FRI BRIEFINGS

Safety of Processed Cheese

A Review of the Scientific Literature

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PASTEURIZED PROCESS CHEESE AND RELATED FOODS AND SPREADS

Background

Pasteurized process cheese and related foods and spreads are prepared by grinding and blending one or more varieties of cheese with an emulsifying agent and are pasteurized by heating to 150ºF (65.6ºC) for 30 seconds. Each process cheese category is defined by its finished product moisture, fat content, percentage of cheese, pH, and other optional ingredients. The standards also describe similar categories with added fruits, vegetables, or meats (21CFR 133, *FDA, 2001*).

Pasteurized process cheese has moisture limited to 1% higher than that of the natural cheese from which it is made and cannot exceed 43% moisture; fat must meet the same standards as for the natural cheese and it must contain at least 47% fat-dry-matter. Emulsifier levels are limited to 3 percent (w/w) phosphate- or citrate-based salts; optional ingredients include cream, anhydrous milkfat or dehydrated cream, acidifying agents to adjust the pH to 5.3 or higher, water, salt, artificial colors, mold inhibitors, enzyme-modified cheese, and lecithin as an antisticking agent.

Pasteurized process cheese food must contain at least 51% cheese, no more than 44% moisture, and ≥23% fat. Acids may be used to adjust the pH to 5.0. In addition to the optional ingredients permitted for process cheese, cheese foods may also contain other dairy products such as milk, whey, and high-moisture cheeses such as skim milk cheese.

Pasteurized process cheese spreads may have a moisture content between 44 and 60%, but must include at least 51% cheese and at least 20% milkfat. The lower pH limit is 4.0, but in practice the pH is usually 5.4 or higher. To maintain product functionality, flavor, and safety, optional ingredients may also include stabilizers, sweetening agents, and nisin.

Process cheese products or cheese sauce are pasteurized process products that do not comply with the above standards, such as products with <50% cheese, <20% fat, >60% moisture, or substantial levels of nondairy ingredients. Many high-moisture cheese sauces are subjected to high-temperature thermal processing.

Imitation cheese or process cheese analogs are nonstandard products that include nondairy proteins or fats, although some include milk derivatives such as casein and whey products. These products are frequently developed as low-cost alternatives for institutional use, for use in formulated foods, or as low-fat or vegetarian alternatives to real cheese.

Outbreaks of foodborne illness associated with process cheese products

Process cheese products have an excellent history of safety. A few outbreaks were associated with high pH or high water activity, highlighting the importance of formulation for process cheese products (*4, 39*). A single case of fatal botulism in California was associated with the consumption of a Liederkranz Brand canned cheese spread in 1951 (*50*). Few details are available about the formulation and conditions under which this product was produced and stored. The product label indicated that it was a pasteurized process soft ripened cheese spread with citric acid and vegetable gum added. Moisture and salt levels were not reported, but pH of the product was 5.9.

Two decades later in 1974, a cheese spread with onions was implicated in an outbreak in Argentina that resulted in six cases of botulism and three deaths (*4*). As with the Liederkranz product, this spread was not thermally processed to be commercially sterile nor was it formulated specifically for safety. Laboratory experiments revealed that botulinal toxin was produced in cheese spread samples with a similar formulation (pH 5.7, a_w 0.97) after 30 to 70 days' storage at 30ºC.

A third outbreak involving process cheese resulted in eight cases of botulism and one death in Georgia in 1993. The implicated cheese sauce was aseptically canned to eliminate *C. botulinum* spores; therefore, it was not formulated to prevent botulinal growth (*39*). The epidemiological investigation suggested that the product was likely recontaminated in the restaurant with *C. botulinum* spores and stored at room temperature for several days before use. Inoculation studies of the implicated cheese sauce (pH 5.8, a_w 0.96) revealed that botulinal toxin was produced after 8 days' storage at 22ºC.

Development of safe formulations for process cheese products

Episodes of spoilage of canned cheese spread used in Army field rations in the 1950s prompted Dack and co-workers at the Food Research Institute, University of Chicago, to conduct a series of experiments designed to determine factors that influenced growth of toxigenic and spoilage *Clostridium* in canned cheese spread (*44–48*).

