ARTICLES

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# Factors That Contribute to the Microbial Safety of Commercial Yogurt

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## SUMMARY

Yogurt with active cultures, at pH of 4.6 or below, and processed in compliance with Good Manufacturing Practices prescribed by the United States Pasteurized Milk Ordinance, is inherently safe and does not support the growth of pathogenic organisms. More specifically, the safety of commercial yogurt is primarily dependent on the use of pasteurized milk to eliminate vegetative bacterial pathogens and spoilage microorganisms, good manufacturing practices and sanitary operating procedures to reduce the potential for recontamination, and a robust fermentation to produce sufficient acid and other antimicrobial metabolites to prevent growth of pathogens, should recontamination occur. High numbers of live and active starter culture organisms assure safety by generating acid and other antimicrobial metabolites during a short fermentation, preventing growth or causing death of pathogens. Chilling of the acid food to < 7°C within four hours after coagulating the milk (pH ~ 4.6) serves to reduce additional acid production and thus to prevent adverse flavor defects, control spoilage, and enhance quality. Data described in this review support the safety of current US industry practices for the production of commercial yogurt when pH values of the finished product is < 4.6 within 24 hours of filling.

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## MANUFACTURING PRACTICES ENSURING THE SAFETY OF YOGURT

Standard commercial yogurt produced in the United States (defined in 21CFR 131.200, 131.203 and 131.206; *62)* is inherently safe because of a number of contributing factors. United States regulations require use of pasteurized milk in yogurt production. Current industry practices typically exceed minimum thermal requirements by pasteurizing to 91°C for 40 to 60 seconds (HTST) or to 85°C for 30 minutes (vat). Heating milk and milk mixes to a high temperature denatures the whey proteins, which improves body and ensures destruction of indigenous thermotolerant microflora that may interfere with the rapid growth and acid production of the starter bacteria *(31, 61, 64)*. Pasteurized homogenized milk or milk mix, and any stabilizers or sweeteners, are then cooled to 42 to 45°C in closed vats before concentrated starter culture is added to yield approximately 6 to 7-log CFU/ml or greater of each *Streptococcus thermophilus* (ST) and *Lactobacillus bulgaricus* (LB) culture. Product mixture may then be filled immediately for cup-set yogurt or vat-fermented before filling for blended-style. During the fermentation, regardless of product packaging, the population of yogurt starter culture increases 100- to 10,000 fold to a final concentration of approximately 9

log CFU/ml and generates lactic acid from the metabolism of lactose. The associated pH reduction causes a destabilization of the micellar casein at a pH of 5.1 – 5.2, with complete coagulation occurring around pH 4.6. At the desired final pH, the coagulated milk is cooled to 4–10°C to slow down the fermentation and retard further acid development. Cultures will continue to metabolize and produce acid after the yogurt is chilled to 7°C or less, although at a slower rate than in yogurt held at elevated temperatures *(35)*.

## EFFECT OF SYNERGISTIC GROWTH OF ACTIVE STARTERS

Lactic acid bacteria starter cultures have long been used to ensure the safety of fermented foods because of their ability to compete with pathogens for nutrients, rapidly produce lactic and other acids to reduce pH, and generate other antimicrobial compounds such as acetaldehyde, diacetyl, hydrogen peroxide, and bacteriocins. If a food substrate is contaminated with high levels of pathogenic bacteria prior to fermentation, such as through cross contamination with raw ingredients, certain pathogens may initially be able to compete with the starter and grow. However, they will be inhibited or die when the level of lactic acid is sufficient to achieve a pH of 4.8 or less *(2, 8, 51)*.

On the other hand, certain factors such as bacteriophages, illegal antibiotic residues, or salt content of 1% or greater inhibit the starter culture activity essential for production of fermented or cultured foods. If starter culture metabolism and the rate of lactic acid production is eliminated or significantly reduced, the resulting environment could permit pathogen growth and toxin production in recontaminated, unfermented substrate stored at ambient temperatures (see reviews *40, 57, 66)*.

