FERMENTED MILK PRODUCTS

YOGURT

Yogurt is probably the most popular fermented milk. It is made in a variety of compositions (fat and dry-matter content), either plain or with added substances such as fruits, sugar, and gelling agents. The essential flora of yogurt consists of the thermophiles *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*. For a satisfactory flavor to develop, approximately equal numbers of both species should be present. They have a stimulating effect on each other's growth. Volatile compounds produced by the yogurt bacteria include small amounts of acetic acid, diacetyl, and most importantly, acetaldehyde.

Yogurt Bacteria

The yogurt bacteria, *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*, grow in milk better when present together than each alone (protocooperation). The proteolytic rods enhance growth of the streptococci by forming small peptides and amino acids, the main amino acid being valine. Milk contains too little of these amino acids and the cocci, which are only weakly proteolytic and form the acids too slowly. The cocci enhance the growth of the rods by forming formic acid out of pyruvic acid under anaerobic conditions and by a rapid production of CO_2 (see Section 13.1). The stimulatory effect of formic acid remains unnoticed in intensely heated milk because in this milk formic acid has been formed by decomposition of lactose. The production of formic acid by the cocci is, however, essential in industrial practice, where more moderate heat treatments of yogurt milk are applied, e.g., 5 to 10 min at 85°C. Due to mutual stimulation during combined growth of the yogurt bacteria in milk, lactic acid is produced much faster than would be expected on the basis of the acid production by the individual pure cultures. Some antibiosis also occurs in yogurt in that the cocci cannot grow after a certain acidity has been reached. The rods are less susceptible to acid and continue to grow. Protocooperation and antibiosis are of great importance in the growth of the yogurt bacteria as well as for the quality of yogurt (see also Figure 22.1).

The cocci as well as the rods contribute significantly to the properties of yogurt. The properties of the bacterial strains used should be matched to each other because not every combination of strains is suitable. Furthermore, both species should be present in large numbers in the product, and hence in the

starter. The mass ratio of the two species depends on the properties of the strains and is often approximately 1:1. This ratio between the yogurt bacteria is best maintained if the inoculum percentage is, say, 2.5, the incubation time is 2.5 h at 45°C, and the final acidity is approximately 90 to 100 mM (pH ≈ 4.2). The growth of cocci and rods in yogurt incubated under these conditions is depicted in Figure 22.2. The ratio between the species keeps changing. Initially, the streptococci grow faster due to the formation of growth factors by the rods and probably also due to the latter compounds being added via the inoculum (especially in the manufacture of set yogurt). Afterwards, the cocci are slowed down by the acid produced. Meanwhile, the rods have started to grow faster because of the growth factors (CO_2 and formic acid) formed by the cocci. As a result, the original ratio is regained. The yogurt should then have attained the desired acidity. Continuing incubation or inadequate cooling causes the rods to become preponderant.

Important Parameters that influence fermentation

1. *Incubation time:* A shorter incubation time, which means a lower acidity, will cause too high a proportion of cocci. Transferring a yogurt starter repeatedly after short incubation times during the production of the starter may cause also the rods to disappear from the culture. Conversely, long incubation times will cause an increasing preponderance of the rods.
2. *Inoculum percentage:* Increasing the inoculum percentage will enhance the rate of acid production. The acidity at which the cocci are slowed down will thereby be reached earlier, resulting in an increased number of rods (incubation time being the same). At a smaller inoculum percentage, the ratio between the bacteria will shift in favor of the cocci.
3. *Incubation temperature:* The rods have a higher optimum temperature than the cocci. Incubation at a slightly higher temperature than 45°C will shift the ratio in favor of the rods; incubation at a lower temperature will enhance the cocci.