By definition, pasteurized process cheese and related products are classified as low acid canned foods (LACF) because they have an equilibrated product pH >4.6 and water activity >0.85. Any

thermally processed low acid food that is packaged in hermetically sealed containers to prevent recontamination must be refrigerated or subjected to a process to render it commercially sterile. Commercial sterility ensures that no viable organisms can be detected by ordinary cultural methods in the food or that the surviving number of microorganisms is so low as to not be of public health significance (*54*). In practice, this translates to: (a) retort thermal processing for a prescribed time to reduce the incidence of botulinal spores to 10^{-12} or (b) formulation preservation techniques, such as acidification or reduction of water activity, to prevent outgrowth of spores.

The minimal heat process to inactivate 12-log *C. botulinum* botulinal spores throughout a food is approximately 2.5–3.0 minutes at 121ºC (*28*). However, temperatures greater than 90ºC significantly diminish the quality of process cheese by creating off flavors and can damage proteins in the cheese that act as natural emulsifiers (*3, 43*). As a result, the microbiological safety of process cheese has traditionally relied on thermal processing at 85–100ºC to destroy vegetative pathogens and on formulation to inhibit growth and toxin production by spore-forming pathogens.

To provide a practical and safe alternative to retort thermal processing, scientists at the Food Research Institute, University of Wisconsin–Madison, evaluated over 300 cheese spread formulations for the ability to support botulinal toxin production and developed a model to predict safety based on formulation (*35*). Statistical analysis of the data revealed that pH, moisture content, sodium chloride, and disodium phosphate (DSP) levels were the primary factors controlling botulinal toxin formation. Lactic acid was found to inhibit botulinal growth and toxin production beyond the effect that could be attributed to pH reduction. Antibotulinal effects of sodium chloride and DSP were similar and additive. The resulting predictive model was reported as a series of graphical representations to easily predict the microbial safety of formulated standard process cheese spreads (*Figure 1*).

To date, the model developed at the Food Research Institute is considered valid for evaluating the safety of standard-of-identity process cheese spread formulations produced with DSP as the emulsifier. However, the model is not applicable to products that use sodium citrate as the sole emulsifier or to reduced-fat and fat-free process cheese products, reduced-sodium products, or other nonstandard-of-identity process cheese products. Process cheese products that vary from the formulations and conditions outlined in the study by Tanaka must verify safety through botulinal challenge tests or by retort thermal process validation. Later researchers have developed models of microbial growth in process cheese (*36*) and other foods under different conditions (*7, 40*).

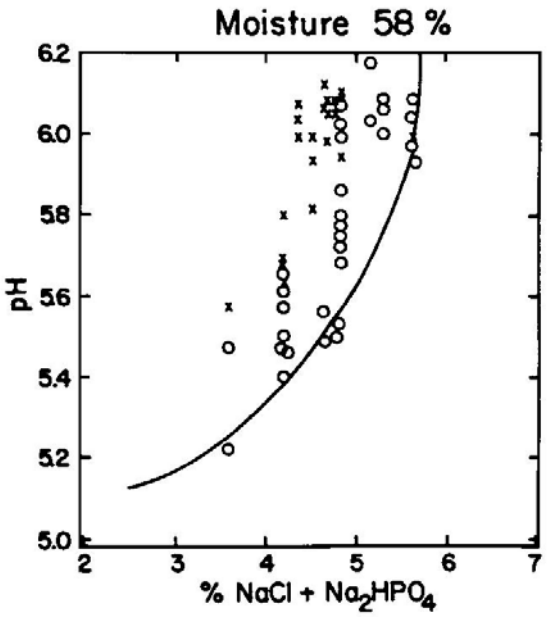

Figure 1. Predictive model (represented by curve on the graph) developed by Tanaka et al. (*35*) to predict the safety of process cheese spreads. Graph shown is for cheese spreads formulated for 58% moisture, pH range 5.0 to 6.2, and NaCl plus Na_2PO_4 levels of 2 to 7%. X represents a formulation that supported botulinal toxin production; O represents a formulation that was negative for toxin production during a 48-week incubation at 30ºC.

