

## ADSA Foundation Scholar Award Fluid Dairy Product Quality and Safety: Looking to the Future

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### ABSTRACT

The fiercely competitive nature of the US beverage industry will drive the fluid milk sector of the dairy industry to improve product quality and shelf life to enable dairy beverages to compete with innovative new introductions as well as with currently popular shelf-stable products. The recent substantial growth in the volume of flavored milk sales specifically suggests that attention is needed to improve these products. Further, increasing public awareness and regulatory attention directed toward food safety issues highlight the need for the dairy industry to proactively address and eliminate emerging food safety issues that may negatively impact the image of dairy products. Shelf life and sensory profiles of high temperature short time pasteurized fluid milk products are presented, illustrating the need for greater attention to controlling contaminating microorganisms in processed fluid milk products. Bacterial spoilage patterns of flavored versus unflavored milks are compared, and suggestions are presented for extending flavored product shelf lives. Strategies currently applied to extend shelf life are reviewed. Food safety issues facing the dairy industry are presented within the context of an overview of foodborne illnesses in the United States. The pressing need to determine thermal resistance characteristics of *Mycobacterium paratuberculosis* is described.

**(Key words:** dairy food safety, extended shelf life products, microfiltration, *Mycobacterium paratuberculosis*)

**Abbreviation key:** BSE = bovine spongiform encephalitis, IBD = inflammatory bowel disease, MF = microfiltration, MQIP = milk quality improvement program, PPC = psychrotrophic plate counts, SPC = standard plate count, TSE = transmissible spongiform encephalopathy.

### INTRODUCTION

The US beverage industry is highly competitive and financially robust, showing an overall annual growth rate of approximately 3% per annum since 1995 (M. P. Anders, 2000, personal communications). Even though US milk production reached a record high in 1998 (Milk Industry Foundation, 1999), fluid milk products have not enjoyed a relative increase in the share of consumption within the past decade. Specifically, fluid milk products have maintained an approximate 10% share of total US liquid consumption since 1990, while the relative shares of soft drinks and juices have increased by nearly 5 and 2%, respectively, during the same period (Milk Industry Foundation, 1999). To date, dairy marketing efforts have aimed to increase consumer awareness of milk and its health benefits, but for the dairy industry to improve its competitive position, milk products must be able to compete with the new, innovative, and convenient shelf-stable beverage products that continue to enter the market. Fluid milk products must compete for sales against popular shelf-stable products such as soft drinks as well as with new products including sports beverages, bottled waters, and ready-to-drink tea beverages. Further, a number of new products, including soy-based beverages and calcium fortified orange juices, appear to directly compete for milk's niche as a highly nutritious beverage.

Consumers expect fluid milk products to be nutritious, fresh tasting, and wholesome. To the consumer, "quality" means that the product tastes good and that it keeps well in their home refrigerator. From a fluid milk processor's or a regulatory point of view, "quality" is measured more objectively by comparing product conformance to established standards on the last day of sale. To maintain or increase market share, however, the processor's goal should be to meet, or perhaps, create, the consumer's quality expectations. To meet these challenges, dairy processors must focus on improving the quality and extending the shelf lives of their products.

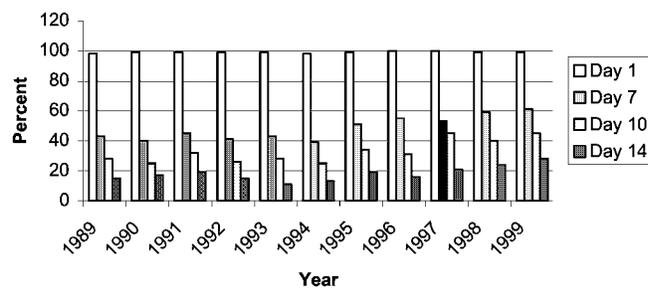
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### Current Shelf-Life Characteristics of Processed Fluid Milk: Results from the New York State Milk Quality Improvement Program

Dairy farmers in New York State recognized the link between processed product quality and market development more than 27 yr ago. In addition to funding state-wide generic dairy product advertising campaigns, since 1972 the New York State Dairy Promotion Advisory Board has funded a Cornell University research and educational program aimed at improving the quality, safety, and shelf life of fluid milk products. The Milk Quality Improvement Program (MQIP) was initiated by Professor David Bandler, who directed the program until 1996. Initially, this program was designed to examine school milk quality. A 1974 survey conducted by the MQIP found a direct correlation between milk flavor and levels of milk consumption by school-age children. This survey revealed that children in districts receiving off-flavored products consumed 30% less milk than did children in school districts that regularly received good tasting milk (Bandler et al., 1975). This clear link between milk quality and product consumption provided the initial impetus for the development of the MQIP. Current objectives of the ongoing MQIP are to monitor initial and keeping quality of commercially processed and packaged fluid milk products in New York State; to monitor raw milk quality; to assist dairy plants in identifying and correcting handling and processing problems affecting dairy product quality; and to assist dairy plants with vitamin A and D fortification. As described below, the MQIP has documented significant improvements in processed fluid milk quality during the last 10 yr; however, the data clearly illustrate the need for further improvements in controlling and reducing bacterial numbers in processed dairy beverages throughout product shelf life.

