Food Biology Series

Fermented Foods

Part II: Technological Intervention

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Technological Innovations in Processing of Fermented Foods

An Overview

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1. Introduction

The last 100 decades have witnessed significant changes in the world's food system. In ancient times fermentation of food was meant for food preservation and flavor improvement. Food processing/fermentations use various technologies and operations to convert relatively perishable, typically bulky and inedible raw materials into palatable foods and potable beverages with high stability and added values (Ray and Joshi 2014). Biotechnological aspects in the industrialization of indigenous fermented foods open the possibilities for exploring these technological interventions

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for improved production methods and quality of the foods. The assurance of the quality and safety of the final product is the main achievement of the technologies applied. The science of food safety provides assurance about the physical, chemical or microbiological hazards being present at a permissible level with respect to health implications of the consumers (Ray 2013). Biotechnology has played a revolutionary role in production, preservation, nutritional enhancement and value addition of foods. Since time immemorial fermented foods have pleased our palates along with increased nutritive values. Understanding the science of microbiology in food applications with identification of new fermenting species was a boon to enhance the quality of our food in a number of ways. Traditional biotechnology has been helpful for production of functional foods, flavor enhancement, biopreservation, probiotics and enzyme modification of foods for long. With advanced technologies in food biotechnology like genetic engineering we have an upper hand in the field of functional foods.

As a technology, food fermentations date back at least 6000 years. The start of industrialization in 16th century initiated technological intervention in food production sector (Truninger 2013). The industrial revolution and blossoming of microbiology at the same time formed the foundation for preparation of bulk quantities and commercialization of processed food to meet the growing food requirement of the masses (Caplice and Fitzgerald 1999). Some of the conventional food processing technologies include salting, drying and fermentation. However, food processing using microorganisms is the most convenient technology for the development of novel fermented food products of commercial significance. Solid state fermentation is used for processing of vinegar, soy sauce, curing of tea and tobacco leaves, ripening of cheese, etc. (Ghosh 2015). Similarly, wine, beer, distilled beverages and yogurt are developed by submerged fermentation. The current article covers the various technological interventions covering the processing of fermented food, past and present.

2. Fermented Foods

The term fermentation comes from Latin word "*fermentum*" (to ferment). The science of fermentation is called zymology and the first zymologist was Louis Pasteur identifying and applying yeast in fermentation (Dubos 1995). Fermented foods are the ones that undergo microbial or enzymatic alterations for quality and sensorial improvement by biochemical changes (Campbell-Platt 1987). It is one of the oldest and most economical methods of production and preservation of foods and various types of fermentations have been used by different civilizations since prehistoric times. Foods are fermented for many reasons, including the enhancement of nutritive value, removal of anti-nutritients and the improvement of sensory characteristics such as flavor and taste. Fermentation with respect to technology and industrial microbiology can be defined as a process of biotransformation of cooked/uncooked food matrices carried out by microorganisms or their enzymes. Many valuable bio-products around the world are the result of fermentation, either occurring naturally or through addition of starter cultures. Presently, modern technologies and large scale production exploit defined species of starter cultures to ensure consistency and quality in the final product.

2.1 History of Fermented Foods

Food preservation and the art of fermentation is centuries old. Since ancient times fermentation has been used to conserve and alter food without understanding the microbial mechanisms. Greeks even attributed fermentation to Dionysus, the god of fruit fermentation (Stanislawski 1975). Cheese making was developed early when plants and animals were just domesticated, 8000 years back in Tigris and Euphrates (Fox 1993). By 2000–4000 B.C., Egyptians and Sumerians developed wine making and brewing with the start of alcoholic fermentation. The fermented beverages appeared in 5000 B.C. in Babylon, 3150 B.C. in ancient Egypt, 2000 B.C. in Mexico and 1500 B.C. in Sudan (Mirbach and El Ali 2005, Ray and Joshi 2014). Also dough fermentation was started by Egyptians for making bread. Evidence suggests that fermented foods were consumed 7,000 years ago in Babylon (Battcock and Azam-Ali 1998). Though for thousands of years the exploitation of fermentation for food and beverages has been taking place but only in the recent past the microorganisms responsible for the metabolic process have been recognized. The knowledge of microorganisms responsible for fermentation, pasteurization and industrial revolution took place simultaneously as massive migrations of local communities to large cities and industrial sectors increased the demand for bulk food production (Caplice and Fitzgerald 1999). This led to large scale fermentation processes for industrial production of fermented foods and beverages.

2.2 Types of Fermented Foods

Broadly, fermentation can be grouped into those that take place using solid foods, that is solid state fermentation and those, that use liquid raw materials called submerged fermentation. The solid state fermentation requires uses of aerobic microorganisms whereas most submerged fermentations using liquid foods require anaerobic conditions. Different production methods, technologies, equipments and protocols are designed to address the different types of fermentation. For solid state fermentation the simplest technologies are tray, bowl or other containers that are used for incubation in room or in cabinet. For small scale production of fermented pickles, beer and dairy products by liquid or submerged fermentation, it consists of covered containers and drums of plastic, aluminum or stain less steel. Equipment known as "Bioreactor" is used for large scale and industrial production. Different types of batch bioreactors include rotating-drum bioreactors, traditional and zymotis packed-bed bioreactors, packed-bed energy balance, intermittently-mixed forcefully-aerated bioreactor, well-mixed bioreactor, etc. (Banks 1984).

