that acute black tea consumption improved coronary vessel function, as assessed by coronary flow velocity reserve (Hirata *et al*., 2004). The beneficial effects of tea consumption on endothelium-dependent vasodilation may be explained by activation of endothelial nitric oxide synthase (eNOS) by tea flavonoids, via an estrogen receptor alpha-dependent pathway (Anter *et al*., 2005). Despite these intriguing results, the potential health benefits of flavonoids in tea and their antioxidant versus non-antioxidant mechanisms of action remain to be fully explored (Williams *et al*., 2004).

Coffee

Several prospective cohort studies have observed significant inverse associations between regular coffee consumption and the risk of type 2 diabetes (van Dam and Feskens, 2002; Rosengren *et al*., 2004; Salazar-Martinez *et al*., 2004; Tuomilehto *et al*., 2004). In a US cohort, a modest inverse association between decaffeinated coffee consumption and the risk of type 2 diabetes also was observed, suggesting that compounds other than caffeine may contribute to risk reduction (Salazar-Martinez *et al*., 2004). High intakes of coffee have been associated with significant reductions in colorectal cancer risk in numerous case-control studies, but prospective cohort studies have not generally observed such significant associations (Giovannucci, 1998; Tavani and La Vecchia, 2004). Coffee and caffeine consumption have been consistently associated with significant reductions in the risk of Parkinson's disease in men (Hernan *et al*., 2002) but not women (Ascherio *et al*., 2004), which may be due to the modifying effects of estrogen. In two large prospective cohort studies, coffee consumption was inversely associated with the risk of Parkinson's disease in women who had never used postmenopausal estrogen, but inverse associations were not observed in women who used postmenopausal estrogen (Ascherio *et al*., 2003, 2004). In the Nurses' Health Study, daily consumption of six or more cups of coffee was

associated with a significant increase in Parkinson's disease risk among postmenopausal estrogen users (Ascherio *et al*., 2003). Two prospective cohort studies in the United States found significant inverse associations between coffee consumption and the risk of suicide (Klatsky *et al*., 1993; Kawachi *et al*., 1996). However, a J-shaped relationship between coffee consumption and the risk of suicide was observed in Finland, where daily consumption of eight or more cups of coffee was associated with a significant increase in the risk of suicide compared with more moderate consumption (Tanskanen *et al*., 2000). The majority of large, prospective cohort studies have not found high intakes of coffee or caffeine to be associated with significantly increased risk of coronary heart disease or myocardial infarction (Kawachi *et al*., 1994; Willett *et al*., 1996; Kleemola *et al*., 2000). In contrast, coffee consumption has been associated with increases of several cardiovascular disease risk factors. The consumption of boiled, unfiltered coffee has been found to increase plasma total and low-density lipoprotein (LDL) cholesterol concentrations, while the consumption of filtered coffee does not appear to have adverse effects on lipid profiles (Jee *et al*., 2001). The diterpenes cafestol and kahweol have been identified as cholesterol-raising factors in roasted coffee beans (Urgert and Katan, 1996). Diterpenes are extracted by hot water when coffee is brewed, and they are trapped by paper filters. Consequently, filtered coffee contains very little cafestol and kahweol, while boiled coffee and espresso may contain significant amounts (Gross *et al*., 1997). Controlled clinical trials have found that high intakes of filtered and unfiltered coffee increase plasma homocysteine concentration, an independent risk factor for cardiovascular diseases (Grubben *et al*., 2000; Verhoef *et al*., 2002). In randomized controlled trials, caffeinated coffee consumption has been found to result in modest but significant increases of systolic (2.0–2.4 mm Hg) and diastolic (0.7–1.2 mm Hg) blood pressure (Jee *et al*., 2001; Noordzij *et al*., 2005). Although coffee consumption was associated with small increases in systolic and diastolic blood pressure in one prospective cohort study, the risk of developing hypertension after an average of 33 years was not affected (Klag *et al*., 2002).

Caffeine intake

There are greater amounts of caffeine in coffee than tea (see Table 18.1). Although caffeine is a mild diuretic, human studies indicate that caffeine consumption of up to about 500 mg/day does not cause dehydration or chronic water imbalance (Armstrong, 2002; Armstrong *et al*., 2005). A caffeinated beverage's fluid content compensates for an acute diuretic effect. At this time, in healthy adults the preponderance of evidence suggests that moderate caffeine intake up to 400 mg/day is not associated with increased risk of heart disease, hypertension, osteoporosis or high cholesterol (Nawrot *et al*., 2003). Some people are more sensitive to caffeine's effects than others and may feel effects at lower doses. Pregnancy and aging may affect one's

Table 18.1 Beverage nutrient composition table (kcals and nutrient per 8 fl oz or 237 ml)

	Calories[1]	Total fat (g)[1]	SAFA (g)[1]	Sugars (g)[1]	Caffeine (mg)[1]	Sodium (mg)[1]	Potassium (mg)[1]	Vitamin A (IU)[1]	Vitamin C (mg)[1]	Calcium (mg)[1]	Vitamin D (IU)[1]	Folate (mcg)[1]
Level 1: Water[2]												
Bottled water												
Level 2: Tea and coffee (unsweetened)	0	0	0	0	0	1	0	0	0	0	0	0
Tea												
Brewed black tea[2]	0	0	0	0	47	7	88	0	0	0	0	3
Decaf brewed black tea[2]	0	0	0	0	2.4	0	88	0	0	0	0	3
Brewed green tea[2]	0	0	0	0	30	0	0	0	0	0	0	0
Decaf brewed green tea[2]	0	0	0	0	3	0	0	0	0	0	0	0
Lipton original (unsweetend)[3]	0	0	0	0	35	0	0	0	0	0	0	0
Herbal tea[2]	0	0	0	0	0	0	0	0	0	0	0	0
Coffee[2]												
Coffee brewed	2	0	0	0	95	5	116	0	0	4.7	0	4.7
Coffee brewed espresso	1	0	0	0	64	0	34.5	0	0	1	0	0
Decaf coffee brewed	0	0	0	0	2.4	0	128	0	0	4.7	0	0
Level 3: Low-fat and skim Milk and soy beverages[2]												
Reduced fat (1.5%& 1%) & skim milk												
Milk (1% fat vitamin A fortified)	102	2	1.5	12.7	0	103	366	478	0	290	127	12.2
Milk (skim vitamin A fortified)	83	0.2	0.3	12.5	0	103	448	499	0	352	98	14.8
Soy beverages[4]												
Silk soy milk, plain	100	4	0.5	6	0	85	300	500	0	300	120	24
Silk soy milk, vanilla	100	3.5	0.5	7	0	130	300	500	0	300	120	24
Silk live 'mango'	230	4	0.5	35	0	120	350	1250	15	350	100	100
Silk soy milk, chocolate	140	3.5	0.5	19	0	100	350	500	0	300	120	24
Level 4: Non-calorically sweetened beverages												
Lipton Green Tea to Go, decaffeinated[3]	0	0	0	0	0	70	0	0	0	0	0	0
Diet Pepsi[5]	0	0	0	0	24	25	20	0	0	0	0	0

