A 100-Year Review: Yogurt and other cultured dairy products¹

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ABSTRACT

The history of the last 100 years of the science and technology of yogurt, sour cream, cultured butter, cultured buttermilk, kefir, and acidophilus milk has been one of continuous development and improvement. Yogurt leads the cultured dairy product category in terms of volume of production in the United States and recent research activity. Legal definitions of yogurt, sour cream and acidified sour cream, and cultured milk, including cultured buttermilk, are presented in the United States Code of Federal Regulations and summarized here. A tremendous amount of research has been done on traditional and novel ingredients, starter cultures and probiotics, mix processing, packaging, chemical aspects, physical and sensory properties, microstructure, specialized products, composition, quality and safety of yogurt and various manufacturing methods, addition of flavorings, viscosity measurements, and probiotic use for sour cream. Over time, there have arisen alternative manufacturing methods, flavor problems, addition of flavorings, and use of probiotics for cultured buttermilk. Many health benefits are provided by yogurt and other cultured dairy products. One hundred years of testing and development have led to wider uses of cultured dairy products and new processing methods for enhanced shelf life and safety. Future research directions will likely include investigating the effects of probiotic dairy products on gut microbiota and overall health.

Key words: cultured dairy products, yogurt, food safety, starter culture and probiotic, 100-year review

INTRODUCTION

Various cultured dairy products are formed by the conversion of lactose in milk to lactic acid by the use of starter cultures. Starter cultures have been used in the sense of inoculating fresh milk with small quantities of

sour milk long before anything about bacteriology was known. Consumption of various fermented milk products by people in different regions of the world living under primitive sanitary conditions, especially in hot climates, was desirable because their high acidity kept these products safe by destroying pathogenic organisms (Nicholls and Nimalasuriya, 1939).

A critically important early advancement in bacteriology was the first observation of bacteria by Antonie van Leeuwenhoek in about 1675 (Orla-Jensen, 1921). Swithinbank and Newman (1903) reported that Andry, as early as 1701, noticed that living organisms are present in sour milk.

Lactic acid was first studied in 1780 by Carl Wilhelm Scheele (Van Slyke and Hart, 1904). Louis Pasteur declared that lactic acid could be formed from sugar fermentation by yeast (probably actually lactic acid bacteria) in 1857, as described and referenced by Manchester (2007). Whether credit should be given to Louis Pasteur or to Pierre Jacques Antoine Béchamp (for his work on inversion of sucrose in 1855) for the first demonstration of fermentation by living organisms was a subject of controversy between these 2 scientists (Manchester, 2007). Joseph Lister, known for his work in showing the critical importance of antiseptic technique during surgery, also studied milk souring by lactic fermentation and believed that milk sugar is converted into lactic acid (Lister, 1878). The older view was that casein was the ferment.

During the 1800s, it was debated whether true fermentation of organic liquids was caused by organisms that they contain. Lister, like Pasteur, believed that organisms caused fermentation but acknowledged that some distinguished physiologists and pathologists at that time believed that fermentation such as putrefaction was not necessarily caused by bacteria (Lister, 1878). Lister (1873) first isolated the organism that he called *Bacterium lactis*, later renamed *Streptococcus lactis* (Orla-Jensen, 1919), and now referred to as *Lactococcus lactis* ssp. *lactis* (Schleifer et al., 1985); this organism converts lactose into lactic acid. Lister was not sure if this was the only bacterium that could undergo lactic acid fermentation.

A history during the 1800s and early 1900s of the use of butter starters for souring that can occur under

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the right conditions was provided by Knudsen (1931). The introduction of the thermometer for use in cream ripening by Segelcke in 1860 and improved recordkeeping for use of definite quantities of starter for definite quantities of cream improved the control of cream souring (Knudsen, 1931). Many other advancements in cultures, ingredients, processing, and packaging were developed over time to improve cultured dairy products. Today, cultured dairy products are fermented with lactic acid bacteria, including species of *Lactobacillus*, *Streptococcus*, *Lactococcus*, and *Leuconostoc* (Appendix Table A1). Yeasts are also used in manufacturing kefir and koumiss.

Some of the more common and well-known cultured dairy products include yogurt, sour cream and dips, cultured buttermilk, acidophilus milk, and kefir. A list of many other lesser-known cultured dairy foods available in specific countries or regions can be found in Chandan (2006) and at https://en.wikipedia.org/wiki/Fermented_milk_products. Consumers generally have a positive perception of yogurt. As a result, annual per capita yogurt consumption increased from 0.1 kg in 1970 to 1.2 kg in 1977 in the United States (Tamime and Deeth, 1980), and production of yogurt has increased from 982.6 million pounds in 1990 to about 4,742.1 million pounds in 2015 (International Dairy Foods Association, 2017) in the United States. Sour cream production has increased from 935.4 million pounds in 2003 to about 1,316.5 million pounds in 2015, and buttermilk sales have decreased from 1,140 million pounds in 1960 to about 513 million pounds in 2015 in the United States (International Dairy Foods Association, 2017). Because of the recent economic importance of yogurt as well as the large number of published articles, the emphasis of this review paper will be on yogurt (Appendix Table A1), with less coverage on other cultured dairy products, including sour cream, cultured butter, cultured buttermilk, kefir, acidophilus milk, and the probiotic dairy beverage Yakult (Yakult Honsha Co., Tokyo, Japan).

YOGURT

History and Legal Description of Yogurt

For an early method of yogurt manufacturing, Heine-man (1921) described the manufacture of yogurt as boiling milk in clean earthenware vessels over a slow fire to reduce the volume of milk from about one-fourth to more than one-half (so that the final volume is between less than 50% to 75% of the original volume), cooling to 45 to 50°C, adding a small amount of product from a previous lot, wrapping the vessels containing this mixture in skins and cloths to maintain a uniform tem-

perature for 10 to 12 h before being ready for consumption. For modern methods of yogurt manufacturing, yogurt mix with an appropriate fat content (typically <0.5, 1, or 3.25%) and total solids content (typically 12 to 15% and adjusted by adding nonfat dry milk) is homogenized, pasteurized [typically at 90 to 95°C for close to a minute to a few minutes (HTST) or at about 85°C for 30 min (vat)], and cooled to 40 to 45°C before pumping into a vat (often cylindrical-shaped on the top and cone-shaped on the bottom). The mix is then inoculated with *Streptococcus salivarius* ssp. *thermophilus* (traditionally and hereafter referred to as *Streptococcus thermophilus*) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (traditionally and hereafter referred to as *Lactobacillus bulgaricus*). The inoculated mix may then be immediately pumped to a yogurt filler for filling into cups, with or without fruit puree already present in the bottom of the cup, and the cups are then sealed. The filled and sealed cups are then transferred to a warm room for incubation. When the yogurt reaches the appropriate pH (typically about 4.5), the yogurt cups are transferred to refrigerated storage (cup or sundae-style yogurt). Instead of immediate pumping to a yogurt filler, the inoculated mix may be allowed to incubate within the vat until the pH reaches the appropriate value, typically about 4.5. The inoculated mix is then stirred and cooled to about 20°C before pumping to a fruit feeder for adding fruit (for fruit-flavored yogurt) and then to a yogurt filler for filling into cups and then cup sealing. The cups are then transferred to refrigerated storage. More details of these procedures are provided by Kosikowski and Mistry (1997).

Yogurt has evolved over time. The industrial production of yogurt incorporating microorganisms was started by Isaac Carasso in 1919 (http://corporate.danone.co.uk/en/discover/our-businesses/fresh-dairy-products/brand-detail/?tx_bidanonesitemarques_pi1%5Buid%5D=324&cHash=0c2732c382f800736405ab84df99142f). During the 1920s and 1930s, yogurt milk was described as having poor flavor because of its high acidity (Winkler, 1929; Nordsiek, 1938). Before the 1960s, yogurt was only found in a few grocery or health food stores (Kroger, 1975). Today, many forms of yogurt can be found including plain yogurt, fruit flavored yogurt (including fruit-on-the-bottom and blended forms), whipped yogurt, granola-topped yogurt, drinkable yogurt, frozen yogurt, and Greek yogurt with varying fat contents (regular, low fat, and nonfat). The order for the US sales of spoonable yogurt flavors includes strawberry, blueberry, vanilla, peach, plain, raspberry, honey, banana and strawberry, black cherry, berry, pineapple, cherry, key lime, lemon, and banana (Cassell, 2014). Earlier in the decade, sales of Greek yogurt grew rapidly. Future trends for yogurt include sugar reduction

(Chollet et al., 2013) and predicted rapid sales growth for drinkable yogurt, as reported by Conick (2016), Shoup (2016), and Kennedy (2016); yogurt with savory flavors (Kennedy, 2016); and whole milk yogurt (Kennedy, 2016).

A legal description of yogurt, lowfat yogurt, and nonfat yogurt is given in the US Code of Federal Regulations in 21CFR131.200 (CFR, 2017a), 21CFR131.203 (CFR, 2017b), and 21CFR131.206 (CFR, 2017c), respectively. Yogurt, lowfat yogurt, and nonfat yogurt contain at least 3.25% milkfat, between 0.5% and 2% milkfat, and less than 0.5% milkfat, respectively, and each of these products contain at least 8.25% milk solids-not-fat before addition of bulky flavors. Optional dairy ingredients, including cream, milk, partially skim milk, or skim milk, used alone or in combination, are cultured with *Lactobacillus bulgaricus* and *Streptococcus thermophilus* and result in a titratable acidity of at least 0.9% expressed as lactic acid. Pasteurization or ultra-pasteurization must be performed before adding the cultures. These yogurt products may contain certain other dairy-based ingredients, nutritive carbohydrate sweeteners, flavoring ingredients, color additives, stabilizers, and vitamins A and D. Also, these yogurt products may be heat treated to kill viable microorganisms after culturing to extend product shelf life.

Ingredients in Yogurt

Some of the added dairy ingredients that increase the milk solids-not-fat content of yogurt mixes have included skim or whole milk powders, milk protein concentrates, condensed milk, buttermilk powder, whey powder, whey protein concentrates, microparticulated whey protein, and caseinates (sodium, calcium, or sodium-calcium). Schulz (1949) stated that addition of condensed skim milk to milk for making yogurt improves yogurt consistency. Addition of milk powder to milk has been used for many years to produce a thicker and firmer yogurt (Pette and Lolkema, 1951). Todoric and Savadinovic (1973) stated that up to 0.3% dried whey can replace dried skim milk in manufacturing yogurt. Horton et al. (1972) mentioned that liquid cottage cheese whey protein concentrates could be used in yogurt production. Modler et al. (1983) added caseinate, milk protein concentrate, skim milk powder, and whey protein concentrates (prepared by ultrafiltration, ion exchange, or electrodialysis/lactose crystallization) to yogurt at concentrations ranging from 0.5 to 1.5% and found that gel firmness tended to be higher and syneresis (defined as liquid whey phase on the top resulting from gel shrinkage) lower with use of the casein-based additives and with the highest incorporation rates. Bong and Moraru (2014) developed a procedure

for manufacturing Greek-style yogurt in which micellar casein concentrate was used to increase the protein content of the milk for making yogurt. Trachoo and Mistry (1998) added ultrafiltered sweet buttermilk and sweet buttermilk powder to fortify skim milk in the manufacture of nonfat and low fat yogurts. Saffon et al. (2013) reported that replacing powdered skim milk with heat-denatured aggregates of mixtures of buttermilk concentrate and whey protein concentrate in set-type yogurt modified the textural properties of the resulting yogurt.