In the case of commercial yogurt, high numbers of live and active ST and LB cultures assure safety through generation of acid and other antimicrobial metabolites during a short (typically 3 to 6 hours) fermentation at 42 to 45°C, thereby preventing growth or causing death of numerous pathogens. Chilling of the acid food to < 7°C after coagulation of the milk (pH ~4.6) serves to diminish adverse flavor defects by reducing excessive acid production. However, rapid cooling (within 4 hours) does not appear to provide any safety advantage over the slow cooling (within 96 hours) currently practiced by US manufacturers because the higher levels of lactic acid production associated with extended fermentation provide an additional barrier to microbial growth.

Numerous studies have demonstrated that symbiotic growth of ST and LB results in greater acid production than when either strain is used individually *(29, 35)*. Both thermophilic bacteria generate lactic acid by fermenting lactose. LB specifically demonstrates mild proteolytic activity in milk and is primarily responsible for production of flavor and aroma components (acetaldehyde, acetone, acetoin, and diacetyl) *(6)*. During the early stages of fermentation, the amino acids released by the proteolysis of casein stimulate growth of ST. The coccus begins to grow faster than the rod and is responsible for the primary acid production. ST utilizes excess oxygen and produces carbon dioxide and formic acid, which in turn stimulates growth of LB. As the acid concentration increases and the pH decreases from 4.4 to 4.2, ST growth is inhibited, but the lactobacilli continue to grow and produce acid until the substrate reaches pH 3.5 to 3.8.

The synergistic growth of ST and LB is important not only to0 the physical, chemical, and sensory characteristics of yogurt, but also to its safety. Dineen et al. reported that *E. coli* O157:H7 was more sensitive to the inhibitory properties exerted by *L. bulgaricus* than to those of *S. thermophilus* but that co-culture of ST-LB reduced populations of *E. coli* O157:H7 more than either culture used alone *(15)*.

## EFFECT OF ACIDITY

Although the use of Good Manufacturing Practices (GMPs) and proper processing are integral to food safety, the acidity of yogurt is a significant factor in inhibiting and inactivating bacterial pathogens should the product be inadvertently recontaminated and stored at non-refrigerator temperatures. Pathogens can survive in yogurt for extended periods if post-fermentation contamination occurs, regardless of storage temperature (see results from Challenge Studies section, following). However, as total acidity increases, survival time decreases *(38)*.

Low pH, by itself, decreases the activity of bacterial enzymes and transport systems. Other factors, such as type of acid and total acidity as well as buffering capacity of the substrate, are also pertinent to bacterial survival and growth capabilities *(10, 29)*. In addition, the lag phase for a microorganism increases if the pH of the substrate is outside the range of optimal growth pH *(29)*. For example, the minimum pH for growth in laboratory media under otherwise ideal conditions for *S. aureus* is 4.0 to 4.3 when inorganic acids are used; range is much higher (pH 4.9 to 5.1) with use of organic acids such as lactic or acetic acid *(27, 37, 39, 57)*. In acidified pasteurized milk stored at 37°C for 24 hours, populations of *S. aureus* decreased > 2 logs in milk acidified to pH 4.5 with lactic acid, but grew > 2.5 log in milk acidified with HCl to the same pH value *(58)*. The pH requirements are more stringent for toxin production than for growth, with the minimum pH for staphylococcal enterotoxin production reported to be 5.1 *(53)*. Enterotoxin production, like growth, is inhibited more effectively when the pH is reduced by lactic acid rather than by hydrochloric acid *(42)*.

Spores of pathogens such as *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens* survive pasteurization. However, they are unlikely to grow at pH < 4.8 when stored at or below typical room temperatures. The USDA-ARS Pathogen Modeling Program 7.0 predicts probability <0.01 for growth of *C. botulinum* at 20°C through 29 days in laboratory media acidified to pH 4.8 (*60)*. Minimum pH for growth of common *B. cereus* strains is 4.8 in media acidified with HCl or 5.6 in media acidified with lactic acid; the organism is reported to die suddenly in yogurt when the pH reaches 4.5 *(27, 42)*. *C. perfringens* growth is limited at pH 5.5, and the organism is reported to be inactivated after several days at pH 5.0 *(27, 42)*.

