Microbiological Spoilage of Dairy Products

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Introduction

The wide array of available dairy foods challenges the microbiologist, engineer, and technologist to find the best ways to prevent the entry of microorganisms, destroy those that do get in along with their enzymes, and prevent the growth and activities of those that escape processing treatments. Troublesome spoilage microorganisms include aerobic psychrotrophic Gram-negative bacteria, yeasts, molds, heterofermentative lactobacilli, and spore-forming bacteria. Psychrotrophic bacteria can produce large amounts of extracellular hydrolytic enzymes, and the extent of recontamination of pasteurized fluid milk products with these bacteria is a major determinant of their shelf life. Fungal spoilage of dairy foods is manifested by the presence of a wide variety of metabolic by-products, causing off-odors and flavors, in addition to visible changes in color or texture. Coliforms, yeasts, heterofermentative lactic acid bacteria, and spore-forming bacteria can all cause gassing defects in cheeses. The rate of spoilage of many dairy foods is slowed by the application of one or more of the following treatments: reducing the pH by fermenting the lactose to lactic acid; adding acids or other approved preservatives; introducing a desirable microflora that restricts the growth of undesirable microorganisms; adding sugar or salt to reduce the water activity (a_w); removing water; packaging to limit available oxygen; and freezing. The type of spoilage microorganisms differs widely among dairy foods because of the selective effects of practices followed in production, formulation, processing, packaging, storage, distribution, and handling.

Types of Dairy Foods

The global dairy industry is impressive by large. In 2005, world milk production was estimated at 644 million tons, of which 541 million tons was cows' milk. The

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W.H. Sperber, M.P. Doyle (eds.), *Compendium of the Microbiological Spoilage of Foods and Beverages*, Food Microbiology and Food Safety,
DOI 10.1007/978-1-4419-0826-1_2,

leading producers of milk were the European Union at 142 million tons, India at 88 million tons, the United States at 80 million tons (20.9 billion gallons), and Russia at 31 million tons. Cheese production amounted to 8.6 million tons in Western Europe and 4.8 million tons in the United States (Anonymous, 2007; Kutzemeier, 2006). The vast array of products made from milk worldwide leads to an equally impressive array of spoilage microorganisms. A survey of dairy product consumption revealed that 6% of US consumers would eat more dairy products if they stayed fresher longer (Lempert, 2004). Products range from those that are readily spoiled by microorganisms to those that are shelf stable for many months, and the spoilage rate can be influenced by factors such as moisture content, pH, processing parameters, and temperature of storage. A short summary of the types of dairy products and typical spoilage microorganisms associated with them is shown in Table 1.

Table 1 Dairy products and typical types of spoilage microorganisms or microbial activity

Food	Spoilage microorganism or microbial activity
Raw milk	A wide variety of different microbes
Pasteurized milk	Psychrotrophs, sporeformers, microbial enzymatic degradation
Concentrated milk	Spore-forming bacteria, osmophilic fungi
Dried milk	Microbial enzymatic degradation
Butter	Psychrotrophs, enzymatic degradation
Cultured buttermilk, sour cream	Psychrotrophs, coliforms, yeasts, lactic acid bacteria
Cottage cheese	Psychrotrophs, coliforms, yeasts, molds, microbial enzymatic degradation
Yogurt, yogurt-based drinks	Yeasts
Other fermented dairy foods	Fungi, coliforms
Cream cheese, processed cheese	Fungi, spore-forming bacteria
Soft, fresh cheeses	Psychrotrophs, coliforms, fungi, lactic acid bacteria, microbial enzymatic degradation
Ripened cheeses	Fungi, lactic acid bacteria, spore-forming bacteria, microbial enzymatic degradation

Types of Spoilage Microorganisms

Psychrotrophs

Psychrotrophic microorganisms represent a substantial percentage of the bacteria in raw milk, with pseudomonads and related aerobic, Gram-negative, rod-shaped bacteria being the predominant groups. Typically, 65–70% of the psychrotrophs isolated from raw milk are *Pseudomonas* species (García, Sanz, Garcia-Collia, & Ordonez, et al., 1989; Griffiths, Phillips, & Muir, 1987). Important characteristics of pseudomonads are their abilities to grow at low temperatures (3–7°C) and to hydrolyze and use large molecules of proteins and lipids for growth. Other important psychrotrophs associated with raw milk include members of the genera *Bacillus, Micrococcus, Aerococcus,* and *Lactococcus* and of the family Enterobacteriaceae.

Pseudomonads can reduce the diacetyl content of buttermilk and sour cream (Wang & Frank, 1981), thereby leading to a "green" or yogurt-like flavor from an imbalance of the diacetyl to acetaldehyde ratio. For cottage cheese, the typical pH is marginally favorable for the growth of Gram-negative psychrotrophic bacteria (Cousin, 1982), with the pH of cottage cheese curd ranging from 4.5 to 4.7 and the pH of creamed curd being within the more favorable pH range of 5.0–5.3. The usual salt content of cottage cheese is insufficient to limit the growth of contaminating bacteria; therefore, psychrotrophs are the bacteria that normally limit the shelf life of cottage cheese. When in raw milk at cell numbers of greater than 10^6 CFU/ml, psychrotrophs can decrease the yield and quality of cheese curd (Aylward, O'Leary, & Langlois, 1980; Fairbairn & Law, 1986; Mohamed & Bassette, 1979; Nelson & Marshall, 1979).

Coliforms

Like psychrotrophs, coliforms can also reduce the diacetyl content of buttermilk and sour cream (Wang & Frank, 1981), subsequently producing a yogurt-like flavor. In cheese production, slow lactic acid production by starter cultures favors the growth and production of gas by coliform bacteria, with coliforms having short generation times under such conditions. In soft, mold-ripened cheeses, the pH increases during ripening, which increases the growth potential of coliform bacteria (Frank, 2001).

Lactic Acid Bacteria

Excessive viscosity can occur in buttermilk and sour cream from the growth of encapsulated, slime-producing lactococci. In addition, diacetyl can be reduced by diacetyl reductase produced in these products by lactococci growing at 7°C (Hogarty & Frank, 1982), resulting in a yogurt-like flavor.