Fermented foods can be classified in a number of ways (Dirar 1993):

- By categories: (Yokotsuka and Sasaki 1985)—(1) alcoholic beverages fermented by yeasts, (2) vinegars/acetic acid fermented by *Acetobacter*, (3) milks fermented by *lactobacilli*, (4) pickles fermented through *lactobacilli*, (5) fish or meat fermented with *lactobacilli* and (6) plant proteins fermented with molds with or without *lactobacilli* and yeasts.
- By classes: (Campbell-Platt 1987)—(1) beverages (alcoholic/lactic acid fermented), (2) cereal bio-products, (3) dairy products, (4) fish products, (5) meat products;
- By commodity: (Kuboye 1985)—(1) cassava and other root crops, (2) cereal, (3) legumes and (4) beverages;
- On functional basis: (Dirar 1993)—(1) Kissar (staples)—porridges and breads such as aceda and kissra, (2) Milhat (sauces and relishes for the staples), (3) marayiss (30 types of opaque beer, clear beer, date wines and meads and other alcoholic drinks) and (4) Akil-munasabat (food for special occasions);
- On basis of types of microorganisms involved, food safety and physical, chemical and nutritive changes occurred: (Steinkraus 1996)—(1) Fermentations producing textured vegetable protein meat substitutes from legume/cereal mixtures. Examples are Indonesian tempe and ontjom (2) High salt/meat-flavored amino acid/peptide sauce and paste fermentations, (3) Lactic acid fermentations, (4) Alcoholic fermentations (5) Acetic acid fermentations, (6) Alkaline fermentations, (7) Leavened breads.

2.3 Microorganisms Associated with Fermented Foods

2.3.1 Yeasts

Yeasts, classified under fungus kingdom are being used since 5000 years ago for the production of bread, wine, beer and other alcoholic beverages. *Saccharomyces* is the most important genus of the yeasts that contribute significantly to the production of fermented foods and beverages of commercial importance (Panda et al. 2014, 2015). *S. cerevisiae*, *S. uvarum* and *S. bayanus* are the popular ones and considered as GRAS (generally regarded as safe). The application of a particular species of the yeast depends on the type of the product to be developed. For example in the production of ale-type beers, top-cropping yeast (ex. *S. cerevisiae*) is applied and in case of lager-type beers bottom-cropping yeast (S. *pastorianus*) is used. Apart from the *Saccharomyces* species other yeasts such as *Hanseniaspora* (*Kloeckera*), *Candida*, *Pichia*, *Metschnikowia*, *Kluyveromyces*, *Schizosaccharomyces*, *Issatchenkia Dekkera* (*Brettanomyces*) species and *Schizosaccharomyces pombe* are known to add to the production of various alcoholic beverages. Furthermore, yeasts are used in production of single cell protein, flavor precursors and colors from cheaper substrates, and also play a vital role in soy sauce fermentation (Fleet 2006).

2.3.2 Molds

Molds are certain multicellular filamentous fungi known as a major spoilage agent of food. However, several genera of the molds are used for the production of value added food products (Lasztity 2009). The most prominent application of molds is in the production of cheeses. *Penicillium camemberti* is used to produce white cheese where as *P. roqueforti* is applied for the production of blue cheese. *P. roqueforti* is known to produce roquefortin, a secondary metabolite that acts against Gram positive bacteria containing hemins. Molds are also used in the fermentation of meats. *P. nalgiovense* and *P. chrysogenum* are generally used for the production of mold-fermented meat products (Holzapfel et al. 2003).

2.3.3 Bacteria: Acetic acid bacteria, Lactic acid bacteria (LAB) and Bacillus

Application of bacteria in food processing is practiced especially for the production of vinegars and dairy products. Vinegars are diluted acetic acid, produced in a two stage fermentation process; in the first step the sugar sources are fermented into alcohol using yeasts and further the alcoholic medium (10–15% v/v, ethanol) is fermented to acetic acid using acetic acid bacteria (Stasiak and Blazejak 2009). In the process ethanol is dehydrogenated to acetic acid and the reduced co-substrates are oxidized simultaneously. Although various bacterial species are known to produce acetic acid but species belonging to *Acetobacter* and *Gluconobacter* are adopted in industries for commercial production of vinegars (Raspo and Goranovic 2009). Vinegars are further classified into different types depending upon the substrate used. Wine vinegar is developed by acetous fermentation of wine, cider vinegar from apple wine, honey vinegar from alcoholic fermented medium of diluted honey. Starchy sources are also used for the production of vinegar. Alpha amylase is used for the breakdown of starch to fermentable sugars such as maltose, dextrins and dextrose. The popular vinegars from starchy sources are malt vinegar, rice vinegar and molasses vinegar.