## EFFECT OF OTHER METABOLITES

In addition to generating lactic acid, the two primary starter cultures required by the Standard of Identity for yogurt (21 CFR 131.200, 131.203 and 131.206, *S. thermophilus* and *L. bulgaricus*) are known to generate other

antimicrobial metabolites *(62)*. Gilliland and Speck demonstrated that lactic streptococci reduced growth of *Salmonella* and *S. aureus* in milk, even when the pH was maintained at pH 6.6 during starter growth *(20)*. These researchers, as well as many others, have reported that metabolites such as hydrogen peroxide, bacteriocins, acetaldehyde, and diacetyl, are antagonistic to bacterial pathogens and spoilage microorganisms *(1, 4, 13, 14, 20, 28, 30)*.

Hydrogen peroxide is a toxic reduction product of molecular oxygen, which inhibits *S. aureus* and other pathogens *(2, 13, 14*; see review *19)*. Diacetyl inhibited growth of *E. coli* O157:H7 and *Salmonella* Typhimurium when added to laboratory media at a concentration of 50 ppm *(30)*. Acetaldehyde (at 500–1000 ppm) has been shown to be inhibitory to other lactic acid starter or probiotic bacteria *(66)*. Levels of these compounds produced by LB cultured alone are lower than those typically considered sufficient for antimicrobial activity individually *(32)*. However, studies have shown that when LB is co-cultured with ST in yogurt, 1400–1700 ppm acetaldehyde and 165–200 ppm diacetyl can be produced *(7)*.

Based on a study that neutralized hydrogen peroxide and acid produced by ST-LB cultures in yogurt, Attaie et al. suggested that bacteriocin or other antimicrobial production by ST and LB may also contribute to the inhibition of *S. aureus (2)*. Numerous bacteriocins that are effective against pathogenic and spoilage bacteria are produced by ST and LB, as well as by adjunct starters *(4)*. Most are active at the low pH values associated with yogurt *(10)*. Several strains of ST produce the bacteriocin thermophilin, which has activity against Gram-positive bacteria such as *L. monocytogenes* and *Clostridium tyrobutyricum (36, 65)*. Adjunct lactic acid bacteria, such as *L. acidophilus*, have been shown to produce the bacteriocin acidophilin, which inhibits *S. aureus*, *E. coli*, *Pseudomonas fluorescens*, and *P. fragi (2, 54, 55)*. Other research demonstrates greater kill of *S. aureus* and *L. monocytogenes* during yogurt fermentation and storage at 4 or 22°C when a bacteriocin-producing ST was used, rather than a starter that did not produce bacteriocins *(5*; N. Benkerroum, personal communication, April 4, 2005).

## INTERNATIONAL OUTBREAKS ASSOCIATED WITH CONTAMINATED YOGURT

To date, no recognized foodborne disease outbreaks have been associated with yogurt in the United States. The enviable record of safety is due primarily to the consistent use of multiple safeguards, including proper GMPs (production in a sanitary environment, use of safe and suitable ingredients such as pasteurized milk) and use of active starter cultures for essential acid development. In contrast, each of the outbreaks associated with contaminated yogurt that have been reported in other countries in the past two decades were associated with improper processing, contamination with raw milk, and/or inadequate acid production *(9, 12, 41, 42, 44, 63)*.

In the United Kingdom, 27 cases of botulism, including 1 death, were associated with the consumption of yogurt that contained insufficient thermally-processed hazelnut puree. Although yogurt itself had been manufactured properly, the preformed botulinum toxin in the contaminated hazelnut puree was stable at the low pH of the product during refrigerated storage *(12, 44)*. Investigations into several outbreaks of *Salmonella* and *E. coli* O157:H7 in the UK, Scotland, and British Columbia similarly revealed violations of good manufacturing practices. Improper practices included using a single pump for transferring raw milk and distributing pasteurized milk for fermentation without intermediate disinfection, failure to record time/temperature for pasteurization, and overall poor hygienic practices *(9, 17, 41, 63)*. Two outbreaks of staphylococcal enterotoxin poisoning, resulting in a total of 47 cases, were reported to New Zealand authorities and linked to yogurt made in institutional kitchens *(42)*. Both outbreaks were attributed to contamination of food by handlers and to slow growth of yogurt starter culture due to fermentation at room temperature (approximately 25°C) rather than at the prescribed 42 to 45°C necessary for rapid acid development (R. Whyte, Institute of Environmental Science & Research Limited, New Zealand, personal communication, April 4, 2005).