Heterofermentative lactic acid bacteria such as lactobacilli and *Leuconostoc* can develop off-flavors and gas in ripened cheeses. These microbes metabolize lactose, subsequently producing lactate, acetate, ethanol, and CO_2 in approximately equimolar concentrations (Hutkins, 2001). Their growth is favored over that of homofermentative starter culture bacteria when ripening occurs at 15°C rather than 8°C (Cromie, Giles, & Dulley, 1987). When the homofermentative lactic acid bacteria fail to metabolize all of the fermentable sugar in a cheese, the heterofermentative bacteria that are often present complete the fermentation, producing gas and off-flavors, provided their populations are 10^6 CFU/g (Johnson, 2001). Residual galactose in cheese is an example of a substrate that many heterofermentative bacteria can metabolize and produce gas. Additionally, facultative lactobacilli can cometabolize citric and lactic acids and produce CO_2 (Fryer, Sharpe, & Reiter, 1970; Laleye, Simard, Lee, Holley, & Giroux, 1987). Catabolism of amino acids in cheese by nonstarter culture, naturally occurring lactobacilli, propionibacteria, and

Lactococcus lactis subsp. *lactis* can produce small amounts of gas in cheeses (Martley & Crow, 1993). Cracks in cheeses can occur when excess gas is produced by certain strains of *Streptococcus thermophilus* and *Lactobacillus helveticus* that form CO_2 and 4-aminobutyric acid by decarboxylation of glutamic acid (Zoon & Allersma, 1996).

Metabolism of tyrosine by certain lactobacilli causes a pink to brown discoloration in ripened cheeses. This reaction is dependent on the presence of oxygen at the cheese surface (Shannon, Olson, & Deibel, 1977). The racemic mixture of L(+) and D(−)-lactic acids that forms a white crystalline material on surfaces of Cheddar and Colby cheeses is produced by the combined growth of starter culture lactococci and nonstarter culture lactic acid producers. The latter racemize the L(+) form of the acid to the L(−) form, which form crystals (Johnson, 2001).

Fungi

Yeasts can grow well at the low pH of cultured products such as in buttermilk and sour cream and can produce off-flavors described as fermented or yeasty. Additionally, yeasts can metabolize diacetyl in these products (Wang & Frank, 1981), thereby leading to a yogurt-like flavor. Contamination of cottage cheese with the common yeast *Geotrichum candidum* often results in a decrease of diacetyl content. *Geotrichum candidum* reduced by 52–56% diacetyl concentrations in low-fat cottage cheese after 15–19 days of storage at 4–7°C (Antinone & Ledford, 1993).

Yeasts are a major cause of spoilage of yogurt and fermented milks in which the low pH provides a selective environment for their growth (Fleet, 1990; Rohm, Eliskasses, & Bräuer, 1992). Yogurts produced under conditions of good manufacturing practices should contain no more than 10 yeast cells and should have a shelf life of 3–4 weeks at 5°C. However, yogurts having initial counts of >100 CFU/g tend to spoil quickly. Yeasty and fermented off-flavors and gassy appearance are often detected when yeasts grow to 10^5–10^6 CFU/g. Giudici, Masini, and Caggia (1996) studied the role of galactose in the spoilage of yogurt by yeasts and concluded that galactose, which results from lactose hydrolysis by the lactic starter cultures, was fermented by galactose-positive strains of yeasts such as *Saccharomyces cerevisiae* and *Hansenula anomala*.

The low pH and the nutritional profile of most cheeses are favorable for the growth of spoilage yeasts. Surface moisture, often containing lactic acid, peptides, and amino acids, favors rapid growth. Many yeasts produce alcohol and CO_2, resulting in cheese that tastes yeasty (Horwood, Stark, & Hull, 1987). Packages of cheese packed under vacuum or in modified atmospheres can bulge as a result of the large amount of CO_2 produced by yeast (Vivier, Rivemale, Reverbel, Ratomahenina, & Galzy, 1994). Lipolysis produces short-chain fatty acids that combine with ethanol to form fruity esters. Some proteolytic yeast strains produce sulfides, resulting in an egg odor. Common contaminating yeasts of cheeses include *Candida*

spp., *Kluyveromyces marxianus*, *Geotrichum candidum*, *Debaryomyces hansenii*, and *Pichia* spp. (Johnson, 2001).

Molds can grow well on the surfaces of cheeses when oxygen is present, with the low pH being selective for them. In packaged cheeses, mold growth is limited by oxygen availability, but some molds can grow under low oxygen tension. Molds commonly found growing in vacuum-packaged cheeses include *Penicillium* spp. and *Cladosporium* spp. (Hocking & Faedo, 1992). *Penicillium* is the mold genus most frequently occurring on cheeses. A serious problem with mold spoilage of sorbate-containing cheeses is the degradation of sorbic acid and potassium sorbate to *trans*-1,3-pentadiene, causing an off-odor and flavor described as "kerosene." Several fungal species, including *Penicillium roqueforti*, are capable of metabolizing this compound from sorbates. Marth, Capp, Hasenzahl, Jackson, and Hussong (1966), who was the first group to study this problem, determined that cheese-spoilage isolates of *Penicillium* spp. were resistant to up to 7,100 ppm of potassium sorbate. Later, Sensidoni, Rondinini, Peressini, Maifreni, and Bortolomeazzi (1994) isolated from Crescenza and Provolone cheeses sorbate-resistant strains of *Paecilomyces variotii* and *D. hansenii* (a yeast) that produced *trans*-1,3-pentadiene, causing off-flavors in those products.

Cream cheeses are susceptible to spoilage by heat-resistant molds such as *Byssochlamys nivea* (Pitt & Hocking, 1999). *Byssochlamys nivea* is capable of growing in reduced oxygen atmospheres, including in atmospheres containing 20, 40, and 60% carbon dioxide with less than 0.5% oxygen (Taniwaki, 1995). Once this mold is present in the milk supply, it can be difficult to eliminate during normal processing of cream cheese. Engel and Teuber (1991) studied the heat resistance of various strains of *B. nivea* ascospores in milk and cream and determined a *D*-value of 1.3–2.4 s at 92°C, depending on the strain. They calculated that in a worst-case scenario of 50 ascospores of the most heat-resistant strain per liter of milk, a process of 24 s at 92°C would result in a 1% spoilage rate in packages of cream cheese.

Spore-Forming Bacteria

Raw milk is the usual source of spore-forming bacteria in finished dairy products. Their numbers before pasteurization seldom exceed 5,000/ml (Mikolajcik & Simon, 1978); however, they can also contaminate milk after processing (Griffiths & Phillips, 1990). The most common spore-forming bacteria found in dairy products are *Bacillus licheniformis, B. cereus, B. subtilis, B. mycoides,*and *B. megaterium.*In one study, psychrotrophic *B. cereus* was isolated in more than 80% of raw milks sampled (Meer, Baker, Bodyfelt, & Griffiths, 1991). The heat of pasteurization activates (heat shock) many of the surviving spores so that they are primed to germinate at a favorable growth temperature (Cromie, Schmidt, & Dommett, 1989). Coagulation of the casein of milk by chymosin-like proteases produced by many of these bacilli occurs at a relatively high pH (Choudhery & Mikolajcik, 1971). Cromie

et al. (1989) reported that lactose-fermenting *B. circulans* was the dominant spoilage microbe in aseptically packaged pasteurized milk. *Bacillus stearothermophilus* can survive ultra-high-temperature treatment of milk (Muir, 1989). This bacterium produces acid but no gas, hence causing the "flat sour" defect in canned milk products (Kalogridou-Vassiliadou, 1992).