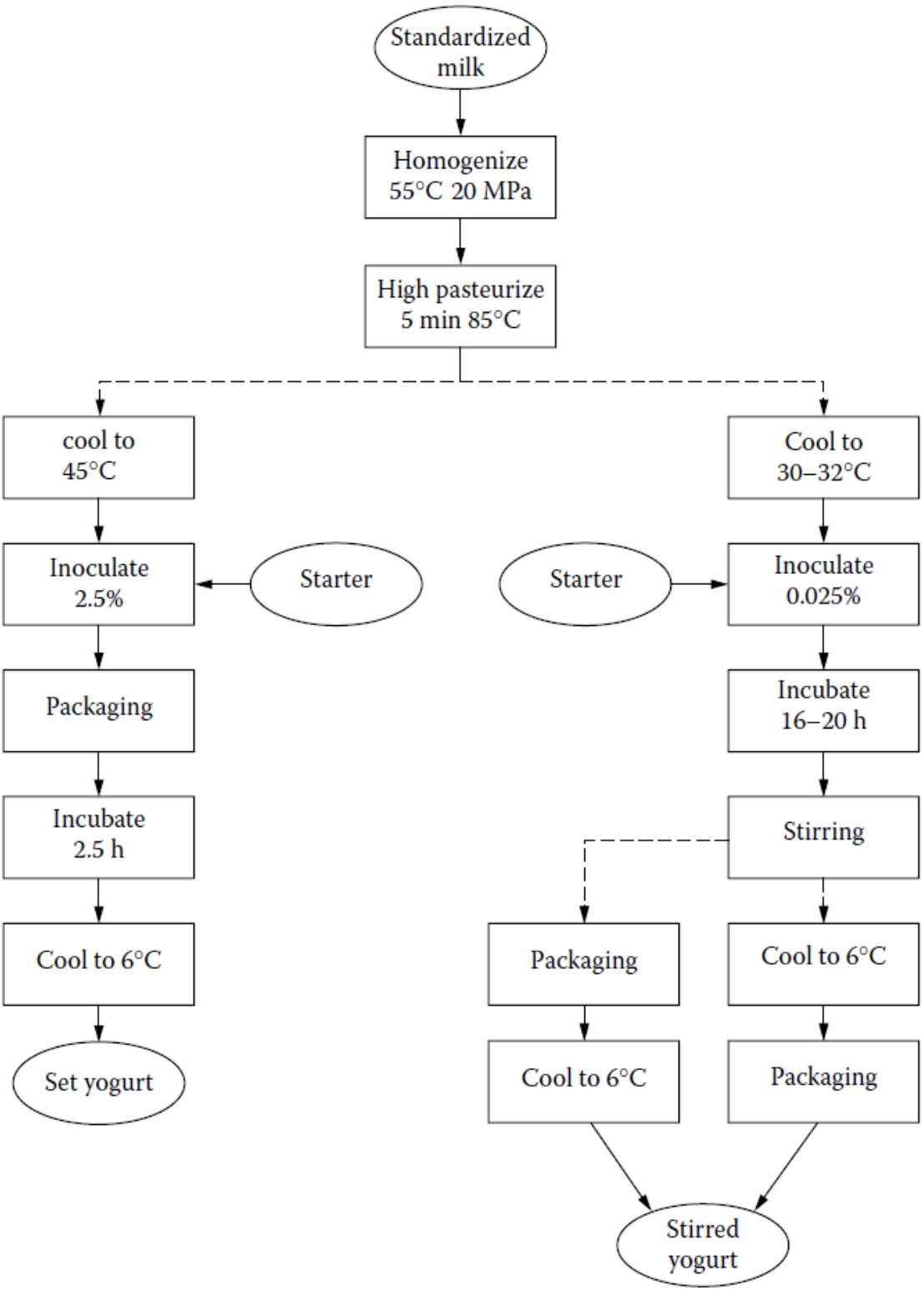
Metabolites produced during yogurt fermentation

S. thermophilus and *L. delbrueckii* ssp. *bulgaricus* form products that contribu to the flavor of yogurt as well as to its structure and consistency. The followi are the main compounds involved:

1. *Lactic acid:* Both bacteria form lactic acid from glucose. Galactose, formed during the decomposition of lactose, is not converted. Hence, the molar concentration of galactose increases just as much as the lactose content decreases (see item 4). Most of the glucose is decomposed in a homofermentative way. *S. thermophilus* forms L(+) and *L. delbrueckii* ssp. *bulgaricus,* D(–) lactic acid. The isomers are produced in almost equal quantities. (Subsection 22.5.2 mentions physiological aspects with respect to the consumption of lactic acid.) CO_2, acetic acid, and ethanol are also produced, though in small amounts. The acetic acid content of yogurt is 30 to 50 $mg \cdot kg^{-1}$ (0.5 to 0.8 mM) and the ethanol content, 10 to 40 $mg \cdot kg^{-1}$ (0.2 to 0.7 mM). Ethanol has a relatively high flavor threshold and it probably does not contribute to the flavor of yogurt. The lactic acid content of yogurt is 0.7% to 0.9% w/w (80 to 100 mM).
2. *Acetaldehyde* (ethanal): This component is essential for the characteristic yogurt aroma. Most of it is formed by the rods. An important precursor is threonine (see Subsection 13.1.2), which is a natural component of milk, even though at low concentration. In addition, proteolysis by the lactobacilli yields threonine. The content of acetaldehyde of yogurt is about 10 $mg \cdot kg^{-1}$ (0.2 mM).
3. *Diacetyl* (CH_3–CO–CO–CH_3): *S. thermophilus* and, to a lesser degree, *L. delbrueckii* ssp. *bulgaricus* form diacetyl in a way that probably corresponds to the mechanism followed by leuconostocs and by *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis* (Subsection 13.1.2). The yogurt

bacteria do not decompose citric acid. Hence, pyruvic acid, formed during sugar fermentation, is the only precursor of diacetyl. The diacetyl content of yogurt ranges from 0.8 to 1.5 $mg \cdot kg^{-1}$ (0.01 to 0.02 mM).

4. *Polysaccharides:* The yogurt bacteria can form a 'hairy' layer or glycocalix, which predominantly consists of polysaccharide chains, made up of galactose and other glucides. They can be partially secreted into the liquid and are then called *exopolysaccharides* (see Subsection 13.1.2.8). The polysaccharides play an important role in yogurt consistency, especially of stirred yogurt (see following text). Although various strains show quite a variation in the amount of polysaccharide produced, this variation does not correlate with the consistency obtained. Presumably, the type of polysaccharide produced is of greater importance.



Flow sheet for the manufacturing of set yogurt and stirred yogurt