**MQIP strategies.** Each processing plant participating in the MQIP is visited two times a year and raw and HTST pasteurized milk samples are collected for analysis. Samples of finished product are chosen to represent the various types of products processed as well as the different packaging machines in operation. The selection of products depends on the operation, but generally includes whole, 2%, 1%, nonfat, chocolate, and a raw milk sample (i.e., a silo sample). Representative containers from each packaging machine (e.g., gallon plastic, half-gallon paperboard, and quart paperboard) are generally collected for one product (e.g., homogenized milk) that has been filled from the same pasteurized milk tank. In general, the remaining products are collected in half-gallon containers. Samples represent the freshest product available.



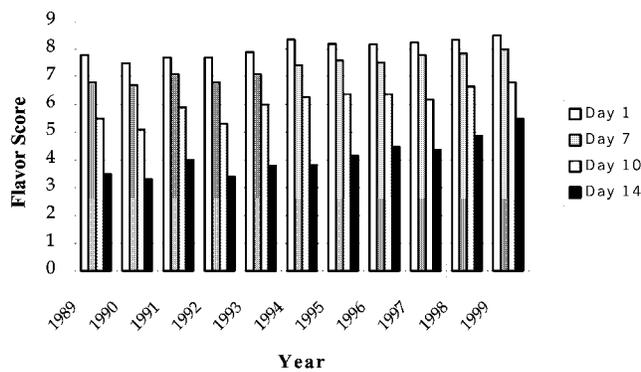
**Figure 1.** Percentage of HTST pasteurized fluid milk products analyzed in the New York State Milk Quality Improvement Program that met the Pasteurized Milk Ordinance (18) regulation of less than 20,000 cfu of total bacteria/ml at 1, 7, 10, and 14 d postprocessing from 1989 to 1999. Milk samples were stored at 6°C.

Samples are transported to the laboratory in coolers kept below 40°F. Initial testing begins within 48 h of collection. Following arrival at the laboratory, the samples are split into four sterile 500-ml bottles. One bottle is used for initial-day testing while the remaining bottles are stored at 6° (±1°) C (43°F) for testing at 7, 10, and 14 d. Ideally, dairy products should be stored at temperatures less than 4°C (40°F) without freezing to achieve the maximum product shelf life and quality. The 6°C temperature is designed to simulate realistic temperatures that may be encountered in retail dairy distribution.

On the initial day, samples are tested for bacterial numbers by standard plate count (SPC) and coliform count (Marshall, 1992), as well as for sensory characteristics (Boor and Nakimbugwe, 1998), acid degree value (Shipe et al., 1980), freezing point (Boor et al., 1998), and vitamin A and D levels (Boor and Nakimbugwe, 1998). Processed milk samples are analyzed for SPC, coliform count, and sensory characteristics at 7, 10, and 14 d postcollection. Raw milk samples are analyzed on the initial day for bacterial numbers by SPC, laboratory pasteurized count, and rapid psychrotrophic count (Marshall, 1992).

**MQIP sample shelf-life characteristics: quality challenges for the dairy processing industry.** United States regulatory standards specify that pasteurized fluid milk must contain less than 20,000 cfu of total bacteria per milliliter by the SPC and fewer than 10 coliforms/ml by analysis on violet red bile agar (FDA, 1995; Marshall, 1992). In general, for fluid products to routinely meet the SPC standard at 14 d postprocessing and beyond, processors must have effective quality control programs in place.

The percentages of MQIP samples that have met the legal standard from 1989 to 1999 are shown in Figure 1. In 1999, of 447 samples tested, 99% had SPC below



**Figure 2.** Organoleptic scores for HTST pasteurized fluid milk products analyzed in the New York State Milk Quality Improvement Program at 1, 7, 10, and 14 d postprocessing from 1989 to 1999. Milks were evaluated on a scale of 1 to 10, with “no defects detected” = 10; “good”, 8–10; “fair”, 6–7.9, and “unacceptable”, <6. Milk samples were stored at 6°C.

20,000 on the initial day of testing. The percentages of samples <20,000 cfu/ml during storage at 6°C were 61, 45, and 28% at 7, 10, and 14 d. The percentage of the 447 MQIP samples tested in 1999 that contained fewer than 10 coliforms/ml were 99% on d 1, and 83, 77, and 79%, respectively, at 7, 10, and 14 d. Although the fraction of samples meeting the regulatory standard for total bacterial numbers has continuously improved in the past decade, our results illustrate the need to further reduce bacterial numbers in pasteurized products.

From a consumer perspective, sensory characteristics most directly influence product acceptability. Average 1999 milk flavor scores for initial day, and for 7, 10, and 14 d postprocessing for 165 products are shown in Figure 2. Milk products were evaluated on a scale of 1 to 10, with no defects detected = 10; good = 8 to 10; fair = 6 to 7.9, and unacceptable <6. The 1999 average initial score for milk flavor (8.5) for MQIP samples has been fairly consistent since 1994. After 7, 10, and 14 d of refrigeration storage (6°C), the average flavor scores for 1999 (8.0, 6.8, and 5.5) were slightly improved over 1998 scores in all categories. Interestingly, when milk samples with scores of 1 (awarded to samples that are not tasted by panelists due to foul odor or visible spoilage, typically attributable to microbial reproduction in the product) were disregarded, the average flavor scores for samples at 1, 7, 10, and 14 d postprocessing became 8.5, 8.0, 7.2, and 6.8, suggesting a bimodal distribution of flavor scores. Further, of the 165 samples analyzed for flavor, 53% had no specific defect other than “lacks freshness” at d 14. These data suggest the feasibility of further shelf-life extension for HTST pasteurized products that are adequately protected from bacterial

contamination. The concept of shelf life extension through control of bacterial contaminants is not novel; however, these data illustrate the scope of improvement needed to allow fluid milk products to compete in the marketplace with beverages with longer shelf lives.