Yogurt may or may not contain fat. The fat content of yogurt is normally adjusted by varying the amount of added cream, milk, partially skim milk, or skim milk. However, unsalted butter can also be used to increase the yogurt fat content (Chandan et al., 1969). A butterfat-free yogurt in which an unsaturated fat or oil such as corn oil, cottonseed oil, coconut oil, soybean oil, or other similar oils in amounts of about 1.5 to 6.4% are added has been patented (Metzger, 1962b). Historical trends of varying yogurt fat contents are discussed in the various types of yogurt section.

Today, most yogurts, other than plain yogurt, are sweetened, usually with sugar. Other sweeteners such as honey can be used, but Popa and Ustunol (2011) found that the flavor of yogurt sweetened with sucrose was preferred by panelists over yogurts sweetened with either high-fructose corn syrup or honey. Various high-intensity sweeteners can be incorporated into yogurt. The sweeteners fructose, xylitol, sorbitol, cyclamate, and a saccharin and xylitol mixture (use of xylitol in this mixture to cover a disturbing bitter aftertaste from saccharin) could be successfully used in yogurt when added after incubation without markedly deteriorating quality (Hyvönen and Slotte, 1983). A method of making yogurt containing pectin and aspartame was patented by Malone and Miles (1984). Keating and White (1990) reported that yogurts sweetened with either aspartame or sorbitol were usually preferred over yogurts sweetened with other alternative sweeteners (calcium saccharin, sodium saccharin, fructose, sucrose plus monoammonium glycyrrhizinate, fructose plus monoammonium glycyrrhizinate, acesulfame-K, and dihydrochalcone) but not as desirable as the sucrose-sweetened yogurt control. When matching the sweetness equivalence of strawberry yogurt containing 11.5% (wt/wt) sucrose with addition of high-intensity sweeteners to yogurt, Reis et al. (2011) found that more aspartame than sucralose was required. Narayanan et al. (2014) found that levels of 0.7 to 5.5% (wt/wt) of various commercial stevia sweeteners were appropriate for sweetening naturally flavored vanilla low-fat yogurt.

Stabilizers are often added to yogurt. Hall (1975) has provided recommendations for usage of stabilizer

depending upon preferences in the type of yogurt to be produced. Common stabilizers for use in yogurt and yogurt drinks include modified starch, gelatin, agar, pectin, locust bean gum, xanthan gum, carrageenan, and carboxymethyl cellulose, used either alone or in combination, depending upon the type of stabilizer (Chandan and O'Rell, 2006).

Fruit is commonly added to yogurt and has been since 1933 (as described at http://ekonomika.idnes.cz/prvni-ovocny-jogurt-se-narodil-u-vltavy-fdi-/test.aspx?c=A020723_103620_test_jan). Davis (1973) stated that the incorporation of real fruit into yogurt led to soaring yogurt sales in Britain. Some of the more common types of fruit added to yogurt include strawberry, blueberry, peach, raspberry, cherry, orange, lemon, and pineapple (Saal and Krebs, 1975); many other fruit and fruit flavors that have been used are listed in Robinson and Tamime (1975).

Many nutritional and functional ingredients have been added to yogurt. Because yogurts are not typically vitamin fortified, studies have reported the addition of vitamins (either with or without other added ingredients) including vitamin C (Maxa, 1949; patented by Metzger, 1962a), vitamin B₁₂ (by addition of *Propionibacterium shermanii* or 2% yeast extract; Karlin, 1961), vitamins A and C (Ilic and Ashoor, 1988), folic acid (Aryana, 2003), vitamin D₃ (Kazmi et al., 2007), and some heart-healthy nutrients (thiamine, riboflavin, niacin, folic acid, manganese, magnesium, and fiber; Cueva and Aryana, 2008). Other examples of adding beneficial ingredients to yogurt during manufacturing include calcium (Tchilinguirian, 1960), nanopowdered eggshells (Mijan et al., 2014), iron (Hekmat and McMahon, 1997), 7 different types of minerals (iron, magnesium, zinc, manganese, molybdenum, chromium, and selenium; Achanta et al., 2007), vegetables (cucumber salad, tomato salad, beet salad, and garden salad; Anonymous, 1976), nuts (walnuts, hazelnuts, almonds, and pistachios; Ozturkoglu-Budak et al., 2016), spice oleoresins (cardamom, nutmeg, and cinnamon; Illupapalayam et al., 2014), fish oil (Estrada et al., 2011; reviewed by Olson and Aryana, 2017), fiber (patented by Reddy, 1989, and Hoyda et al., 1990), oat fiber (Fernández-García et al., 1998), inulin (Robinson, 1995), resistant starch (Anonymous, 1997), lutein (Aryana et al., 2006), plant sterols (Volpe et al., 2001), and green tea and green coffee powders (Dönmez et al., 2017).

Yogurt Cultures and Added Probiotics

Although current standard procedures and regulations call for the addition of both *Streptococcus thermophilus* and *Lactobacillus bulgaricus* as the starter

culture to be used in the production of yogurt (or possibly yogurt-like products) because of their symbiotic relationship (as will be discussed in more detail below), other cultures or combinations have been suggested over time. Rosell (1933) described 3 types of bacteria in preparing yogurt milk: “thermo- or plocamo-bacterium yoghourtii (Jensen), the *Bacterium bulgaricum* (Grigoroff) and the *Streptococcus lacticus thermophilus* (Jensen, Weigman, and Henneberg).” Davis (1973) used the phrase “a culture of *L. bulgaricus* and other lactic acid bacteria in milk or concentrated milk” to describe traditional yogurt. Adjunct cultures and probiotics can also be added to yogurt.

Orla-Jensen (1919) described *Streptococcus thermophilus*. It is the most frequently occurring *Streptococcus* in milk pasteurized at low temperatures. The most rapid growth occurs at 40 to 45°C, and the best growth is in milk. *Streptococcus thermophilus* forms longer chains at 45°C than at 30°C. Thick capsules, or even slime (probably exopolysaccharides and discussed in more detail below), can be formed by several strains. It does not markedly dissolve casein and it slightly ferments maltose and mannose but not salicin (Orla-Jensen, 1919). Radke-Mitchell and Sandine (1986) found that temperature for optimal acid production by *S. thermophilus* ranged from about 2 to 8°C higher than the temperature for its optimal growth. *Streptococcus thermophilus* was later reclassified as *Streptococcus salivarius* ssp. *thermophilus* (Farrow and Collins, 1984).

Grigoroff described *L. bulgaricus* in 1905 as documented and referenced by Kulp and Rettger (1924). Orla-Jensen (1919) called the bacteria isolated from Bulgarian yogurt *Thermobacterium bulgaricum*. *Lactobacillus bulgaricus* was later reclassified as *Lactobacillus delbrueckii* ssp. *bulgaricus* (Weiss et al., 1983).

Complete genomic sequences of various strains of *S. thermophilus* and *L. bulgaricus* have been published. Bolotin et al. (2004) reported the genome sequences of *S. thermophilus* CNRZ1066 and LMG13811 isolated from yogurt and found that these strains contain a single circular chromosome of about 1,800,000 bp with 39% G+C content for about 1,900 coding sequences. As of August 2015, the genomes of 17 *S. thermophilus* strains are publicly available (Vendramin et al., 2017). van de Guchte et al. (2006) reported the genome sequence of the *L. bulgaricus* ATCC11842 that Orla-Jensen originally isolated from Bulgarian yogurt in 1919. This strain contains 1,864,998 bp with an overall G+C content of 49.7%. A draft genome sequence of *L. bulgaricus* LBB. B5, a strain that originates from homemade Bulgarian yogurt and forms a strong symbiotic relationship with *S. thermophilus*, was published by Urshev et al. (2016).

Genetic modification of *S. thermophilus* has been performed. Somkuti and Steinberg (1988) genetically

transformed intact cells of *S. thermophilus* with plasma DNA by electric field pulses. Blomqvist et al. (2010) demonstrated site-directed mutagenesis on *S. thermophilus* LMG 18311 that could allow construction of starter strains that possess novel or improved properties.

Various culture media have been developed or improved over time to isolate and enumerate streptococci and lactobacilli. Heinemann and Hefferan (1909) found that *Bacillus bulgaricus* (*L. bulgaricus*) does not grow well on ordinary laboratory media but grows most favorably on milk or a medium containing whey, peptone, and glucose. To overcome the difficulties in preparing the whey and digest media, Kulp (1927) prepared an agar by adding peptone, tomato juice, and water, adjusting the pH to 7.0, and adding agar and found optimum colony development of various strains of *L. bulgaricus* after incubation in an atmosphere containing 10% CO₂ for 72 h. Davis (1935) found that yeast dextrose litmus milk was the best medium for supporting growth of bacteria important for dairying, including strains of *Thermobacteria bulgaricus* (*L. bulgaricus*). McLaughlin (1946) devised trypticase sugar agar to cultivate lactobacilli. Briggs (1953) developed a medium containing tomato juice, Tween 80 (polyoxyethylene sorbitan mono-oleate), and other ingredients that supported growth of a collection of lactobacilli including strains of *L. bulgaricus*. An agar developed by Elliker et al. (1956) supports growth of fastidious strains of streptococci and lactobacilli. De Man, Rogosa, and Sharpe (MRS) agar, a commonly used, general nonselective growth medium for lactobacilli, was described by de Man et al. (1960). Terzaghi and Sandine (1975) developed M17 medium and found that both *S. thermophilus* and *L. bulgaricus* grew well in M17, especially with adjustment of pH to 6.8 before inoculation. Dave and Shah (1996) recommended *Streptococcus thermophilus* agar for selectively enumerating *S. thermophilus* and either MRS agar adjusted to pH 5.2 or reinforced clostridial agar adjusted to pH 5.3 for selectively enumerating *L. bulgaricus*. Tharmaraj and Shah (2003) described *S. thermophilus* colonies as round and yellowish and 0.1 to 0.5 mm in diameter when plated on *Streptococcus thermophilus* agar, and *L. bulgaricus* colonies as white, cottony, rough, and irregular and 1.0 mm in diameter when plated on MRS agar adjusted to pH 4.58.

Molecular-based techniques have been developed to identify *S. thermophilus* and *L. bulgaricus*. Lick and Teuber (1992) synthesized a species-specific DNA-oligonucleotide probe allowing rapid identification of *S. thermophilus* using DNA hybridization. Afterward, Lick et al. (1996) developed a primer-specific PCR technique based on the *lacZ* gene sequence for rapid and accurate identification of *S. thermophilus*. Both *L. bulgaricus* and

S. thermophilus can be identified quickly and accurately with PCR using methionine biosynthesis and 16S rRNA genes (Cebeci and Gürakan, 2008).

Traditional production of fermented products used a mother culture. A mother culture is a culture of small volume that is transferred to a larger container or vat of milk for preparing a larger volume of starter for ripening milk or cream in the manufacture of fermented milk products. Starter cultures can be obtained as frozen pellets or in the freeze-dried form. Rogers (1914) was able to prepare *B. bulgaricus* (*L. bulgaricus*) cultures with high activity by a freezing-vacuum method. Concentrated cultures frozen in liquid nitrogen have been commercially developed for use for manufacturing various types of dairy products including yogurt (Christensen, 1969). The history of the development of dairy cultures is summarized by Wigley (1977) and Jespersen (1977).

