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High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk

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ABSTRACT

The grade A Pasteurized Milk Ordinance specifies minimum processing conditions of 72°C for at least 15 s for high temperature, short time (HTST) pasteurized milk products. Currently, many US milk-processing plants exceed these minimum requirements for fluid milk products. To test the effect of pasteurization temperatures on bacterial numbers in HTST pasteurized milk, 2% fat raw milk was heated to 60°C, homogenized, and treated for 25 s at 1 of 4 different temperatures (72.9, 77.2, 79.9, or 85.2°C) and then held at 6°C for 21 d. Aerobic plate counts were monitored in pasteurized milk samples at d 1, 7, 14, and 21 postprocessing. Bacterial numbers in milk processed at 72.9°C were lower than in milk processed at 85.2°C on each sampling day, indicating that HTST fluid milk-processing temperatures significantly affected bacterial numbers in fluid milk. To assess the microbial ecology of the different milk samples during refrigerated storage, a total of 490 psychrotolerant endospore-forming bacteria were identified using DNA sequence-based subtyping methods. Regardless of processing temperature, >85% of the isolates characterized at d 0, 1, and 7 postprocessing were of the genus *Bacillus*, whereas more than 92% of isolates characterized at d 14 and 21 postprocessing were of the genus *Paenibacillus*, indicating that the predominant genera present in HTST-processed milk shifted from *Bacillus* spp. to *Paenibacillus* spp. during refrigerated storage. In summary, 1) HTST processing temperatures affected bacterial numbers in refrigerated milk, with higher bacterial numbers in milk processed at higher temperatures; 2) no significant association was observed between genus isolated and pasteurization temperature, suggesting that the genera were not differentially affected by the different processing temperatures; and 3) although typically present at low numbers in raw milk, *Paenibacillus* spp. are capable of growing to numbers that can exceed Pasteurized Milk Ordinance limits in pasteurized, refrigerated milk.

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INTRODUCTION

In the United States, *Bacillus* spp. and *Paenibacillus* spp. have been identified as the biological barriers currently limiting HTST pasteurized fluid milk shelf life (Fromm and Boor, 2004; Durak et al., 2006; Huck et al., 2007b, 2008). *Bacillus* spp. and *Paenibacillus* spp. are capable of forming heat-resistant spores that can survive HTST pasteurization (Collins, 1981; Huck et al., 2007b), and some strains are able to germinate and grow at refrigeration temperatures, ultimately causing spoilage of processed products (Washam et al., 1977; Huck et al., 2008). Because *Bacillus* spp. and *Paenibacillus* spp. spores are ubiquitously present in nature, it is difficult to exclude them from the milk supply. Representatives from both genera have been isolated from dairy farms (Crielly et al., 1994; Sutherland and Murdoch, 1994; Lukasova et al., 2001; Huck et al., 2008), processing plants (Lin et al., 1998; Huck et al., 2007b), and pasteurized packaged products (Griffiths and Phillips, 1990; Lin et al., 1998; Douglas et al., 2000; Fromm and Boor, 2004; Durak et al., 2006; Huck et al., 2007b).

In recent years, both food safety concerns and the desire to extend fluid milk shelf life have prompted many dairy processors to increase HTST pasteurization temperatures above the minimum conditions specified by the Pasteurized Milk Ordinance (72°C for 15 s; Pasteurized Milk Ordinance, 2005; Gandy et al., 2008). Anecdotal reports from multiple milk processors of higher bacterial numbers and more rapid spoilage in fluid milk products after an increase in processing temperatures prompted this investigation of the effects of commonly applied HTST pasteurization temperatures on aerobic plate counts (**APC**) in refrigerated fluid milk products. To test the hypothesis that higher HTST processing temperatures result in higher bacterial numbers in fluid milk products during refrigerated storage, the objectives of this study were 1) to determine the effect of processing temperature on APC in milk samples that had been processed under a range of

commonly applied HTST temperatures and held under refrigerated storage conditions (6°C); and 2) to monitor the microbial ecology of pasteurized products processed at each temperature during storage.