### Shelf-Life Extension in Flavored Milk Products

Although the largest fraction of fluid milk products currently sold (93%) comprises unflavored products, with flavored milk products constituting only about 6% of total volume, flavored milk sales have shown substantial growth since 1995, with a nearly 8% increase in volume in 1998 and an additional 7% increase in 1999 (Milk Industry Foundation, 1999). Further, this increased sales volume is at least partially due to an increased number of households purchasing flavored milk products (Milk Industry Foundation, 1999). Thus, timely improvements in flavored milk quality and shelf life could provide a growth opportunity for the US fluid milk industry.

Several fluid milk manufacturing companies participating in the MQIP had reported reduced fluid product shelf lives for their flavored products in contrast to their unflavored products, despite somewhat higher processing times, temperatures, or both for their flavored milk products. To examine this problem, the MQIP collected matched flavored (chocolate) and unflavored milk products from four processing plants over four periods and compared SPC and psychrotrophic plate counts (PPC) from the samples on the initial day, and at 7, 10, and 14 d postprocessing. Bacterial numbers within 24 h of processing were not significantly different in the unflavored and in the chocolate milk samples ( $P > 0.001$ ). SPC and PPC were less than 1000 and 10 cfu/ml, respectively, for all products. However, both SPC and PPC were higher in chocolate milk samples than in unflavored milk samples after 14 d of storage at 6°C ( $P < 0.001$ ) in products collected from all four processing plants (Douglas et al., 2000). These findings confirmed the previously reported observations of reduced product shelf lives among flavored products relative to unflavored products.

We thus sought to identify the source(s) of the contaminating microorganisms. We first examined the possibility of bacterial contaminant entry through the addition of the various cocoa formulations. Bacteria present in the cocoa formulations were below the detection limits of our analytical procedures (<10 cfu/g) for products manufactured in all four plants, suggesting that these cocoa powders did not serve as major sources of contamination for these products. With one exception, isolation and identification of bacteria present in the various products showed that the profiles of the predominant

organisms were the same for flavored and unflavored products processed in the same plants, suggesting that the spoilage organisms were not unique to either set of products. The predominant organisms present in 73% of the samples examined were Gram-positive, catalase-positive, oxidase-negative, spore-forming rods identified as *Bacillus* spp. by 16s rDNA sequencing and by Biolog analyses (Douglas et al., 2000). These organisms were found to be present in raw and in processed milk samples and were capable of surviving laboratory pasteurization procedures. These findings are consistent with our previous observation of the widespread presence in the raw milk supply (albeit in low numbers) of heat-resistant, spore-forming psychrotrophic bacteria that are capable of increasing in number when held at 7°C for 10 d (Boor et al., 1998).

To further investigate the effects of chocolate milk components on bacterial numbers in processed products, SPC were monitored in experimentally prepared and aseptically collected unflavored milk, milk with chocolate powder and sucrose (chocolate milk), milk with sucrose only, and milk containing chocolate powder only on the initial day and after storage at 6°C on d 7, 14, and 21 postprocessing. At d 14 and 21, SPC were higher ( $P < 0.001$ ) in both chocolate milk and in milk with chocolate powder only than in either milk with sucrose or in unflavored milk (Douglas et al., 2000), suggesting that the chocolate powder stimulates increases in bacterial numbers among the residual bacteria surviving HTST pasteurization. From these studies, we conclude that extension of flavored milk shelf life and improved milk quality will require that bacterial spores be removed from raw milk (e.g., through microfiltration strategies, such as those described below) or by the application of processing conditions that utilize increased time or temperature treatments relative to those used for unflavored milk products or through a combination of these strategies.

**Other quality parameters that may limit fluid product shelf life extension: SCC.** To date, fluid product shelf life extension has focused primarily on reducing and controlling the presence of bacterial contaminants to achieve better product quality for longer periods. It is possible, however, that other factors intrinsic to the raw product may begin to limit fluid product quality as strategies are implemented to reduce bacterial contaminants to the extent that HTST pasteurized fluid products approach and exceed 21-d shelf lives. Thus, David Barabano's research group at Cornell University examined the effects of various levels of somatic cells resulting from intramammary infection of *Streptococcus agalactiae* (mastitis infection) on fluid milk quality (Ma et al., 2000).

Milk was collected before and after cows were artificially infected with *S. agalactiae*. Following infection, milk samples were commingled to achieve average SCC of 45,000 cells/ml (preinfection) and 849,000 cells/ml (postinfection). Pasteurized, homogenized 2% milk samples from pre- and postinfection periods were analyzed for proteolysis, lipolysis, bacterial numbers, and sensory attributes on the initial day and following 7, 14, and 21 d of storage at 5°C.

Bacterial numbers (SPC, coliform counts, and PPC) were generally low in both high and low SCC milk samples throughout the 21 d. However, the average rates of FFA increase and casein hydrolysis were two and three times higher, respectively, in high SCC milk than in low SCC milk. Although low SCC milk maintained acceptable organoleptic quality throughout the 21-d period, sensory defects were identified between 14 and 21 d in the high SCC milk samples, resulting in low overall quality ratings for these milks. The detected sensory defects were predominantly "rancid" and "bitter," which was consistent with the increased lipolysis and proteolysis observed in the high SCC milks (Ma et al., 2000). Thus, from these studies, fluid milk processors are urged to use milk with low SCC numbers when seeking to extend pasteurized fluid product shelf life and quality beyond 14 d of storage.