INGREDIENTS THAT AFFECT PRODUCT SAFETY

Effect of salt and water activity

Growth of Group I (proteolytic) *C. botulinum* is inhibited by a_w values <0.935 and Group II (non-proteolytic) *C. botulinum* is inhibited by a_w <0.97. This corresponds to 10% and 5% salt in the aqueous phase, respectively. Salt levels used in process cheese are generally 5–8%, resulting in water activities of 0.94–0.96 in standard commercial process cheese spreads and 0.91–0.93 in commercial process cheese slices. These a_w are generally significantly lower than those required to prevent proteolytic botulinal growth and

toxin production (*37*). When a_w of cheese spreads was at or below 0.944, no toxin was detected, whereas all formulations with a_w >0.957 supported toxin production (*35*). In the range between 0.944 and 0.957, botulinal safety was best predicted by the moisture, pH, NaCl and disodium phosphate (DSP) levels as specified by the model. In combination with other ingredients that depress a_w and other factors such as pH, temperature, and antimicrobials, salt levels are an important factor in controlling pathogen growth.

Potassium salts have been considered as alternatives to sodium to produce low sodium process cheese products. One study of potassium-based salts did not provide conclusive evidence on their efficacy (*20*). Although DSP appeared to impart somewhat greater antibotulinal activity than dipotassium phosphate (DKP), ionic strength was not considered. The product formulated with DKP had an a_w of 0.97 compared with 0.96 for that formulated with DSP. This likely decreased the botulinal safety of the DKP product.

Experiments at the Food Research Institute compared the antibotulinal activity of sodium- and potassium-based salts in reduced-fat process cheese products (5% fat). Empirically, the results suggested that safe reduced-fat process cheese products may be formulated with potassium salts as a partial replacement (up to 75% replacement calculated on a molar-basis) for sodium salts. However, the statistical significance of the effect of potassium salts on the development of botulinal toxin was considered inconclusive because of the relatively small number of toxic formulations in the experiment.

Effect of emulsifiers

In the U.S., sodium monophosphates (orthophosphates) are widely used as emulsifiers in process cheese spreads; citric acid salts and disodium phosphate (DSP) are also utilized. Orthophosphate and polyphosphate emulsifiers may inhibit growth and toxin production by *C. botulinum* by sequestering iron, magnesium, and calcium metal ions. Polyphosphates may also interact physically with bacterial cells by forming channels, increasing their permeability to inhibitory compounds, and promoting leakage and cell lysis.

Several studies have compared the antimicrobial effects of phosphate emulsifiers. Botulinal toxin production was delayed in media supplemented with 2.0% BekaPlus FS, HBS, and T_{new} compared with controls and with media supplemented with DSP or other polyphosphates (*13*). Trials in full-fat, high-moisture process cheese spread (56% moisture, pH 5.75) suggest that 2% HBS+S9 polyphosphates exhibited equivalent or slightly greater antibotulinal activity than DSP.

Process cheeses manufactured with two polyphosphates, S9 and S9H, supported less botulinal growth than those made with DSP (*8*). However, the equilibrated pH values of the products with polyphosphates were 0.15 to 0.35 pH units lower than those with DSP. Most polyphosphates hydrolyze over time to disodium orthophosphate (*49*); therefore, the pH of process cheese spreads containing polyphosphates may take as many as three days to equilibrate. Because pH has a significant effect on the inhibition of *C. botulinum* in process cheese, standardization of pH is essential to accurately assess equivalence in antibotulinal properties.

Polyphosphates HBS, HBS-1, and HBS-9 also demonstrated antimicrobial activity against *Clostridium tyrobutyricum*, which causes late-blowing defect in hard and semi-hard cheeses (*22*). Growth and gas-formation by *C. tyrobutyricum* were prevented by the addition of 0.5 to 1.0% polyphosphate to process cheese. Other studies also reported delayed gassy spoilage of process cheese blends by polyphosphates compared with orthophosphates, whereas sodium citrate emulsifiers provided the least safety (*38*).