Table 18.1 *Cont'd*

	Calories[1]	Total fat (g)[1]	SAFA (g)[1]	Sugars (g)[1]	Caffeine (mg)[1]	Sodium (mg)[1]	Potassium (mg)[1]	Vitamin A (IU)[1]	Vitamin C (mg)[1]	Calcium (mg)[1]	Vitamin D (IU)[1]	Folate (mcg)[1]
Diet Coke[6]	0	0	0	0	31	70	0	0	0	0	0	0
Level 5: Caloric beverages with some nutrients												
Fruit and vegetable juices												
Orange juice (Minute Maid)[7]	110	0	0	24	0	15	450	0	72	20	0	60
Tropicana Light'n Healthy[8]	50	0	0	10	0	10	450	1000	72	200	0	28
Concord grape juice (Welch's)[9]	170	0	0	40	0	20	0	0	60	0	0	0
Apple Juice (Minute Maid)[7]	110	0	0	26	0	0	0	0	72	0	0	0
Fruit Medley (Minute Maid)[7]	170	0	0	36	0	20	340	0	60	0	0	0
Cranberry Juice Cocktail[2]	137	0	0	31	0	5	0	0	52	0	0	0
Apple juice (unsweetened)[2]	112	0	0	24	0	7	295	100	12	170	0	0
Vegetable juices												
V8 tomato juice[10]	50	0	0	8	0	590	470	2000	60	20	0	0
Carrot juice[2]	94	0	0	9	0	68	689	45130	21	57	0	9
Whole (full fat/higher fat) milk												
Milk (whole, 3.25% fat)[2]	146	8	4.5	13	0	98	350	249	0	276	98	12
Milk (2% fat vitamin A fortified)[2]	122	4.8	3.1	13	0	100	366	461	0	285	105	12
Wendy's Frosty[11]	217	5	4	27	0	129	0	520	0	12	0	0
Sports drinks												
Gatorade X Factor[12]	50	0	0	14	0	110	30	0	0	0	0	0
POWERade Lime (Coca-Cola)[6]	64	0	0	15	0	53	32	0	0	0	0	0
POWERade Raize (Coca-Cola)[6]	110	0	0	29	36	46	32	0	0	0	0	0
Alcoholic beverages[2]												
Beer (regular) 12 fl oz	139	0	0	0	0	14	96	0	0	14	0	21
Beer light (Bud Light) 12 fl oz	110	0	0	0	0	11	92	0	0	11	0	0
Beer, ale	155	0	0	13	0	14	77	0	0	17	0	0
Red table wine (3.5 fl oz)	74	0	0	0	0	0	115	0	0	8	0	2

White table wine (3.5 fl oz)	70	0	0	0	0	5	82	0	0	9	0	0
Level 6: Calorically sweetened beverages												
Pepsi Cola[5]	100	0	0	27	25	25	10	0	0	0	0	0
Coca-Cola Classic[6]	105	0	0	26	23	33	0	0	0	0	0	0
Tropicana Fruit Punch (3% Juice)[8]	110	0	0	29	0	50	0	0	0	0	0	0
Fruitopia (10% juice varieties)[13]	110	0	0	29	0	75	0	0	100	0	0	0
Nestea Cool[14]	82	0	0	22	11	68	0	0	0	0	0	0
Lipton Original Iced Tea[3]	60	0	0	17	20	50	0	0	0	0	0	0
Arizona Green Tea1[15]	70	0	0	17	10	20	0	0	0	0	0	0
Kool-Aid Splash Grape Berry Punch[2]	116	0	0	30	0	35	12	0	0	0	0	0
Jamba Juice, Banana Berry Smoothie[16]	149	0.5	0	31	0	36	0	62	5	62	0	0
Sweetened coffee drinks[17]												
Starbucks Frappuccino, coffee flavored	160	2.5	1.7	25	70	93	0	100	0	220	0	0
Starbucks Caffe Mocha, no whipped cream	240	10	5	24	65	125	0	0	0	0	0	0

[1] Amount per 8 fl oz (237 ml) serving.
[2] data from USDA, Agricultural Research Services, Nutrient Data Laboratory: National Nutrient Database for Standard Reference. See website: http://www.nal.usda.gov/fnic/foodcomp/search/.
[3–17] Data from various manufacturer's website. See individual listings below.
[3] lipton.com.
[4] silkissoy.com.
[5] pepsi.com.
[6] coca-cola.com.
[7] minutemaid.com.
[8] tropicana.com.
[9] welchs.com.
[10] v8jiuce.com.
[11] wendys.com.
[12] gatorade.com.
[13] fruitopia.com.
[14] neatea.com.
[15] arizonabev.com.
[16] jambajuice.com.
[17] starbucks.com.

sensitivity to caffeine. Pregnant women are often advised to limit caffeine consumption because caffeine intakes higher than 300 mg/day have been associated with increased risk of miscarriage and low birth weight (Dlugosz *et al*., 1996; Hinds *et al*., 1996; Rasch, 2003). It is unclear whether caffeine has adverse effects in children, but concerns regarding its effects on the developing nervous system have led to recommendations that daily caffeine intake by children should be limited to 2.5 mg per kg of body weight (Nawrot *et al*., 2003).

Interestingly, a variety of investigations report an 'inverted U' relationship when a physiological or psychological response is plotted versus caffeine intake in a graph. That is, the magnitude of caffeine's effect is smaller at low and high levels, but greater at intermediate levels. Such a relationship has been reported for exercise performance time (Cadarette *et al*., 1983; Graham and Spriet, 1995), reaction time (Jacobson and Edgley, 1987), vigilance (Frewer and Lader, 1991), information processing (Battig and Buzzi, 1986), and mood state (Lieberman *et al*., 2002) but may not exist for all physiological and psychological responses. Further, this graphic relationship may shift left or right, with caffeine habituation or naivety.

Added calories

Addition of milk, cream, or caloric sweeteners to coffee and tea increases the energy density of these beverages. This might be particularly important for gourmet coffee users who consume a lot of high-energy coffee drinks. For instance, Shields *et al*. (2004) found in one very small sample of college women that gourmet coffee drinkers consumed 206 more calories per day than non-gourmet coffee drinkers. The high caloric content of some gourmet coffee drinks is shown in Table 18.1. Sweetened tea provides smaller amounts of energy than gourmet coffee, as noted in Table 18.1.