**Processing strategies profoundly influence fluid product shelf life.** One significant barrier to extending the shelf lives of various dairy products is the difficulty of balancing the removal or destruction of spoilage microorganisms and spores present in raw milk while limiting product color changes, vitamin destruction, and milk protein denaturation. Strategies currently employed in the United States to extend dairy product shelf lives include the application of high-temperature processing technologies such as UHT thermal processing and ultrapasteurization. Because UHT milk is considered to be a shelf-stable product, thermal processes for UHT milk in the US must comply with FDA requirements for sterilizing low acid foods. Although UHT and ultrapasteurized milks are exposed to similar processing times and temperatures (135 to 140°C for a few seconds for UHT and 138°C (280°F) for at least 2 s for ultrapasteurized; FDA, 1995), UHT products are aseptically packaged, and the resulting products are thus shelf stable for several months at room temperature. In contrast, ultrapasteurized milk products are not aseptically packaged. The resulting products are generally coded for a shelf life of 2 to 3 mo under refrigerated conditions (Boor and Nakimbugwe, 1998). In previous work in our laboratory, we determined that ultrapasteurized 2% fat milk remained organoleptically, chemically, and microbiologically stable for at least 10 wk of storage at 7° ( $\pm 1^\circ$ ) C (Boor and Nakim-

bugwe, 1998). HTST pasteurized milk, which must be heated to 72°C (161°F) for a minimum of 15 s or the equivalent, is usually coded for a last day of sale between 10 and 21 d of refrigerated storage after the date of processing. Thus, with existing technology, processed fluid milk products in the US typically have shelf lives ranging from a minimum of approximately 10 d for HTST products and up to 6 mo for UHT products.

### Microfiltration

One problem limiting consumer acceptance of high-temperature processed milk (UHT and ultrapasteurized products) in the United States is the development of a distinct cooked flavor, which may be objectionable to those accustomed to pasteurized milk (Hill, 1988). Other common limitations to UHT product acceptability among consumers include oxidized and stale flavors (Hill, 1988), creaming or fat separation (Schroder and Bland, 1984), gelation or sediment formation (Hill, 1988), and proteolytic or lipolytic deterioration from enzymes surviving the heat treatment (Cox, 1993; Hill, 1988; Lopez-Fandino et al., 1993). In general, with the exception of cooked flavor, most flavor and texture defects tend to increase in severity with increased storage time and temperatures (Boor and Nakimbugwe, 1998; Hill, 1988; Lopez-Fandino et al., 1993).

Microfiltration may provide a lower temperature approach for the production of dairy products with extended shelf lives (Daufin et al., 1993; Eckner and Zottola, 1991; Kosikowski and Mistry, 1990; Madec et al., 1992; Olesen and Jensen, 1989). This approach, followed by a mild pasteurization treatment, has already been adopted by a Canadian fluid milk processor. The company reports production of “fresher-tasting” milk products with extended shelf lives. These types of products may meet with consumer approval in the United States, as well.

Microfiltration (MF) is the passage of product under relatively low pressure (approximately 1 bar) through a semipermeable membrane with pore sizes ranging from 0.2 to 5  $\mu\text{m}$  (Olesen and Jensen, 1989). As bacteria generally range from 1 to 3  $\mu\text{m}$ , under some circumstances MF should be able to completely remove bacteria from the fluid permeate. MF might provide a lower temperature option, and thus, a less pronounced cooked flavor than UHT processing for extended shelf-life dairy products.

Very little published work exists on microfiltration for milk processing, and among the existing reports, the results are sometimes contradictory. Olesen and Jensen (1989) found that the initial content of *Bacillus cereus* spores in milk had a significant effect on the content of spores in the microfiltered milk, but that

concentration ratio and circulation pressure had no effects under the conditions studied. Varying operating temperature between 30 and 50°C did not appear to affect bacterial retention in one study (Eckner and Zottola, 1991); however, increasing microfiltration temperatures from 35 to 50°C was reported to significantly increase *Salmonella*, but not *Listeria* retention (Madec et al., 1992). Eckner and Zottola (1991) concluded that different membranes with the same molecular weight cut-off have different bacterial retention characteristics, but that bacterial morphology did not affect the ability of the organism to pass through the membrane. In contrast, Madec et al. (1992) reported that *Listeria* and *Salmonella* had differing retention characteristics, but that these characteristics were not influenced by initial contamination levels, suggesting that bacterial size and shape may play a role in the passage of a particular organism through a membrane. This report also found retention of *Listeria* cells to be much lower when cells were inoculated into milk that had been previously microfiltered. In summary, microfiltration operating parameters must be established to meet the specific goals of a given processing operation.

### Foodborne Illnesses in the United States: an Overview

In addition to contributing to increased rates of product spoilage, the presence of microbes with pathogenic potential can render foods unfit and harmful for human consumption. In the following sections, the current status of foodborne illnesses in the United States is reviewed, and emerging issues of relevance to the dairy industry are highlighted.

