Short communication: Postpasteurization hold temperatures of 4 or 6°C, but not raw milk holding of 24 or 72 hours, affect bacterial outgrowth in pasteurized fluid milk

Alexis D. Andrus, Brynne Campbell, Kathryn J. Boor, Martin Wiedmann, and Nicole H. Martin¹
Milk Quality Improvement Program, Department of Food Science, Cornell University, Ithaca, NY 14853

ABSTRACT

As fluid milk processors continue to reduce microbial spoilage in fluid milk through improved control of postpasteurization contamination and psychrotolerant sporeformer outgrowth, it is necessary to identify strategies to further improve the quality and extend the shelf life of fluid milk products that are high-temperature, short-time pasteurized. Solutions that optimize product quality, and are economically feasible, are of particular interest to the dairy industry. To this end, this study examined the effects of raw milk holding time and temperature of pasteurized milk storage over shelf life on bacterial growth. In 3 independent replicates, raw milk was stored for 24 and 72 h before pasteurization at 76°C for 25 s and then incubated at 3 different storage conditions: (1) 4°C for 21 d; (2) 4°C for the first 48 h, then 6°C for the duration of the 21-d shelf life; or (3) 6°C for 21 d. Total bacteria counts were assessed initially and on d 7, 14, and 21. No substantial difference in bacterial growth over shelf life was observed between samples processed from raw milk held for 24 versus 72 h. A significantly lower bacterial load was seen at d 21 after pasteurization in samples held at 4°C, versus 4°C for the first 48 h followed by 6°C for the duration of the 21-d shelf life and samples held at 6°C for 21 d. This work demonstrates the importance of maintaining control of the fluid milk cold chain throughout postpasteurization, transportation, and retail storage on fluid milk microbial quality.

Key words: milk, shelf life, cold chain

Short Communication

The quality of HTST-pasteurized fluid milk has been improving over the last 2 decades, as specifically reported for New York State (Carey et al., 2005; Martin et al., 2012a). These quality improvements are largely due to more stringent good manufacturing practices,

enhanced routine preventative maintenance, and improved cleaning and sanitization protocols, which all reduce the incidence of postpasteurization contamination. Furthermore, many fluid milk manufacturers have taken steps to control the entry and outgrowth of psychrotolerant sporeforming bacteria (e.g., *Paenibacillus* sp.) that enter the fluid milk continuum on the farm and subsequently germinate and grow to spoilage levels in pasteurized, refrigerated fluid milk (Ranieri and Boor, 2009; Martin et al., 2012a; Masiello et al., 2014). Additional strategies to improve fluid milk quality and thereby extend shelf life include, among others, novel processing technologies (Sepulveda et al., 2009; Walkling-Ribeiro et al., 2011) and the addition of antimicrobial agents (Woodcock et al., 2009). Although these strategies may provide significant improvements in fluid milk quality, many processors are unable or unwilling to invest capital and other resources into these sometimes expensive endeavors. Alternative methods for improving shelf life that take advantage of the manufacturer's existing equipment and technology would be of interest to the dairy industry as a whole.

A promising area of quality improvement and shelf-life extension is in process and storage optimization, whereby existing equipment and resources are used in more efficient ways. One example of processing optimization that has resulted in improved fluid milk quality is reducing the outgrowth of psychrotolerant sporeformers through reductions in HTST pasteurization temperature. For example, Ranieri et al. (2009) found significantly lower bacterial counts on all days of refrigerated shelf life when HTST pasteurization temperature was lowered from 85.2°C to 72.9°C, and Martin et al. (2012b) similarly found significantly lower bacterial counts in commercially processed fluid milk pasteurized at 76.1°C compared with that pasteurized at 79.4°C. In addition to pasteurization parameters, previous work has examined the effect of raw milk storage time on pasteurized milk quality (Ravanis and Lewis, 1995). Ravanis and Lewis (1995) found that pasteurized fluid milk processed from raw milk stored for 3 to 4 d before processing had lower total bacteria counts after 43 d of refrigerated storage than those processed after 1, 7, or

Received March 4, 2015.

Accepted July 18, 2015.

¹Corresponding author: nhw6@cornell.edu

9 d of raw milk storage. The authors hypothesized that the inherent raw milk lactoperoxidase enzyme system is most active in raw milk after 3 to 4 d of refrigerated storage before pasteurization, leading to better pasteurized-fluid-milk keeping quality. The lactoperoxidase enzyme system and its bactericidal activity have been well studied in raw and pasteurized milk (Seifu et al., 2005). In the United States, however, activation (i.e., the addition of low levels of hydrogen peroxide and thiocyanate to fluid milk) of the lactoperoxidase system is prohibited (USDA, 2014). Leveraging the inherent raw milk lactoperoxidase system as it naturally occurs in raw milk by storing raw milk until the peak lactoperoxidase activity is reached before pasteurization may offer a low cost method for improving fluid milk quality.