LAB are beneficial microorganisms, popularly used for fermentation of milk. They are gram-positive, catalase-negative, acid tolerant, aerotolerant, non-sporulating, and they are strictly fermentative rods or cocci which produce lactic acid as the major product from the energy-yielding fermentation of sugars (Temmerman et al. 2004, Wessel et al. 2004). The genus *Lactobacillus* is sub-divided into three groups based on sugar fermentation: facultative hetero-fermentative (*Group I*), obligated hetero-fermentative (*Group II*) and obligated homo-fermentative (*Group III*), *respectively* (Bernardeau et al. 2008). Lactobacilli from *Group I* are known to ferment hexoses to lactic acid and pentoses to lactic acid and acetic acid, and gas is not produced from glucose, but from gluconate. *Group II* bacterial species produce carbon dioxide, lactic acid, acetic acid and/or ethanol from hexoses, and produces gas from glucose. Lactobacilli from *Group III* do not ferment gluconate or pentoses, but ferment glucose to lactic acid. *Lactobacillus* spp. from all three of these groups participate in food fermentation. Keeping in view the health promoting and anti-microbial properties of probiotic LAB, currently they are the prime interest of researchers (Khandelwal et al. 2016). Hence, the modern food market is witnessing the innovative dairy and non-dairy probiotic products. Probiotic juices, yogurts and ice creams are being developed by using probiotic LAB and are getting acceptance in the market.

Similarly, bacteria belonging to *Bacillus* species are involved in preparation of different types of fermented foods. Especially, the unpalatable legumes are fermented using *Bacillus* to improve the organoleptic properties for consumption (Reddy et al. 1983). Several traditional fermented legumes are prepared in different regions such as *natto* of Japan, Nigerian *dawadawa* or *iru*, Nepalese *kinema* and Thai *thua nao*. *Natto* in Northern Japan is prepared by fermentation of cooked soybean seeds by pure cultures of *B. subtilis*. Fermentation of legumes is mostly carried out in solid state fermentation and each product has a unique distinct flavor and aroma. The *Bacillus* fermented foods are generally used as meat substitute and as a flavoring agent in soups or consumed directly. *Dawadawa* is produced and sold in African market by Nestle (Leejeerajumnean 2003).

3. Era of Starter Cultures

A starter culture may be defined as a preparation containing large numbers of desired microorganisms, used for accelerating the fermentation process. The preparations may contain some unavoidable residues from the culture substrates and additives (such as antifreeze or antioxidant compounds), which support the vitality and technological functionality of the microorganisms. A typical starter after being adapted to the substrate facilitates improved control of a fermentation process and predictability of its products (Holzapfel 1997). Basically there are three categories of starter cultures: (1) Single strain culture-contains only one strain of a species, (2) Multi-strain cultures-contain more than one strains of a single species, (3) Multi-strain mixed cultures-contain different strains from different species.

3.1 History and Subsequent Development of Starter Culture

Microorganisms are naturally omnipresent and hence observed in raw food materials. This was the basis of the idea of spontaneous fermentation. Backslopping was the important technological phenomenon used in spontaneous fermentation by inoculating the raw material with a small quantity of a previously performed successful fermentation. Hence, the dominance of the best adapted strains resulted in backslopping. This technology is still used for production in foods where the ecology and concrete knowledge about microbial population and role is not clearly known (Harris 1998). This is also an economical and reliable method of production of fermented foods.

3.2 Application of Functional Starter Cultures in Food Fermentations

The presence of at least one inherent functional property in the starter makes it a functional starter culture. Functional foods refer to the food with health promoting and disease prevention properties. The food fermentation industries have started to explore the application of functional starter cultures in the last two-to-three decades (De Vuyst 2000, Gasper and Crespo 2016). The careful selection of strains as starter cultures or co-cultures and its implementation in fermentation processes can achieve the desired natural and healthy product. Many probiotic strains are also considered for its application in food fermentation as functional starter or co-cultures (Jahreis et al. 2002, Khandelwal et al. 2016). Nowadays most leading starter culture manufacturers produce and market common Gastro Intestinal (GI)-based LAB and bifidobacteria commercially (Picard et al. 2005).

3.3 Recent Technologies and Use of Starter Cultures

The performance of the starter culture is regulated by the strain's growth, type of sugars it ferments and the nature of the final product formed. Biochemical and functional properties of yeasts used in bread, beer and wine manufacturing have been extensively studied (Montet and Ray 2016). LAB are intensively studied for the development of starter cultures

(Ray and Joshi 2014). Some examples of functional starters with technological advances are cited below:

3.3.1 Food grade genetic engineering

Genetically manipulated microorganisms used as starter cultures in food fermentation should be safe and regarded as acceptable ingredients in our food. One example of engineering for food safety was the removal of the D-lactate dehydrogenase (*ldhD*) gene from *Lactobacillus johnsonii* La1, which demonstrated the exclusion of the undesired D-isomer of lactate and leaving only the desired L-lactate (Mollet 1999).

3.3.2 Phage-resistant starters for the dairy industry

In large scale production in dairy industry bacteriophages possess a serious problem. Hence Phage-resistant starters with rotation of use and proper sanitation will overcome the issue. Natural resistance mechanisms (restriction and modification enzymes), phage adsorption and absorptive phage infection that prevent intracellular phage development, intracellular defense strategies are the usual cause of phage resistance (Forde and Fitzgerald 1999). Large scale application of strains with acquired natural mechanisms of phage resistance either by *in vivo* recombination (conjugation) or *in vitro* self-cloning, is highly desirable in dairy industry (Moineau 1999).