18.2.3 Low-fat (1.5% or 1%) and skim (non-fat) milk and soy beverages

For children, milk is the current key source of vitamin D and calcium and is an excellent source of high-quality protein. Low-fat and skim milk, including low-fat yogurt drinks, can contribute to a healthy diet but are not essential. Fortified soy milk is a good alternative for individuals who prefer not to consume cow's milk, although consumers should be aware that soy milk cannot be legally fortified with vitamin D and provides about 75% of the calcium bioavailable from milk (Heaney and Skillman, 1971). Yogurt drinks have lower lactose content than milk and may be preferred by individuals with reduced lactose tolerance. In general, low-fat dairy beverages and fortified soy milk provide an important source of protein, calcium, and other essential micronutrients.

A number of beneficial, and some detrimental, health effects have been attributed to the consumption of cow's milk. The role of milk intake on weight control has been explored in a number of studies. Teegarden and

Zemel (2003) found that higher consumption of milk appeared to induce weight loss, but their study was of small sample size and had a high dropout rate. In larger randomized trials, those assigned to higher intake of low-fat milk experienced greater weight gain that was either statistically significant (Barr, 2003) or not statistically significant (Gunther *et al*., 2005). In a longitudinal study of many thousand adolescents, low-fat milk consumption was positively associated with a gain in BMI (body mass index); this was accounted for by a higher energy intake among those who consumed more milk (Berkey *et al*., 2005). The 2005 Dietary Guidelines for Americans Committee performed a detailed review of this topic and concluded that there was not sufficient evidence that milk consumption reduced, or prevented, weight gain (Dietary Guidelines Advisory Committee Report, 2005). Subsequent published research has found that milk did not prevent a weight gain, including one 48 week clinical trial funded by the National Dairy Council (Clifton, 2005; Thompson, 2005).

A second issue relates to bone health. The Dietary Guidelines Committee also evaluated 7 randomized trials and 32 observational studies exploring the relationship between milk intake and bone health. All 7 randomized trials and 25 of the observational studies showed a positive relationship between milk consumption and bone mineral density in one or more skeletal sites (Standing Committee on the Scientific Evaluation of Dietary Reference Intakes, 1997). However, the benefits of higher calcium intake on bone mineral density are not maintained if the high intake is reduced. In one trial with children, milk intake, but not calcium supplement, had a continuing effect on bone mineral density 3.5 years after termination of the intervention (Bonjour *et al*., 2001). The duration of the randomized studies were too brief to validly assess fracture incidence. Large prospective studies in adults have consistently shown no significant relation between milk intake and risk of fractures.

Milk is an important source of calcium and is the key source for vitamin D due to fortification, particularly for ages 6–18 years, where calcium requirements are higher. Milk products are also important contributors to the intake of essential nutrients in the diet of children and adolescents. Data from the National Health and Nutrition Examination Survey (NHANES) and Continuing Survey of Food Intakes by Individuals (CSFII) indicate that as consumption of milk products increases, so does the intake of calcium, magnesium, potassium, zinc, iron, vitamin A, riboflavin, and folate (Weinberg *et al*., 2004). Conversely, eliminating milk products from the US Department of Agriculture (USDA) dietary pattern would substantially reduce intake of those essential nutrients (Dietary Guidelines Advisory Committee, 2005). Nevertheless, while it would require a careful selection of foods, milk products could be replaced with soy-based products and items from other food groups, particularly fruits and vegetables – some of which are also good sources of calcium. The essential micronutrients in milk products could also be replaced by daily multivitamin/mineral and calcium

supplements. Fortification of milk with vitamin D has reduced rickets in children, but other sources of supplemental vitamin D can be used.

Some studies have reported a beneficial effect of milk consumption in reducing the risk for the metabolic syndrome, a cluster of disorders that includes insulin resistance, glucose intolerance, hypertension, hypertriglyceridemia, and low levels of high-density lipoprotein. In the Coronary Artery Risk Development in Young Adults (CARDIA) study, milk consumption was inversely associated with the 10 year cumulative incidence of the metabolic syndrome in overweight individuals (Pereira *et al*., 2002). A pooled analysis of 10 prospective studies also indicated a beneficial effect of milk consumption in reducing the risk of coronary heart disease and ischemic stroke (Elwood *et al*., 2004). In a short-term clinical trial, two dietary patterns were used – one emphasizing fruits and vegetables and the other emphasizing fruits and vegetables, low-fat dairy products, higher protein and fiber intake and lower fat intake (Dietary Approaches to Stop Hypertension [DASH] Diet); both significantly reduced blood pressure in normotensive or stage I hypertensive men and women of diverse ethnic backgrounds. The DASH diet had a significantly greater effect on reducing blood pressure than the fruits and vegetables diet, and is one of the dietary patterns recommended by the recent US Dietary Guidelines (Appel *et al*., 1997; Sacks *et al*., 2001). It is interesting that the DASH diet actually recommended more fruits and vegetables than the fruit and vegetable diet so there might be confounding factors involved. Moreover, in a carefully conducted multicenter trial, an increase of three glasses of low-fat milk per day had no effect on blood pressure (Barr *et al*., 2000).

Among the evidence for possible adverse effects of milk consumption, a meta-analysis of case-control studies reported a 70% greater risk of prostate cancer in men with the highest milk consumption levels (Qin *et al*., 2004). Other studies have suggested an increased risk of aggressive ovarian cancer in persons consuming >3 fl oz of dairy products per day, although this literature is not consistent (Fairfield *et al*., 2004). It has been speculated that this adverse effect of milk may be related to its well-documented effect on circulating levels of insulin-like growth factor (IGF)-1 (Barr *et al*., 2000; Holmes *et al*., 2002; Giovannucci *et al*., 2003), which has been associated with increases of many cancers in both humans and animals (Giovannucci *et al*., 2003).

18.2.4 Non-calorically sweetened beverages

The non-calorically sweetened beverages (diet sodas and other 'diet' drinks) are preferable to calorically sweetened beverages because they provide water and sweetness, but no calories. Non-caloric sweeteners approved by the US Food and Drug Administration (FDA) are considered safe, although other than FDA surveillance data there is no evidence from long-term studies in humans available to this group and is most likely lacking.

Raben *et al*. (2002) showed that beverages sweetened with non-caloric sweeteners were associated with weight loss when ingested in amounts similar to calorically sweetened beverages where weight gain and increased blood pressure occurred. A new literature is emerging that seems to suggest that the high sweetness in these beverages may contribute to conditioning for a high preference for sweetness (Sclafani, 1997; Davidson and Swithers, 2004), and thus these non-calorically sweetened beverages would be less desirable than water, tea, or coffee.