From a consumer perspective, foodborne illnesses are sometimes thought to be “nuisance” diseases that cause temporary discomfort but have no serious long-term ill effects. However, recent evidence suggests that some food-related illnesses may cause lingering health problems, ranging from reactive arthritis as a consequence of salmonellosis to miscarriage resulting from listeriosis (Donnelly, 1990). One recent estimate suggests that up to 76 million food-related illnesses, 325,000 hospitalizations, and 5000 deaths may occur in the United States on an annual basis (Mead et al., 1999). Medical expenses and lost work productivity caused by seven major foodborne pathogens may cost between \$6.6 and \$37.1 billion annually (Crutchfield et al., 1999).

**Food safety challenges for the dairy processing industry.** Although fluid milk and milk-based products are more closely regulated at state and federal levels than many other food products, occasional outbreaks of foodborne illness resulting from the consumption of

contaminated dairy products do occur. In fact, the largest single salmonellosis outbreak in US history—over 23,000 culture-confirmed cases from March to April 1985—resulted from consumption of *Salmonella*-contaminated whole and 2% milk that had been produced by a suburban Chicago processor (Lecos, 1986). A few months later, in June 1985, an outbreak of listeriosis resulting in 145 cases and 46 deaths was linked to the consumption of Mexican-style white cheese produced in California (Donnelly, 1990). More recently, bacterially contaminated dairy products were implicated as the source of the most widespread outbreak of foodborne illness in Japan since the central government began collecting statistics of this nature in 1975. Processed fluid milk samples containing *Staphylococcus aureus* enterotoxin or *Bacillus cereus* or both sickened at least 14,000 people in June and July, 2000. The economic, medical, and emotional consequences of large-scale, food-related illnesses are devastating. For example, as a consequence of the US-based outbreaks described above, the factories involved permanently ceased operations.

**Pasteurization: a false sense of security?** Currently, the most common methods of destroying pathogenic organisms and of reducing or eliminating spoilage organisms in US dairy products are through pasteurization by the HTST method. These thermal treatments are designed to destroy the most heat-resistant of the non-spore-forming pathogens, specifically, *Mycobacterium tuberculosis* and *Coxiella burnetii*. Some microbes can survive pasteurization (Hammer et al., 1995). Spore-forming bacteria, including those of the *Bacillus* and *Clostridium* genera (e.g., *Bacillus cereus*, *Clostridium botulinum*, and *Clostridium perfringens*), are among the heat resistant organisms that can be isolated from pasteurized milk. Some studies suggest the possibility that *Mycobacterium paratuberculosis*, a bacterium that causes Johne's disease in cattle and that may be linked to Crohn's disease in humans, can survive pasteurization (Mechor, 1997). This issue is addressed in detail below. In addition, minimal pasteurization conditions will not inactivate the causative agent of bovine spongiform encephalitis (BSE), also known as mad cow disease. The agent responsible for this disease, an infectious protein, shows little loss of infectivity, even after prolonged exposure to temperatures up to 176°F (80°C) (Asher et al., 1986). Fortunately, no evidence exists linking transmission of this disease to consumption of milk from cows with BSE. For example, mice injected with milk from BSE-infected cattle did not develop this disease nor have epidemiological analyses suggested transmission of BSE to calves via milk (Hilberton, 1997). The issue of disease transmissibility through consumption of animal products is likely to

haunt the US dairy industry, however. To illustrate, on July 18, 2000, Jan Charney, Vermont's health commissioner, in consultation with the US Centers for Disease Control and Prevention advised consumers not to eat cheese made from the milk of three flocks of Vermont sheep that may have a form of transmissible spongiform encephalopathy (TSE). The precedent established by this recommendation and the implications for perceived food safety risks associated with dairy product consumption are very sobering for the US dairy industry.

Although some pathogens can survive pasteurization, as described above, the presence of most pathogenic microbes (e.g., *Salmonella* or *L. monocytogenes*) in processed dairy products implies either failure of the pasteurization process or postpasteurization contamination. Postpasteurization contamination occurs when microbes are reintroduced into the pasteurized product as a consequence of product contact with contaminated processing or packaging equipment or workers. In general, postpasteurization contamination contributes the majority of microorganisms that contaminate and spoil pasteurized milk (Griffiths et al., 1984). We demonstrated the extension of HTST product shelf life from <7 d to >21 d through identification and elimination of a single, specific source of bacterial contamination in the product filling unit (Ralyea et al., 1998). Rapid trouble-shooting of postpasteurization contamination sites can be accomplished effectively through the application of adenosine triphosphate-bioluminescence hygiene monitoring kits (Murphy et al., 1998). In addition to the benefits of shelf life extension, the importance of protecting processed products from recontamination is further illustrated by the demonstration that the pathogen *Escherichia coli* O157:H7 can persist for at least 35 d as a postpasteurization contaminant in buttermilk with a pH of 4.1 (Dineen et al., 1998).

Multiple routes exist for the entry of contaminating microbes into the dairy processing environment. For example, microbes can be introduced into a dairy-processing environment along with the raw milk. The abundance of nutrients and moisture in a processing plant can facilitate the survival and growth of these contaminants. For example, *Listeria innocua*, *Listeria monocytogenes*, and *Yersinia enterocolitica* are commonly isolated from dairy processing plant locations that involve "wet traffic," including the floors of coolers, freezers, and processing rooms; cases and case washers; floor mats and foot baths; and beds of paper fillers (Donnelly, 1990). Thus, processed product contact with a contaminated surface or employee downstream of the pasteurizer can render the pasteurization step useless.