Similarly, the effect of low temperature storage on pasteurized fluid milk has also been studied (Muir et al., 1987; Phillips and Griffiths, 1987; Griffiths and Phillips, 1988). Although there is a consensus that lower storage temperatures lead to slower and reduced bacterial growth over time, the fluid milk manufacturer can do very little to control this factor once the milk has left the processing facility. Examination of the effect of storage temperature on pasteurized fluid milk is necessary to determine whether lowering the storage temperature during the first 2 d after processing (a time period when the product typically remains at the processing facility) is a worthwhile optimization. To that end, this study had 2 aims: (1) to assess the effect of raw milk holding time on microbial quality of pasteurized fluid milk and (2) to assess the effect of pasteurized fluid milk storage temperature on bacterial growth over refrigerated storage.

High quality raw milk (i.e., history of low total bacteria count and low SCC) was obtained on 3 separate occasions from the Cornell Teaching and Research farm (Harford, NY) and transported to the Cornell University Food Processing and Development Laboratory (Ithaca, NY) at or below 6°C. A raw milk sample (approximately 100 mL) was aseptically collected for microbiological analysis. Raw milk samples were transported on ice to the Milk Quality Improvement Program Laboratory (Cornell University, Ithaca, NY) and plated on Coliform Petrifilm (3M, St. Paul, MN), SPC agar for total bacteria count and crystal violet tetrazolium agar for total gram-negative count. Raw milk was stored at 4°C before processing at either 24 or 72 h as follows. Raw milk held for 24 and 72 h was homogenized in 2 stages, yielding 13,789 kPa total (first stage 3,447 kPa, second stage 10,342 kPa). Homogenized raw milk was pasteurized at 76°C for 25 s on a Microthermics unit (model 25 DH, MicroThermics Inc., Raleigh, NC) and transferred to the laboratory on ice.

Upon arriving at the laboratory, samples processed from raw milk stored for 24 or 72 h were split into 3 sterile glass Pyrex bottles (Corning Inc., Corning, NY) with approximately 200 mL in each. Two bottles were placed in a 4°C incubator, one to be stored for 21 d and the second to be moved after 2 d at 4°C to a 6°C incubator for the remainder of the 21-d shelf life, simulating the cold chain from processor to consumer. The third bottle was stored in a 6°C incubator for 21 d. On the initial day and on d 7, 14, and 21 of storage all samples were plated on SPC to determine total bacteria count.

Statistical analysis was completed using the lmerTest package in R; a multilevel mixed effect model was fit with the log SPC as the response. The lsmeans package in R was then used to perform a Tukey's honestly significant difference test with the least squares means to identify combinations of refrigeration temperatures and days of shelf life with significantly different log SPC.

Raw milk samples were held for either 24 or 72 h before pasteurization. Although a slightly lower total mean bacterial count was observed at the end of shelf life in the pasteurized milk that was stored for 72 h before pasteurization, these samples and those held for 24 h were not significantly different ($P = 0.55$; Figure 1). Previous work has suggested that various raw milk hold times have an effect on pasteurized milk quality (Ravanis and Lewis, 1995). Ravanis and Lewis (1995) specifically reported that raw milk storage times of 3 to 4 d before pasteurization have the greatest positive effect on pasteurized milk quality, as assessed by bacterial growth over refrigerated shelf life. The authors observed that this raw milk hold time corresponds to when the inherent raw milk lactoperoxidase system is at its peak. The current study did not corroborate the previous findings.

The inherent raw milk lactoperoxidase system has been studied extensively (Haddadin et al., 1996; Fonteh et al., 2005; Seifu et al., 2005) and has been theorized to influence bacterial outgrowth in refrigerated fluid milk (Ranieri and Boor, 2009; Martin et al., 2012b). In one study, approximately 70% of the raw milk lactoperoxidase enzyme has been reported to remain active after minimum pasteurization time-temperature combinations (72°C for 15 s), whereas temperatures exceeding 76°C were reported to completely eliminate lactoperoxidase activity found in raw milk (Barrett et al., 1999). Levels of lactoperoxidase, however, vary greatly from cow to cow and even from day to day within the same cow (Fonteh et al., 2002). This variability may explain the differences seen between this study and previous work (Ravanis and Lewis, 1995). The intrinsic variability in the raw milk lactoperoxidase system limits the practical use of this enzyme system for improving quality in a standardized and consistent manner.