3.3.3 Mild yogurt by lactose-negative starters

Lactose-negative mutants of *Lb. delbrueckii* subsp. *bulgaricus* only grow in the presence of actively lactose fermenting *Streptococcus thermophilus* cells and hence enable production of mild yogurts (Mollet 1996). This is a technological achievement against the undesirable bitter taste due to acidification during yogurt production by *Lb. delbrueckii* subsp. *bulgaricus.*

3.3.4 Acceleration of cheese maturation

Aroma, flavor and taste in cheese production are enhanced by lactic acid production by LAB. Hence optimal activity of endogenous and exogenous enzymes that modify the cheese by rational selection of LAB starter and co culture and also *in situ* autolysis gives alternate solutions (Fox et al. 1996).

3.3.5 Metabolic engineering of starter culture

The possibility of using metabolic engineering to alter or optimize various aspects of this metabolic network have been reviewed by several researchers (Swindell et al. 1996, Daly et al. 1998). The currently known metabolic pathways of immediate practical importance are the ones described for the conversion of sugars *via* pyruvate to acids and metabolites with distinct flavors (Ray and Joshi 2014).

3.3.6 Bacterial genomics and high throughput technologies

This is the era of genomics in biological sciences. Acquiring and analyzing biological data and obtaining the whole genome sequence for a large number of microorganisms including LAB (Klaehammer et al. 2002, 2005) will provide a rich source to guide physiological studies, mutant selections and the use of genetic and protein engineering for starter culture preparations. For example, the *Lactococcus lactis* genome sequence revealed a number of unexpected genetic and metabolic potentials (Bolotin et al. 2001).

3.3.7 Safety in microbial cultures

Over the last decade, directly or indirectly microbial food cultures have come under various regulatory frameworks. They emphasise the history of use, traditional foods, or general recognition of safety. Foods and food additives are regulated according to the Food Drug and Cosmetic Act (1958), in the United States. The status of Generally Recognized as Safe (GRAS) was introduced by FDA in 2010. GRAS substance is adequately shown to be safe under the conditions of its intended use generally recognized, among qualified experts. A substance recognized for such use prior to 1958 is by default GRAS (like food used in the EU prior to May 15, 1997, not being Novel Food) (Anon. 1997, ILSI Europe Novel Food Task Force 2003). Microbial food cultures are covered by general food laws. They must be considered safe and suitable for their intended use. An organism or a product with GRAS status is exempt from the statutory premarket approval requirements. If a microorganism is GRAS for one food usage, it does not make it necessarily GRAS for all food usages.

Similarly the Qualified Presumptions of Safety (QPS) started in November 2007 by European Food Safety Authority (EFSA), was applied in evaluating microorganisms that requires a market authorization (like feed cultures, production of enzymes, etc.) (Anon. 2005, Vogel et al. 2011). The QPS assessment is based on the taxonomic level, body of knowledge, history of use and identification of potential safety concerns. After the assessment a list of microorganisms was prepared at the species level that are presumed safe for use, independent of media, fermentation conditions and intended use. The list of microorganisms submitted to EFSA for safety assessment is being updated annually. The factors like undesirable properties of the microorganisms for food fermentation, opportunistic infection, toxic metabolites and virulence factors, antibiotic resistance are taken into consideration when considering a microorganism as safe for human use (Bourdichon et al. 2012).

3.3.8 Recent list of organisms that can be used

In order to document microorganisms, traditionally used as food ingredients, the International Dairy Federation (IDF) in collaboration with European Food and Feed Cultures Association (EFFCA) has compiled a non exhaustive inventory of microorganisms with a documented history of use in foods. The "2002 IDF Inventory" listed 82 bacterial species and 31 species of yeast and molds whereas the present "Inventory of MFC" contains 195 bacterial species and 69 species of yeasts and molds belonging to 62 genera (Bourdichon et al. 2012).

4. LAB as Cell Factories

LAB are used all over the world in a large variety of industrial food fermentations because of their enormous potential for the biosynthesis of a number of compounds as metabolic end products or secondary metabolites (LeBlanc et al. 2012, Ray and Joshi 2014). LAB comprise different groups of microorganisms, such as *Carnobacterium, Enterococcus, Tetragenococcus, Vagococcus, Weissella*. The industrial core species of LAB belong to the genera, like *Lactococcus, Lactobacillus, Streptococcus, Pediococcus* and *Leuconostoc* (Klaenhammer et al. 2002). LAB produce lactic acid as an anaerobic product of glycolysis with high yield and productivity. They play important roles in the production of food and feed and are increasingly used as health-promoting probiotics. LAB also have the ability to enhance flavor, texture and nutrition. Hence this technology is widely used for food processing in dairy industries, fermentation of meat and vegetables.