## VERIFICATION OF YOGURT SAFETY BY CHALLENGE STUDIES

Numerous studies have evaluated the survival of pathogens during production and storage of yogurt; however, all conditions have not been tested for each pathogen. Table 1 summarizes inactivation or survival rates for pathogens in yogurt at various temperatures from representative studies. In all reported studies, pathogens died in yogurt with pH ≤ 4.6, in contrast to the growth predicted at pH 4.6 with use of the USDA-ARS Pathogen Modeling Program 7.0 *(60)*. As described above, the enhanced inhibitory properties of yogurt compared with laboratory media are due to several factors: lactic acid as the predominant acid, generation of antimicrobial metabolites, and active competition of the starter cultures with pathogens for nutrients.

Few pathogenic bacteria are able to survive extended periods in the harsh, acidic environment of yogurt. Although the pH of commercial yogurt is generally less than 4.4, some unusual varieties may have pH values that exceed 4.6. Data from multiple challenge studies suggest that if the pH is < 4.6 within 24 hours of the beginning of fermentation, the probability of pathogen growth in yogurt at the nonstandard pH values is very low.

The enteric pathogen *E. coli* O157:H7 is noted to be particularly acid resistant *(16)* and therefore would pose the greatest risk of extended survival in yogurt. Factors that would control growth or survival of *E. coli* O157:H7 should be sufficient to ensure the overall safety of these products with regard to other pathogens. Any potential risks associated with *E. coli* O157:H7 can be mitigated by standard pasteurization of raw ingredients to eliminate the pathogens and good manufacturing practices to prevent any recontamination of the milk *(9, 41)*. However, numerous research studies have demonstrated that, should the product be inadvertently contaminated, fermentation by the ST-LB culture and prolonged exposure to the high-acid environment (pH ≤ 4.6) provide an additional hurdle to inactivate pathogens, especially when the product is stored at non-refrigeration temperatures. Research conducted using other acidic foods, such as apple cider and

**TABLE 1. Pathogen inactivation in yogurt, minimum pH values for pathogen growth, and predicted growth potential in laboratory media adjusted with HCl to pH 4.6 and with no competitive microflora**

| | Modeled time to 1-log increase to pH 4.6 laboratory media at 20°C[a] | Yogurt storage temperature | Initial pH | Log reduction (time) in pH < 4.6[b] yogurt | Ref. |
|---|---|---|---|---|---|
| *L. monocytogenes* | 2.85 days | 4°C | 4.54 | 4 (12 wk) | *52* |
| | | 4°C | 4.44 | 4 (3 wk) | *52* |
| | | 4°C | 4.19 | 3 (2 wk) | *52* |
| | | 4°C | 4.02 | 2 (1 wk) | *52* |
| | | 8°C | 4.35 to 4.52 | 2 to 3.5 (28 d) | *59* |
| | | 4°C | 3.8 to 4.1 | 4 (13 to 27 d) | *11* |
| *S. aureus* | 3.18 days | 7°C | 4.3 | 2 (10 d) | *5* |
| | | 7°C | 3.7 to 4.1 | 2 to 3 (1 d) | *38* |
| | | 23°C | 3.7 to 4.1 | 2 to 3 (1 d) | *38* |
| *Salmonella* | not modeled | 42°C | 4.54 | 3 (6 h) | *49* |
| | | 37°C | 3.85 | 6 (1 h) | *49* |
| *E. coli* O157:H7 | 0.88 days | 4°C | 4.65 | 0.8 (7 d) | *25* |
| | | 4°C | 4.65 | >3 (35 d) | *25* |
| | | 12°C | 4.65 | 1.0 (7 d) | *25* |
| | | 12°C | 4.65 | >3 (35 d) | *25* |
| | | 4°C | 4.47 | 4 (16 d) | *26* |
| | | 10°C | 4.47 | 4 (13 d) | *26* |
| | | 4°C | 4.4 to 4.5 | 1 to 2 (7 d) | *34* |
| | | 4°C | 4.39 | 6 (17 d) | *26* |
| | | 10°C | 4.39 | 6 (15 d) | *26* |
| | | 4°C | 4.2 | 1 (5 d) | *43* |
| | | 25°C | 4.2 | 5 (48 h) | *43* |
| | | 4°C | 4.17 | 6 (8 d) | *26* |
| | | 10°C | 4.17 | 6 (5 d) | *26* |
| | | 4°C | 4.1 | 0.8 (72 h) | *3* |
| | | 8°C | 4.1 | 2.7 (72 h) | *3* |
| | | 17°C | 4.1 | 3 (72 h) | *3* |
| | | 22°C | 4.1 | 4 (74 h) | *3* |