Several studies reported that sodium citrate emulsifiers had less inhibitory effect on *C. botulinum* growth compared with phosphate-based emulsifiers (*20, 34, 37*). However, cheese spreads can be safely formulated with citrate-based emulsifiers provided the moisture, pH, and NaCl+DSP levels comply with the model described by Tanaka et al. (*35*).

Monolaurin is a monoglyceride that has antimicrobial effects and is used as an emulsifier. The minimum inhibitory concentration against *C. botulinum* varies from 40 ppm in media to 5000 ppm in turkey wiener and meat slurries. Lauricidin delayed botulinum toxin production for two weeks in process cheese products made with skim milk compared to a similar product without monolaurin (*9*). Lauri-Lac™, a blend of 0.2% lactic acid and 0.1% monolaurin, has also been proposed for use as an emulsifier and preservative in cheese sauce. Usage of Lauri-Lac™ substantially increased the shelf-life of a cheese sauce compared with usage of lactic acid or monolaurin alone. The shelf-life of a cheese sauce was increased from 10 days to 35 days when supplemented with 1% of the preservative combination.

Effect of fat

Research at FRI during the early 1990s revealed that botulinal toxin production is delayed in high moisture reduced-fat and fat-free formulations compared with full-fat products with similar levels of moisture, NaCl, emulsifier, and pH. For example, no botulinal toxin was detected during 56 weeks in process cheese products containing <1% or 5% fat, while botulinal toxin was detected in a similar full-fat (20% fat) product after only 4 weeks' storage at 27ºC. Statistical analysis indicated that fat level and changes to other ingredients had significant effects in delaying botulinal toxin production in process cheese products.

Further research at FRI demonstrated a significant reduction of the antibotulinal effects of nisin and the free fatty acids caprylic, capric, lauric, myristic, oleic, and linoleic in media with 20% fat. Lauric acid (C12) was the most active fatty acid against *Clostridium*. Fat also reduced the inhibitory effects of enzyme-modified cheese, potassium sorbate, sorbic acid, monolaurin and polyphosphate (*10, 12*).

Growth of anaerobic bacteria was delayed in reduced-fat process cheese products compared with higher fat products having the same moisture, salt, and pH (*37*). A mathematical model, based on anaerobic plate counts at different storage temperatures, pH, and concentrations of sodium chloride, emulsifiers, and lactate in moisture, was presented (*36*).

There have been several explanations for the apparent protective effects of fat. Fat may provide protected microenvironments for bacteria and may protect bacteria from antimicrobials in the water phase of a product. Reduced-fat and fat-free process cheese products often include ingredients to enhance the flavor, and some of these may reduce water activity or contribute antimicrobial free fatty acids, aldehydes, or peroxides.

A more likely mechanism for the reduced antibotulinal activity in full-fat process cheese products is that the lipophilic portion of antimicrobials may interact with the fat molecules rather than with the phospholipids in bacterial cell membranes, thereby decreasing their antimicrobial effects (*23*). Fat has been shown to decrease the efficacy of several antimicrobials, including monolaurin, nisin, sorbic acid, and the antioxidant BHA.

Reduced fat cheeses may also be less hospitable to many bacteria. Populations of *Listeria monocytogenes* and *Salmonella* were inactivated more rapidly in a 50% reduced-fat cheese produced with a carbohydrate-based fat-replacer than in full-fat cheese. In a similar study, a population of *Salmonella* decreased 5.16 $\log_{10}$ CFU/g in one-third reduced-fat Cheddar compared with 4.8 $\log_{10}$ CFU/g in full-fat Cheddar during a 20-week aging period. However, there was no difference in the inactivation of *L. monocytogenes* in this study (*24*).

Certain fatty acids, such as C_{10}, C_{12}, $C_{18:1}$, and $C_{18:2}$, have antimicrobial effects in media and in cheese. Since fatty acid profiles vary among different cheese types, substituting standard Cheddar cheese with another cheese type may affect microbial safety of process cheese (*41*). Feeding lactating cows diets supplemented with soybean or sunflower oil results in cheese with higher levels of oleic and linoleic acids than control cheese. This modified fatty acid profile correlated with decreased survival of *L. monocytogenes* and *Salmonella* compared with the control cheese (*29*).