LAB has a long history of use in food and beverages without any detrimental health effects. Commercially, important LAB strains such as *Lb. plantarum*, *Lb. fermentum, Lb. rhamnosus* and *Lb. acidophilus*, have been useful due to their high acid tolerance and their ability to be engineered for the production of D- or L-lactic acid (Kyla-Nikkila et al. 2000, Abdel-Rahman et al. 2013). However in specific fermentation, particular species, strain or variant is determined in the food substrate that is used, the temperature of the process, other environmental conditions. *L. lactis* a mesophilic bacterium is used for Gouda cheese that has ability to grow in 35–38ºC where as thermoplilic LAB such as *Streptococcus thermophilus* and *Lb. helveticus* are used for production of yogurt and parmesan cheese where heating of 50ºC is employed (Delcour et al. 2000).

Lactic acid fermentation, the metabolism of LAB is completely geared towards production of a single metabolite—lactic acid. Such a focused metabolism seems to be a perfect basis for creating cell factories of single metabolites. This potential has been demonstrated by many successful technological interventions and metabolic engineering. The development of the adequate gene expression system called NICE (Nisin controlled gene expression) for the production of the antimicrobial compound nisin is produced by *L. lactis* (Kuipers et al. 1993). Such cell factories can overproduce proteins (enzymes) a thousand folds or more. Production of low calorie sweeteners by conversion of lactose into sugar alcohols like mannitol is accelerated by LAB (Korakli et al. 2000, Ladero et al. 2007). Manufacture of various vitamins and flavors are also enhanced with LAB. Also it plays a novel role of efficient cell factory for the production of functional biomolecules and food ingredients to enhance the quality of cereal-based beverages (Waters et al. 2015). Genomics techniques also have been very productive in utilizing LAB as cell factory. Thus, keeping in view the extensive application of LAB in the food industry coupled with consumer demand for healthier and functional foods, the use of these food grade microorganisms as cell factories would be of great advantage in the near future.

5. Immobilization of Cell and Encapsulation of Bacteria

The application of encapsulated and immobilized cell technologies has been of interest to fermentation industries recently. Encapsulation technique is defined as a process whereby cells are embedded or enrobed within a gel-matrix, wherein the metabolic activity of encapsulated cells is completely responsible to carry out the fermentation process. Several research groups have attempted whole cell immobilization as a viable alternative to the conventional free cells fermentations. Immobilized enzymes play a pivotal role in food processing, for example, lactose hydrolysis, whey processing, skimmed milk production, production of high fructose corn syrups have been greatly facilitated by the use of immobilization technology. Immobilization has been carried out by employing *Bacillus brevis* MTCC 7521 for the production of α-amylase and calcium alginate was used as the immobilization matrix (Ray et al. 2008). There are various advantages in the use of encapsulated and immobilized cell technologies (Hutkins 2008) like re-use of cells for several cycles, continuous extraction of metabolites, low cost of the process as also to produce continuous cell biomass from encapsulated cells. Recent fusion proteins (Ushasree et al. 2012) and nanotechnology are used for encapsulation of bacteria in food processing because of their efficiency in increasing enzyme loading and diffusion properties and reduction in mass transfer limitation. The encapsulation of bacteria is an improved technology in food science that basically concentrates on economic, fast, non destructive and food grade purity which will help the food industries with improved quality, aroma and fine taste to the final product (Doleyres and Lacroix 2005).

The technology of micro-encapsulation has developed from a simple immobilization or entrapment to sophisticated and precise micro capsule formation. Microencapsulation helps to separate a core material from its environment until it is released. There are different methods for micro-encapsulation like spray drying, extrusion, emulsion and phase separation. The advances in the field of nutraceuticals and food ingredients have been tremendous; however, the focus on the micro-encapsulation of live probiotic bacterial cells, in fermented food processing is recent (Mortazavian et al. 2016).

6. Probiotics, Prebiotics and Synbiotics

Human health and efficient nutrition absorption are maintained to a larger extent by the microbes of the gastrointestinal tract. Various end products of nutritional substrates like organic acids, vitamins, short chain fatty acids are metabolized by the gut bacteria through fermentation. Probiotics, prebiotics and synbiotics (a combination of probiotics and prebiotics) are new technologies developed to modulate the target gastrointestinal microflora balance. Probiotic therapy (or microbial intervention) is based on the concept of healthy gut microflora.

6.1 Probiotics

The word "probiotic" was first used in 1954 to indicate substances that were required for a healthy life. Out of several definitions, the most widely used and accepted definition is the one proposed by a joint FAO/WHO panel (FAO/WHO 2001): "Live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host". Probiotics helps in enhancing resistance to colonization by exogenous, potentially pathogenic organisms (Elmer et al. 1996, Helland et al. 2004). They produce compounds such as lactic acid, hydrogen peroxide and acetic acid increasing the acidity of the intestine that inhibit the reproduction of many harmful bacteria; they also out compete the pathogenic organisms preventing latter's survival in the gastro intestinal tract (Reid et al. 1999, Helland et al. 2004). The intestinal microbial balance of the consumer is maintained by these live microbial food supplements (Fuller 1991). Starting about 20 years ago functional foods with probiotics are now well established and presently known to most consumers. Probiotic microorganisms are mostly of human or animal origin. The dairy industry, in particular, has found probiotic cultures. Yogurts and fermented milks are the main vehicles for probiotic cultures (Trabelsi et al. 2013). New fermented products such as milk-based desserts, powdered milk for newborn infants, ice creams, butter, mayonnaise, various types of cheese are also being introduced in the international market (Cruz et al. 2009); however, some studies show that strains recognized as probiotics are also found in non-dairy fermented substrates (Martins et al. 2013, Panda and Ray 2016). With the revolution in sequencing and bioinformatics technologies well under way, it is timely and realistic to launch genome sequencing projects for representative probiotic microorganisms. Increasing knowledge of genomes important for the technological functionality and rapid development of the toolboxes for the genetic manipulation of *Lactobacillus* and *Bifidobacterium* species will help in tailoring the technological properties of probiotic strains in future. In addition to the dietary supplementation approach of directly introducing live bacteria named as probiotics to the colon, another approach to increase the number of beneficial bacteria in the intestinal microbiota is through the use of prebiotics.