[a]Based on Pathogen Modeling Program 7.0 *(60)*

[b]Based on yogurt fermented at 42°C with standard ST-LB starter cultures

mayonnaise, further supports the contention that *E. coli* O157:H7 demonstrates lower tolerance of acid conditions when held at ambient temperatures than when refrigerated *(7, 67)*. Multiple challenge studies in yogurt confirm that acid content and temperature both have effects on pathogen survival.

Hudson et al. suggested that survival of *E. coli* O157:H7 in commercial yogurt with live cultures was dependent on both pH and storage temperature *(26)*. Shorter survival times were reported in yogurt with initial pH of 4.17 than yogurt at pH 4.39 or 4.47. Similarly, at any given pH, pathogen viability was lower in yogurt stored at 10°C than at 4°C. Populations of *E. coli* O157:H7 decreased 6 log units, to undetectable levels, within 5 and 8 days at 10 and 4°C respectively for yogurt with pH 4.17, within 7 and 15 days at 10 and 4°C respectively for yogurt with pH 4.39, and within 17 days at both 10 and 4°C for yogurt with pH 4.47. Similar trends were observed for strawberry-flavored full-fat and nonfat yogurt *(21)*. Populations of *E. coli* O157:H7 decreased by > 2.5 and 1 log CFU/g after two weeks in nonfat and full-fat yogurt, respectively, when cooled slowly from 27 to 7°C over 96 hours and then held at 7°C. The pathogen was more stable in products stored at a constant 7°C, with approximately 0.7 log decrease for both yogurt types at the end of the two-week testing interval. The pH values of the products decreased from an initial 4.4 to 4.2 when stored at a constant 7°C, whereas the products that were cooled slowly had final pH values of approximately 4.1 due to extended acid production.

Govaris et al. *(22)* inoculated milk with ST-LB starter culture and 4.8 log *E. coli* O157:H7 prior to preparation of set yogurt *(22)*. Products were fermented

at 42°C for 3 hours to coagulate the milk and then stored at 4 or 12°C. Populations of *E. coli* O157:H7 decreased approximately 1 log unit during the fermentation to pH 4.4 and to undetectable levels in yogurt after 5 and 7 days storage at 12 and 4°C, respectively.

Bachrouri et al. *(3)* similarly observed accelerated inactivation at higher storage temperatures. The researchers inoculated finished, retail, plain yogurt (with live ST-LB cultures; initial pH 4.1) with > 4 log CFU *E. coli* O157:H7 per g yogurt and stored the product at 4, 8, 17, and 22°C *(3)*. Populations of *E. coli* O157:H7 decreased 0.8 and 2.7 log in yogurt stored 72 hours at 4 and 8°C, respectively. Storage at ambient temperatures increased the death rate, yielding a 3 and 4 log decrease in yogurt stored at 17 and 22°C, respectively.

Ogwara et al. *(43)* compared the behavior of *E. coli* O157:H7 in African yogurt and in recontaminated milk fermented at 43, 37, 30, and 25°C, and then stored at 4 or 25°C *(43)*. Data revealed that in spite of the recontamination, *E. coli* O157:H7 did not grow in milk rapidly fermented at 43°C (final pH 4.0 at 24 h), but did increase in recontaminated milk during slow fermentation at the lower temperatures (final pH at 24 h was 5.1, 4.6, and 4.4, for 25, 30, and 37°C, respectively). In yogurt stored at 4°C, populations of pathogen decreased approximately 8 and 2 log CFU/g for product fermented at 43 and 25°C, respectively. In all fermented milk samples stored at 25°C, no viable *E. coli* O157:H7 were recovered after 5 days regardless of fermentation temperature.

