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# *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

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## 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

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processing had lower total bacteria counts after 43 d of refrigerated storage than those processed after 1, 7, or 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 Pertrifilm (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 lactoperoxide 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