Product protection from contaminating microbes should be a top priority for all dairy and food processing

establishments. The consequences of product linkage with an outbreak of foodborne illness will result in the need to recall the remaining contaminated product that is still on the market. A product recall can be very expensive and time consuming, depending on the scope of product distribution. Once product has been recovered, it must then be stored until disposal can be completed. In general, once a product has been linked with human illness, the media begin to cover the outbreak. Negative media coverage can result in loss of consumer confidence in the product and brand name. Consumer confidence in product safety plays an important role in food purchasing decisions. Consumers may avoid product types or specific labels following widely publicized episodes of product contamination and food-related illnesses. Reduced sales can further compound the economic distress a company faces following a product recall. In general, regulatory agencies responsible for controlling the product in question will rapidly arrive at the plant as the outbreak unfolds. Regulatory investigations usually require that the plant cease operations until the cause of the outbreak can be identified and eliminated. These closures can be brief, prolonged, or permanent. In summary, product protection must be a top priority to protect the consumer from illness and to prevent the financial losses associated with troubleshooting and preventing further product contamination.

Attention to process controls, raw product quality, and plant sanitation will greatly contribute to production of high quality, safe dairy products. However, the dairy industry must be prepared to handle challenges imposed by emerging pathogens as they are identified. As described below, *Mycobacterium paratuberculosis* is one such microbe that may threaten the perception of dairy product safety.

### ***Mycobacterium paratuberculosis*: An Emerging Pathogen Affecting the Dairy Industry?**

*Mycobacterium paratuberculosis* is the causative agent of Johne's disease in dairy cows. This transmissible disease, which currently affects ~33% of US dairy herds (Collins, 1997), dramatically reduces milk production, reproductive performance, and animal condition (Stabel, 1997) and thus has a significant negative economic impact on the dairy industry. This organism, which is excreted in feces and milk, is reportedly not as easily inactivated by pasteurization and thermal treatments as other bacteria infecting humans and animals (Sung and Collins, 1998). Recent studies suggest that humans with Crohn's disease, a chronic intestinal disorder, are more likely to show indications of the presence of *Mycobacterium paratuberculosis* than humans

not suffering from Crohn's disease (Thompson, 1994). *M. paratuberculosis* might, therefore, be considered a foodborne pathogen; thus, it is critical to establish the thermal resistance characteristics of this organism. Experiments of this nature do not represent a trivial undertaking because culturing techniques for this organism are labor intensive and very slow, taking up to 16 wk to produce visible colonies on appropriate microbiological media (Collins, 1997).

***Mycobacterium paratuberculosis as an animal pathogen.*** Paratuberculosis, or Johne's disease, is a chronic progressive enteric disease of ruminants caused by infection with *M. paratuberculosis* (Stabel, 1997). Cattle can become infected with *M. paratuberculosis* as calves but often do not develop clinical signs until 2 to 5 yr of age. Clinical symptoms of paratuberculosis include chronic and intermittent diarrhea, rapid weight loss, failure to respond to treatment and death. Animals with clinical paratuberculosis are often culled from the herd early in their productive lives, while animals with subclinical disease show reduced milk production and poor reproductive performance. These consequences of *M. paratuberculosis* infections are estimated to lead to economic losses exceeding \$1.5 billion/yr for the national dairy industry (Jones, 1989).

Eradication and control of Johne's disease in infected herds is generally difficult. The primary route of infection is through the ingestion of fecal material, milk, or colostrum containing *M. paratuberculosis*. Cattle may shed low levels of *M. paratuberculosis* in their fecal material during subclinical infection, but over time, shedding can cause significant contamination of the environment and, thus, subsequent infection of other animals. *M. paratuberculosis* can also be transmitted to calves through feeding of colostrum from infected cows. One of the significant problems for control of paratuberculosis is the fact that diagnosis of this disease is difficult and time consuming. Bacteriologic culture is the most definitive method of diagnosis, but it is slow and very labor intensive (Stabel, 1997; Sung and Collins, 1998). Furthermore, animals with subclinical disease may shed the organism intermittently so that, at any given time, fecal culture alone may detect as few as 50% of these animals (Sanftleban, 1990). The lack of sensitive and rapid methods for the detection of *M. paratuberculosis* also presents a problem for the detection of this organism in milk and colostrum. Fast and sensitive detection methods are essential to make it feasible to screen for the presence of *M. paratuberculosis* in milk or colostrum prior to feeding it to calves and thus risking the spread of infection to additional animals in a herd. Serologic tests for the diagnosis of paratuberculosis appear to lack the necessary sensitivity to be applicable for reliable eradication or diagnosis

of infection (Stabel, 1997). More recently, DNA-based methods for the detection of *M. paratuberculosis* (e.g., PCR) have been developed. PCR assays are generally combined with fecal culture to allow more rapid detection of this organism. However, one study found that a commercial PCR assay was only able to detect 60% of infected cows determined positive by standard culturing methods (Whipple et al., 1992). Clearly, to enhance our ability to limit the spread of paratuberculosis, improved, rapid detection methods for *M. paratuberculosis* are needed.