A symbiotic relationship between *S. thermophilus* and *L. bulgaricus* has been observed. This symbiotic relationship within yogurt and other cultured dairy products was reported by Orla-Jensen (1921). Pette and Lolkema (1950a) described the relationship between *L. bulgaricus* and *S. thermophilus* as symbiosis and then as antibiosis. *Lactobacillus bulgaricus* stimulated *S. thermophilus* growth when *S. thermophilus* was in the logarithmic phase of growth but later, *L. bulgaricus* inhibited *S. thermophilus* because of the large amount of lactic acid produced by *L. bulgaricus*. However, Jankov and Stoyanov (1966) reported that certain strains of *L. bulgaricus* were clearly antagonistic with *S. thermophilus*, and therefore not suitable for yogurt production. They isolated only 6 strains of *L. bulgaricus* out of 442 isolations of lactic acid bacteria from raw milk, and only 2 of these strains were suitable for making yogurt. Also, they reported that the most suitable *L. bulgaricus* to *S. thermophilus* ratios to result in desirable yogurt were between 2:1 and 1:5 (Jankov and Stoyanov, 1966). A symbiotic relationship between *L. bulgaricus* and *S. thermophilus* was also shown by the higher production of acetaldehyde in milk by a 1:1 mixture of these microorganisms than by either of the microorganisms alone (Hamdan et al., 1971). Although Radke-Mitchell and Sandine (1986) found optimum growth temperatures of 35 to 42°C for *S. thermophilus* and 43 to 46°C for *L. bulgaricus*, the growth of mixed cultures of *L. bulgaricus* and *S. thermophilus* in milk was not affected by these optimum growth temperatures of the strains grown individually but appeared to be dependent on the concentration or type of stimulatory factors produced by *L. bulgaricus*. When growing equal volumes of *S. thermophilus* and *L. bulgaricus* together in milk at 37, 42, and 45°C, Radke-Mitchell and Sandine (1986) found that *S. thermophilus* usually obtained higher cell

counts than *L. bulgaricus* with rod to coccus ratios ranging from 1:2.2 to 1:8.

Bacteriophages (viruses that infect bacteria and then replicate within them, leading to bacterial destruction by lysis) have been reported for yogurt cultures. Pette and Kooy (1952) isolated bacteriophages active against *S. thermophilus* in slow-souring yogurt. They also reported that different strains varied in their susceptibility to these bacteriophages. In addition, these phage-infected cultures regained normal acid production, leading to products with normal flavor upon continued cultivation, and becoming insensitive to subsequent bacteriophage attack (Pette and Kooy, 1952). Deane et al. (1953) observed the microstructure of a bacteriophage that attacks *S. thermophilus* that was isolated from Swiss cheese whey and found head sizes of 90 to 95 μm in diameter and tail lengths ranging from 225 to 275 μm . Both Deane et al. (1953) and Stolk (1955) measured the heat resistance of bacteriophage active against *S. thermophilus* and the latter study found that heat treatments of 80°C or 85°C for 10 s were necessary to destroy these bacteriophages from yogurt. Mocquot and Hurel (1970) reported that bacteriophage active against *L. bulgaricus* is rare, but Pette did find one, as reported by Humphreys and Plunkett (1969). Peake and Stanley (1978) obtained bacteriophage plaques for a bacteriophage of *L. bulgaricus* isolated from commercial yogurt and showed, by electron microscopy, that this bacteriophage had an approximately 60-nm hexagonal head and an approximately 210-nm tail with a variable number of cross-bar structures.

Starter cultures produce exopolysaccharides, and exopolysaccharides enhance the body of yogurt and provide health benefits to consumers. In 1973, Groux, as reported by Zourari et al. (1992), found that the slime secreted by a strain of *L. bulgaricus* consists primarily of galactose but also contains some glucose, mannose, and arabinose. *Lactobacillus bulgaricus* has been shown to produce exopolysaccharides that contain galactose, glucose, and rhamnose in an approximately 4:1:1 molar ratio and with a molecular weight of approximately 500,000 (Cerning et al., 1986), and *S. thermophilus* has been shown to produce exopolysaccharides consisting primarily of galactose and glucose with smaller amounts of xylose, arabinose, rhamnose, and mannose (Cerning et al., 1988). The structures and properties of exopolysaccharides produced by various strains of *S. thermophilus* have recently been described by Pachekrepapol et al. (2017). Galesloot and Hassing (1968) found that mixing a high slime-producing *L. bulgaricus* culture with a commonly used *S. thermophilus* culture during yogurt making led to a stirred yogurt with increased viscosity. Skim milk gels produced by slime-producing strains had decreased susceptibility

to syneresis and higher viscosities than skim milk gels produced by non-ropy strains (Schellhaass and Morris, 1985). Exopolysaccharides provide potential health benefits including prebiotic effects, immunostimulatory activity, anti-tumoral activity, and reduced blood cholesterol levels, as described by O'Connor et al. (2005).

The biochemistry, including production of volatile compounds by these starter cultures, has been studied extensively. Pette and Lolkema (1950b) found that acetaldehyde production was linked to the true yogurt aroma. Renz and Puhon (1975) concluded that bitterness in yogurt largely resulted from proteolysis by *L. bulgaricus* during storage. Dan et al. (2017) used solid-phase microextraction and gas chromatography-mass spectrometry methods to identify 53, 43, and 32 volatile compounds produced by *S. thermophilus* alone, *L. bulgaricus* alone, and both species together in fermented milk and confirmed the presence of some important flavor compounds including acetic acid, acetaldehyde, acetoin, 2,3-butanedione, ethanol, and 1-heptanol.

Other cultures can be added to yogurt to improve the flavor. Rašić and Milanović (1966) found that the flavor of yogurt could be improved with the addition of a *Streptococcus diacetylactis* culture. Lawrence and Perry (1962) reported that adding a flavor-forming strain of a psychrotrophic *Leuconostoc* could improve the flavor of some fermented milk products, including yogurt.

Addition of unique strains of starter cultures results in novel, value-added products. Anbukkarasi et al. (2014) used galactose-positive strains to produce low-galactose yogurt. Consumption of food products high in galactose is harmful to individuals with galactosemia. Linares et al. (2016) used *S. thermophilus* APC151 to produce yogurt that contains γ -aminobutyric acid (GABA) and that has comparable properties of a reference yogurt. γ -Aminobutyric acid promotes health by its anti-stress, anti-hypertensive, and anti-diabetic properties.

Probiotics are beneficial organisms. Lilly and Stillwell (1965) used the term "probiotics" to describe the production of growth-promoting factors produced by one species of protozoa that assists the growth of another species of protozoa. More recently, probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). The health benefits of a probiotic can be shown by well-designed double-blind, randomized, placebo-controlled trials. Health benefits provided by various probiotic microorganisms have been summarized by Fijan (2014).

Yogurt is often a carrier of probiotics (Lourens-Hattingh and Viljoen, 2001). *Lactobacillus acidophilus* (described by Moro in 1900, as documented and referenced by Kulp and Rettger, 1924) with or without *Bifidobacteria* spp. (described by Tissier in 1900, as

documented and referenced by Kulp and Rettger, 1924) are commonly added to yogurt. *Lactobacillus casei* DN-114001 (*L. casei* strain CNCM I-1518 or *L. casei* Immunitas) has been patented (Cayuela et al., 2007) and is used in the probiotic yogurt-type drink DanActive (Actimel; Groupe Danone, Paris, France; <https://www.actimel.co.uk/the-story-so-far>) that became commercially available in 1994. *Bifidobacterium animalis* DN-173 010 is a probiotic used in the yogurt brand Activia (Groupe Danone) that was launched in France in 1987 (<https://www.activia.ca/en-us/explore-science/history-activia>). *Lactobacillus rhamnosus* GG (ATCC 53103; also known as *Lactobacillus* GG) is a probiotic patented as *L. acidophilus* GG and later reclassified as *L. rhamnosus* GG (Gorbach and Goldin, 1989). Hekmat et al. (2009) reported that *L. rhamnosus* GR-1 and, to a lesser extent, *Lactobacillus reuteri* RC-14 were able to survive in yogurt.

Milk and Mix Processing and Yogurt Packaging

Membrane filtration has been applied to making yogurt. Ultrafiltration of whole milk by a volume reduction of 2 was successfully used to manufacture a high-quality yogurt containing 21% total solids without the use of nonfat dry milk or homogenization (Chapman et al., 1974). Davies et al. (1977) successfully manufactured yogurt from milk concentrated to 12.5 to 15% total solids by reverse osmosis. Jepsen (1979) noted that a French factory used reverse osmosis to concentrate skim milk to 13% total solids at 5°C for the manufacture of yogurt.

Various heat treatments of yogurt milk or mixes reduce the manufacturing time and improve some physical properties of the resulting yogurt. Pette and Lolkema (1951) stated that the yogurt curd is improved by milk pasteurization, which is preferably performed at 85°C for 5 min because overheating of the milk can weaken the curd. By holding milk for 30 min at 85°C instead of cooling it immediately before souring the milk, Grigorov (1966a) reported that the time for coagulation decreased from 2.43 to 2.01 h, with the pH of the sour milk at the time of coagulation increasing from 4.70 to 5.16. Grigorov (1966b) recommended a pasteurization temperature of 85°C with a holding time of 20 to 30 min to minimize syneresis in the resulting yogurt, and reported a deterioration of product quality at pasteurization temperatures of 90 and 95°C with similar holding times. Yogurt made by vat heating (85°C for 10 to 40 min) of the mix was firmer and more viscous than yogurt made by either HTST (98°C for 0.5 to 1.87 min) or UHT (140°C for 2 to 8 s) heating of the mix (Parnell-Clunies et al., 1986). Heating yogurt mixes at 100°C, 110°C, or 120°C for 4 or 16 s led to yogurts characterized with a

firmer gel and increased viscosity compared with yogurt manufactured by heat treatments of the mix at 140°C for 4 or 16 s (Savello and Dargan, 1995). Also, Savello and Dargan (1997) found less syneresis in yogurt manufactured from UF-concentrated skim milk heat treated between 100°C and 130°C than in yogurt manufactured from UF-concentrated skim milk that was heat treated at 140°C or vat heated at 82°C for 20 min.

Various pressure treatments (homogenization, microfluidization, and high hydrostatic pressures) have been performed on yogurt mixes and on the finished product. Pette and Lolkema (1951) reported increased curd firmness and prevention of creaming and whey separation in yogurt that was made from pasteurized milk that was homogenized. Schmidt and Bledsoe (1995) found that a yogurt containing 1.5% fat can be manufactured to have similar physical and sensory properties, except for syneresis and water-holding capacity, to a full-fat (3.5% fat) yogurt by using homogenization pressures of either 10.344 or 34.48 MPa during manufacture. Ciron et al. (2012) used low-fat milk microfluidized at 50 to 150 MPa to manufacture low-fat yogurt that had creaminess and textural properties resembling full-fat yogurt manufactured with conventional valve homogenization. Harte et al. (2003) manufactured low-fat set yogurt from fortified skim milk subjected to high hydrostatic pressure processing between 300 to 676 MPa for 5 min and thermal processing at 85°C for 30 min and found that the yogurt manufactured using the 400 to 500 MPa pressure treatments of the fortified skim milk had increased yield stress, increased normal penetration resistance, and increased elastic modulus but less syneresis compared with yogurt manufactured without the high hydrostatic pressure processing. These improved properties of yogurt will allow yogurt to be manufactured with less stabilizers and thickeners (Harte et al., 2003). de Ancos et al. (2000) subjected stirred low-fat yogurts to high hydrostatic pressures (100 to 400 MPa) for 15 min at 20°C and prevented post-acidification of yogurt during refrigerated storage when using pressures above 200 MPa, increased yogurt viscosity after refrigerated storage, reduced counts of viable lactobacilli when using pressures of 300 and 400 MPa, and improved the general acceptability, flavor, and texture using pressures of 200 and 300 MPa compared with the control yogurt.