6.2 Prebiotics and Synbiotics

The concept of prebiotics emerged during mid nineties of the twentieth century (Gibson and Roberfroid 1995). The prebiotic concept for modulation of gut microbiota was introduced in 1995. After a meeting of the International Scientific Association of Probiotics and Prebiotics (ISAPP) in 2010; the definition summarized was "A dietary prebiotic is a selectively fermented ingredient that results in specific changes, in the composition and/or activity of the gastrointestinal microbiota, thus conferring benefit(s) upon host health" (Chapman et al. 2011). Prebiotics doesn't breakdown by digestive enzymes while passing through digestive system. Hence this non-digestible carbohydrate serves as feast for the probiotic bacteria in the large intestine. It stimulates indigenous beneficial flora of the gut while inhibiting the growth of pathogenic bacteria. Target organisms of species belonging to the *Lactobacillus* and *Bifidobacterium* genera are generally preferred for prebiotics. Multipronged beneficial effects including gut health, higher mineral absorption, lowering of cholesterol, immune system stimulation, pathogen exclusion make prebiotic oligosaccharides the centre of attraction against other functional foods. Also prevention and treatment of hypertension with prebiotics has been proved (Rycroft et al. 2001, Roberfroid et al. 2002, Samanta et al. 2007). Most prebiotics as known today are fermentable, non-digestible carbohydrates. Some examples are lactulose, galacto- and fructo-oligosaccharides and resistant starch. Finding of new source and types of natural prebiotics might explore new areas of research. A combination of these probiotics and prebiotics for human endeavor is often referred to as synbiotics (Gibson and Roberfroid 1995, Collins and Gibson 1999). Basically functional foods with both probiotics and prebiotics are called synbiotics (Roberfroid 1998).

New and improved technological interventions are necessary to study the mechanisms and action of probiotics in the gastro intestinal (GI) tract, its effects in GI-diseases, GI-infections and allergies, to develop diagnostic tools and biomarkers for assessment of the GI-tract, to develop technology for non-diary probiotics (Bansal et al. 2016).

7. Ultrasonic Sounds Applications in Fermented Foods

Ultrasound is an unconventional innovative technology for processing and preservation of food. Ultrasound is a sound energy (really pressure) wave of high frequencies that are too high to be audible by human ears, i.e., above 16 kHz. However, the science of application of the technology in food fermentation is a recent one (Ojha et al. 2016).

Ultrasound can be used in fermentation processes to either monitor the progress of fermentation or to influence its progress. High frequency ultrasound (>2 MHz) has been extensively reported as a tool for the measurement of the changes in chemical composition during fermentation providing real time information on reaction progress. Low frequency ultrasound (20–50 kHz) can influence the course of fermentation by improving mass transfer and cell permeability leading to improved process efficiency and production rates. It can also be used to eliminate microorganisms which might otherwise hinder the process. Ojha et al. (2016) reviewed the key applications of high and low frequency ultrasound in food fermentation applications.

Milk is often pasteurized prior to its use in various fermented dairy products. Application of low frequency ultrasound alone or in combination with external pressure (manosonication), heat (thermosonication) or both (manothermosonication) is reported to improve the safety profile of milk and can achieve the desired 5 log reduction of pathogenic microorganisms including *Listeria innocua* and *Escherichia coli* (Nguyen et al. 2012). Also, low frequency ultrasound processing of milk is reported to improve homogenization, pasteurization, reduction in yogurt fermentation time (Wu et al. 2000) and improved rheological properties of yoghurt (Vercel et al. 2002). Similarly, low frequency ultrasound applications help in controlling spoilage of wine and malolactic fermentation (Ojha et al. 2016).

8. GMO for Food Processing

Genetically Modified Foods have been around for years, but how many of us actually know that almost 70% of foods in the grocery shelves are genetically modified (Teisl et al. 2003). There are several rising concerns about the upcoming push of genetically modified foods, due mainly to the emergence of new products from GM companies. The use of genetic modification has become relatively common in today's technologically expanding world. By selecting specific long strands of DNA (genes) of our interest and inserting them into other host cells, it is possible for the new cells to carry useful traits. These new cells that emerge with foreign genes are called transgenic organisms, and are also known as genetically modified organisms (GMOs). Genetically modified microbial enzymes were the first application of GMOs in food production and were approved in 1988 by the US Food and Drug Administration (FDA 1990). In the early 1980s, Gist-brocades began investigating the possibility of producing chymosin from a microorganism using the genetic engineering approach (van Dijck 1999). The chymosin preparation, registered under the brand-name Maxiren®, has been commercially produced since 1988.