If extensive proteolysis occurs during aging of ripened cheeses, the release of amino acids and concomitant increase in pH favors the growth of clostridia, especially *Clostridium tyrobutyricum,* and the production of gas and butyric acid (Klijn, Nieuwendorf, Hoolwerf, van der Waals, & Weerkamp, 1995). Spores are concentrated in cheese curd, so as few as one spore per milliliter of milk can cause gassiness in some cheeses (Myhara & Skura, 1990). Spore numbers of more than 25/ml were required to produce this defect in large wheels of rindless Swiss cheese (Dasgupta & Hull, 1989). Cheeses most often affected, e.g., Swiss, Emmental, Gouda, and Edam, have a relatively high pH and moisture content, and low salt content. An example of gassing caused by *C. tyrobutyricum* in Swiss cheese is shown in Fig. 1.

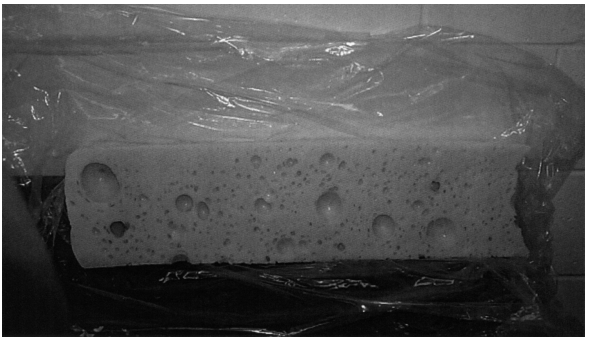

Fig. 1 Gassy Swiss cheese caused by *Clostridium tyrobutyricum*. L. H. Ledenbach photo

Occasionally, gassy defects of process cheeses are also caused by *C. butyricum* or *C. sporogenes.* These spores are not completely inactivated by the normal cooking treatment of process cheeses. Therefore, they may germinate and produce gas unless their numbers are low, the pH is not higher than 5.8, the salt concentration is at least 6% of the serum, and the cheese is held at 20°C or lower (Kosikowski & Mistry, 1997). The products of fermentation in these cheeses are butyric and acetic acids, carbon dioxide, and hydrogen. A summary of known causes of gassiness in cheese products is shown in Table 2.

Thermoduric and thermophilic spore-forming bacteria are the common causes of spoilage of concentrated milks. They survive pasteurization and the extended high temperatures of evaporative removal of moisture to increase the milk solid content to 25.5–45%. When these foods are contaminated, the survivors are heat-resistant *Bacillus* spp. (Kalogridou-Vassiliadou, 1992).

Table 2 Causes of gassiness in different types of cheese

Organism	Cheese affected	Time to defect
Coliforms	Raw milk pasta filata cheese	Early blowing
Yeasts	Raw milk Domiati (Egyptian), Camembert, blue-veined, Feta	Early blowing
Lactobacillus fermentum	Provolone, mozzarella	Late blowing
Heterofermentative Lactobacilli	Cheddar, Gouda, Saint Paulin, Oka	Late blowing
Propionibacteria	Sbrinz (Argentinean)	Late blowing
Clostridium tyrobutyricum	Gouda, Emmental, Swiss, Cheddar, Grana	Late blowing
Eubacterium sp.	Cheddar	Late blowing

Sources: Bottazzi and Corradini (1987); Dennien (1980); El-Shibiny, Tawfik, Sharaf, and El-Khamy (1988); Font de Valdez, Savoy de Giori, Ruiz Holgado, and de Oliver (1984); Johnson (2001); Klijn et al. (1995); Laleye et al. (1987); Myhr et al. (1982); Melilli et al. (2004); Roostita & Fleet (1996); Vivier et al. (1994)

Other Microorganisms

Eubacterium sp., a facultative anaerobe that is able to grow at pH 5.0–5.5 in the presence of 9.5% salt (Myhr, Irvine, & Arora, 1982), can cause gassiness in Cheddar cheese. An unusual white-spot defect caused by a thermoduric *Enterococcus faecalis* subsp. *liquefaciens* has occurred in Swiss cheese. This bacterium is inhibitory to propionibacteria and *Lactobacillus fermentum*, resulting in poor eye development and lack of flavor in the cheese as well (Nath & Kostak, 1985).

Enzymatic Degradation

An indirect cause of dairy product spoilage is microbial enzymes, such as proteases, phospholipases, and lipases, some of which may remain active in the food after the enzyme-producing microbes have been destroyed. Populations of psychrotrophs ranging from 10^6 to 10^7 CFU/ml can produce sufficient amounts of extracellular enzymes to cause defects in milk that are detectable by sensory tests (Fairbairn & Law, 1987). Adams, Barach, and Speck (1975) reported that 70–90% of raw milk samples tested contained psychrotrophic bacteria capable of producing proteinases that were active after heating at 149°C (300°F) for 10 s. Others have verified this observation (Griffiths, Phillips, & Muir, 1981).

Extracellular proteases can affect the quality of milk products in various ways, but largely by producing bitter peptides. Thermally resistant proteases have caused spoilage of ultra-high-temperature (UHT) milk (Shah, 1994; Sørhaug & Stepaniak, 1991). In addition, phospholipases can be heat stable. Experimentally, phospholipase production in raw milk can result in the development of bitter off-flavors due to the release of fatty acids by milk's natural lipase (Fox, Chrisope, &

Marshall, 1976; Chrisope & Marshall, 1976). Heat-stable bacterial lipases have been associated with the development of rancid flavors in UHT milk (Adams & Brawley, 1981). *Pseudomonas fluorescens* is the most common producer of lipases in milk and milk products, but lipases can also be produced by Gram-negative psychrotrophic bacteria. Products that may be affected by residual lipases include UHT milk, butter, some cheeses, and dry whole milk. The release of short-chain fatty acids, C4 through C8, results in the occurrence of rancid flavors and odors, whereas the release of long-chain fatty acids results in a soapy flavor. Oxidation of free unsaturated fatty acids to aldehydes and ketones results in an oxidized flavor (Deeth & Fitz-Gerald, 1983), and fruity off-flavor results from lipolysis of short-chain fatty acids by *Pseudomonas fragi* followed by esterification with alcohols (Reddy, Bills, Lindsey, & Libbey, 1968).