Guyara and collaborators reported a > 3 log reduction of *E. coli* O157:H7 in inoculated retail yogurt (pH 4.2 or lower) stored at either 4 or 12°C for 7 days *(25)*. For pH 4.65 yogurt, populations of the pathogen declined 0.8 and 0.1 log when stored at 4 and 12°C, respectively, for 7 days. At day 35, a >3 log reduction was observed regardless of storage temperature.

The effect of the adjunct culture, *Bifidiobacterium bifidum*, used in addition to the standard ST-LB cultures was evaluated by co-inoculating high and low levels of *E. coli* O157:H7 with yogurt starters in pasteurized milk *(34)*. Product was fermented at 42°C for 5 hours until the pH was 5.1–5.2, and then stored at 4°C for 7 days. As seen with traditional yogurt, the pH continued to decrease during refrigerated storage to achieve a final pH 4.5–4.6; a concomitant decrease in viable *E. coli* O157:H7 was observed. No significant difference was observed between the traditional yogurt and the bifido yogurt, but continued acid production and pH decrease were deemed important in reducing pathogen populations.

Dineen et al. *(15)* demonstrated that populations of *E. coli* O157:H7 decreased from 2 log CFU/g to less than detectable levels in three brands of retail low-fat yogurt during storage for 6 to 14 days at 4°C. The acidity remained constant during the 2-week refrigerated storage with pH values of 4.0, 4.0, and 4.2 for the varieties made with ST-LB only, ST-LB with *L. acidophilus*, and ST-LB with *L. acidophilus* and *L. bifidus*, respectively. These data suggest that survival of this pathogen is diminished in an acidic environment, even at refrigeration temperatures.

Survival of *E. coli* O157:H7 in yogurt has been shown to be influenced by the presence of colanic acid (CA), which is polysaccharide slime on the surface of the bacterial cell that increases the pathogen's resistance to acid *(33)*. Wild-type cells with CA demonstrate the longer survival in yogurt (initial pH 4.7) stored at 15°C than at 4°C, whereas there was little difference in survival in mutant strains without CA. However, *E. coli* O157:H7 declined in all treatments during the 3-week storage period.

*Salmonella* Typhimurium grows in laboratory media acidified to pH 4.4 with lactic acid, but is inactivated in cultured skim milk with the same pH value *(45)*. In spite of the potential to tolerate extreme pH values, challenge studies reveal that *Salmonella* will not grow during early stages of yogurt production and will be inactivated during extended fermentation *(49)*. Populations of *Salmonella* Typhimurium remained constant during the first 4 hours of fermentation in the presence of ST-LB culture as the pH decreased from 6.25 to 4.54 in plain yogurt (0.34% lactic acid). *Salmonella* died rapidly thereafter, decreasing > 3 log CFU/g to undetectable levels during the next 3 hours at 42°C as the pH continued to decline to 4.15. Other research noted bactericidal activity when lactic acid reduced the pH of the environment to 4.5, causing the internal pH of the cell to be reduced to 5.3 and causing cell death *(48)*.

A study evaluating the survival of several serotypes of *Salmonella* in Egyptian yogurt demonstrated that *Salmonella* Typhimurium was the serotype most resistant to adverse pH conditions *(18)*. As reported for many of the *E. coli* O157:H7 studies, *Salmonella* survival was lower when yogurt was stored at elevated temperatures (30–32°C) than at refrigeration temperatures (4°C). The pathogen was inactivated to less than detectable levels at 16 and 23 days (final pH 4.5) or 11 and 19 days (final pH 4.0) when stored at 4°C and room temperature, respectively.

The behavior of Gram-positive bacteria, including spore-formers, which can survive pasteurization, is similar to that of the enteric pathogens in the presence of extreme acid conditions. While pathogens may be able to survive or grow in laboratory media with pH adjusted to < 4.8 under otherwise optimal conditions, few can grow or produce toxin in acidic foods such as yogurt.