Effect of enzyme-modified cheese and other flavor enhancers

Fatty acid profiles also differ among enzyme-modified cheeses (EMC) used as flavor enhancers. EMC added to process cheese products made with skim milk (<1% fat) significantly delayed botulinum toxin production. However, EMC was not an effective antibotulinal agent in reduced fat (13% fat) or full fat (24%) cheese products (*9*).

Botulinal toxin production in media was inhibited for at least 6 weeks at 30ºC by 10% soy-based flavor enhancer (Beta-Trim), 10% Parmesan EMC, and 5 and 10% Cheddar EMC. EMC derived from Blue Cheese did not inhibit botulinal growth and toxin production at 30ºC for one week (*11*).

Effect of nisin

Nisin is a compound produced by certain strains of *Lactococcus lactis*, which has demonstrated activity against certain gram-positive bacteria (*5, 6*). The United States Food and Drug Administration affirmed GRAS status for nisin in 1988 and approved its use as a preservative in process cheese spreads at a usage rate of 250 ppm nisin preparation (21CFR184.1538, *FDA, 2004*).

Nisin is water-soluble and can also bind to the phospholipids of the bacterial cell membrane and may generate ion channels, depleting a cell's proton motive force (*5*). Although spores are much more resistant to bacteriocins than vegetative cells, nisin has also been reported to inhibit spore germination at the pre-emergent swelling stage and to sensitize spores to heat so that thermal processing can be reduced (*6*).

Use of 100 to 250 ppm nisin preparation (4000 to 10,000 IU nisin/g) was required to prevent botulinal

growth and toxin production in cheese spreads formulated to 57% moisture, 1.2% added NaCl, and 1.4% DSP (*33*). Spreads formulated to 52% moisture, pH 5.9, and 2.5% DSP required only 12.5 ppm to inhibit toxin production. Both pH and food constituents affect the antibotulinal activity of nisin. Nisin is 228 times more soluble at pH 2 than at pH 8; therefore, its efficacy is greatly diminished in food products with neutral pH.

Cheddar cheese manufactured with nisin-producing lactococci contained up to 1200 IU nisin per gram of cheese. Cheese spreads made from this cheese and containing 300 to 390 IU nisin/g had a significantly longer shelf-life (when inoculated with *C. sporogenes* PA 3679) compared to control spreads without nisin. When incubated at 22ºC, 300 IU/g nisin delayed spoilage to 87 days in a 60% moisture product compared to 14 days for control product without nisin (*42*). Processing changes in reduced-fat and fat-free cheeses may affect bacteriocin production by wild or starter lactic acid bacteria. There may also be slower acid production during cheese manufacture by the bacteriocin-producing starter.

Effect of pH and acids

The minimum pH requirement for Group I *C. botulinum,* under otherwise ideal conditions, is 4.6 to 4.8 and for nonproteolytic *C. botulinum* is 5.0. However, resistance to acids depends on the type of acidulant. Many studies have reported *C. botulinum* growth at values of 4.4 or below in high-protein substrates acidified with HCl or citric acid. The efficacies of monocarboxylic acids as antimicrobials depend on their dissociation at a given pH. *In vitro* studies indicate that the inhibitory effects of acids (most to least) are acetic > lactic > citric or HCl (*53*).

Lactic acid has been demonstrated to prevent botulinal toxin formation beyond that attributed to pH reduction (*35*,*37*). Sodium and potassium lactate provide protection against toxin production without decreasing pH appreciably. The addition of 1.5% sodium lactate significantly delayed botulinal toxin production in high moisture, low salt process cheese products, compared to product without lactate (*12*). In a limited study, the inhibitory activity of sodium lactate empirically appeared to be equivalent to NaCl.