Lipase tends to partition into cream instead of the nonfat milk portion when cream is separated from milk (Downey, 1980; Stead, 1986). The large concentration of fat globules and the activation of lipase caused by some disruption of the fat globule membrane increase the probability of enzyme–substrate interactions. In the production of butter, lipolysis can cause excessive foaming during churning of cream (Deeth & Fitz-Gerald, 1983), hence increasing the time of churning. Rancidity of butter may result from the activity of lipase in the raw milk or the residual heat-stable microbial lipase in the finished butter. Although short-chain fatty acids from rancid cream, being water-soluble, are partially lost in the buttermilk and wash water during manufacture (Stead, 1986), microbial lipases remaining in the butter can hydrolyze the fat even during frozen storage (Nashif & Nelson, 1953). Low pH limits the rate of lipase activity, but in some cheeses, e.g., Brie and Camembert, the pH rises to near neutrality as ripening progresses, making them especially susceptible to lipolysis (Dumont, Delespaul, Miquot, & Adda, 1977). For Cheddar cheese, however, a high concentration of lipase is needed to create the desired flavor (Law, Sharpe, & Chapman, 1976). Products such as whole milk powder may be affected by residual heat-resistant bacterial lipases. Residual lipases in nonfat dry milk and dry whey products can hydrolyze fats in products into which they are added as ingredients (Stead, 1986).

Sources of Spoilage Microorganisms

Contamination of Raw Milk

The highly nutritious nature of dairy products makes them especially good media for the growth of microorganisms. Milk contains abundant water and nutrients and has a nearly neutral pH. The major sugar, lactose, is not utilized by many types of bacteria, and the proteins and lipids must be broken down by enzymes to allow sustained microbial growth. In order to understand the source of many of the spoilage microflora of dairy products, it is best to discuss how milk can first become contaminated, via the conditions of production and processing.

The mammary glands of many very young cows yield no bacteria in aseptically collected milk samples, but as numbers of milkings increase, so do the chances of isolating bacteria in milk drawn aseptically from the teats. The stresses placed on the cow's teats and mammary glands by the very large amounts of milk produced and the actions of the milking machine cause teat canals to become more open and teat ends to become misshapen as time passes (Fig. 2). These stresses may open the teat canal for the entry of bacteria capable of infecting the glands.

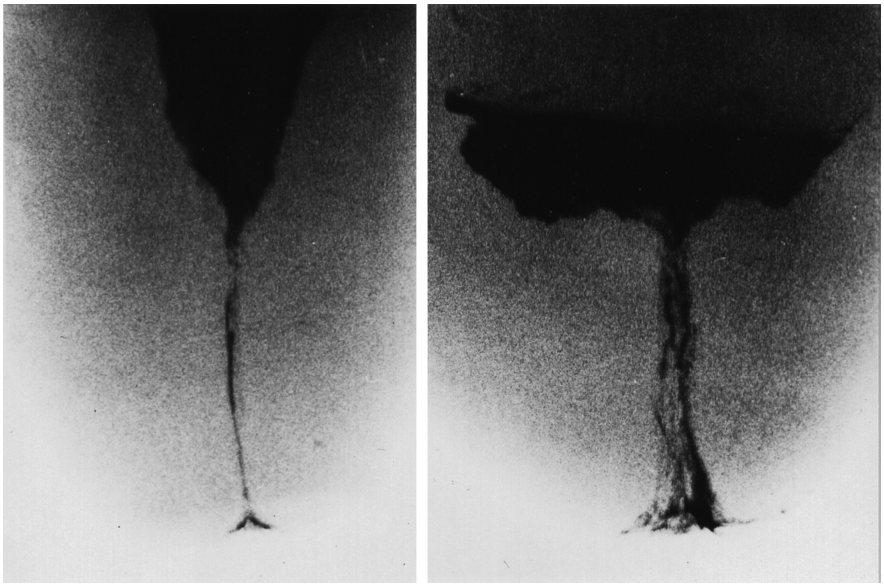

Fig. 2 X-ray photographs showing an increase in the diameter of the teat canal of the same teat of a milking cow between the first lactation (*left*) and a later lactation (*right*). Courtesy Dr. J. S. McDonald, National Animal Disease Laboratory, U. S. Department of Agriculture, Ames, Iowa

Environmental contaminants represent a significant percentage of spoilage microflora. They are ubiquitous in the environment from which they contaminate the cow, equipment, water, and milkers' hands. Since milking machines exert about 38 cm (15 in.) of vacuum on the teats during milking, and since air often leaks into the system, bacteria on the surfaces of the cow or in water retained from premilking preparation can be drawn into the milk. Also, when inflation clusters drop to the floor, they pick up microorganisms that can be drawn into the milk. The pumping or agitation of milk supplies the oxygen needed by aerobes for growth and breaks chains and clumps of bacteria. Single cells, having less competition than those in colonies, have the opportunity for more rapid multiplication. Bacteria recontaminating pasteurized milk originate primarily from water and air in the filling equipment or immediate surroundings and can be resident for prolonged periods of time (Eneroth, Ahrne, & Molin, 2000). In a study performed in Norway and Sweden, Ternstrom, Lindberg, and Molin (1993) investigated nine dairy plants and found that five taxa of psychrotrophic *Pseudomonas* spp. were involved in the

spoilage of raw and pasteurized milk and that the same strains were recovered from both the raw and pasteurized milk, suggesting that recontamination originated from the raw milk. Additionally, the investigators found that *Bacillus* spp. (mainly *B. cereus* and *B. polymyxa*) were responsible for spoilage in 77% of the samples that had been spoiled by Gram-positive bacteria. The spoilage *Bacillus* spp. grew fermentatively, and most were able to denitrify the milk, which has implications for cheeses that contain added nitrate/nitrites for protection against clostridia. Sporeforming bacteria are abundant in dust, dairy feed concentrates, and forages; therefore, they are often present on the skin and hair of cattle from which they can enter milk. The presence of sporeformers such as *C. butyricum* in milk has been traced to contaminated silage (Dasgupta & Hull, 1989).

Contamination of Dairy Products

Washed curd types of cheeses are especially susceptible to growth of coliforms (Frank, Marth, & Olson, 1978), so great care must be taken to monitor the quality of water used in these processes. A high incidence of contamination of brine-salted cheeses by yeasts results from their presence in the brines (Kaminarides & Lakos, 1992). Many mold species are particularly well adapted to the cheese-making environment and can be difficult to eradicate from a production facility. Fungi causing a "thread mold" defect in Cheddar cheeses (Hocking & Faedo, 1992) were found in the cheese factory environment, on cheese-making equipment, in air, and in curd and whey. In a study of cheese-making facilities in Denmark, *Penicillium commune* persisted in the cheese coating and unpacking areas over a 7-year period (Lund, Bech Nielsen, & Skouboe, 2003). Ascospores of *B. nivea* and other heat-resistant species shown to be able to survive pasteurization, such as *Talaromyces avellaneus*, *Neosartorya fischeri* var. *spinosa*, and *Eupenicillium brefeldianum*, have also been found in raw milk (Pitt & Hocking, 1999).