Many projects dealing with inoculation have described factors affecting counts of *L. acidophilus* during incubation and storage, as low survival of probiotics during storage has been observed. Shah et al. (1995) found *L. acidophilus* counts of 10^7 to 10^8 viable cells/g of yogurt in 3 out of 5 brands and less than 10^5 cells/g in the remaining 2 brands when fresh. Gilliland and Speck (1977) reported low survival of *L. acidophilus*

incorporated into yogurt after incubation, and this low survival was explained by production of hydrogen peroxide by *L. bulgaricus*. Hull et al. (1984) found a decrease of *L. acidophilus* counts by approximately half after 3 wk of storage when adding *L. acidophilus* culture with the starter culture, but *L. acidophilus* survival was less than 1% after 4 d of 5°C storage when added after incubation. Olson and Aryana (2008) found that *L. acidophilus* counts in yogurt could be increased by increasing its inoculation level to a point, but excessively high inoculation of *L. acidophilus* levels lowered counts during storage and adversely affected yogurt quality, including decreased apparent viscosity and sensory scores but increased syneresis compared with yogurts manufactured with lower *L. acidophilus* inoculation levels.

Yogurt packaging has changed over time. Yogurt was originally packaged in glass containers (Crawford, 1962). Due to the expense involved with returning glass jars, a nonreturnable container in the form of a paper cup was needed (Sanderson, 1957). However, filling paper cups with warm milk to be incubated into yogurt led to a reaction on the coating of these cups. Therefore, the yogurt manufacturing procedure was modified based on changing the culture symbiosis to allow it to be performed at a lower temperature (Sanderson, 1957). A pliable plastic container with a push-on lid also became available (Crawford, 1962). Although most yogurt is currently packaged in plastic containers (polypropylene, polystyrene, or high-density polyethylene), some specialized yogurt products are packaged in flexible tubes or glass jars. Yogurt can be packaged in single-serve containers of varying size or in multi-pack containers.

Protein Denaturation and Acid Coagulation

Heating milk to a sufficiently high temperature denatures whey proteins, and the denatured whey proteins interact with casein micelles. Rowland (1934) determined the amount of denaturation of albumin and globulin upon heating milk as a function of temperature ranging from 63 to 80°C with variable heating times and reported that 83.4% of the total albumin and globulin became denatured after 30 min at 80°C. More recently, Dannenberg and Kessler (1988a) made a graph containing curves of equal degrees of β -lactoglobulin B denaturation in milk at various heating time and temperature combinations. Using this graph, an increasing degree of β -lactoglobulin denaturation led to a relatively uniform decrease in susceptibility of syneresis, regardless of the heating temperature, but the volume of drained whey did not continue to decrease with a longer holding time than is needed to obtain a

calculated 99% denaturation at heating temperatures of 85, 90, and 95°C (Dannenberg and Kessler, 1988b). The gel firmness of set-style nonfat yogurt increased with increasing degree of β -lactoglobulin denaturation up to 60%, and to a lesser degree up to 90%, but not between 90 and 99% denaturation (Dannenberg and Kessler, 1988c). However, a slight reduction in firmness occurred with increasing holding times beyond the times needed to obtain 99% denaturation.

Various mechanisms for acid coagulation of milk have been proposed over time. Acid coagulation of the casein micelles occurs because of the lactic acid that is formed during incubation that lowers the pH. Scheele in 1780 and Millon and Commaille in 1865 believed that lactic acid reacting with casein forms an insoluble compound that precipitates while Rochleder in 1843 and Hammarsten in 1877 believed that no definite chemical compound was formed upon acid precipitation of milk casein (Van Slyke and Hart, 1904). Hammarsten in 1874 reported that calcium phosphate is removed from the milk casein upon treatment with acid leading to casein precipitation (Van Slyke and Hart, 1904). Van Slyke and Hart (1904) concluded that casein monolactate (13 to 14% of the casein) and casein dilactate (86 to 87% of the casein) were the compounds that formed upon first visual coagulation of casein with lactic acid. Van Slyke and Hart (1904) expected that milk coagulation would occur upon reaching 0.8 to 0.9% total acidity, whereas Jenness and Patton (1959) stated that casein precipitates upon reaching pH 5.2 to 5.3. The isoelectric point of casein, meaning the pH at which there is no net electrical charge on the casein micelles, is 4.6. A newer model of acid milk gel formation involves more than just aggregation of the original casein micelles (Heertje et al., 1985). Micellar disaggregation with release of β -casein and solubilization of colloidal calcium phosphate occurs followed by β -casein reabsorption to form new particles that differ in structure and composition compared with the original casein micelles. A detailed description of acid coagulation of milk is provided by Lucey and Singh (2003).

Finished Product Characteristics

Titrateable acidity is an important characteristic for yogurt. Crawford (1962) found that a titrateable acidity of 0.74 to 0.83% expressed as lactic acid when placing glass-bottled yogurt into cold storage and a titrateable acidity of 0.91 to 0.93% during cold storage resulted in the most desirable yogurt. An insipid, unattractive yogurt resulted when yogurt with a lower titrateable acidity is cooled, whereas a slightly over-acid yogurt resulted when cooled at higher titrateable acidities. Galesloot (1958) recommended a titrateable acidity of 95° to 100°N

(equivalent to 0.85 to 0.90% lactic acid; Crawford, 1962) when ready for consumption. Rosell (1933) reported that yogurt milk acidities can occasionally reach 3.5% and pH can drop to nearly 3 in 24 to 36 h.

Surveys have been conducted to determine chemical composition and microbiological quality of yogurt. O'Neil et al. (1979) analyzed 7 brands of plain yogurt and found total solids contents of 13.3 to 16.5%, fat contents of 0.9 to 3.7%, protein contents of 4.4 to 5.3%, titratable acidities of 1.20 to 1.76%, and pH values of 3.72 to 4.14, whereas Kroger and Weaver (1973) analyzed 3 plain and 41 fruit yogurts from central Pennsylvania and found total solids contents of 15.10 to 30.73%, fat contents of 0.82 to 2.04%, protein contents of 3.09 to 5.38%, and pH values of 3.80 to 4.35. Arnott et al. (1974) analyzed 15 plain and 137 fruit or fruit-flavored yogurts manufactured in Ontario and found 15.1% containing the desired 1:1 ratio of *Lactobacillus* to *Streptococcus*, 27.6% containing staphylococci, 13.8% containing coliforms, 26.3% containing yeast counts >1,000/g, 17.8% containing mold counts >10/g, and 11.8% containing psychrotrophic counts >1,000/g.

Sensory properties and acceptance of yogurt, including drinkable yogurt and Greek yogurt, have been described. Nelson and Trout (1964) claimed that yogurt should have a fine and smooth texture and a firm body so it can hold its shape when it is spooned. The flavor should be clean, distinct, and acidic. Duthie et al. (1977) proposed a scorecard for evaluating the flavor, body and texture, and color of yogurt. Ryan et al. (1984) reported that a red raspberry-flavored yogurt drink and a strawberry-flavored yogurt drink were accepted more often than not by a large-scale consumer taste survey, and Thompson et al. (2007) found only slight differences in preferences in strawberry drinkable yogurts by consumers of different ethnic groups. Harper et al. (1991) found that samples of plain yogurt were judged as too sour and not sweet enough by a consumer panel. Coggins et al. (2008) developed a sensory lexicon for effectively characterizing and differentiating plain yogurt and found that taste and texture characteristics were more important than aroma and appearance in differentiating yogurt treatments. They also reported that fat content of yogurt and whether the milk source was conventional or organic could not be differentiated by descriptive sensory attributes. Hekmat and Reid (2006) reported that the sensory properties of a yogurt containing *Lactobacillus rhamnosus* and *Lactobacillus reuteri* were comparable to those of a conventional yogurt. Desai et al. (2013) found that Greek yogurt manufactured both by traditional straining and centrifugation and by addition of dried dairy ingredients were well liked in blinded acceptance testing. A texture that was firm and dense and flavors described as mod-

erate sweet aromatic, milkfat, and dairy sour were the preferred attributes of Greek yogurt as determined by external preference mapping (Desai et al., 2013).

Gel strength and viscosity are important yogurt parameters. Firmness of yogurt depends upon the concentration of solids in milk (Pette and Lolkema, 1951). Haque et al. (2001) found that gel strength of set-style yogurt measured by compression testing and viscosity and G' (solid-like character) of stirred samples increase with increasing fermentation temperature during manufacture of yogurt. Walstra et al. (2006) stated that the firmness of set-style yogurt increases when pH is lowered. Yogurt is a non-Newtonian fluid because it displays decreased apparent viscosity upon increased shear rate.

Syneresis, defined as formation of a top liquid phase (whey) resulting from shrinkage of a gel, is a common problem in yogurt. Yogurt syneresis can be measured by centrifugation and drainage methods (Harwalkar and Kalab, 1983). The latter authors found that susceptibility to syneresis decreased with increasing total solids content in yogurt and with use of preheated milk instead of unheated milk in making yogurt, but only insignificant effects for susceptibility to syneresis were observed with yogurt pH within the 3.85 to 4.5 range. Lee and Lucey (2004) found that casein particle rearrangement within the gel network and colloidal calcium phosphate solubilization rate affect whey separation and gel strength. They recommended manufacturing yogurt using intermediate to high inoculation rates (2%) and then incubating at low temperatures (40°C) to decrease whey separation and textural defects in the resulting yogurt.

The microstructure of yogurt has been observed by scanning and transmission electron microscopy and by confocal microscopy. Kalab et al. (1975) used scanning and transmission electron microscopy to observe differences in microstructure of unsupplemented yogurt and yogurt containing gelatin, carrageenan, and pregelatinized waxy maize starch. Yogurt was shown to be formed by fusion of casein micelles, forming long chains and, rarely, clusters. Although gelatin addition did not alter the yogurt microstructure, addition of carrageenan led to a fibrillar microstructure without free terminations, and addition of pregelatinized waxy maize starch led to formation of short fibers with frequent free terminations and sheets. Hassan et al. (1995) used confocal scanning laser microscopy to observe gel formation as a continuous process without sample preparation procedures. By observing casein micelle aggregation and loss of bacterial cell mobility, they were able to determine that coagulum formation starts at pH 5.35. Contraction of the casein network was noted with a further decrease in pH.

Various Types of Yogurt

Perceptions about desired fat content of yogurt have changed over time. Davis (1956) stated that yogurt at that time did not have a legal definition in English food law. It was assumed that yogurt was made from whole milk unless it was clearly labeled that it was made with reduced fat or skim milk. However, some reports suggested that the amount of total fat in the diet affects the serum cholesterol level (Hildreth et al., 1951). Therefore, Davis (1956) suggested that it would be better for some people to consume skim milk yogurt instead of whole milk yogurt. Over time, most yogurts became either nonfat or low fat. Low-fat diets increased in popularity in the 1980s and 1990s, and much effort went into producing foods with lower fat contents and improving the flavor and body and texture of foods with lower fat contents so that they more closely resemble their full-fat counterparts. Many low-fat or calorie yogurts were produced and patented about this time, including a low-calorie, low-fat fruit-containing yogurt (Baker, 1983). More recently, it was shown in a large multiethnic cohort study that a higher intake of saturated milk fat was associated with a lower risk of cardiovascular disease (de Oliveira Otto et al., 2012). Even though recent sales of whole milk yogurt are relatively low compared with sales of nonfat and low-fat yogurt, sales of whole milk yogurt are growing rapidly.