***M. paratuberculosis*—a potential human food-borne pathogen?** Over recent years, concern has arisen over a possible role of mycobacterial species (including, but not limited to, *M. paratuberculosis*) in the etiology of human Crohn's disease. Crohn's disease is chronic, incurable, inflammatory bowel disease (IBD) with symptoms similar to those of Johne's disease (Sung and Collins, 1998). Because of their similar clinical symptoms, Crohn's disease and ulcerative colitis are often confused. The term IBD is used to refer to both diseases (Chiodini, 1989). An estimated 2 million patients in the United States alone appear to be afflicted by IBD (Chiodini, 1989).

In 1984, Chiodini et al. (1984; 1986) reported the first isolation of *M. paratuberculosis* from a Crohn's patient. These isolates were subsequently shown to be genetically identical to bovine *M. paratuberculosis* strains and were shown to be able to cause Johne's disease by oral inoculation in goats (Collins, 1997; Van Kruiningen et al., 1986). Since then, a variety of studies have been conducted to determine whether there is a correlation between the presence of *M. paratuberculosis* or other *Mycobacterium* species and human Crohn's disease (reviewed in Chiodini, 1989). These studies used culturing techniques to isolate *Mycobacterium* species from Crohn's patients or DNA-based approaches to screen for the presence of mycobacterial DNA in tissues obtained from Crohn's patients. Many studies have shown that a variety of *Mycobacterium* species, including *M. paratuberculosis*, can be isolated from patients with Crohn's disease. For example, Chiodini et al. (as cited in Chiodini, 1989) isolated *M. paratuberculosis* from four of 26 Crohn's patients but from none of 26 control samples. Additionally, Sanderson et al. (1992) found that 65% of the intestinal samples from Crohn's disease patients (n = 40) tested positive for the presence of *M. paratuberculosis* DNA by PCR compared with 10% of the samples from controls (no IBD or ulcerative colitis; n = 63). Fidler et al. (1994) reported that four of 31 Crohn's disease tissues, but none of 30 control and ulcerative colitis-derived samples were positive for *M. paratuberculosis* DNA by PCR. Additional studies also found a higher incidence of *M. paratuberculosis* DNA in tissue from

Crohn's patients compared with control samples (e.g., Dell'Isola et al., 1994). A variety of other studies did not find any evidence for the presence of mycobacterial DNA in tissues from Crohn's disease patients using PCR (Frank and Cook, 1996; Rowbotham et al., 1995; Wu et al., 1991). In summary, although some studies described an association between the presence of *M. paratuberculosis* and Crohn's disease, a role for *Mycobacterium* species and *M. paratuberculosis* in the etiology of this human disease remains highly controversial.

**Thermal tolerance of *M. paratuberculosis*.** Excretion of *M. paratuberculosis* from infected dairy cattle occurs primarily in feces and, to a lesser extent, in milk (Collins, 1997). For example, Sweeney et al. (1992) found low concentrations of *M. paratuberculosis* from asymptomatic cows (2 to 8 cfu/50 ml of milk). Grant et al. (1998) suggested that concentrations of *M. paratuberculosis* in milk might be as high as  $10^4$  cfu/ml due to fecal contamination of the raw milk. In one study designed to examine commercially pasteurized milk for the presence of *M. paratuberculosis*, Millar et al. (1996) found that at least 15 of 312 milk samples collected at retail stores contained viable *M. paratuberculosis*. These results do not necessarily prove that *M. paratuberculosis* survives commercial pasteurization procedures, but could also suggest the possibility of postpasteurization contamination with this organism.

Heat treatment represents the most important microbial killing step in dairy product processing. Because of the possibility of the presence of *M. paratuberculosis* in raw milk, determination of this organism's potential for survival of thermal inactivation during pasteurization is of considerable importance. Various studies have attempted to determine thermal death curves for *M. paratuberculosis*. Grant et al. (1996) obtained a concave thermal death curve at 63.5 C, showing rapid death at 10 min of heating and slow death ("tailing") after 10 min of heating, possibly due to the presence of clumped cells. Sung and Collins (1998) determined D values for human and animal strains of *M. paratuberculosis* using the sealed tube method and found linear death curves for both single and clumped *M. paratuberculosis* cells by a radiometric counting method. Their results suggest that *M. paratuberculosis* may survive HTST time and temperature treatments when the initial organism concentration is >10 cells/ml.

In studies designed to simulate commercial thermal processing conditions, Chiodini and Hermon-Taylor (1993) reported that 5 to 9% of inoculated *M. paratuberculosis* cells survived heat treatment at 63°C for 30 min (batch pasteurization conditions) and 3 to 5% survived 72°C for 15 s (HTST pasteurization conditions). Grant et al. (1996) also isolated *M. paratuberculosis* after inoculated milk samples were heat treated by either HTST

or by low temperature holding, finding that, with an inoculum density of  $10^3$  to  $10^4$  cfu/ml, 50 and 58% of the inoculated samples were positive for *M. paratuberculosis* after low temperature holding and HTST, respectively. More than 85% of the samples were positive when the inoculum level was between  $10^6$  and  $10^7$  cfu/ml. In a more recent study, Grant et al. (1998) found viable *M. paratuberculosis* in 14.8 and 10% of statically heated HTST-pasteurized milk samples inoculated at  $10^3$  or  $10^2$  cfu/ml, but no viable organisms from samples inoculated with either 10 cfu/ml or 10 cfu/50 ml. Stabel et al. (1997) found that use of a laboratory-scale pasteurizer unit resulted in the destruction of *M. paratuberculosis* at 72°C for 15 s, while viable bacteria were recovered from static test tubes held at 65, 72, 74, or 76°C for 0 to 30 min. The results of Stabel et al. (1997) imply that turbulent-flow HTST pasteurization conditions may be effective for destruction of *M. paratuberculosis*, but that currently applied processes for batch pasteurization and HTST time and temperature treatments under static conditions are likely to be inadequate. Using heat treatment in capillary tubes, Keswani and Frank (1998) found that three strains of *M. paratuberculosis* appeared to not survive treatment conditions equivalent to pasteurization treatment when plated on Middlebrook 7H11 agar.