A major cause of failure of processing and packaging systems is the development of biofilms on equipment surfaces. These communities of microorganisms develop when nutrients and water remain on surfaces between times of cleaning and reuse. Bacteria in biofilms (sessile form) are more resistant to chemical sanitizers than are the same bacteria in suspension (planktonic form) (Mosteller & Bishop, 1993). Chemical sanitizers may be rendered ineffective by biofilms leaving viable bacteria to be dislodged into the milk product (Frank & Koffi, 1990).

Factors Affecting Spoilage

Spoilage of Fluid Milk Products

The shelf life of pasteurized milk can be affected by large numbers of somatic cells in raw milk. Increased somatic cell numbers are positively correlated with

concentrations of plasmin, a heat-stable protease, and of lipoprotein lipase in freshly produced milk (Barbano, Ma, & Santos, 2005). Activities of these enzymes can supplement those of bacterial hydrolases, hence shortening the time to spoilage. The major determinants of quantities of these enzymes in the milk supply are the initial cell numbers of psychrotrophic bacteria, their generation times, their abilities to produce specific enzymes, and the time and temperature at which the milk is stored before processing. Several conditions must exist for lipolyzed flavor to develop from residual lipases in processed dairy foods, that is, large numbers ($>10^6$ CFU/ml) of lipase producers (Stead, 1986), stability of the enzyme to the thermal process, long-term storage and favorable conditions of temperature, pH, and water activity.

Spoilage of Cheeses

Factors that determine the rates of spoilage of cheeses are water activity, pH, salt to moisture ratio, temperature, characteristics of the lactic starter culture, types and viability of contaminating microorganisms, and characteristics and quantities of residual enzymes. With so many variables to affect deteriorative reactions, it is no surprise that cheeses vary widely in spoilage characteristics. Soft or unripened cheeses, which generally have the highest pH values, along with the lowest salt to moisture ratios, spoil most quickly. In contrast, aged, ripened cheeses retain their desirable eating qualities for long periods because of their comparatively low pH, low water activity, and low redox potential.

For fresh, raw milk pasta filata cheeses, Melilli et al. (2004) determined that low initial salt and higher brining temperature (18°C) allowed for greater growth of coliforms, which caused gas formation in the cheese. Factors affecting the growth of the spoilage microorganisms, *Enterobacter agglomerans* and *Pseudomonas* spp. in cottage cheese, were higher pH and storage temperature of the cheese (Brocklehurst & Lund, 1988). Some of the spoilage microorganisms were able to grow at relatively low pH values (4.6–4.7) when incubated at 7°C and were able to grow at pH 3.6 when grown in media at 20°C. Rate of salt penetration into brined cheeses, types of starter cultures used, initial load of spores in the milk used for production, pH of the cheese, and ripening temperature affect the rate of butyric acid fermentation and gas production by *C. tyrobutyricum* (Stadhouders, 1990c). Fungal growth in packaged cheeses was found to be most significantly affected by the concentration of CO_2 in the package and the water activity of the cheese (Nielsen & Haasum, 1997). Cheddar cheese exhibiting yeast spoilage had a high moisture level (39.1%) and a low salt in the moisture-phase value (3.95%) (Horwood et al., 1987). Roostita and Fleet (1996) determined that the properties of yeasts that affected the spoilage rate of Camembert and blue-veined cheeses were the abilities to ferment/assimilate lactose, produce extracellular lipolytic and proteolytic enzymes, utilize lactic and citric acid, and grow at 10°C.

Prevention and Control Measures

Prevention of Spoilage in Milk

In the early days of development of the commercial dairy industry, milk was produced under much less sanitary conditions than are used today, and cooling was slow and inadequate to restrict bacterial growth. Developments during the first half of the twentieth century created significant reductions in the rate of spoilage of raw milk and cream, by making it possible for every-other-day pickup of milk from farms and shipments of raw milk over long distances with minimal increases in bacterial cell numbers. Rapid cooling and quick use of raw milk are accepted as best practices and can affect the spoilage ability of *Pseudomonas* spp. present in milk. Pseudomonads that had been incubated in raw milk for 3 days at 7°C (44.6°F) had greater growth rates and greater proteolytic and lipolytic activity than those isolated directly from the milk shortly after milking (Jaspe, Oviedo, Fernandez, Palacios, & Sanjose,1995).

As the quality of raw milk improved, so did that of pasteurized milk. Heating of milk to 62.8°C (145°F) for 30 min or to 71.7°C (161°F) for 15 s kills the pathogenic bacteria likely to be of significance in milk as well as most of the spoilage bacteria. However, processors learned that long shelf life of pasteurized fluid milk products requires a higher temperature treatment as well as prevention of contamination between the pasteurizer and the sealed package. In particular, it is imperative that filling equipment be sanitary and that the air in contact with the filler, the milk, and the containers be practically sterile. Whereas in the early to mid-twentieth century, milk was delivered daily to homes because of its short shelf life, today's fluid milk products are generally expected to remain acceptable for 14–21 days. Pasteurization standards for several countries are listed in Table 3.

A shelf life of 21 days and beyond can be attained with fluid milk products that have been heated sufficiently to kill virtually all of the vegetative bacterial cells and protected from recontamination. Ultra-pasteurized milk products, heated at or above 138°C for at least 2 s, that have been packaged aseptically can have several weeks of shelf life when stored refrigerated. Ultra-high-temperature (UHT) treatment destroys most spores in milk, but *B. stearothermophilus* can survive. Aseptic processing, as defined in the Grade A Pasteurized Milk Ordinance (2003), means that the product has been subjected to sufficient heat processing to render it commercially sterile and that it has been packaged in a hermetically sealed container. These dairy foods are stable at room temperature.