Many specialized types of yogurt have been described, including carbonated yogurt, concentrated yogurt, instant yogurt, lactose-free yogurt, aseptic (sterile) yogurt, and directly acidified yogurt. Choi and Kosikowski (1985) developed sweetened plain and strawberry-flavored carbonated yogurt beverages without free whey and found that the latter beverages were well accepted by a consumer panel. A concentrated yogurt obtained by whey drainage was described by Robinson (1977). Greek yogurt (a concentrated yogurt) is now very popular. Ferguson (1963) patented an instant yogurt with half the fat content of whole milk; such a yogurt has desirable sensory properties and a long shelf life, and can be formed upon reconstitution with ordinary temperature tap water. Engel (1973) described sweetening yogurt with the use of lactase. A sterile yogurt that will not degrade within 6 mo of unrefrigerated storage was patented by Egli and Egli (1980). Yogurt prepared by direct acidification of milk containing a thickening blend has been patented by Igoe (1979).

Keeping Quality and Safety of Yogurt

Microbiological factors can affect quality and shelf life of yogurt. Gassem and Frank (1991) pointed out

the importance of using milk with low microbial contamination levels and storing milk at low temperatures. Salih et al. (1990) reported that manufacturers indicate an average of 40 d as shelf life for yogurt. Schulz (1949) stated that yogurt at that time often suffered from high "Coli aerogenes" counts. Hervet et al. (2017) concluded that the hygienic status of yogurt can be appropriately monitored by *Enterobacteriaceae* testing.

Yogurt and yogurt-like products may contain yeast. Ram Ayyar (1928) observed yeast cells in dahi. Although yeasts are considered contaminants in yogurt, Souliades (1955) isolated non-lactose-fermenting *Torulopsis* yeasts from yogurt and found that they assisted the survival of *S. thermophilus* and *L. bulgaricus*, possibly through the removal of some lactic acid by the yeasts. The growth of yeast was found to limit the microbiological shelf life of unflavored and strawberry yogurt during storage at 8°C or 20°C, but lower yeast counts in strawberry yogurt compared with unflavored yogurt were likely related to the presence of potassium sorbate in strawberry puree in the study of Tirloni et al. (2015). Microgard (Wesman Foods Inc., Portland, OR) has been used to inhibit yeast and, later, gram-negative psychrotrophs that appear after extended storage in strawberry-flavored yogurt (Salih et al., 1990).

Although yogurt starter cultures and added probiotics resist pathogens (as will be discussed below in the health benefits section), food poisoning outbreaks involving yogurt have been reported. Verotoxin-producing *Escherichia coli* O157:H7 in yogurt, most likely due to contamination after pasteurization, led to an outbreak with 16 cases of illness, including 5 cases of hemolytic uremic syndrome after consumption of locally produced flavored yogurt made from pasteurized whole milk in England in 1991 (Morgan et al., 1993). In one case of foodborne botulism involving yogurt, 27 patients in the UK in June 1989 became affected, with 1 death, after consumption of hazelnut yogurt, in which the hazelnut conserve was inadequately processed for destruction of *Clostridium botulinum* spores, leading to the production of *Clostridium botulinum* type B toxin (O'Mahony et al., 1990). In another case of foodborne botulism involving yogurt, 10 patients in Turkey in September 2005 developed botulism from type A botulinum toxin in süzme (condensed) yogurt buried under soil (Akdeniz et al., 2007). An outbreak caused by *Salmonella typhimurium* DT 170 occurred by eating kebabs, doner kebabs, and kebabs with yogurt-based relish, and it was found that raw minced lamb tested positive for *Salmonella* and that blood-stained, unsealed yogurt pots were stored under a rack of raw lamb (Evans et al., 1999). A food poisoning outbreak at a military hospital in Strasbourg, France, in 1964 was caused by consumption of a raspberry-flavored yogurt that contained 1

$\times 10^8$ staphylococci per gram of yogurt (Mocquot and Hurel, 1970). One of the risk factors involved in the epidemic of 149 cases of diphtheria in Yemen Arab Republic between August 1981 and January 1982 was the consumption of factory-made yogurt (Jones et al., 1985). One case of *Geotrichum* sp. in yogurt has been reported, resulting from mishandling of food in Canada in 1975 (Todd, 1978). A food-borne fungal pathogen, *Mucor circinelloides* f. *circinelloides*, caused more than 200 consumers to become ill with nausea, vomiting, and diarrhea after consumption of Greek yogurt in the United States in 2013 (Lee et al., 2014).

A pathogen of major concern to the dairy industry is *Listeria monocytogenes*. This organism appeared to be observed as early as 1891 (Gray and Killinger, 1966). *Listeria monocytogenes*, as it is known today, was first described in detail by Murray et al. (1926). Gray and Killinger (1966) believed that more emphasis should be placed on the seriousness of *L. monocytogenes*. The outbreak of listeriosis from pasteurized milk in Massachusetts (Fleming et al., 1985) and from Mexican-style cheeses (Linnan et al., 1988) increased the awareness of *L. monocytogenes* and listeriosis within the dairy industry. As a result, the number of research papers involving *L. monocytogenes* in dairy products, including yogurt and other cultured dairy products, has increased substantially since the 1980s.

Various techniques have been used to detect *L. monocytogenes* in yogurt. Siragusa and Johnson (1988) compared various agars for recovering *L. monocytogenes* that was inoculated into yogurt and proposed use of *Listeria* selective isolation agar and modified acriflavin ceftazidime esculin agar. They also found that use of an enrichment procedure lead to recovery of *L. monocytogenes* on various agars that were not found by direct plating. Cox et al. (1998) developed a PCR-based fluorogenic 5'-nuclease assay for the rapid and sensitive detection of *L. monocytogenes* in various types of dairy products including yogurt and buttermilk.

Surveys of presence of *Listeria* in yogurt have been conducted. A survey of various milk and milk products in Ethiopia revealed that 2% of yogurt samples were contaminated with *L. monocytogenes* and 2% were contaminated with *Listeria innocua* (a nonpathogenic species; Muhammed et al., 2013). Another survey found 10.71% of yogurt samples in Sri Lanka to be contaminated with *L. monocytogenes* (Wijendra et al., 2014). However, *Listeria* spp. were not found in the studies of Kerr et al. (1992), Ubeyratne et al. (2014), or Abdimoghadam et al. (2015).

Challenge studies in which *Listeria* have been added to yogurt to determine their survival have been performed. In an early study, Ikononov and Todorov (1964) found no *L. monocytogenes* serotypes 1 and 4b

at 24 to 40 h when storing ewe milk yogurt at 18 to 22°C but found a survival period of more than 6 d at a storage temperature of 10°C. Choi et al. (1988) found that *L. monocytogenes* will survive 13 to 27 d in vanilla-flavored yogurt after inoculation of 10^4 to 10^5 cells, whereas Tirloni et al. (2015) reported that *L. monocytogenes* with a starting concentration of either 2 or 5 log cfu/g was still detected in 25-g samples of unflavored and strawberry yogurt at 68 d of storage. Szczawiński et al. (2016) developed linear and polynomial models to describe the inactivation rate of *L. monocytogenes* in yogurt as a function of temperature and found D-values (decimal reduction time) ranging from 87.0 h at a storage temperature of 15°C to 243.9 h at a storage temperature of 6°C when calculated from linear models.

Acidity affects the survival of *L. monocytogenes* in yogurt. Siragusa and Johnson (1988) found that *L. monocytogenes* in yogurt with a pH of 4.1 could survive up to 9 d when using enrichment steps for their recovery. Hill et al. (1995) inoculated an acid-tolerant mutant strain of *L. monocytogenes* and its parental strain into yogurt with a pH of 4.3 and stored at 4°C and found that the mutant strain had a higher survival rate than its parental strain due to acid adaptation of the mutant strain.

High-pressure processing of ayran, a popular traditional yogurt drink in Turkey, applied at 600 MPa for 300 s at ambient temperature decreased *L. monocytogenes* and *L. innocua* counts by greater than 5 log units without significantly changing the pH, water activity, color, or serum protein separation of ayran (Evrendilek and Balasubramaniam, 2011). The same extent of inactivation can be accomplished with a pressure reduction of 100 to 300 MPa or with a time reduction of 210 s with the addition of mint essential oil.

Salmonella is another pathogen of major concern to the dairy industry. Emanuiloff (1941) reported the viability of *Salmonella* in yogurt. Ghoniem (1971) showed that *Salmonella typhimurium* survived for 68 d during freezing (−1°C), 23 d at 4°C storage, and 19 d at 30 to 32°C storage, whereas *S. typhi* survived for only 30 d during freezing, 16 d at 4°C storage, and 11 d at room temperature storage when cans of buffalo milk yogurt were inoculated with 5 mL of *Salmonella*. Álvarez-Ordóñez et al. (2013) showed that counts of both acid-adapted and non-acid-adapted cells of *Salmonella enterica* serovar *typhimurium* only decreased between 1.3 and 1.9 log units when stored at 4, 10, or 25°C for 43 d but decreased about 4 log units at 37°C for 43 d.

Surveys and investigations for presence of other pathogens in yogurt have been conducted. *Yersinia enterocolitica* was found in homemade yogurt (made from pasteurized milk) that was involved in an outbreak in

Pennsylvania in 2011 (Centers for Disease Control and Prevention, 2011). Omara et al. (2015) analyzed yogurt samples in Egypt for the presence of *Campylobacter jejuni* and found 4.4% to be positive by isolation and biochemical identification and 1.2% to be positive by PCR detection of the *MapA* gene. Amer et al. (2010) found that enterohemorrhagic *Escherichia coli* and *Yersinia enterocolitica* were no longer present during storage for 10 and 4 d, respectively. *Bacillus cereus* was isolated from 1 of 30 yogurt samples (San et al., 2012).

Challenge studies in which various pathogens have been inoculated into yogurt to determine the extent of survival have been performed. Dineen et al. (1998) found *E. coli* O157:H7 in yogurt for up to 12 d after inoculation of 10^3 cfu/mL of this pathogen into yogurt. Hudson et al. (1997) reported that post-processing contamination of yogurt could lead to survival of enterohemorrhagic *E. coli* O157:H7, even in highly acidic environments at refrigeration temperatures. They reported survival between 5 and 17 d after inoculating a bioluminescent strain of *E. coli* O157:H7 into commercial yogurt. Tirloni et al. (2015) reported that *E. coli* was still detected in 25-g samples of unflavored yogurt at 26 d of storage with a starting concentration of 2 log cfu/g and up to 47 d with a starting concentration of 5 log cfu/g. Aytaç and Özbas (1994) found that *Yersinia enterocolitica* survived at least 26 d when inoculated into milk to be made into yogurt but *Aeromonas hydrophila* did not survive 5 d after inoculation. *Enterobacter sakazakii* grew during the early stages of fermentation in the manufacture of plain yogurt, but their population significantly decreased during the cooling and storage steps, due to the low pH (Shaker et al., 2008).