18.2.5 Caloric beverages with some nutrients

Fruit juices (100% juice) provide most of the nutrients of their natural source, but are also relatively high in energy content and may lack fiber and other beneficial non-nutrient compounds present in the whole produce. There is no specific need to consume fruit juices, and consumption of whole fruits should be encouraged for satiety and energy balance. The US Dietary Guidelines Committee (Dietary Guidelines Advisory Committee, 2005) recommended that no more than one-third of the daily intake of fruits be in the form of juices. Fruit smoothies are usually high-calorie versions of fruit drinks and therefore are not recommended.

Vegetable juices (e.g. tomato and multi-vegetable juices) are a healthy alternative to fruit juices. They have fewer calories per 100 ml (3.4 fl oz) than orange juice but usually have significant amounts of added sodium. For example, tomato juice and vegetable cocktails have over 975 mg of sodium per 12 fl oz (357 ml). As with fruit juices, whole tomatoes and vegetables should be encouraged for satiety and energy balance rather than vegetable juices.

Whole (full-fat) milk contains 236 kcal/12 fl oz (255 ml) and has higher energy density and saturated fat content than reduced fat milk (2% fat, 180 kcal/12 fl oz), low-fat milk (1%, 150 kcal/12 fl oz), and skim or non-fat milk (135 kcal/12 fl oz). The adverse health effects of saturated fats have been well documented in numerous studies, especially with respect to increased risk of cardiovascular diseases (Temme and Mensink, 1998). Whole fat dairy products are a significant source of saturated fat in the American diet. Whole fat milk contributes significantly to US saturated fat intake, which has been found in NHANES III data to be 20% higher than the desirable level of 10% or less of daily energy intake.

Sports drinks contain from 50 to 90% of the energy (75–140 kcal/12 fl oz [255 ml]) contained in calorically sweetened soft drinks (158 kcal/12 fl oz) and provide small amounts of sodium, chloride, and potassium. Although a well-balanced nutritious diet provides the same ingredients, the carbohydrates, water, and sodium in sports drinks are advantageous during endurance activities (i.e. when sweat rate exceeds 8 liters per day, strenuous exercise that lasts longer than 60 minutes, or a deficiency exists for sodium or carbohydrates) (Armstrong, 1994). We recommend sports drinks be consumed sparingly except for endurance athletes because these beverages

Table 18.2 Energy content comparison of alcoholic beverages

Beverage	Energy (kcal)	Amount (fl oz)
Beer	140	12 (355 ml)
Light beer	100	12 (355 ml)
Wine cooler	110–275	12 (355 ml)
Wine	115	5 (148 ml)
Spirits, 80 proof	100	1.5 (44 ml)
Standard alcoholic drink	98	14 (414 ml)
Soft drink	150	12 (355 ml)

provide calories. Alcoholic beverages consumed in moderation have some health benefits for adults. Moderate intake is defined as the daily consumption of no more than one drink for women and two for men (US Department of Agriculture/US Department of Health and Human Services, 1990; Meyerhoff *et al*., 2005). Alcoholic beverages contain calories. A standard alcoholic drink is defined as one that contains approximately 14 g of alcohol (National Institute on Alcohol Abuse and Alcoholism, 2004). Table 18.2 provides the amount of each beverage and the related kcal for beer, wine, an 80 proof distilled beverage, and the standard alcoholic drink (i.e. 14 g of alcohol). Alcohol provides approximately 7 kcal/g (approximately 100 kcal) per standard alcoholic drink. Wine-, malt- and spirit-based coolers containing 3–7% alcohol are widely available. They are often marketed to young people and packaged to look like sodas. Many of these beverages contain added sugars. A 8 fl oz (237 ml) cooler may contain more alcohol than 8 fl oz of beer, and some coolers contain more than 250 kcal (compared with 104 kcal in a 8 fl oz soft drink). The health impact of coolers has not been studied.

While excessive alcohol (ethanol) consumption has been linked to serious health and social problems, moderate alcohol consumption has been associated with some health benefits (Klatsky, 2003). The relationship between alcohol consumption and mortality is often described as J-shaped, meaning that light to moderate consumption compared with abstention or high consumption is associated with lower rates of mortality – mostly from coronary heart disease (Rimm *et al*., 1996) and ischemic stroke (Reynolds *et al*., 2003) – while heavy alcohol consumption is associated with higher rates of mortality from a number of causes. The benefits of moderate alcohol consumption, which, in addition to cardiovascular diseases, may include reduced risk of type 2 diabetes (Ajani *et al*., 2000; Conigrave *et al*., 2001) and gallstones (Leitzmann *et al*., 1999; Leitzmann *et al*., 2003), appear to be derived mainly from alcohol itself. While short-term studies have shown beneficial effects of red wine on blood pressure, platelet aggregation, and serum lipids, epidemiological evidence does not support added health benefits specific to flavonoids in red wine or dark beer (Rimm *et al*., 1996; Mukamal *et al*., 2003). Alcoholic beverages, even at moderate intakes, are linked with

increased risk of birth defects (American Academy of Pediatrics Committee on Substance Abuse and Committee on Children With Disabilities, 2000) and breast cancer (Smith-Warner *et al*., 1998; Hamajima *et al*., 2002). The increased risk of breast cancer appears to be caused, at least in part, by the interference of alcohol with the absorption and metabolism of folate. Therefore, pregnant women should not drink alcoholic beverages and other women who consume alcohol should also consume adequate folate, preferentially from a supplement (400 mcg/day) (Zhang *et al*., 1999, 2003). Heavy alcohol consumption is associated with several cancers, in addition to breast cancer (Bagnardi *et al*., 2001), and other significant health problems such as cirrhosis of the liver (Mann *et al*., 2003), hypertension (Xin *et al*., 2001), hemorrhagic stroke (National Institute on Alcohol Abuse and Alcoholism, 2004), cardiomyopathy (Piano, 2002), atrial fibrillation (Ruigomez *et al*., 2002), and dementia (Zuccala *et al*., 2001).

18.2.6 Calorically sweetened beverages

Calorically sweetened beverages, including carbonated (fizzy) and non-carbonated (still) beverages, usually sweetened with high-fructose corn syrup or sucrose, have high energy density and no, or very small amounts of, other nutrients. The authors recommend that calorically sweetened soft drinks and 'juice drinks' are consumed sparingly. Caloric sweeteners have been linked to dental caries, increased energy intake, weight gain, and type 2 diabetes (Jones *et al*., 1999; Ludwig *et al*., 2001; Raben *et al*., 2002; Schulze *et al*., 2004; Dietary Guidelines Advisory Committee, 2005).