Figure 1. Mean SPC (log cfu/mL) for all days of shelf life by raw milk hold time. Dark gray bars represent samples held for 24 h before pasteurization, and light gray bars represent 72 h of hold time. Error bars represent ± 1 standard deviation for data representing 3 independent trials.

Holding raw milk for long periods of time before pasteurization should also be cautioned against because of the potential for growth of psychrotolerant gram-negative bacteria such as *Pseudomonas* sp. Some *Pseudomonas* and other gram-negative contaminants in raw milk may produce heat-stable enzymes capable of deteriorating the sensorial quality of pasteurized fluid milk and other dairy products (Sørhaug and Stepaniak, 1997). Furthermore, although raw milk quality and parameters play an important role in pasteurized fluid milk quality, a myriad of other factors affect fluid milk quality (e.g., postprocessing contamination), which highlights the fact that a grass to glass approach should be taken to ensure high quality fluid milk.

Pasteurized milk samples were held under the following conditions: (1) 4°C for 21 d; (2) 4°C for the first 48 h, then 6°C for the duration of the 21-d shelf life; or (3) 6°C for 21 d. Samples were evaluated for total bacterial count initially and on d 7, 14, and 21 after processing. Results indicated that at d 21, bacterial numbers significantly differed between samples held at 4°C versus both the samples held at 6°C and those held at 4°C for 48 h followed by 6°C for the remainder of the 21-d shelf life ($P = 0.0004$, $P = 0.023$, respectively; Figure 2). These significant differences between samples held at the lowest temperatures and samples held at higher temperatures for all or a portion of their shelf life are consistent with the observation that bacterial generation time is slowed at lower temperatures (Phillips and Griffiths, 1987). The magnitude of difference in bacterial counts observed between samples held at 4°C

Figure 2. Mean SPC (log cfu/mL) at d 21 of refrigerated shelf life by storage temperature. ^{a,b}Differing letters indicate statistically significant differences between means. Error bars represent ± 1 standard deviation for data representing 3 independent trials.

versus 6°C (or 4°C and then 6°C) is noteworthy though with, for example, a greater than 2.0 log difference in d-21 bacterial counts between milk stored at 4 and 6°C for 21 d (which equals an approximately 100-fold higher bacterial count in the milk stored at 6°C). Overall, our results indicate that holding pasteurized milk at 4°C for the first 2 d after processing before holding it at 6°C for the remainder of storage does not have a significant effect on bacterial growth when compared with samples held at 6°C for the entire shelf life, suggesting no significant long-lasting effect exists from holding product at low temperatures initially after pasteurization if the product is temperature abused during the majority the of shelf life.

Results of this study emphasize how critical maintaining the fluid milk cold chain, from processor to consumers, is to optimize fluid milk microbial quality. Processors have little control over the storage temperature of their product once it leaves the processing facility. Retailers and, more importantly, consumers can be a major contributor to temperature abuse of fluid milk. Studies have shown that many consumers' home refrigerators are set to temperatures above the recommended 4.4°C (Kosa et al., 2007). The extension of fluid milk shelf life and improvement of quality not only enhances consumer acceptance of fluid milk, but has the potential to drastically reduce food waste due to spoilage. Dairy products, in particular, have one

of the highest rates of waste, with current estimates reaching 19% of product lost in retail, food service, and households (Gunders, 2012). This study highlights both the importance of cold chain management for processors as well as how critical consumer education will be in maximizing fluid milk quality.

ACKNOWLEDGMENTS

This work was supported by the New York State Milk Promotion Advisory Board through the New York State Department of Agriculture and Markets—NYS dairy farmers committed to the production of high quality dairy products. The authors acknowledge the staff and students of the Milk Quality Improvement Program and the Cornell Food Product Development Laboratory (Ithaca, NY) for their support in this project.