Pathogens leading to tuberculosis and Crohn's disease have been studied in yogurt. Tuberculosis originating from yogurt is a concern in areas where unpasteurized milk is used. DeGrolier (1939) isolated virulent bovine tubercle bacilli (now *Mycobacterium tuberculosis*) from 1 out of 8 yogurt samples. Van Brandt et al. (2011) reported that *Mycobacterium avium* ssp. *paratuberculosis* (the likely causative agent in Crohn's disease) could survive in yogurt for 6 wk. Nasr et al. (2014) inoculated *Mycobacterium bovis* (the causative agent for bovine tuberculosis) at 10^3 cfu/mL and 10^5 cfu/mL into yogurt and found that it could survive for 7 d.

OTHER CULTURED DAIRY PRODUCTS

Sour Cream and Acidified Sour Cream

Sour cream and acidified sour cream are legally described in 21CFR131.160 (CFR, 2017d) and 21CFR131.162 (CFR, 2017e), respectively. Sour cream is soured by lactic acid bacteria and acidified sour

cream is prepared with safe and suitable acidifiers, with or without use of lactic acid bacteria, and the titratable acidity of both of these products is at least 0.5%, expressed as lactic acid. These products contain at least 18% milkfat before addition of nutritive sweeteners and bulky flavors, but at least 14.4% milkfat after addition of these ingredients. Optional ingredients include ingredients that improve texture, prevent syneresis, or prolong shelf life as well as rennet, nutritive sweeteners, salt, and flavoring ingredients either with or without coloring. Additionally, sour cream may contain up to 0.1% sodium citrate added before culturing as a flavor precursor.

An old method of producing sour cream was to hold cream at a suitable temperature to allow native bacteria to produce acid, allowing the cream to sour (Page and Lavalie, 1955). However, this procedure results in the separation of whey from the curd and in off-flavors due to the growth of undesirable bacteria, yeasts, and molds.

Doan and Dahle (1928) studied the effects of various parameters on the body of the resulting sour cream and developed a recommended manufacturing procedure. They found that a thicker body could be obtained by using higher pressures during homogenization and higher temperatures during pasteurization; increasing fat content; adding extra solids, rennet, or gelatin; and ripening after packaging. Therefore, their recommended manufacturing procedure was standardizing cream to at least 18% fat, pasteurizing at a temperature of at least 82°C for at least 10 min, homogenizing at this temperature using a pressure of at least 13.8 MPa, cooling to 21°C, adding a sufficient amount of starter to ripen the product to about 0.6% acidity, packaging with minimal agitation, cooling to 4°C, and aging for 12 to 24 h before marketing.

More recently, Kosikowski and Mistry (1997) described sour cream production by standardizing fresh cream to about 19% fat, adding up to 0.5% stabilizer and about 0.2% citric acid, pasteurizing and homogenizing, cooling to about 22°C, inoculating with a commercial mesophilic lactic acid starter (*Lactococcus lactis* ssp. *cremoris* and *L. lactis* ssp. *lactis*), possibly adding a rennet extract, agitating gently for a few minutes, incubating until the desired pH (about 4.5 to 4.6) and acidity (about 0.70%) are reached, chilling, and packaging. Dual homogenization may be performed to obtain a heavy-bodied sour cream. Alternatively, the incubation may be performed in the consumer package.

Research has been performed on sour cream flavor and flavor-active compounds. Hempenius et al. (1969) determined that a volatile acidity requiring 1.2 mL of 0.1 N NaOH to neutralize and 1 ppm of diacetyl were the flavor thresholds for these compounds in sour

cream. Harper (1968) reported that 0.65 to 0.80% lactic acid was the optimal level to achieve the desired taste of sour cream. Shepard et al. (2013) performed a study to explain consumer acceptance of sour cream by their chemical and sensory properties. They found that sour creams characterized by the flavor-active compounds of diacetyl, acetoin, δ -decalactone, and 2-methyl-3-furanthiol were judged as the most desirable. Also, they reported that the characteristics of moderate to high levels of diacetyl, milk fat, and cooked/milky flavors but low to moderate levels of sour taste and sour aromatics with moderate degrees of denseness and firmness for sour cream would be desirable to most consumers.

Viscosity is an important property for sour cream. Hilker (1947) developed a procedure for relating the resistance of penetration of a plummet to the visual viscosity of sour cream. Little (1967) reported that the ideal viscosity ranges from 140,000 to 250,000 cP. Manufacturing cultured sour cream with a lactic culture that has a slight tendency toward ropiness will lead to a sour cream with a high viscosity and a very smooth texture (Babel, 1967).

Values for shelf life of sour cream have been reported. Page and Lavalie (1955) developed a procedure for producing a protein-fortified sour cream-like product with a firm body that is not too susceptible to syneresis, and the shelf life was prolonged from about 4 d to about 20 d by incorporating a pasteurization and homogenization step after the product reached the proper degree of acidity. More recently, Salih et al. (1990) found that manufacturers reported an average shelf life of 31 d for sour cream.

A probiotic, low-calorie sour cream containing *L. acidophilus*, *Bifidobacterium*, and *S. thermophilus* was developed by Fayed et al. (2006). A fat substitute called Simplese 100 (CP Kelco, Atlanta, GA) was added to mimic milk fat, and dried whey protein concentrate was used as a bulking agent to replace the total solids.

Commercial production of sour cream by direct acidification started in 1962 (Little, 1967). A stabilizer was found to effectively promote a firm body in this type of sour cream. Acidified sour cream did not receive complaints about its flavor.

Cultured Butter and Cultured Buttermilk

Some butter is made from cultured cream. Conn (1889) described the advantages of ripening cream for buttermaking as a better separation of butter from ripened cream than from sweet cream, better keeping quality of butter, and improved butter flavor. Early methods of ripening cream for butter making include spontaneous souring (no addition of special souring methods) or addition of either buttermilk (either fresh

or from the previous day) or sour cream (cream that was ready for churning), but problems with contamination often occurred. Afterward, acid-producing cultures were prepared and shipped to dairymen in liquid form, but the problem of long transport of liquid cultures under unfavorable conditions led to production of cultures in the dry state in the 1890s (Knudsen, 1931). An improved manufacturing procedure for cultured butter from ripened cream was patented by Vilhelm Storch (1893) by performing a 70°C to 80°C heat treatment on the cream or milk, adding a pure culture of certain types of flavor producing bacteria obtained from fresh butter, fully ripened cream, or fresh buttermilk for ripening, and finally churning. Storch (1893) reported that the flavor of cultured butter results more from the action of bacteria than from the feed consumed by the cows. Adding butter culture directly to butter was also discussed by Babel and Hammer (1944).

It was generally thought before 1919 that butter cultures were pure lactic acid streptococci cultures (Hammer and Babel, 1943). In 1919, 3 separate studies (Boekhout and Ott de Vries, 1919; Hammer and Bailey, 1919; Storch, 1919; cited in J. Dairy Sci., 1943, 26:160–166) showed that other cultures were also present, as discussed by Hammer and Babel (1943). Cultures that are now used include *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* biovar *diacetylactis*, and *Leuconostoc mesenteroides* ssp. *cremoris* (Frede, 2003).

Due to the disappearance of citric acid within 2 to 4 d in milk, Templeton and Sommer (1929) added citric acid and sodium citrate to ordinary commercial cultures, leading to increased production of volatile acidity by about 50%, with only a 10% increase in acidity, and these cultures in milk were often preferred organoleptically over untreated milk cultures. Likewise, Templeton and Sommer (1935) reported that use of citric acid and sodium citrate in the cream or in the starter in manufacturing butter leads to a more desirable flavor and aroma.

Much research has been performed on identifying flavor compounds in butter cultures and improving flavor of butter. Michaelian et al. (1933) reported that diacetyl was detected by Schmalfuss in 1928 by the detection of its aroma in a milk culture and was found by van Niel, Kluyver, and Derx in 1929 to be responsible for, or at least the main component of, the aroma of butter. Satisfactory butter cultures were found to contain considerable amounts of acetylmethylcarbinol (also known as acetoin or 3-hydroxy-2-butanone) and diacetyl, whereas butter cultures lacking flavor contained either smaller amounts or none of these compounds (Michaelian et al., 1933). Citric acid can be broken down to acetylmethylcarbinol, which can be oxidized to diacetyl. Because

acetylmethylcarbinol does not directly contribute to the aroma of butter, it would be advantageous to convert acetylmethylcarbinol to diacetyl. The yield of diacetyl from acetylmethylcarbinol can be increased by subjecting the butter cultures to pressure and stirring as explained by Werkman and Hammer (1940). Lindsay et al. (1965b) identified many volatile flavor components (aldehydes, methyl ketones, primary and secondary alcohols, methyl and ethyl esters of the normal aliphatic acids, and sulfur compounds) of a commercial mixed-strain butter culture.

Michaelian et al. (1933) found a large effect of acidity on acetylmethylcarbinol plus diacetyl production by citric acid-fermenting streptococci. This observation supports the finding of very rapid formation of acetylmethylcarbinol plus diacetyl, and the occurrence of butter flavor and aroma, late in the ripening of the butter cultures. This effect of acid also explains the characteristic odor that developed when lactic acid bacteria were added to a milk culture that was observed in 1919 by Bockhout and Ott de Vries, as reported by Michaelian et al. (1933).

The aroma bacteria used in starter cultures for making butter in the Netherlands were originally *Leuconostoc cremoris* (Badings and Galesloot, 1962). Starting around about 1950, *Streptococcus diacetylactis* (now *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*; described by Matuszewski et al., 1936) or *S. diacetylactis* plus *L. cremoris* also began to be used. A flavor defect referred to as "yogurt flavor" started occurring. Badings and Galesloot (1962) found that the use of the *S. diacetylactis* cultures led to this flavor defect. Lindsay et al. (1965a) reported that the green flavor (yogurt-like) occurs when the diacetyl-to-acetaldehyde ratio becomes less than 3:1.

Cultured milk, including cultured buttermilk, is described in 21CFR131.112 (CFR, 2017f). Optional dairy ingredients, including cream, milk, partially skim milk, or skim milk, either alone or in combination, are cultured with characteristic microbial organisms so that a titratable acidity of at least 0.5%, expressed as lactic acid, is obtained. Cultured milk must be pasteurized or ultra-pasteurized before adding culture. Other optional ingredients that may be used include vitamin A and D, certain other dairy ingredients, nutritive carbohydrate sweeteners, flavoring ingredients, color additives that do not simulate color of milkfat or butterfat, stabilizers, butterfat or milkfat, aroma- and flavor-producing microbial cultures, salt, and up to 0.15% citric acid (or an equivalent amount of sodium citrate) as a flavor precursor.

To more effectively use creamery by-products for human consumption, Finkelstein (1917) reported that addition of 25% *Bacillus bulgaricus* (*L. bulgaricus*) cul-

tures to buttermilk derived from pasteurized ripened cream reduces a tendency for wheying off and improves the body and flavor of buttermilk. Although real buttermilk is the by-product of ripened cream after churning, cultured buttermilk is the product resulting from the addition of cultures to milk.