The addition of carbon dioxide to milk and milk products reduces the rates of growth of many bacteria (Dixon & Kell, 1989). King and Mabbitt (1982) demonstrated improved keeping quality of raw milk by the addition of CO_2. Loss and Hotchkiss (2002) found lowered survivor rates of both *P. fluorescens* and the spores of *B. cereus* during heating of milk containing up to 36 mM CO_2. McCarney, Mullen, and Rowe (1995) determined that carbonation may be a desirable treatment for cheese milk when on the day of collection populations of psychrotrophic

Table 3 Dairy product heat treatment standards in different countries

Treatment	Temperature	Time
United States[a]		
Pasteurization of milk	63°C/145°F	30 min*
	72°C/161°F	15 s*
Ultra-pasteurization of milk	138°C/280°F	2 s
Ultra-high temperature (UHT)-treated milk	140–150°C/ 284–302°F	Few seconds
*If fat content >10% or contains sweeteners, increase the temperature by 3°C/5°F		
Product	Temperature	Time
Australia[b]		
Pasteurization of milk and liquid milk products (includes milk used for production of cream/cream products, fermented milks, yogurt, dried, condensed, and evaporated milks, butter, and ice cream)	72°C/162°F	15 s
Pasteurization of milk for cheese production	72°C/162°F	15 s
	62°C/144°F	15 s*
*__and__ cheese is stored at ≥2°C/36°F for 90 days prior to sale __or__ curd is heated to ≥48°C/119°F and moisture is ≤36% after storage at ≥10°C/50°F for ≥6 months prior to sale		
European Union[c]		
Raw milk and raw milk for production of dairy products	Milk is not heated beyond 40°C/104°F	
Thermized milk and thermized milk for production of dairy products	57–68°C/135–155°F	≥15 s
Pasteurization of milk	71.7°C/161.1°F	15 s
UHT-treated milk	>135°C/275°F	>1 s

[a]Source: USPHS/FDA Pasteurized Milk Ordinance, 2003
[b]Source: Australia Food Code Standard 1.6.2, 2001
[c]Source: EU Council Directive 92/46/EEC, 1992

bacteria are approximately 10^5 CFU/ml. Rajagopal, Werner, and Hotchkiss (2005) demonstrated that treatment with CO_2 at a pressure of 689 kPa and temperature of 6.1°C produced a substantial decrease in bacterial counts, resulting in milk that was within the grade A raw milk limits for up to 8 days of storage. A disadvantage can be that an acidic flavor note may be produced in a CO_2-treated milk product. When CO_2 is dissolved in milk, the pH decreases (Ma, Barbano, Hotchkiss, Murphy, & Lynch, 2001) and does not return to the original pH value following the removal of CO_2 before pasteurization (Ruas-Madiedo, Bascaran, Brana, Bada-Gancedo, & Reyes-Gavilan, 1998).

High hydrostatic pressure treatments of milk are effective in killing vegetative bacterial cells, but spores are mostly refractory to this treatment (McClements, Patterson, & Linton, 2001). The phase of growth of the bacteria and the temper-

ature of incubation are significant variables affecting the sensitivities of bacterial cells to high pressures. Cells in the stationary phase are more resistant than those in the exponential phase of growth. Survivor curves have shown resistant tailing populations (McClements et al., 2001; Metrick, Hoover, & Farkas, 1989). Other alternative treatments for the pasteurization of milk, such as ohmic heating, microwave heating, UV radiation, electron beam irradiation, pulsed electric fields, infrared processing, and high voltage arc discharge, may have the potential to be used alone or in combination with other treatments. However, all pasteurization processes need to be validated through the combined use of process authorities, challenge studies, and predictive modeling, and must be verified to ensure that critical processing limits are achieved (NACMCF, 2006).

Prevention of Spoilage in Cultured Dairy Products

Cultured products such as buttermilk and sour cream depend on a combination of lactic acid producers, the lactococci, and the leuconostocs (diacetyl producers), to produce the desired flavor profile. Imbalance of the culture, improper temperature or ripening time, infection of the culture with bacteriophage, presence of inhibitors, and/or microbial contamination can lead to an unsatisfactory product. A buttery flavor note is produced by *Leuconostoc mesenteroides* subsp. *cremoris*. This bacterium converts acetaldehyde to diacetyl, thus reducing the "green" or yogurt-like flavor (Lindsey & Day, 1965). A diacetyl to acetaldehyde ratio of 4:1 is desirable, whereas the green flavor is present when the ratio is 3:1 or less. Proteolysis by the lactococci is necessary to afford growth of the *Leuconostoc* culture, and citrate is needed as substrate for diacetyl production.

Although cooking of the curd destroys virtually all bacteria capable of spoiling cottage cheese, washing and handling of the curd after cooking can introduce substantial numbers of spoilage microorganisms. It is desirable to acidify alkaline waters for washing cottage cheese curd to prevent solubilization of surfaces of the curd. However, more pseudomonads can be adsorbed onto cottage cheese curd from wash water when adjusted to pH 5 (40–45%) rather than adjusted to pH 7 (20–30%) (Wellmeyer & Marshall, 1972). Flushing packages of cottage cheese or sour cream with CO_2 or N_2 suppressed the growth of psychrotrophic bacteria, yeasts, and molds for up to 112 days, but a slight bitterness can occur in cottage cheese after 73 days of storage (Kosikowski & Brown, 1973).

Cheesemakers can use the addition of high numbers of lactic acid bacteria to raw milk during storage to reduce the rate of growth of psychrotrophic microbes. For fresh, raw milk, brined cheeses, gassing defects can be reduced by presalting the curd prior to brining and reducing the brine temperature to <12°C (Melilli et al., 2004). Pasteurization will eliminate the risk from most psychrotrophic microbes, coliforms, leuconostocs, and many lactobacilli, so cheeses made from pasteurized milk have a low risk of gassiness produced by these microorganisms. Most bacterial cells, including spores, can be removed from milk by centrifugation at

about 9,000*g*. The process, known as bactofugation, removes about 3% of the milk, called bactofugate. Kosikowski and Mistry (1997) invented and patented a process for recovering this bactofugate which is heated at 135°C for 3–4 s, then added back to the cheese milk. The process can reduce the population of butyric acid-producing spores by 98% (Daamen, van den Berg, & Stadhouders, 1986). Spore-forming bacterial growth and subsequent gas production in aged, ripened cheeses can be minimized with a salt to moisture content of $\geq$3.0% (Stadhouders, 1990c). Other potential inhibitors of butyric acid fermentation and gas production in cheese are the addition of nitrate (Stadhouders, 1990b), addition of lysozyme (Lodi, 1990), cold storage of cheese prior to ripening, direct salt addition to the cheese curd, addition of hydrogen peroxide, or use of starter cultures that form nisin or other antimicrobials (Stadhouders, 1990a).