In the quantities consumed today, soft drinks and fruit drinks most likely contribute to the obesity epidemic by facilitating excess energy intake. As noted in the Introduction, animal and human literature exists showing that these beverages are not satiating, and compensation in terms of reduction in the intake of other foods and beverages is poor, with the net effect being increased energy intake and obesity (Hulshof *et al*., 1993; Mattes, 1996; Harnack *et al*., 1999; Ludwig *et al*., 2001; Ludwig, 2002; Raben *et al*., 2002; Berkey *et al*., 2004; Schulze *et al*., 2004; Davidson and Swithers, 2005). It is possible that the fructose content has an added effect (Bray *et al*., 2004).

There is also evidence linking calorically sweetened beverages with an increased risk of type 2 diabetes. One recent prospective study using data from the Nurses' Health Study found that women consuming one or more servings of sugar-sweetened soft drinks per day had a significantly higher risk of developing type 2 diabetes than those who consumed fewer than one serving per month (Schulze *et al*., 2004). Others suggest soft drinks are replacing milk in the diet (Harnack *et al*., 1999; Nielsen and Popkin, 2004).

We note that soft drinks and fruit drinks are not the only high-calorie beverages. New drinks are constantly being offered that fit the same profile. Examples are some of the very high-calorie beverage smoothies. We

have not systematically reviewed these newer beverages. At the same time we recommend significantly reduced intake of calorically sweetened beverages.

18.3 Guidelines for beverage consumption

We have published guidelines for beverage consumption, which focus on substances that affect the energy density (kcal/100 ml) and nutrient density of each beverage (Popkin *et al*., 2006). In this proposed guidance system, we have ranked beverages with water at the bottom (Level 1), to be consumed frequently, and calorically sweetened beverages at the top (Level 6), which should be consumed sparingly.

18.4 Overview of different beverage terms and definitions

We defined beverages as all fluids consumed by humans, including water. However, we excluded liquid meal replacement products aimed at weight management as well as soups. In assessing each beverage category, we considered the following factors:

- Energy and nutrient density. Energy density was defined as kcal/100 ml. Nutrient density was defined as nutrient content (in nutrient-specific units) per 8 fl oz (237 ml) and per 100 ml (3.4 fl oz).
- Contribution to total energy intake and body weight.
- Contribution to the daily intake of essential nutrients.
- Evidence for beneficial health effects.
- Evidence for adverse health effects.

We used 8 fl oz (237 ml) as reference unit. Eight ounces is the official FDA portion size used for food labels; however, the actual portion size served and consumed is larger. For instance, for soft drinks this was 19.9 fl oz for the average American aged 2 and older in 1994–96 (Nielsen and Popkin, 2003). The USDA Food Composition Table utilizes 8 fl oz (237 ml). We also recommend that calorically sweetened beverages move back to the 8-ounce beverage size. Table 18.3 presents a set of definitions for all the key concepts utilized in discussing beverages in this review.

There are thousands of beverages in the marketplace. We can, however, group them into a number of logical groupings based on their health and nutritional benefits and consequences. We use the following categories, some of which are defined in Table 18.3.

- Water – all bottled and tap sources of water.
- Tea and coffee – all non-calorically sweetened, or with use of a small amount of caloric sweetener.
- Skim-reduced fat milk – Non-fat and 1% milk.

Table 18.3 Glossary of definitions of the key concepts and beverages

Added caloric sweeteners	All the composite sugars added to a food including sucrose, high-fructose corn syrup, honey, molasses, and other syrups.
Calorically sweetened beverages	Any beverage to which a caloric sweetener has been added. These beverages include carbonated or noncarbonated soft drinks, fruit punch, fruit drinks, lemonade, sweetened powder drinks, or any other non-artificially sweetened beverages. We exclude from this definition sugars naturally present in fluids, and that are not added in processing, preparation, or at the table.
Energy density	Kilocalories per 8 fl oz (237 ml) of beverage.
Fruit and vegetable juices	Beverages that are composed exclusively of an aqueous liquid or liquids extracted from one or more fruits or vegetables with no added caloric sweeteners.
Fruit drinks	Calorically sweetened beverages with a small percentage of a fruit juice or juice flavoring containing carbonated water and flavoring.
Metabolic water	Water formed during the metabolism of food.
Naturally occurring sugars	Sugars occurring in food and not added in processing, preparations, or at the table.
Non-calorically sweetened beverages	Soft drinks (diet sodas) or fruit drinks sweetened with FDA-approved non-caloric sweeteners. Non-caloric sweeteners do not provide calories, but they do provide the sweet taste. Non-caloric sweeteners currently include aspartame (Equal or NutraSweet), acesulfame K (Sunett), saccharin or benzosulfamide (Sweet 'n Low) and sucralose (Splenda). All are many times sweeter than sugar per gram.
Nutrient density	Amount of each nutrient in 8 fl oz (237 ml) of beverage. The health benefits and risks to be considered include non-communicable diseases such as obesity, type 2 diabetes, heart disease, various cancers, dental caries, and bone health.
Potable water	Whether supplied from ground water or underground aquifers, water suitable for human consumption, free of pathogens and major pollutants, containing less than 50 mg nitrates/l (European standard), and not having toxic amounts of any mineral.
Soft drinks	Non-alcoholic carbonated or noncarbonated beverages containing caloric sweeteners and flavorings.

- Diet beverages – all non-calorically sweetened beverages with no or minimal calories.
- Caloric beverages with nutrients – we include alcohol here as it provides some health benefits when consumed in moderate amounts. Also included are sports drinks, all 100% juices, 2% and whole milk.

- Calorically sweetened beverages – both soft drinks and fruit drinks, smoothies, calorically sweetened coffees and teas, new caloric energy drinks.

18.4.1 Our focus

The focus of the guidance for beverages is on caloric and non-caloric sweeteners and other substances that affect the energy density (kcal/100 ml) and nutrient density of each beverage. It is recognized that the concept of 'energy density' for solid and liquid foods may not be equivalent, particularly when focusing on hunger and satiety responses; however, the concept is used by some scholars for solid foods, soups, and beverages (Seagle *et al*., 1997; Marti-Henneberg *et al*., 1999; Cox and Mela, 2000; Gibson, 2000; Grunwald *et al*., 2001), whereas others do not use this concept in their measurement (Ledikwe *et al*., 2005). In this chapter, we use a simple, operational definition based on caloric content per unit volume. Relative to most foods, beverages have low energy density (<350 kcal/12 fl oz [355 ml]) because water is the item that reduces energy density the most (Drewnowski, 1998, 2003; Rolls *et al*., 1999, 2005). Thus, relative energy density within each beverage category was compared to other beverage categories.