The contradictory nature of the results described above illustrates the need for definitive studies to clearly define the thermal tolerance characteristics of *M. paratuberculosis* strains. While thermal inactivation curves have been described for some *M. paratuberculosis* strains, heat treatment procedures that will reliably inactivate *M. paratuberculosis* have not been established. Our current understanding of the inactivation of *M. paratuberculosis* during heat treatment processes is limited largely because of a lack of rapid and sensitive methods for detection of viable cells (Collins, 1997). Furthermore, questions of recovery of heat injured *M. paratuberculosis* cells on selective agar media have not been addressed in most experiments intended to determine the heat resistance of this organism.

**Detection and enumeration methods for *M. paratuberculosis*.** Detection of *M. paratuberculosis* represents a significant challenge and is very time consuming. Bacteriologic culture is currently the most definitive method of diagnosis, but typically takes up to 6 wk. Incubation times are often further extended (up to 8 mo) to ensure detection of heat-injured cells, e.g., after heat treatment of milk.

Because of the difficulties in detection and enumeration of *M. paratuberculosis*, alternative methods have been used to monitor for the presence of this organism. For example, Sung and Collins (1998) used a radiomet-

ric culture method (BACTEC) to monitor the survival of *M. paratuberculosis* after heat treatment of inoculated milk samples. In this method, the sample is inoculated into a selective medium and subsequently incubated at 37°C. The presence of viable *M. paratuberculosis* cells is indirectly determined by measuring the production of  $^{14}\text{CO}_2$  produced by the metabolism of [ $^{14}\text{C}$ ] palmitate.

In an attempt to improve detection methods for *M. paratuberculosis*, a variety of groups have also developed DNA-based methods, including various PCR protocols. A commercially available PCR test for *M. paratuberculosis* (IDDEX, Westbrook, ME) allows this organism to be detected at levels of 1000 cells/g of sample or higher (van der Giessen et al., 1992; Vary et al., 1990). This approach provides approximately a 100-fold lower sensitivity than current culturing methods, which can detect as few as 10 cells/g of sample (van der Giessen et al., 1992). Although a variety of researchers with extensive paratuberculosis experience continue to work toward development of various *M. paratuberculosis* PCR assays, so far, all published studies have reported assay detection limits ranging between 1000 and 10,000,000 *M. paratuberculosis* cells/g of fecal material. In evaluations on both high and low shedding animals, PCR assays have detected only 3 to 54% of the positive samples (van der Giessen et al., 1992; Whitlock et al., 1996). In addition to the current inability to apply PCR assays for detection of low numbers of *M. paratuberculosis*, another significant disadvantage of PCR methodology is the inability to reliably determine whether positive results arise from the existence of living or dead bacteria (Collins, 1997). This disadvantage is likely to be a particular problem when one attempts to detect the presence of *M. paratuberculosis* following a heat treatment or any other killing step. For example, Millar et al. (1996) found that 22 of 312 commercially processed milk samples were PCR positive for *M. paratuberculosis*, while only nine of 18 PCR positive samples tested positive by standard culturing methods, suggesting that, in some samples, the PCR methodology detected the presence of residual DNA from dead *M. paratuberculosis* cells. In the same study, six of 36 PCR negative samples tested positive by standard culturing methods. While improved PCR-based methods are ultimately likely to be appropriate for some *M. paratuberculosis* applications, current results clearly show that these methods are unlikely to be useful for monitoring the survival of *M. paratuberculosis* during heat treatments. Particular problems with PCR-based assays for the detection of *M. paratuberculosis* include 1) low sensitivity in comparison with culturing methods; 2) the potential for the detection of dead organisms, coupled with the unreliability of the use of current PCR-based strategies designed to distinguish between dead and

living cells (e.g., strategies that target mRNA rather than DNA, etc.); and 3) the inability to apply short enrichments before PCR assays due to the extremely slow growth of the organism on bacteriological media.

Conclusive determination of the destruction of *M. paratuberculosis* during milk pasteurization hinges upon our ability to devise effective, specific, and relatively rapid methods for detecting cell viability. This information is essential to ensure consumer confidence in the safety of US dairy products.

## CONCLUSIONS

The ultimate goal of the US dairy industry is to sell wholesome, nutritious dairy products to the consuming public. To remain economically competitive, the dairy industry must move toward production of fluid products that can compete in distribution channels and in the marketplace with popular, shelf-stable beverages. To that end, processors must focus on product shelf-life extension. These strategies will ultimately improve product quality as well.

To ensure public confidence in the safety of the US milk supply, the dairy industry must act aggressively to monitor, minimize, and ultimately eliminate, food safety risks that may be associated with emerging pathogens such as *M. paratuberculosis* and TSE. Dairy manufacturers must not rely solely on pasteurization to protect product safety, but must evaluate their entire processing operations with a view to maximizing product protection from microbial contamination at all points.

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