The most popular mold inhibitors used on cheeses are sorbates and natamycin. Sorbates tend to diffuse into the cheese, thereby modifying flavor and decreasing their concentration, whereas very little natamycin diffuses (de Ruig & van den Berg, 1985). Electron beam irradiation, studied by Blank, Shamsuzzaman, and Sohal (1992) for mold decontamination of Cheddar cheese, can reduce initial populations of *Aspergillus ochraceus* and *Penicillium cyclopium* by 90% with average doses of 0.21 and 0.42 kGy, respectively. Since nearly all mold spores are killed by pasteurization (Doyle & Marth, 1975), practices that limit recontamination and growth, although difficult, are vital in prevention of moldy cheeses. Modified atmosphere packaging (MAP) of cheeses can retard or prevent the growth of molds, and optimum MAP conditions for different types of cheeses were described by Nielsen and Haasum (1997). For processed cheeses containing no active lactic acid starter bacteria, low O_2 and high CO_2 atmospheres were optimum; for cheeses containing active starter cultures, atmospheres containing low O_2 and controlled CO_2 using a permeable film provided the best results. For mold-ripened cheeses requiring the activity of the fungi to maintain good quality, normal O_2 and high, but controlled, CO_2 atmospheres were best. In Italian soft cheeses such as Stracchino, vacuum packaging decreased the growth of yeasts, resulting in a shelf life extension of >28 days (Sarais, Piussi, Aquili, & Stecchini, 1996).

Processing times and temperatures used in the manufacture of cream cheese and pasteurized process cheese are able to eliminate most spoilage microorganisms from these products. However, the benefit of the presence of competitive microflora is also lost. It is very important to limit the potential for recontamination, as products that do not contain antimycotics can readily support the growth of yeasts and molds. Sorbates can be added; however, their use in cream cheese is limited to amounts that will not affect the delicate flavor.

Prevention of Spoilage in Other Dairy Products

The high salt concentration in the serum-in-lipid emulsion of butter limits the growth of contaminating bacteria to the small amount of nutrients trapped within

the droplets that contain the microbes. However, psychrotrophic bacteria can grow and produce lipases in refrigerated salted butter if the moisture and salt are not evenly distributed (Deeth & Fitz-Gerald, 1983). When used in the bulk form, concentrated (condensed) milk must be kept refrigerated until used. It can be preserved by addition of about 44% sucrose and/or glucose to lower the water activity below that at which viable spores will germinate (a_w 0.95) (Jay, 1996). Lactose, which constitutes about 53% of the nonfat milk solids, contributes to the lowered water activity. When canned as evaporated milk or sweetened condensed milk, these products are commercially sterilized in the cans, and spoilage seldom occurs. Microbial growth and enzyme activity are prevented by freezing. Therefore, microbial degradation of frozen desserts occurs only in the ingredients used or in the mixes prior to freezing.

Methods for Detection and Isolation

It has been a long-standing practice to use microbiological standards for indicator microorganisms as a predictor of the safety and quality of dairy products, and many countries have regulations or guidelines for these microbes (Table 4). While these tests can be useful as a general indication of the cleanliness of the dairy processing operation, they may not necessarily correlate with the shelf life of the products. Boor, Carey, Murphy, and Zadoks (2005) reported results of audits of pasteurized milk quality collected from 23 plants in New York State over a 10-year period. On an annual basis, the percentage of samples that met the Grade A Pasteurized Milk Ordinance Standard Plate Count limit of 20,000 CFU/ml after 14 days of storage at 6.1°C ranged from 12 to 32%. Tests for coliform bacteria were positive for 5–15% of the samples on initial testing and increased up to 34% after 14 days of storage. Sensory tests on the 14th day of storage revealed that 33–59% of the milks were still acceptable. After about 17 days of storage, the dominant spoilage bacteria belonged to the spore-forming genera *Paenibacillus* (39%) and *Bacillus*(32%) and to heat-tolerant *Microbacterium lacticum* (14%).

As an outgrowth of the efforts in the early twentieth century to improve the safety and quality of milk products, the American Public Health Association standardized the methods for detection of spoilage indicators and published them in the Standard Methods for the Examination of Dairy Products (Marshall, 2001). Recommended methods for various microorganisms are listed in Table 5. Common tests in use today for the prediction of shelf life of fluid milk products use a preliminary incubation or keeping quality step followed by standard microbiological testing. These methods are designed to determine low levels of thermoduric Gram-negative bacteria, such as psychrotrophic coliforms and pseudomonads, that have survived pasteurization and are most likely to grow under typical storage conditions. The recommended methods have the disadvantage of taking several days to complete. There is a vast array of rapid test methods available for use (Entis et al., 2003) in dairy product testing. The preferred method for assaying for specific spoilage

Table 4 Regulatory standards for indicator organisms in different countries

Product	Test	Limits			
		n	c	m	M
United States[a]					
Grade A raw milk and milk products for further processing	Standard plate count (SPC)	100,000/ml max individual bulk tank 300,000/ml max commingled milk			
	Somatic cell count (SCC)	750,000/ml max individual bulk tank (1,000,000/ml max. goat's milk)			
Grade A pasteurized milk and milk products	SPC	20,000/ml max.			
	Coliforms	10/ml max. (100/ml max. bulk)			
Grade A aseptically packaged dairy products		No growth			
Grade A nonfat dry milk	SPC	30,000/gm max.			
	Coliforms	10/gm max.			
Grade A condensed whey and whey products, dry whey and whey products, dry buttermilk and buttermilk products	Coliforms	10/gm max.			
European Union[b]					
Raw cow's milk for production of heat-treated drinking milk, fermented milk, junket, jellied or flavored milk and cream	Aerobic plate count (APC)	100,000/ml max.			
	SCC	400,000/ml max.			
Raw cow's milk for manufacture of milk-based products other than above	APC	400,000/ml max.			
	SCC	500,000/ml max.			
Raw buffalo's milk for manufacture of milk-based products	APC	1,000,000/ml max.			
	SCC	500,000/ml max.			
Raw buffalo's milk for "product with raw milk" not involving further heat treatment	APC	500,000/ml max.			
	SCC	400,000/ml max.			

(Continued)

Table 4 (Continued)

Product	Test	Limits			
		n	c	m	M
Raw goat and sheep's milk for manufacture of milk-based products not involving heat treatment	APC	1,000,000/ml max.			
Raw goat and sheep's milk for "products with raw milk" not involving heat treatment	APC	500,000/ml max.			
Raw cow's milk for drinking	APC	50,000/ml max.			
Pasteurized milk	APC at 21°C (after incubation for 5 days at 6°C)	5	2	50,000	500,000
	Enterobacteriaceae	5	2	0	5
Liquid milk-based products	Enterobacteriaceae	5	2	0	5
Butter made from pasteurized milk or cream	Enterobacteriaceae	5	2	0	10
Soft cheese made from heat-treated milk	Enterobacteriaceae	5	2	10,000	100,000
Powdered milk-based products	Enterobacteriaceae	5	2	10	100
Liquid heat-treated unfermented milk-based products	APC at 21°C (after incubation for 5 days at 6°C)	5	2	50,000	100,000
Frozen milk-based products, including ice cream	APC	5	2	100,000	500,000
	Enterobacteriaceae	5	2	10	100
UHT milk	APC at 30°C (after incubation for 30 days at 30°C)				10/0.1 ml max.