Our recommendations are aimed at the population over 6 years of age. Below that age, there are a number of additional factors, such as development of taste preferences and early imprinting of food choices that may have an impact on beverage choice and intake.

18.4.2 Current trends in consumption

We know that in the United States, South Africa, Australia, and Sweden where studies have focused on intake of sweetened foods, there have been large increases of caloric sweeteners in our diet (Popkin and Nielsen, 2003; Bray *et al*., 2004; Nielsen and Popkin, 2004). In one study we showed that globally between 1962 and 2000, caloric sweeteners available for consumption on a daily basis increased by 74 for all persons globally (Popkin and Nielsen, 2003). Although based on imperfectly measured food balance information, this trend shows a very large increase in the use of caloric sweeteners globally.

Studies of added sugar in the diets based on individual food intake from the United States, South Africa, and Australia revealed a different picture when they looked at intake trends using detailed food composition tables to examine added caloric sweetener. Depending on the reference point, the average caloric intake for all Americans aged >2 years has increased by about 150–300 kcal/day for different age-gender groups (Nielsen *et al*., 2002; CDC, 2004). Data also show that about half of that increase is contributed by the consumption of calorically sweetened beverages. Between 1977 and 2001, the proportion of energy obtained from calorically sweetened soft

drinks and fruit drinks (which – as defined later – are different from fruit juices) has increased threefold, from 2.8 to 7.0% (50 to 144 kcal/day), with a concurrent reduction in milk intake (Nielsen and Popkin, 2004). Portion sizes of calorically sweetened beverages for all ages increased from 13.6 fl oz (402 ml) to 21.0 fl oz (621 ml) between 1977 and 1996 – a proportionately larger increase than the increase in the number of servings (Nielsen and Popkin, 2004). At the same time that portion sizes have increased, Americans have also increased the number of servings of calorically sweetened beverages from 1.96 in 1977 to 2.39 in 1996. Servings are measured for beverages according to USDA standards. Our proposed guidance thus focuses on obtaining as much of the daily fluid needs as possible from beverages that have lower amounts of energy and an improved nutrient profile. A more recent study has shown that the percentage of calories from beverages significantly increased between 1965 (11.8%), 1977 (14.2%), 1989 (17.2%) and 2002 (21.0%); an overall increase of 250 calories from beverages per day among US adults with over two-thirds of this increase coming from calorically sweetened beverages (Duffey and Popkin 2007).

South African data showed intake levels for added sugar similar to those for US children and adolescents but no data for adults (Steyn *et al*., 2003). In Australia in a nationally representative survey of children, average daily intake of refined sugar ranged from 6.6 to 14.8% of total energy intake for girls and 8.0–14.0% for boys [both aged 2–18] (Somerset, 2003).

18.5 Guidelines for beverage consumption for different consumer groups: what is the proportion of energy from beverages a person should consume?

Elsewhere we have summarized the beverage intake pattern in the United States from the 1999–2002 NHANES surveys of a nationally representative population sample (Popkin *et al*., 2006). The pattern for adults aged 19 years and older was selected. Water, tea and coffee intake – the unsweetened beverages – constitute 70% of the total volume and contributed only 2% of the calories. In contrast, the calorically sweetened soft drinks and fruit drinks provide 46% of the calories. As noted earlier, the proportion of energy from beverages for the average American aged >2 years is 21%. Hence, US adults aged 19 years and older daily consume 464 calories from beverages (Fig. 18.1b). Reduced intake of caloric beverages that provide no nutritional benefits is needed to reduce this high energy intake from beverages; these are not needed to fulfill the daily energy intake of any individual.

A number of IOM/Food and Nutrition Board dietary requirements panels, as well as the 2005 Department of Health and Human Services (DHHS)–USDA Dietary Guidelines Advisory Committee have developed