No data for challenge studies evaluating the behavior of sporeforming pathogens have been published. However, the safety of yogurt related to these hazards can be predicted based on "worse-case scenarios" reported for growth in laboratory media. The addition of competitive microflora (starter cultures) will further inhibit growth or toxin production by these pathogens. *B. cereus* generally does not grow at pH 4.8 in media adjusted with HCl, or at pH 5.6 when lactic acid is used as the acidulant *(27)*. The pathogen has been reported to be inactivated by 0.1 M acetic, formic and lactic acids in nutrient broth and will die suddenly in yogurt when the pH reaches 4.5 *(42)*. The minimum pH for growth for Group I (proteolytic) *Clostridium botulinum* is considered to be 4.6; however, growth would be slow *(27)*. Outgrowth of Group II (nonproteolytic) spores, which are also able to grow at refrigeration temperatures, are prevented at pH 5.0 or lower. *C. perfringens* growth is slight at pH 5.5, and vegetative cells will die at pH 5.0.

More extensive research has been completed that studies the fate of *S. aureus* and *L. monocytogenes* in yogurt and acidified dairy products. The lag phase of *S. aureus* at 27°C is over 25 hours and generation time is 2 hours in laboratory

media adjusted to pH 4.5 with HCl *(60)*. If the pH of the substrate is less than 4.4, *S. aureus* will die at both refrigeration (7°C) and ambient temperatures (23°C) *(39)*. Neither growth nor toxin formation was detected in milk acidified to pH 4.5 with lactic acid *(58)*, but additional reports suggest that growth is slight in milk acidified to pH 5.1 to 5.2 *(37)*. The minimal pH for enterotoxin production is more stringent than that required for multiplication and is generally limited to values greater than 5.1 *(37, 53, 57, 58)*.

*S. aureus* is noted for being a poor competitor. However, staphylococcal food intoxications are possible if a food is recontaminated, if acid development by starters is inadequate, and if inhibitory pH is not reached quickly *(40)*. Although acid production is important in preventing staphylococcal growth, Reiter et al. *(47)* reported that even when lactic acid in milk was neutralized, lactic acid bacteria starter culture still retained inhibitory activity against *S. aureus*. If starter activity was poor because of bacteriophage infection, the pathogen was able to multiply. For this reason, hygienic manufacturing practices are essential to prevent recontamination, and starter activity should be monitored to verify proper fermentation.

Several published studies provide evidence demonstrating the control of *S. aureus* in properly fermented yogurt *(2, 5, 38)*. For example, when *S. aureus* was added as a post-fermentation contaminant in retail yogurt (pH 3.7 to 4.1), populations of *S. aureus* decreased by > 3 log within 1 day, regardless of whether it was stored at 7 or 23°C *(38)*. In another study, yogurt was produced in the laboratory by co-inoculating milk with *S. aureus*, *S. thermophilus*, *L. bulgaricus*, and *L. acidophilus (2)*. *S. aureus* grew approximately 1.5 log during the first 4 hours of fermentation until the pH reached 4.8. After the yogurt had reached pH 4.8, populations of *S. aureus* decreased > 3 log during an additional 4 hours at 42°C. To further demonstrate the effect of cultures, beyond acid production, acidified yogurt was produced by adding lactic acid to milk, mimicking the pH changes during fermentation of standard yogurt. Although the populations of *S. aureus* also decreased when the pH 4.8 was reached, the decline was much less dramatic. The greater bactericidal activity associated with standard yogurt and acidophilus yogurt was attributed to high levels of hydrogen peroxide (0.88 µg/ml) produced by the starters. Results for initial growth and subsequent kill of the pathogen during refrigerated storage were confirmed by Pazakova et al. *(46)*. Trends were comparable regardless of the concentration of *S. aureus* introduced at the onset of fermentation.

Similar results were observed when yogurt was produced with bacteriocin-producing ST and a non-producing strain of LB *(5)*. *S. aureus* grew 1.5 log during the early stages of fermentation at 40°C, but decreased > 3.5 log when the mixture reached pH 4.4 at the end of an 8-hour fermentation. Differences in storage temperature appeared to have little effect on viability after fermentation. Populations of *S. aureus* continued to decrease during storage at 7 and 22°C and were undetectable (additional 2 log decrease) at 10 days at both temperatures (N. Benkerroum, personal communication, e-mail April 4, 2005).