As with lactic acid, the inhibitory activity of sorbic acid is more than that associated with lowered pH. Sorbic acid is a short-chain unsaturated fatty acid with a water solubility of 0.16%. It is about three-fold more soluble in fat. Therefore, the amount of sorbic acid in the aqueous phase of a food system is lowered when the lipid content is increased. The degree of partitioning of sorbic acid between the water and fat phases of foods depends on pH, type and amount of fat, and other ingredients. Potassium sorbate is more soluble in water and has obvious advantages over sorbic acid in foods. The antimicrobial activity of sorbate is pH dependent and increases as the pH approaches its dissociation constant (*32*).

Sorbic acid may be added to process cheese in an amount not to exceed 0.2% (w/w). Numerous studies reported that 0.13–0.26% potassium sorbate delayed botulinal growth and toxin production in cured and uncured meat and poultry products. Although no published study has specifically evaluated the effect of sorbic acid in process cheese, data from the studies described above suggest that sorbic acid is part of the safety system; therefore, sorbate should not be removed from a formulation unless studies demonstrate it is safe to do so.

Effect of other additives

Several antimicrobials have been shown to inhibit botulinal growth and toxin production in foods but have not yet been approved for wide use in dairy products in the United States. Sodium and potassium nitrites are used in meat-curing for the development of color and flavor and for their antibotulinal properties (*9*). Although nitrite and nitrate are not approved for use in dairy products in the United States, nitrate is used in Europe to prevent late blowing of certain cheeses due to *Clostridium butyricum* and *C. tyro-butyricum* (*31*). The efficacy of nitrite is affected by a variety of factors. Early studies reported a phenomenon referred to as the Perigo factor that demonstrated that combining heat and nitrite increased the antibotulinal activity 10-fold compared with nitrite alone (*31*). As with many other preservatives, the additional hurdles of salt and acidic pH act synergistically with nitrite to provide antibotulinal activity.

Lysozyme, an enzyme present in milk, eggs, tears and other secretions, is most active against gram-positive bacteria by degrading the cell wall. Certain strains of *C. botulinum* retained their refractile properties when treated with lysozyme alone, but EDTA enhanced its activity and lysed the bacteria. As with nitrite, lysozyme is used to prevent gas formation of Edam and Gouda by *C. tyrobutyricum* (*16*, *17*). However, reports that lysozyme enhanced the recovery of nonproteolytic *C. botulinum* in heated foods indicate that lysozyme should be used as an antimicrobial only after verifying safety (*14*, *15*).

Sodium diacetate, commonly referred to as dry vinegar, has been shown to inhibit growth of *Listeria*

monocytogenes in meats and has been approved for use at 0.3% in processed poultry and meat products (*9*). Few studies have evaluated the anticlostridial effect of sodium diacetate in foods (*51, 52*) and no work has been published on dairy products. Although its usage is limited because of its impact on flavor, additional studies are warranted to evaluate its antibotulinal properties in other foods.

Effect of Maillard reaction products: Maillard reaction products include brown melanoidins as well as compounds with antioxidant and antimicrobial properties. Water-soluble, unfractionated Maillard reaction products have been shown to inhibit the enzymatic activity of trypsin, and it has been suggested that they may prevent or reduce formation of active botulinal toxin. Late Maillard reaction products obtained from a mixture of glucose and glycine inhibited growth of *Aeromonas hydrophila* but did not inhibit growth of *Staphylococcus aureus*, *Listeria monocytogenes*, or *Salmonella* Typhimurium. One study suggested that synthetic melanoidins can enhance the *in vitro* growth of clostridia isolated from human gastrointestinal tracts (*1*). No study specifically evaluating the effect of Maillard reaction products on the growth and toxin production of *Clostridium botulinum* has been described.

Botulinal safety of process cheese analogs: Eleven imitation cheeses were obtained from commercial sources, cold-inoculated with botulinal spores, repackaged, and incubated at 26°C (*21*). Products represented included those manufactured into loaves, slices, spreads, and shredded. The water activities ranged between 0.942 to 0.973 and pH values ranged from 5.53 to 6.14. Only the Mozzarella-substitute loaf product, having pH 5.86 and the highest water activity of 0.973, supported toxin production within 4 weeks' storage. All other products having water activities less than 0.961 did not support toxin production. However, mold growth or gas production was observed for many of the other products.