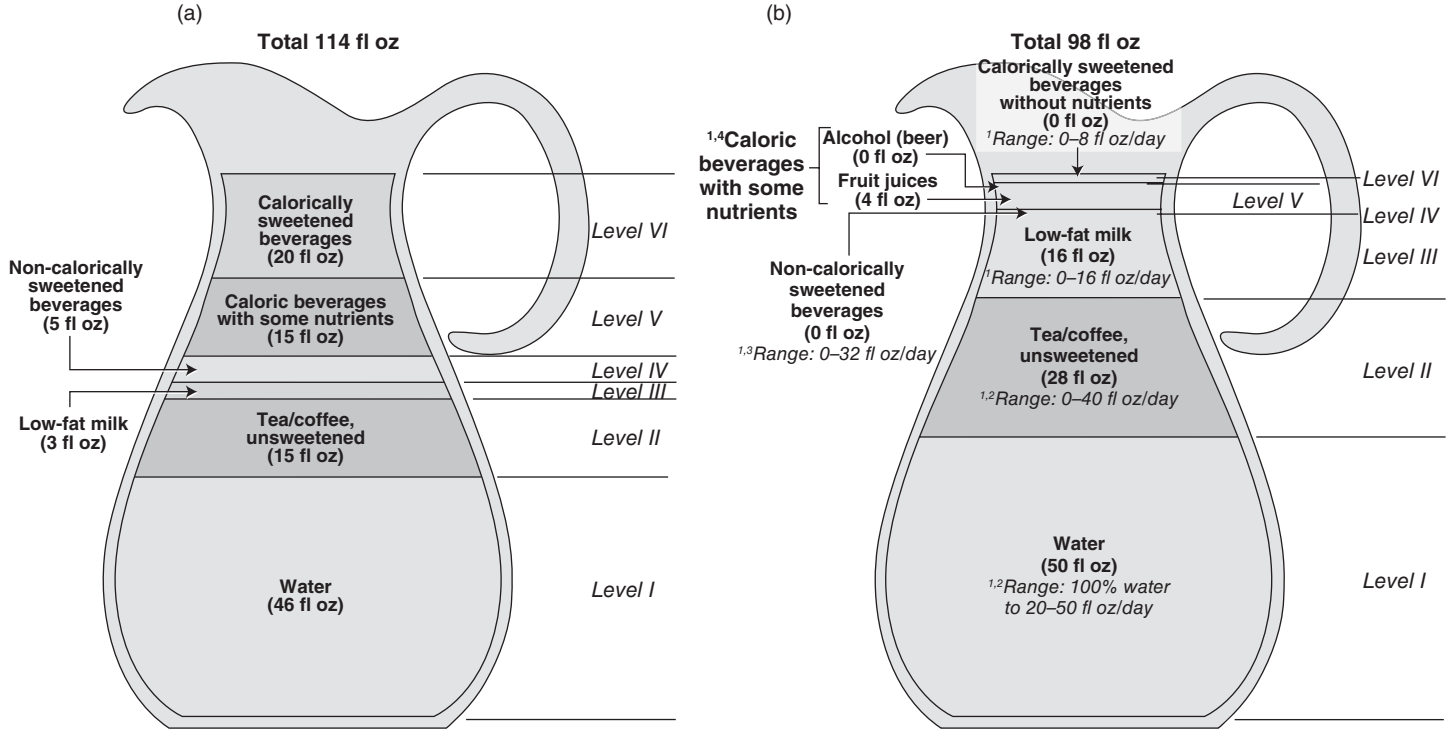

Fig. 18.1 Actual and suggested beverages intake for US adults: (a) average beverage intake patterns for US adults aged 19 and older, 1999–2002; (b) suggested beverage consumption patterns (10% of energy from beverages) for a person with a 2200 kcal energy requirement per day. The values of 50, 28, 16 and 4 fluid ounces are shown for illustrative purposes only and that the total should sum to 98 fluid ounces, as shown at the top of part (b). [1]The suggested range for each beverage from us. [2]Range: caffeine is a limiting factor up to 400 mg/day or about 32 fl oz/day of coffee (can replace water). [3]Can substitute for tea and coffee with the same limitations regarding caffeine. [4]100% fruit juices 0–8 fl oz/day, alcoholic beverages 0–1 drink per day for women, and 0–2 drinks per day for men, whole milk 0 fl oz/day. 1 fl oz = value.

sample healthful menus that fulfill the recommended intakes for macronutrients, micronutrients, fiber, and water of average adults. The total beverage requirement is based on the overall composition of an individual's diet and his/her physiological needs for water. Our review utilizes one such sample menu (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004) to estimate the contribution of beverages to nutrient intake (Table 18.1). In this and similar examples, the contribution of beverages to total energy intake is 14%. These calories are contributed primarily by low-fat milk (9%), which is a naturally nutrient-rich beverage. Excluding milk, the other caloric beverages contributed 5% to the total caloric intake or about 114 kcal per day.

A sample average male from the IOM review has an energy requirement of 2200 kcal, which requires a total beverage intake of about 98 fl oz (2.9 l) (Panel on Dietary Reference Intakes for Electrolytes and Water *et al*., 2004). In his diet, water contributed about 25 fl oz (26%), an additional 52 fl oz (54%) came from tea and coffee, leaving less than 20 fl oz (21%) from milk and juice or other calorically sweetened beverages. This distribution is ideal for an adult male with low levels of activity, in particular the high consumption of non-sweetened beverages – water, tea and coffee. This could be 100% water or any one of many combinations, with the goal to get approximately 80% of beverage intake from very low-calorie beverages. Thus, we suggest a distribution in which about 80% of beverages consist of water, unsweetened tea, and unsweetened coffee and only about 20% of low fat milk, juice, alcohol, and calorically sweetened beverages (Figs 18.1 and 18.2).

The graphic design (Fig. 18.1) developed by us summarizes the relative importance of each beverage presented in this review. We suggest that the proportions of beverages shown in Fig. 18.2 should be consumed by any person, but the actual amounts of fluids shown are based on a person with an energy intake requirement of 2200 kcal and a dietary intake pattern presented by the IOM in its publication and summarized in Table 18.1. The suggested pattern shown in Fig. 18.2 would provide at most 10% of total energy from beverages. An acceptable intake pattern (Fig. 18.2) would provide 14% of energy from beverages. We recommend, based on this review and our knowledge of health and nutrition, the following range of intake for beverages:

- Top priority: water 20–50 fl oz/day.
- Priority 2: tea and coffee (unsweetened) 0–40 fl oz/day (can replace water; caffeine is a limiting factor – up to 400 mg/day or about 32 fl oz/d of coffee).
- Priority 3: low-fat and skim milk and soy beverages 0–16 fl oz/day.
- Priority 4: noncalorically sweetened beverages 0–32 fl oz/day (could substitute for tea and coffee with the same limitations regarding caffeine).
- Priority 5: caloric beverages with some nutrients: 100% fruit juices 0–8 fl oz/day, alcoholic beverages 0–1 drink per day for women and

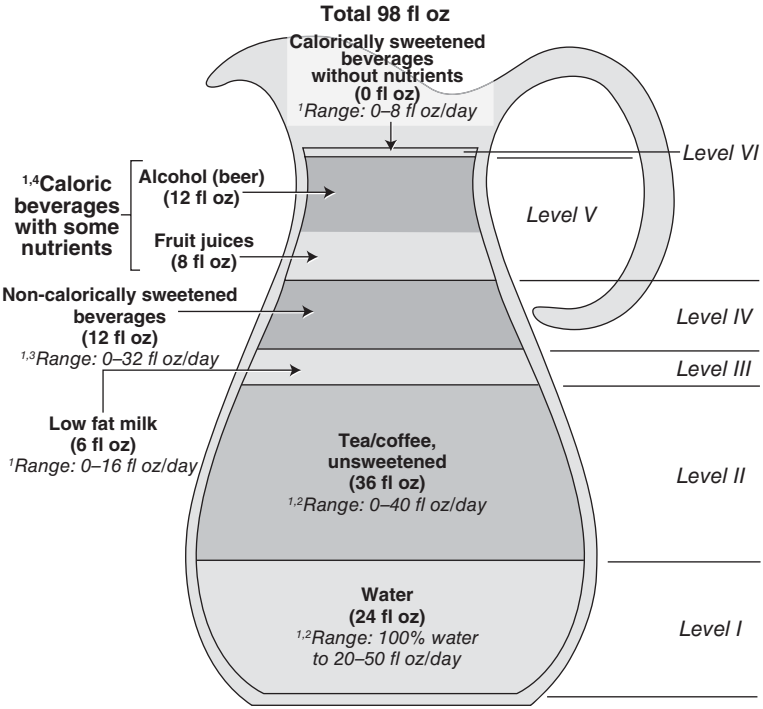

Fig. 18.2 Acceptable beverages consumption patterns (14% of energy from beverages) for a person with a 2200 kcal energy requirement per day. The values of 24, 36, 6, 12, 8, and 12 fluid ounces are shown for illustrative purposes only and that the total should sum to 98 fluid ounces, as shown at the top of the figure. [1]The suggested range for each beverage from us. [2]Range: caffeine is a limiting factor up to 400 mg/day or about 32 fl oz/day of coffee (can replace water). [3]Can substitute for tea and coffee with the same limitations regarding caffeine. [4]100% fruit juices 0–8 fl oz/day, alcoholic beverages 0–1 drink per day for women, and 0–2 drinks per day for men, whole milk 0 fl oz/day. 1 fl oz = value.