With the advancement of recombinant deoxyribonucleic acid (rDNA) technology, the metabolic potentials of microorganisms are being explored and harnessed in a variety of new ways. Today, genetically modified microorganisms (GMMs) have found tremendous applications in food industries (Arvanitoyannis and Krystallis 2005). Genetic engineering and different technologies in molecular biology are utilized to manipulate the microorganisms for the expression of desired traits. GMMs will be used to produce enzymes with optimized properties regarding activity, specificity or stability (Roller and Goodenough 1999). Technologies are (1) gene transfer methods to deliver the selected genes into desired hosts; (2) cloning vectors; (3) promoters to control the expression of the desired genes; and (4) selectable marker genes to identify recombinant microorganisms (Arvanitoyannis and Krystallis 2005).

8.1 Pros and Cons

Genetic engineering is still a relatively young technology, about 25 years old, and many of the predictions about it, for better or worse, have yet to be verified in practice. There are various pros and cons that have been debated for years like two sides of the same coin.

8.1.1 Pros

There are many pros or merits of using GMO in food application discussed from day to day. With the discovery of DNA, unreveiling the genetic code it contains and its possibility to be transferred from one organism to another and design organisms at will has been a boon for the industrial aspect of food technology. Genetic engineering allows introducing desirable traits to economically useful organisms by gene targeting and gives desired economic and industrial benefits in bulk production of enzymes or other food applications. It has tremendously alleviated the food and nutritional requirement of the world with other functional properties in pharmaceuticals, agriculture and feed. These are supposed to be beneficial to people in countries that do not have an adequate supply of these nutrients. And it is safe for human consumption too as so far there has been no case to prove them unsafe.

8.1.2 Cons

There has not been any long term testing to detect possible problems of the use of GMO in food application with respect to long term disorder as it is a new concept, a mere 25 years. Major cons may be allergic reactions as the genetic modification mixes or adds proteins that are not endemic which may cause new allergic reactions in consumers. Some GMO's have antibiotic features added to them so as to make them resistant to certain contaminations. Hence human consumption of these products may lead to some antibiotic features persisting in human body that may actually make the antibiotic medication less effective in future. The loss of the diversity of gut microflora endemic in humans is a major concern. Also GMO is not natural and scrambling genomes may lead to total chaos in evolution. Genetic engineering exposes people to the increased dangers of horizontal gene transfer, a process whereby genes are passed not 'vertically' down the generations in the usual way but 'horizontally' from organism to organism and from species to species (Arvanotoyannis and Krystalles 2005). GE is potentially dangerous and therefore involves taking risks. The consequences could be devastating and irreversible. Furthermore, the adverse consequences could take years to show and the company liable for any damages may have long since ceased trading.

8.2 Ethics and Legislation

GMO has been a very controversial topic since many years. Researchers have been accused numerous times of manipulating the genetic make-up of organisms. The food prepared by GMO was named as "Franked-food" and researchers were condemned to be unethical and playing god. They were criticized for hampering nature and for loss of biodiversity and natural immunity. Hence strict regulations were imposed for using GMO, such as safety aspects, thresholds, labeling, detection and coexistence. Many traditional and cultural beliefs were ignored for the sake of commercialization of GMO. Hence with respect to ethics, beliefs, customs and traditions there are a number of organizations who govern and monitor the regulations for use of GMO in food processing.

8.2.1 Structure of pertinent legislation

There is no comprehensive federal legislation specifically addressing GMOs. GMOs are regulated under the general statutory authority of environmental, health, and safety laws. The three main agencies involved in regulating GMOs are the US Department of Agriculture's Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA), and the Environmental Protection Agency (EPA). The FDA's primary statutory authority is the Federal Food, Drug, and Cosmetic Act (FFDCA), Under the FFDCA, substances added to food can be classified either as "food additives", which require approval from the FDA that they are safe before they can be marketed (21 U.S.C. §348 2012) and substances added to food classified as "generally recognized as safe" (GRAS), as to which preapproval is not needed (21 U.S.C. §321 2012). The EPA has established regulations specifically for microorganisms that require submission of a Microbial Commercial Activity Notice (MCAN) before they are used for commercial purposes (40 C.F.R. §725.100 2013). The Notice must include information describing the microorganism's characteristics and genetic construction, byproducts of its manufacture, use, and disposal, health and environmental effects data and other information (40 C.F.R. §§725.155, 725.160 2013). All of the various statutory schemes under which GMOs are regulated in the US provide for civil and criminal penalties, and different countries have their own set of rules with respect to the same.

9. Patented Approaches

For improved food safety and enhanced health benefits more attention has been given to standardize the protocols and microorganisms used in fermentation. Fermented foods are patented using novel mechanisms and microorganisms that may enhance nutritional composition and safety of fermented food products. These patented approaches may improve the quality of fermented foods with a promising strategy for the prevention, control and treatment of both infections and chronic diseases. Various cultures are invented for fermented milk products like infant formula, yogurt or ice-cream composition, i.e., LAB culture for *Lactobacillus acidophilus* species that provide multiple uses of the species (Izvekova et al. 2000, 2002). Also from vegetable proteins various inventions have been made and patented (Flambard 2011). Patents involving fermented tea generally focus on the methods involved in the fermentation process that helps to provide a consistent product for mass distribution (Toba et al. 2001, 2005). Also some inventions of fermented tea claim to improve flavor and safety with additional nutritional values (Kwach et al. 2011). Different food crops have been patented and marketed as fermented foods in Soy and Rice (Ghoneum and Maeda 1996). This is comprehensively summarized in Table 1 (Borresen et al. 2012).