Table 4 (Continued)

Australia/New Zealand[c]					
Unpasteurized milk	SPC	5	1	25,000	250,000
	Coliforms	5	1	100	1,000
	E. coli	5	1	3	9
Pasteurized milk, cream	SPC	5	1	50,000	100,000
	Coliforms	5	1	1	10
	Psychrotrophs	5	1	10	100
Butter from unpasteurized milk and/or unpasteurized milk products	SPC	5	0	500,000	
	Coliforms	5	1	10	100
	E. coli	5	1	3	9
Pasteurized butter – salted and unsalted	SPC	5	1	50,000	100,000
	Coliforms	5	1	10	100
	Psychrotrophs	5	1	10	100
Yogurt and other fermented milk products	Coliforms	5	2	10	100
	E. coli	5	0	0	
All cheeses	*E. coli*	5	1	10	100
Ice cream and edible ices	SPC	5	2	10,000	50,000
	Coliforms	5	2	10	100
	E. coli	5	0	0	
Dried milk powder	SPC	5	2	50,000	200,000
	Coliforms	5	2	10	100
UHT/Sterilized milk		Commercially sterile			

[a]Source: USPHS/FDA Pasteurized Milk Ordinance, 2003
[b]Source: Australia/New Zealand Food Standards Code-Microbiological Limits for Foods-Standard 1.6.1, 2001
[c]Source: EU Council Directive 92/46/EEC, 1992

Table 5 Recommended methods for testing of dairy products (Entis, et al., 2003; Richter & Vedamuthu, 2003)

Product	Property	Method – Reference
Raw milk	General quality	Direct microscopic count – SMEDP[a]
		Direct microscopic somatic cell count – SMEDP
		Electronic somatic cell count – SMEDP
	Shelf life	Preliminary incubation – SMEDP
	Microorganism counts	Standard plate count – SMEDP
		Thermoduric count – SMEDP
		Coliform count – SMEDP
		Psychrotrophic count – SMEDP
Pasteurized milk	Shelf life	Preliminary incubation – SMEDP
		Mosley keeping quality – SMEDP
	Microorganism counts	Standard plate count – SMEDP
		Coliform count – SMEDP
		Psychrotrophic count – SMEDP
Dried milk products	Microorganism counts	Standard plate count – SMEDP
		Coliform count – SMEDP
		Direct microscopic clump count – SMEDP
		Thermoduric count – SMEDP
		Psychrotrophic count – SMEDP
		Yeast and mold count – SMEDP
Butter products	Microorganism count	Standard plate count – SMEDP
		Coliform count – SMEDP
		Lipolytic count – SMEDP
		Proteolytic count – SMEDP
		Psychrotrophic count – SMEDP
		Yeast and mold count – SMEDP
Frozen dairy products	Microorganism counts	Standard plate count – SMEDP
		Coliform count – SMEDP
		Thermoduric Count – SMEDP
		Yeast and mold count – SMEDP
Concentrated milk products	Microorganism counts	Standard plate count – SMEDP
		Coliform count – SMEDP
		Thermoduric count – SMEDP
		Thermophilic count – SMEDP
		Yeast and mold count – SMEDP
Cheeses, yogurt, fermented milk products	Microorganism counts	Coliform count – SMEDP
		Yeast and mold count – SMEDP
		Psychrotrophic count – SMEDP

[a]Standard Methods for the Examination of Dairy Products, 2001

microorganisms can often depend on the product characteristics, such as amount of competing microflora, pH, and water activity.

Fungi can be particularly troublesome, because they can adapt to the environment of the food and can be difficult to detect on conventional plating media within the standard incubation times. In yogurts, yeasts often grow slowly in conventional laboratory plating methods, but as few as 10 CFU/ml were detectable after 16 h of incubation by PCR amplification of the conserved region of their 18S rRNA (García et al., 2004). Several investigators (Ingham and Ryu, 1995; Vlaemynck, 1994; Beuchat, Nail, Brackett, & Fox, 1990) have made comparisons of a number of alternative yeast and mold detection methods in shredded cheese, hard and soft cheeses, cottage cheese, yogurt, and sour cream, and found that, while results for all of the methods were statistically similar, price, speed, and convenience of use are often overarching considerations when users choose a method. Rapid genomic subtyping methods, such as RAPD, RFLP, and AFLP, can be used to determine the sources of fungal contamination in a manufacturing environment (Lund et al., 2003).

Laleye et al. (1987) compared four plating media for recovery of spoilage lactococci from gassing cheeses and determined that MRS agar and APT agar gave the best results. For detection of *C. tyrobutyricum* in gassing cheeses, the classical method of most-probable-number testing in RCM-lactate or BBMB-lactate medium followed by confirmation on LATA or DRCM medium, and gas chromatographic analysis of volatile and nonvolatile organic acid by-products was determined to be both lengthy and difficult to perform (Bergere & Sivela, 1990). Herman et al. (1995) and Lopez-Enriquez, Rodriguez-Lazaro, and Hernandez (2007) have developed PCR-based detection methods that are reported to detect less than one spore of *C. tyrobutyricum* per milliliter of milk. Cocolin, Innocente, Biasutti, and Comi (2004) developed a PCR-denaturing gradient gel electrophoresis method that could detect 10^4 CFU of *Clostridium* spp. per milliliter in gassing cheeses.

Conclusion

While the introduction of pasteurization has helped to ensure the safety of dairy products, progress has been slower in preventing the microbial spoilage of cheese and dairy products. Worldwide standardized pasteurization practices would be an effective first step in eliminating or reducing the levels of many spoilage microorganisms. However, preventing postprocess contamination by spoilage microorganisms and retarding the growth of surviving organisms remain a challenge. Novel technologies and preservatives are needed to prevent the growth of spoilage microorganisms and extend the shelf life of dairy products. Limited applicability of current approved antimycotics such as sorbic acid and natamycin provides a major opportunity to expand the arsenal of preservatives available for today's dairy processor. In addition, studies to determine the interaction of current preservative technologies against spoilage microorganisms are also needed. Improved methods for detecting spoilage microbes, especially the slow-growing psychrotrophs and fungi, could assist in finding the niche environments in processing facilities that lead to

postprocess contamination. The next century will bring many challenges to the dairy processor, but maintaining the quality and shelf life of this highly nutritious food should not be one of them.

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Report originally adopted August 27, 2004. Viewed on October 18, 2007. Also available at: www.fsis.usda.gov/Regulations/National_Advisory_Committee_on_Microbiologcal/index.asp

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http://www.springer.com/978-1-4419-0825-4

Compendium of the Microbiological Spoilage of Foods and Beverages

Sperber, W.H.; Doyle, M.P. (Eds.)
2009, XII, 369 p., Hardcover
ISBN: 978-1-4419-0825-4