CONTAMINATING BACTERIA OF CONCERN

A comprehensive review of the microbiological safety of cheese considered many pathogens that might be associated with raw milk and cheese and their susceptibility to thermal treatments and acidity (*53*).

Pathogenic sporeforeformers

Clostridium botulinum

Spores of *C. botulinum* are widespread in the environment and can contaminate dairy products. Reported minimum temperature and pH for growth are 5.0ºC and pH 4.7 (*53*).

Other clostridia

Clostridium butyricum, producing botulinum toxin type E, has been implicated in cases of foodborne botulism in China, India, and Italy. Lowest temperature and pH that allowed growth of this bacterium in pesto and soft cheese (mascarpone) was 12ºC and pH 4.8. Toxin production was observed after 5 days at 25ºC (*2*).

Clostridium perfringens is widespread in the environment and can contaminate dairy products. Spores have been reported in raw milk and cheese but there have been few reports of illness associated with this bacterium in milk (*53*). Reported minimum temperature and pH for growth are 15ºC and pH 5.0.

Bacillus spp.

Spores of *B. cereus* are widespread in the environment and can contaminate dairy products (*53*). Reported minimum temperature and pH for growth are 4.0ºC and pH 5.0. Recent tests using skim milk demonstrated that spores of both *B. anthracis* and *B. cereus* were highly resistant to pasteurization temperatures from 72–150ºC (90–0.5 min) (*26*).

A recent review presents up-to-date information on food poisoning associated with *B. cereus* (*30*).

Post-pasteurization pathogenic contaminants

Listeria monocytogenes

L. monocytogenes is a widespread environmental contaminant and has been detected in raw milk with a greater frequency during cold weather months. *L. monocytogenes* is present in cheese factories even when good sanitation and hygiene are practiced. It frequently contaminates drains and surfaces and may be present in cooling systems. Reported minimum temperature and pH for growth are 1.0ºC and pH 4.8 (*53*). Surveys for *L. monocytogenes* in cheese plants in Switzerland yielded about 5% positive samples, with more frequent positives detected in cheese ripening plants (7.5%). The highest proportion of positive samples was for water used for washing cheese (9.5%) (*27*).

Populations of *L. monocytogenes* on pasteurized process cheese slices decreased during storage at 30ºC for 4 days (*13*).

Salmonella spp.

Salmonella spp. have often been detected in raw milk but are not often detected in cheese. At 65ºC, it takes 19–43 seconds to kill *Salmonella* in milk depending on species. Reported minimum temperature and pH for growth are 6.5ºC and pH 4.5 (*53*).

Populations of *Salmonella* spp. on pasteurized process cheese slices decreased during storage at 30ºC for 4 days (*13*).

Staphylococcus aureus

Toxigenic staphylococci are present in raw milk but do not survive pasteurization. However, their enterotoxins are not destroyed by pasteurization. At 65ºC, it takes 63 seconds to kill *S. aureus* in milk. Reported minimum temperature and pH for growth are 7.0ºC and pH 4.0 (*53*). *S. aureus* is often present on human skin and in nasal passages and is potentially a post-processing contaminant. Conditions required for growth are temperature of 7–46ºC, pH 5.2–9.0, and a_w >0.86. *S. aureus* can grow at 15–20% NaCl but is generally not a good competitor under conditions that allow growth of other bacteria.

Pasteurized process cheese slices inoculated with *S. aureus* and stored for 4 days at 30ºC allowed survival but not rapid growth of these bacteria (*13*). Sorbate (0.1%) does inhibit growth of this bacterium in process cheese slices.

E. coli O157:H7

Although *E. coli* O157:H7 is more often associated with meat, pathogenic *E. coli* have been detected in raw milk. This bacterium is not as widespread in the environment as some other pathogens and may be less likely to be a post-processing contaminant. At 65ºC, it takes 18 seconds to kill *E. coli* in milk. Reported minimum temperature and pH for growth are 2.5ºC and pH 4.6 (*53*).

Populations of *E. coli* O157:H7 on pasteurized process cheese slices decreased during storage at 30ºC for 4 days (*13*).

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