Conventional and alternative procedures for manufacturing cultured buttermilk have been reported. Cultured buttermilk is produced by adding salt and citric acid to milk, heating to 85°C for 30 min or 88°C for 2 min, cooling to 22°C, inoculating with lactic starter culture (*Lactococcus lactis* ssp. *lactis* or *L. lactis* ssp. *lactis* biovar. *diacetylactis*), incubating for about 14 to 16 h, breaking the curd when the titratable acidity reaches 0.80%, cooling to 10°C or lower with gentle agitation, and packaging as described by Kosikowski and Mistry (1997). Buttermilk can also be made by direct acidification by lowering the pH to about 4.8 to 5.1 from the first addition and then to about 4.2 to 4.5 in the second addition (Corbin and Long, 1967). Buttermilk made this way does not whey off after 2 wk of refrigerated storage, and this product can retain its original viscosity, body, flavor, and appearance for up to about 1 mo under refrigeration. Corbin and Long (1967) claimed that otherwise cultured buttermilk has a shelf life of only about 5 d because of the difficulty in controlling the bacterial fermentation within the cultured buttermilk. Roberts et al. (1971) reported that manufacturing cultured buttermilk by preacidifying milk to pH 5.2 using concentrated hydrochloric acid before inoculation and incubation with lactic starter cultures resulted in a more consistent product and this product was preferred by experienced judges compared with the control cultured buttermilk. Flakes or granules of butter can be added to buttermilk to make flake buttermilk by the Vogt method as described by Guthrie (1935).

Flavor, spoilage, and shelf life of cultured buttermilk have been studied. The quality of cultured buttermilk in terms of flavor has often been criticized (Keenan et al., 1968). Wang and Frank (1981) isolated various species of *Pseudomonas*, *Enterobacter*, *Acinetobacter*, *Escherichia*, and *Actinobacillus* and detected yeast and mold in commercial cultured buttermilk. They found that these psychrotrophic bacteria and yeast can reduce diacetyl to varying degrees. Salih et al. (1990) reported that manufacturers indicate an average of 17 d as the shelf life for buttermilk.

Methods to improve cultured buttermilk have been reported. Kosikowski (1969) reported that concentrated purees or fruit juices could be added to cultured buttermilk to form orange-, pineapple-, strawberry-, raspberry-, lemon-, and lime-flavored cultured buttermilks and found that the most acceptable flavor was strawberry. Rodas et al. (2002) prepared probiotic

buttermilk by adding *Lactobacillus reuteri* without significantly affecting composition, proteolysis, or sensory quality compared with the control. The buttermilk maintained greater than 10^6 cfu/mL for 10 d of storage when adding this probiotic strain at 1% before buttermilk fermentation. Antunes et al. (2012) achieved *Bifidobacterium animalis* log counts of 8.5 cfu/mL in strawberry-flavored buttermilk sweetened with sucrose and sucralose.

Acidophilus Milk

Acidophilus milk has been referred to either as milk fermented with *L. acidophilus* or an unfermented milk containing *L. acidophilus*. Cheplin and Rettger (1920b) described *Bacillus acidophilus* (*L. acidophilus*) milk as possessing desirable sensory properties and it was prepared by growing these organisms in milk to produce a soft curd within 24 h. However, Myers (1931) claimed that ordinary cultured acidophilus milk has an unpleasant taste, and lactic acid was not important in transforming the intestinal flora. To utilize acidophilus therapy without the unpleasant taste, Myers (1931) described an unfermented acidophilus milk in which high concentrations of viable *L. acidophilus* were suspended while retaining the taste and physical properties of rich whole milk. *Lactobacillus acidophilus* counts in unfermented acidophilus milk do not decrease as rapidly as in sour acidophilus milk, and the milk may be kept sweet for up to 7 d when stored at 2 to 5°C (Myers, 1931). Acidophilus milk products were reviewed by Gililand (1989).

Kefir

Kefir is a fermented milk product that has been manufactured traditionally for centuries in the Caucasus Mountains (Stepaniak and Fetliński, 2003). Kefir grains, resembling miniature cauliflower florets and consisting of a wide variety of symbiotic yeasts, *Lactobacillus*, *Lactococcus*, and *Leuconostoc*, are added to milk in kefir manufacturing. The detailed manufacturing procedures for traditional and industrially produced kefir are diagrammed in Stepaniak and Fetliński (2003). Although lactic acid is the main fermentative metabolite in kefir, a small amount of alcohol is also produced. Pronounced effervescence from carbon dioxide is present in traditional kefir. Kolakowski and Pawlikowska (2012) successfully incorporated the probiotics *Bifidobacterium lactis* HN019, *L. acidophilus* NCFM, and *L. rhamnosus* HN001 into kefir. Recently, O'Brien et al. (2017) reported that kefir was acceptable to cancer survivors after exercising.

Yakult

Yakult (Yakult Honsha Co.) was launched in 1935 and is a Japanese milk drink fermented by *Lactobacillus casei* Shirota until a titratable acidity of 2% is reached (Tamime et al., 2007). The resulting curd is broken, sweetened, flavored, homogenized at 15 MPa, and diluted with water before being packaged into plastic bottles. The *L. casei* Shirota strain is indigenous in the human intestinal tract (Akuzawa and Surono, 2003).

HEALTH BENEFITS PROVIDED BY YOGURT AND OTHER CULTURED DAIRY PRODUCTS

There are various early claims of the therapeutic value of yogurt and other cultured dairy products. People from the Middle East and Asia have consumed yogurt—and likely have known that yogurt consumption provided health benefits—for thousands of years. The severe diarrhea of King Francis I of France was successfully treated by prescribing yogurt back in the 1500s (Hume, 2011). Jagielski (1871) mentions the therapeutic value of koumiss without describing the scientific background in his patent dealing with the production of an artificial koumiss from milk of various species.

Leeds (1888) did not see health benefits that would be provided by fermented milk products for invalids and infants. He stated that the effect of matzoon (an Armenian fermented milk product similar to yogurt) on treatment of severe gastric disorders and general debility was not strong enough to form a favorable opinion of its effectiveness. Also, he did not believe that matzoon was suitable as an infant food based on its analysis, and he noted that the milk sugar was converted into lactic acid, alcohol, and other fermentation products.

Elie Metchnikoff (1908) thought that many ailments in people were caused by toxins produced by putrefactive bacteria in the intestinal tract. If these putrefactive bacteria could be limited or eliminated in the intestinal tract, he believed that people could live longer. He noted that many Balkan peasants live very long lives and thought that it was due to their high consumption of sour milk containing large numbers of lactobacilli. The acid production by these lactobacilli in the human intestinal tract was thought to discourage putrefactive bacterial growth, leading Metchnikoff to encourage the consumption of sour milk to promote growth of *L. bulgaricus* in the intestinal tract.

Because of Metchnikoff's beliefs, the survival of cultures within the gastrointestinal tract from ingested sour milk became an important research topic. Herter and Kendall (1908) fed milk fermented with *Bacillus bulgaricus* (*L. bulgaricus*) to a rhesus monkey and

found that lactic acid could be detected, especially above the ileo-cecal region, but this microorganism did not establish itself in relatively large numbers. Also, Cheplin and Rettger (1920b) were not able to implant *Bacillus bulgaricus* into the intestines of man. However, they were able to implant *Bacillus acidophilus* (*Lactobacillus acidophilus*) into the intestinal tract of human subjects by daily consumption of 500 mL to 1 L of acidophilus milk culture. Therefore, Cheplin and Rettger (1920a) believed that Metchnikoff might have confused *L. bulgaricus* with *L. acidophilus* because they closely resemble each other. Also, Cheplin and Rettger (1920a) claimed that beneficial effects provided by consumption of yogurt and other sour milk products likely resulted from the milk itself instead of their acid-producing bacteria.

Rotch and Kendall (1911) noted that *Bacillus acidophilus* grew better below the ileo-cecal valve compared with above it, as is the case for *B. bulgaricus*. They suggested the therapeutic use of *B. acidophilus* based on many factors including its ability to inhibit and replace foreign invading bacteria in the large intestines.

However, recent studies have indicated that yogurt bacteria can survive transit in the human gastrointestinal tract because these organisms can be recovered in feces (Mater et al., 2005; Elli et al., 2006). Conway et al. (1987) believed that the adhesion mechanism of *L. bulgaricus*, *L. acidophilus*, and *S. thermophilus* to both human and pig intestinal cells was probably nonspecific. Berrada et al. (1991) emphasized the importance of choosing an acid-resistant strain of *Bifidobacterium* in manufacturing bifidus-fermented milks because they showed that *Bifidobacterium* in one brand of commercial fermented milk survives well in vitro and in vivo, whereas *Bifidobacterium* from another brand did not survive well in these tests.

Although food poisoning outbreaks involving yogurt have been reported, as discussed above, some protection against pathogens and intestinal infections from yogurt consumption is provided by its acidity or by inhibitory substances produced by added cultures. Emanuiloff (1941) reported that the degree of acidity affects the viability of *Salmonella* in yogurt, and Ozek (1942) reported that *Salmonella* (including *S. typhi*), *Shigella*, and *Brucella* spp. could be killed in yogurt because of yogurt acidity. Likewise, Rubin and Vaughan (1979) reported that almost all of the bactericidal activity of yogurt against *Salmonella typhimurium* at 37°C was caused by lactic acid under the conditions of their experiment, but this pathogen was partially protected by the casein fraction of yogurt. However, Todorov (1967) reported that exposure to milk products, including yogurt, changed the morphological, cultural, biochemical, and serological properties of various strains

of *Salmonella*, and this change was caused by inhibitory substances produced by starter cultures. Also, Lucca (1975) concluded that antibacterial activity was due more to substances of an antibiotic nature formed by organisms within the yogurt than due to the yogurt acidity. Attaie et al. (1987) reported that *Staphylococcus aureus* was inhibited in acidophilus yogurt more than in regular yogurt, probably because of bacteriocin production by *L. acidophilus*.

Lactobacillus acidophilus and *L. reuteri* have been shown to inhibit pathogens. White and Hill (1949) found that *L. acidophilus* inhibits the growth of *Aerobacter aerogenes* (*Enterobacter aerogenes*), possibly due to lactic acid production. Shahani et al. (1976) noted strong inhibitory effects of an inhibitory compound from *L. acidophilus* against 8 pathogens, and Shahani et al. (1972) patented the antibiotic acidophilin that is produced by various strains of *L. acidophilus*, especially *L. acidophilus* DDS 1. Recently, Ortiz-Rivera et al. (2017) showed that reuterin produced by *L. reuteri* ATCC 53608 can inactivate pathogenic (*S. aureus*, *Salmonella enterica* ssp. *enterica*, and *L. monocytogenes*), pathogen indicator (*E. coli* DH5α), and spoilage (*Penicillium expansum*) microorganisms.

Studies have shown that yogurt and other cultured dairy products can alleviate diarrhea and shorten colonic transit time. Milk artificially soured by lactic acid organisms (Marriott, 1919) and whole milk acidified by lactic acid (Marriott and Davidson, 1923) have been recommended as a routine infant food to treat gastrointestinal disturbances. Consumption of yogurt containing *Lactobacillus* GG decreased incidents of antibiotic (erythromycin)-associated diarrhea, abdominal distress, stomach pain, and flatulence in healthy volunteers compared with consumption of pasteurized yogurt (Siitonen et al., 1990). Likewise, consumption of a fermented milk product containing *Lactobacillus* GG compared with a pasteurized yogurt was effective in promoting recovery from acute rotavirus diarrhea in young children (Kaila et al., 1992). Marteau et al. (2002) performed a double-blind crossover study for determining effect of ingestion of a milk fermented with *B. animalis* DN-173 010 on colonic transit time in healthy women and found that the total and sigmoid transit times were reduced with *B. animalis* consumption without significantly affecting other transit times or fecal weight, pH, bacterial mass, and bile acids.