On the basis of the potential for *L. monocytogenes* as an environmental contaminant, comprehensive studies have also evaluated the behavior of *L. monocytogenes* in fermented milk products and yogurt *(5, 11, 23, 24, 50, 52, 56, 59)*. Two studies by Schaack and Marth demonstrated that the behavior of *L. monocytogenes* during the fermentation and storage of yogurt was similar to that of the other pathogens described in this review *(50, 52)*. Slow growth of *L. monocytogenes* (1 log increase) was observed during the initial 5-hour fermentation of yogurt with use of either ST alone or ST-LB cultures. After the pH reached 4.8, populations declined as the pH continued to decrease to 4.5 and to 4.0 during additional time at fermentation temperature and during storage at 4°C, respectively. Greater acid production and greater kill of *L. monocytogenes* were reported for yogurt fermented with ST-LB cultures than with ST alone. The pH decreased more rapidly when product was fermented at 42°C than at 37°C, which translated to decreased survival time of *L. monocytogenes* during refrigerated storage. *L. monocytogenes* survived 12 hours in refrigerated product previously fermented with 1.0% ST-LB culture at 42°C (final pH 3.8–3.9) but survived 1–2 weeks in similar product fermented at 37°C (final pH 4.0).

In addition, two research groups compared the differences in listerial survival in retail plain yogurt versus vanilla yogurt with sugar *(11, 59)*. In one study, the type of yogurt (plain vs. with vanilla with sugar) had no obvious effect on pathogen survival when yogurt was stored at 4°C *(11)*. *L. monocytogenes* decreased 2–3 logs during the first 8–12 days, while the pH values of 3.8–4.2 remained similar to 0-time samples. A second study evaluated the survival of *L. monocytogenes* that was inoculated into low-fat and nonfat plain or flavored yogurt (pH ranging from 4.35 to 4.52) and stored at 8°C *(59)*. In the latter study with higher-pH yogurt, listerial populations decreased more gradually, demonstrating a < 1 log decrease in 14 days at 8°C. The most significant decrease was observed at 28 days; populations of *L. monocytogenes* decreased 2.5 log in low-fat plain and vanilla yogurt and in fat-free plain yogurt, whereas a 3.5 log decrease was observed for the fat-free vanilla. Slight additional inhibitory effect by vanillin was observed.

Benkerroum et al. *(5)* reported that storage at either 7 or 22°C had no effect on survival of *L. monocytogenes* in pH 4.4 yogurt, but survival of *L. monocytogenes* was significantly decreased when yogurt was fermented with a bacteriocin-producing strain of ST ($Bac^+$ ST). Populations of *L. monocytogenes* decreased > 8 log after 8 to 24 hours fermentation with $Bac^+$ST, but only 1 log in the $Bac^-$ST yogurt.

## CONCLUSIONS

Multiple factors contribute to the microbiological safety of commercial yogurt. Assuming that the milk used in yogurt production is pasteurized and adjunct ingredients are free of vegetative pathogens, good manufacturing practices and sanitation will minimize the risk of post-processing contamination. Rapid acid production to pH values ≤ 4.8 will prevent the outgrowth of any surviving spores of mesophilic and psychrotrophic strains of *Clostridium botulinum* and *Bacillus cereus* during refrigerated or ambient temperature storage. Similarly, *S. aureus* will not produce enterotoxin at these low pH values. While certain vegetative pathogens such as *E. coli* O157:H7 and *L. monocytogenes* are more acid tolerant than the sporeformers, research has demonstrated that as the pH

decreases to pH 4.6, the substrate will inhibit growth and can be bactericidal. Studies comparing the effect of fermentation and storage temperatures in yogurt further suggest that storage temperatures greater than 4°C will enhance the demise of vegetative pathogens by increasing acid production.

Acidity is one of the principal factors in controlling growth, but other metabolites produced during the ST-LB fermentation contribute to the overall safety of yogurt. Although strains may vary in their ability to produce bacteriocins or the level of hydrogen peroxide accumulated in the substrate during fermentation, utilization of nutrients by the high populations of added starter bacteria will compete with low levels of contaminants.

Scientific studies confirm that the current US practice of cooling yogurt to 7°C over 96 hours does not cause any additional safety risks, provided the pH is at or below 4.6 within 24 hours of filling. However, products should be cooled as rapidly as possible to decrease over-production of acid that may reduce quality of the product and to control spoilage. Environmental controls are essential to prevent recontamination with acid-tolerant microorganisms that may have long survival times.

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