REFERENCES

- Barrett, N. E., A. S. Grandison, and M. J. Lewis. 1999. Contribution of the lactoperoxidase system to the keeping quality of pasteurized milk. *J. Dairy Res.* 66:73–80.
- Carey, N. R., S. C. Murphy, R. N. Zadoks, and K. J. Boor. 2005. Shelf lives of pasteurized fluid milk products in New York State: A ten year study. *Food Prot. Trends* 25:102–113.
- Fonteh, F. A., A. S. Grandison, and M. J. Lewis. 2002. Variations of lactoperoxidase activity and thiocyanate content in cows' and goats' milk throughout lactation. *J. Dairy Res.* 69:401–409.
- Fonteh, F. A., A. S. Grandison, and M. J. Lewis. 2005. Factors affecting lactoperoxidase activity. *Int. J. Dairy Technol.* 58:233–236.
- Griffiths, M. W., and J. D. Phillips. 1988. Modelling the relation between bacterial growth and storage temperature in pasteurized milks of varying hygienic quality. *J. Soc. Dairy Technol.* 41:96–102.
- Gunders, D. 2012. Wasted: How America is losing up to 40 percent of its food from farm to fork to landfill. Natural Resources Defense Council Issue Paper IP:12–06-B. Nat. Resour. Def. Council, New York, NY.
- Haddadin, M. S., S. A. Ibrahim, and R. K. Robinson. 1996. Preservation of raw milk by activation of the natural lactoperoxidase systems. *Food Contr.* 7:149–152.
- Kosa, K. M., S. C. Cates, S. Karns, S. L. Godwin, and D. Chambers. 2007. Consumer home refrigeration practices: results of a web-based survey. *J. Food Prot.* 70:1640–1649.
- Martin, N. H., N. R. Carey, S. C. Murphy, M. Wiedmann, and K. J. Boor. 2012a. A decade of improvement: New York state fluid milk quality. *J. Dairy Sci.* 95:7384–7390.
- Martin, N. H., M. L. Ranieri, M. Wiedmann, and K. J. Boor. 2012b. Reduction of pasteurization temperature leads to lower bacterial outgrowth in pasteurized fluid milk during refrigerated storage: A case study. *J. Dairy Sci.* 95:471–475.
- Masiello, S. N., N. H. Martin, R. D. Watters, D. M. Galton, Y. H. Schukken, M. Wiedmann, and K. J. Boor. 2014. Identification of dairy farm management practices associated with the presence of psychrotolerant sporeformers in bulk tank milk. *J. Dairy Sci.* 97:4083–4096.
- Muir, D. D., M. E. Kelly, and J. D. Phillips. 1978. The effect of storage temperature on bacterial growth and lipolysis in raw milk. *J. Soc. Dairy Technol.* 31:203–208.
- Phillips, J. D., and M. W. Griffiths. 1987. The relation between temperature and growth of bacteria in dairy products. *Food Microbiol.* 4:173–185.
- Ranieri, M. L., and K. J. Boor. 2009. Short communication: Bacterial ecology of high-temperature, short-time pasteurized milk processed in the United States. *J. Dairy Sci.* 92:4833–4840.
- Ranieri, M. L., J. R. Huck, M. Sonnen, D. M. Barbano, and K. J. Boor. 2009. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. *J. Dairy Sci.* 92:4823–4832.
- Ravanis, S., and M. J. Lewis. 1995. Observations on the effect of raw milk quality on the keeping quality of pasteurized milk. *Lett. Appl. Microbiol.* 20:164–167.
- Seifu, E., E. M. Buys, and E. F. Donkin. 2005. Significance of the lactoperoxidase system in the dairy industry and its potential applications: A review. *Trends Food Sci. Technol.* 16:137–154.
- Sepulveda, D. R., M. M. Gongora-Nieto, J. A. Guerrero, and G. V. Barbosa-Canovas. 2009. Shelf life of whole milk processed by pulsed electric fields in combination with PEF-generated heat. *Food Sci. Technol. (Campinas)* 42:735–739.
- Sørhaug, T., and L. Stepaniak. 1997. Psychrotrophs and their enzymes in milk and dairy products: Quality aspects. A review. *Trends Food Sci. Technol.* 8:35–40.
- USDA. 2014. Direct food substances affirmed as generally recognized as safe. 21 CFR, part 184. Accessed Nov. 24, 2014. <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=184.1366>.
- Walkling-Ribeiro, M., O. Rodriguez-Gonzalez, S. Jayaram, and M. W. Griffiths. 2011. Microbial inactivation and shelf life comparison of 'cold' hurdle processing with pulsed electric fields and microfiltration, and conventional thermal pasteurisation in skim milk. *Int. J. Food Microbiol.* 144:379–386.
- Woodcock, N. H., B. H. Hammond, R. D. Ralyea, and K. J. Boor. 2009. Short communication: N α -lauroyl-L-arginine ethylester monohydrochloride reduces bacterial growth in pasteurized milk. *J. Dairy Sci.* 92:4207–4210.