Some studies have shown that immunity can be enhanced by consumption of yogurt or other cultured dairy products. Hatakka et al. (2001) found a reduction in the number of respiratory infections and its severity in young children whom consumed a probiotic milk containing *Lactobacillus* GG in a randomized, double blind, placebo controlled study. Although consumption

of milk fermented with yogurt cultures and *L. casei* DN-114001 for 3 wk did not reduce the incidence of winter infections in elderly people, the duration of infections was reduced compared with the control group (Turchet et al., 2003). Parra et al. (2004) concluded that daily consumption of fermented milk containing *L. casei* DN-114001 by healthy middle-aged adults improved their innate immune defense. However, Spanhaak et al. (1998) performed a double-blind placebo-controlled study to determine the effect of consumption of *L. casei* Shirota-fermented milk on the intestinal microflora and immune parameters in healthy subjects and found increases in the *Lactobacillus* and *Bifidobacteria* counts in their fecal microflora; these authors could not show distinct effects on immune responses.

The nutritional value and protein digestibility of yogurt have been reported. Forster (1931) found that contents of vitamins A and D are higher but contents of vitamins B and C are lower in yogurt compared with an equal weight of milk used to prepare it. Hernandez and Park (2014) quantified the contents of 20 minerals in both cow milk yogurt and in goat milk yogurt. Lee et al. (1988) showed that digestibility of protein in fermented yogurt is higher than that of heated milk when fed to rats.

Lactose digestion can be improved by consumption of yogurt and other fermented milk. Bayless and Huang (1969) reported that people in Mashona tribes in Salisbury, Rhodesia (now Harare, Zimbabwe) could tolerate large amounts of milk soured by a traditional ritual even though they are lactose intolerant. Baer (1970) suggested that yogurt consumption might be suitable for people who cannot digest lactose because of the reduced lactose content in yogurt resulting from fermentation. Goodenough and Kleyn (1976) found that lactase present in yogurt might be active in the small intestine of rats upon yogurt consumption. Kim and Gilliland (1983) were able to show improved lactose utilization in individuals who were lactose malabsorbers by their consumption of milk with added *L. acidophilus*. Kolars et al. (1984) reported enhanced absorption of lactose from yogurt compared with lactose from milk as determined by breath hydrogen measurements in lactase-deficient subjects and fewer cases of diarrhea or flatulence from yogurt consumption compared with consumption of similar amounts of lactose from either milk or a water solution. Hertzler and Clancy (2003) showed that kefir consumption improved lactose digestion and tolerance in adults who have lactose maldigestion.

Serum cholesterol can be decreased by yogurt and fermented milk consumption. Mann and Spoerry (1974) found decreases in blood cholesterol levels in young Maasai men (a tribe from Kenya) after excessive intake of milk fermented with a wild culture of *Lactobacillus*

and suggested this decrease was likely caused by a factor in sour milk that affects cholesterol metabolism. Later, Mann (1977) reported that the lower cholesterolemia resulting from consumption of a large amount of yogurt was likely from the inhibition of the regulatory enzyme hydroxymethyl glutaryl CoA reductase (EC 1.1.1.3.4) by hydroxymethyl glutarate. Consumption of yogurt containing the probiotics *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 led to a 4.54% decrease in total serum cholesterol and a 7.45% decrease in low density lipoprotein cholesterol compared with consumption of conventional yogurt in individuals with type 2 diabetes mellitus (Ejtahed et al., 2011).

Many studies have been performed to investigate the effect of yogurt consumption on risk of becoming overweight or obese or developing metabolic syndromes. Mozaffarian et al. (2011) performed a study to relate dietary and lifestyle habits to weight changes for 3 separate cohorts and found a 4-yr multivariable-adjusted loss of 0.82 lb per increased daily serving of yogurt. Sayon-Orea et al. (2017) concluded that cohort studies tended to find an inverse relationship between yogurt consumption and becoming overweight or obese and a decreased risk of developing metabolic syndrome.

Yogurt consumption may reduce the likelihood of tumor formation and certain types of cancer. Reddy et al. (1973) found lower total tumor cell counts and DNA content of cells in yogurt-fed male Swiss mice with Ehrlich ascites tumor transplants compared with their control. In a later study, Reddy et al. (1983) reported that yogurt had its greatest anti-tumor effect in the early stages of tumor growth and that tumor cell progression was not affected by milk or by lactic acid. Also, they centrifuged the yogurt into solids and supernatant and found that the anti-tumor activity was localized in the solids fraction rather than in the supernatant fluid fraction. Ayebo et al. (1981) fractionated yogurt and concluded that components having a molecular weight less than 14,000 and not chemically bound to any larger compounds may be responsible for the antitumor activity of yogurt. Pala et al. (2011) found that increased yogurt consumption was associated with decreased risk of colorectal cancer in a cohort study. Shida and Nomoto (2013) concluded that *L. casei* Shirota protects against bladder cancer.

A few additional health benefits are obtained by consumption of yogurt and other fermented milks. Hilton et al. (1992) found that candidal colonization and infection in women could be decreased by daily consumption of 227 g of yogurt containing *L. acidophilus*. Hata et al. (1996) found that consumption of sour milk fermented with *Lactobacillus helveticus* and *Saccharomyces cerevisiae* lowered blood pressure in elderly hypertensive patients. Yadav et al. (2007) found that consumption

of probiotic dahi containing *L. acidophilus* and *L. casei* had antidiabetic effects in rats. Recently, Rungsri et al. (2017) found that daily consumption for 4 wk of a fermented milk containing *L. rhamnosus* SD-11 was beneficial for oral health by reducing mutans streptococci levels in saliva.

SUMMARY AND FUTURE DIRECTIONS

Manufacturing of yogurt and other cultured dairy foods has changed from crude and elementary procedures to more controlled procedures over time. A few of the advances in our knowledge that made this possible include use of ingredients in addition to milk, use of starter cultures, characterizing and sequencing the starter cultures for their improvement, and improved processing and packaging methods. A better understanding of these products has resulted in the development of a wider range of desirable products and increased opportunities for providing health benefits to consumers.

Because of improvements in the available techniques, one interesting area of future research would deal with the role that yogurt and other cultured dairy products play in the human microbiome. Escherich in 1886, as described and referenced by Kendall (1910), first published application of procedures considered modern at the time, including both aerobic and anaerobic culture methods, in investigating fecal bacteria. Many molecular biological techniques for studying these bacteria as well as the gut microbiota have since been developed. Recently, characterizing and altering the gut microbiota and metabolic alterations using these techniques have become active research topics, especially in terms of providing benefits to our health, including our mental health. Tillisch et al. (2013) showed that the activity of brain regions controlling central processing of emotion and sensation was altered in women who consumed a fermented milk product containing a probiotic for 4 wk. Also, Marin et al. (2017) was able to reduce behavioral abnormalities in mice by feeding them food pellets containing *Lactobacillus reuteri* ATCC 23272. Although the latter study did not specifically involve yogurt or cultured dairy products, research could be extended to incorporating microorganisms into yogurt or other cultured dairy products to see if the human microbiome can be altered in a way as to reduce behavioral abnormalities and depression in people. Many other possible health benefits resulting from altering the human microbiota with dairy products containing prebiotics or probiotics can also be investigated.

There are also many other exciting directions for future research in yogurt and other cultured dairy products.

Although yogurt and other cultured dairy products are relatively safe, additional research on pathogens, especially emerging pathogens, in these products can be expected. Further research on incorporation of additional probiotics and functional ingredients for developing value-added products can be expected. Incorporation of novel ingredients and use of novel processing and packaging technologies, possibly including clear packaging, can lead to improved products as well as a greater variety available to the consumer and with improved sustainability. Continuous improvements in yogurt and other cultured dairy products and the continued development of value-added products made possible by ongoing research as well as increased knowledge of their health benefits and additional health benefits not yet identified will increase consumer acceptability of these products as well as profits for manufacturers.

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APPENDIX

Table A1. Timeline of significant advances in the science and technology of yogurt and other cultured dairy products

Date	Milestone	Reference
1873	Lister describes <i>Bacterium lactis</i> (now <i>Lactococcus lactis</i> ssp. <i>lactis</i>).	Lister, 1873
1900	Tissier describes <i>Bifidobacteria</i> .	Cited in Kulp and Rettger, 1924
1900	Moro describes <i>Lactobacillus acidophilus</i> .	Cited in Kulp and Rettger, 1924
1905	Grigoroff describes <i>Lactobacillus bulgaricus</i> .	Cited in Kulp and Rettger, 1924
1908	"The Prolongation of Life" is published.	Metchnikoff, 1908
1919	Orla-Jensen describes <i>Streptococcus thermophilus</i> .	Orla-Jensen, 1919
1920	Ability to implant <i>L. acidophilus</i> but not <i>L. bulgaricus</i> into intestinal tract of man is discovered.	Cheplin and Rettger, 1920b
1921	Symbiosis in cultures for yogurt and other cultured dairy products is reported.	Orla-Jensen, 1921
1931	Unfermented acidophilus milk is described for acidophilus therapy.	Myers, 1931
1933	First addition of fruit to yogurt.	https://ekonomika.idnes.cz/prvni-ovocny-jogurt-se-narodil-u-vltavy-fdi-/test.aspx?c=A020723_103620_test_jan
1935	A probiotic drink with <i>Lactobacillus casei</i> Shirota is developed.	Described in Tamime et al., 2007
1950	Symbiosis and antibiosis between <i>L. bulgaricus</i> and <i>Streptococcus thermophilus</i> are described.	Pette and Lolkema, 1950a
1950	Acetaldehyde is described as a true yogurt aroma compound in yogurt.	Pette and Lolkema, 1950b
1960	de Man, Rogosa, and Sharpe medium is developed.	de Man et al., 1960
1969	Discovery that lactose-intolerant people could tolerate fermented milk.	Bayless and Huang, 1969
1973	Discovery of inhibition of cancer.	Reddy et al., 1973
1974	Discovery of decrease in blood cholesterol levels with consumption of fermented milk.	Mann and Spoerry, 1974
1975	M17 medium is developed.	Terzaghi and Sandine, 1975
1975	Microstructure of yogurt is observed.	Kalab et al., 1975
1984	Use of aspartame in yogurt is patented.	Malone and Miles, 1984

Continued

Table A1 (Continued). Timeline of significant advances in the science and technology of yogurt and other cultured dairy products

Date	Milestone	Reference
1987	Yogurt containing <i>Bifidobacterium animalis</i> DN 173 010 is launched.	https://www.activia.ca/en-us/explore-science/history-activia
1988	Genetic transformation of <i>S. thermophilus</i> by electroporation.	Somkuti and Steinberg, 1988
1989	Patent awarded for <i>Lactobacillus rhamnosus</i> GG.	Gorbach and Goldin, 1989
1989	Method for making fiber-enriched yogurt is patented.	Reddy, 1989
1994	Yogurt drink containing <i>L. casei</i> DN-114001 is launched.	https://www.actimel.co.uk/the-story-so-far
1995	Inulin is first added to yogurt.	Robinson, 1995
2004	Genomic sequence of <i>S. thermophilus</i> is reported.	Bolotin et al, 2004
2006	Genomic sequence of <i>L. bulgaricus</i> is reported.	van de Guchte et al., 2006
2008	Identification of <i>S. thermophilus</i> and <i>L. bulgaricus</i> using methionine biosynthesis and 16S rRNA.	Cebeci and Gürakan, 2008
2010	Site-directed mutagenesis of <i>S. thermophilus</i> .	Blomqvist et al., 2010
2014	A low-galactose yogurt is developed.	Anbukkarasi et al., 2014