0–2 drinks per day for men (one drink = 12 fl oz of beer, 5 fl oz of wine, or 1.5 fl oz of distilled spirits), whole milk 0 fl oz/day.

- Priority 6: calorically sweetened beverages 0–8 fl oz/day.

18.6 Recommendations to beverage manufacturers (beverage formulation, labeling, health claims)

18.6.1 Conclusions and recommendations

While this beverage guidance system provides a sense of the relative energy density, nutrient density, health benefits, and health risks linked with each

category of beverages (and also the relative importance of each beverage), it is not possible to provide clear guidance regarding specific quantities. The current high intake of calorically sweetened beverages contributes importantly to the excess caloric intake and is an important factor underlying the development of obesity in the United States. The evidence from nationally representative surveys shows that both portion sizes and the number of servings of these beverages have increased. If caloric intake is to be reduced, decreased intake of these beverages should be part of the solution. We have identified some research and development issues that the food industry could address. For example, reducing the caloric content of sweetened beverages and developing low-calorie alternatives, such as calorically sweetened beverages, with a 75–80% caloric reduction versus current levels could be useful.

We wish to note that the evidence seems to point to calorically sweetened beverages having replaced milk in the US diet, reducing net intake of key essential nutrients. There is a need among children and adolescents to reverse this trend. The fortification of non-caloric beverages with essential nutrients, such as flavored bottled water, is also of concern. Many vitamins and minerals are in the FDA's Generally Recognized As Safe (GRAS) list, and therefore have little restrictions for their addition to foods. However, international guidelines for food fortification clearly state that it should be based on demonstrated need. The Food and Agriculture Organization (FAO) guidelines state that to justify fortification 'there should be a demonstrated need for increasing the intake of an essential nutrient in one or more target groups' (Food and Agriculture Organization, 1996). Furthermore, although these fortified beverage products may provide comparable micronutrient levels to some natural foods, they lack fiber, phytochemicals, and other natural compounds that come from naturally nutrient-rich beverages. Although not fully characterized, these may contribute to the demonstrated health benefits of whole foods such as fruits and vegetables. Thus, these fortified non-caloric beverages should not be regarded as equivalent to other foods that are naturally rich in micronutrients. In the case of calorically fortified beverages, their consumption may even further increase the already excessive caloric intake of the American population.

There is a potential need for adding minerals to bottled water. For example, a careful review of the level of fluorine in bottled waters should be undertaken to determine if these waters might need added fluorine. Currently, maximum levels of fluorine exist for bottled water (domestic and imported) but minimum levels do not.

Many government documents have discussed the benefits and risks of various beverages, but the results are often too vague or general and are affected by lack of a clear focus on the calorically sweetened beverages that provide a significant source of calories in our diet. This group recommends that these beverages be replaced over time by other beverages with more nutritional value and fewer calories.

We also note the need for further research regarding the health effects of dairy products and reduced or non-calorically sweetened beverages. Figure 18.1 provides what we consider an ideal beverage intake pattern as well as one that is less ideal but acceptable. Further, in our view and that of the IOM it is important that over 60%, if not 100%, of fluid needs are provided by calorie-free beverages. This is important since – as we recognize – fluid needs vary widely among people, and those with higher than average needs should increase their fluid intake from calorie-free beverages, preferably water.

18.7 Future trends

There are a number of positive but many equally disturbing trends in the beverage sector. Among these are the following:

- *The push to hydrate*. Advertisements encourage us all to hydrate, energize, nourish, relax or enjoy every drop of life. Our research has shown that while water intake has remained constant in the United States since 1995, calorically sweetened beverage intake has increased by 20 ounces per day (Duffey and Popkin, 2007).
- *New energy drinks*. The new category of beverages personified by Red Bull, Adrenaline Rush, 180 and many others. These highly-caffeinated, high-energy drinks have exploded on the beverage market in the United States and globally and generated a whole new generation of copycat caloric or – in many cases – sweetened beverages. Surprisingly, these new energy drinks are potentially dangerous. Consider the 18-year-old at an emergency room in Berkeley, California, with sudden heart arrhythmia who drank eight 16-ounce cans of Rockstar every evening to stay awake for his night job (Mason, 2006). These drinks are particularly dangerous when combined with alcohol, a most common practice. Consider the drinkers who combine these energy drinks and alcohol, only to find as they did in a recent Brazilian study that this commonly used combination reduces ability to perceive one's own inebriation (Ferreira *et al*., 2004, 2006; Kalus, *et al*., 2007). Since these energy drinks are stimulants and alcohol is a depressant, the combination of effects may be dangerous. The stimulant effects can mask how intoxicated a person is and prevent him or her from realizing how much alcohol he or she has consumed. The energy drink stimulant provides the person with the impression that he or she is not impaired.
- *Calorie-burning beverages*: Coca Cola and Nestlé have partnered to produce Enviga, one of the new 'calorie-burning beverages'. They do not explicitly tout this as a beverage to help you lose weight, but the advertising campaign of Coke promotes a beverage that burns calories and discusses negative calories, implying that it will. Coke also says Enviga

will burn extra calories. Many other similar beverages follow this advertising style. *These beverages have not been shown to reduce weight or prevent weight gain.* These new 'calorie-burning beverages' all utilize elements from tea as a basic mechanism to achieve their goal. For decades, scholars had studied tea catechins in combination with caffeine. The tea catechin (epigallocatechin gallate [EGCG]) has a small thermogenic property (Higdon and Frei, 2003). That is, it is likely to generate some heat and possibly burn some calories. This effect is really small and is slightly enhanced by caffeine, which is also slightly thermogenic (Higdon and Frei, 2003). However, there is no evidence that these effects will reduce body fat in humans, as the Obesity Society (2006; Rudelle *et al*., 2007) has stated.

- *Healthy waters and other drinkable elixirs*: there are also the new drinkable skincare or 'healthier functional beverages.' Go to a local Sephora (US beauty retailer) and you will find many of these drinkable skincare beverages shelved in refrigerators. The plastic bottles contain mixtures of vitamins and plant extracts that promise to enhance the skin. These are supposed to be replenishing waters. There is one that improves moisture levels by 66% and improves the elasticity (i.e. firmness of your skin) by 24%. If any of these beverages were supported by research, they would revolutionize the skincare industry – but *the research is not there*.

In other words, a vast array of new beverages are entering the market with an array of health claims. When adolescents and others begin to be harmed by these beverages, there will be a call for major legislation to regulate these labels. It would behoove the beverage industry to handle these issues ahead of time.

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