Table 1. Patents in foods.

Patent Title	Health Utility	Mechanism/Novelty	Patent ID (Year)
Fermented Milk Products			
Fermented milk Nutraceuticals	Treat diseases or conditions resulting from opportunistic and pathogenic microorganisms	Novel cultures of *Lactobacillus* acidophilus combinations of group Er-2 strain and *L. acidophilus* N.V. Er 317/400	US6,156,320 (2000) and 6, 357,521, B1 (2002)
Fermented milk proteins comprising receptor ligand and uses thereof	Reduce and/or stabilize heart rate in CVD. Treat or relieve benign prostrate hypertrophy	Comprised of LAB strain DSM 14998 and a receptor ligand	EP1796480B1 (2011)
Fermented milk or vegetable protein comprising receptor ligand and uses thereof	Reduce and/or stabilize heart rate in CVD. Treat or relieve benign prostrate hypertrophy	Comprised of LAB strain DSM 14998 and a receptor ligand	US20110195891A1 (2011)
Fermented Tea			
Method of producing fermented milk containing manganese and tea	Prevent diseases caused by active oxygen	Bacteria with catalase activity (*Lactobacillus plantarum*) with manganese-containing natural material	US6228358B1 (2001)
Antioxidant food product, antioxidant preparation, and antioxidation method	Prevent diseases caused by active oxygen	Antioxidation to express superoxides dismutase like activity and catalase activity Preferred that tea or other natural material is added to the food product in the form of powder	US6884415B2 (2005)
Method of preparing fermented to using *Bacillus* sp. strains	Improve flavor and safety	Fermenting tea leaves by treating with stabilized *Bacillus* sp. Strains from Korean traditional fermented foods	US20110250315A1 (2011)

Fermented Soy			
Fermented Soy nutritional supplements including mushrooms components	Wide ranging-malnutrition to mood related disorders to metabolic support	Comprised of a mushroom grown in fermented soy growth medium and curcuminoids	US20110206721A1 (2011)
Methods for inhibiting cancer growth, infection and promoting general health with a fermented soy extract	Promote general health, prevent and/or treat cancer, prevent infections, reduce incidence of infections, treat infections, asthma, and inflammation. Modulate the immune system and treat immune disorders	Fermented soy extracts	US6855350B2 (2005)
Fermented Rice			
Methods and compositions employing red rice fermentations products	Treat or prevent hyperlipidemia and associated disorders and symptoms (CVD, cerebrovascular diseases, diabetics, hypertension, obesity, etc.)	Fermentation of atleast one *Monascus* strain with red rice products to be used as a dietary supplement	US6046022 (2000)
Method for prevention and treatment of Alzheimer's disease	Treat and prevent Alzheimer's disease	Administration of effective amount of *Monascus* fermented product extracted from red mold rice (powder or beverage)	US8097259B2 (2012)

Source: Borresen et al. (2012) with permission

10. Conclusion and Future Scope

Recent scientific advances have revealed the important role of microorganisms particularly of LAB and yeasts in fermented food industries with novelties and added values. Rationalized use of microorganisms in our diet as evident from either ancient and traditional fermented foods or new advanced patented foods opens up new perspectives. Though this remains undoubtedly promising, one should not forget that man has not yet finished characterizing all traditional fermented foods consumed for centuries, with often numerous isolates belonging to species with undefined roles. The use of novel technological interventions in bioengineering, food processing and fermentation, system biology and bioinformatics shall open vast avenues for new generation microorganisms with enhanced functional features. This will not only provide several health benefits but also the technological advances and marketing shall profusely contribute to the development of small and medium sized enterprises on the one hand, and product diversification of large companies which directly or indirectly contributes to the economy as a whole.

Keywords: Genetically modified foods, food grade GMO, patented approaches, lactic acid bacteria, molds, *Bacillus* sp., fermented foods, bacterial genomics, probiotics, prebiotics, synbiotics

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21 U.S.C. §321(s) (excluding substances from definition of "food additive" that are "generally recognized. . . to be safe.").

21U.S.C. §348 (2012) http://uscode.house.gov/view.xhtml?req=granuleid:USCprelimtitle21 section348& num=0&edition=prelim.

40C.F.R. §§725.155,725.160 (2013) http://www.ecfr.gov/cgibin/textidx?SID=7c33b229782bc8 24885546d 25f6cf057&node=40:32.0.1.1.13&rgn=div5#40:32.0.1.1.13.4.1.5.

40C.F.R. §725.100 (2013) http://www.ecfr.gov/cgibin/textidx?SID=7c33b229782bc82488554 6d25f6cf057& node=40:32.0.1.1.13&rgn=div5#40:32.0.1.1.13.4.1.1.

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