MATERIALS AND METHODS

Experimental Design

To measure the effects of HTST pasteurization temperatures on APC in pasteurized fluid milk, 4 independent batches of raw 2% fat homogenized milk were each processed for 25 s at one of the following temperatures: 72.9, 77.2, 79.9, or 85.2°C. A complete block design was used to allow all combinations of position in the processing order (first, second, third, or fourth) for each processing temperature (72.9, 77.2, 79.9, or 85.2°C) to ensure that each temperature held a different position in processing in each replicate.

Pasteurization of Raw Milk

Raw bovine milk from approximately 300 cows in Hartford, New York, was pooled, and representative samples were collected and transported to the Cornell University Food Processing and Development Laboratory (Ithaca, NY). The raw milk was cold-separated into cream and skim fractions with a DeLaval separator (Model 590; DeLaval, Poughkeepsie, NY), and then held refrigerated at 5°C for up to 72 h. On the day of processing, raw skim milk and cream were blended to make 2% fat milk. Approximately 5 gallons (18.93 L) of 2% fat raw milk was added to a jacketed steam kettle, heated to 60°C, and then homogenized with 2 passes through a Gaulin APV homogenizer (Model 200 E; Gaulin, Everett, MA). After homogenization, the milk was pasteurized at 72.9, 77.2, 79.9, or 85.2°C for 25 s as described by Ma and Barbano (2003). Milk samples (2 L) pasteurized at each temperature were collected aseptically and held on ice until all processing treatments were completed. Immediately postprocessing, 8 aliquots of approximately 200 mL were poured from each of the 2-L containers into sterile 250-mL screw-capped Pyrex bottles. The pasteurized milk samples were held at 6°C for up to 21 d.

Microbiological Testing of Milk Samples

To determine the microbiological quality of the raw milk before pasteurization, a sample was taken from the standardized 2% fat milk before heating and homogenization. Milk samples were assessed for total APC, coliform count, psychrotrophic bacteria count (**PBC**), and laboratory pasteurized count (**LPC**) according to the Standard Methods for the Examination of Dairy Products (Frank and Yousef, 2004), except that the samples for APC, PBC, and LPC tests were spread-plated onto brain heart infusion agar (**BHI**; Difco, BD Diagnostics, Franklin Lakes, NJ). Preliminary incubation counts were performed on raw 2% fat milk samples according to the Standard Methods for the Examination of Dairy Products (Richardson, 1985), except that the raw 2% fat samples were spread-plated onto BHI agar and incubated at 32°C for 24 h. For the mesophilic spore count (**MSC**) and psychrotrophic spore count (**PSC**) tests, milk samples were heated at 80°C for 12 min, and then the milk was cooled rapidly, incubated overnight at 6°C, and spread-plated the following day on BHI agar, with subsequent incubation at 32°C for 24 h (for the MSC) or at 6°C for 10 d (for the PSC).

Two aliquots of milk that had been processed at each temperature were tested for APC at d 1, 7, 14, and 21 postpasteurization. Samples were serially diluted in PBS (Weber Scientific, Hamilton, NJ), spread-plated onto BHI agar, and incubated at 32°C for 24 h. On d 1, plating was performed by spreading 1 mL of milk sample over 5 plates to allow bacterial enumeration in samples with low bacterial counts.

Statistical Analyses

A mixed model was used to analyze the APC data (JMP Version 7.0; SAS Institute Inc., Cary, NC). For all analyses, log-transformed bacterial count data were used as a response. The model included temperature of processing and time of refrigerated storage of pasteurized milk as independent fixed variables. Replicate and temperature were random variables in the model. Temperature was a between-replicate effect and day was a within-replicate effect. Models were tested for an interaction between time and temperature. When an effect was significant, multiple comparisons were done with a Tukey correction. Distribution of bacterial subtypes was analyzed in JMP using a chi-square test for independence.

Bacterial Isolation

For each milk sample, bacterial colonies present on BHI that had been plated at d 0, 1, 7, 14, and 21 postprocessing were visually examined and a colony representative of each distinct morphology present on 1 plate that had been used for colony enumeration for each sample was chosen for isolation and later identification. Typically, 5 to 10 colonies per sample were selected and streaked for purity on BHI agar. Purified isolates were characterized for gram reaction using a 3-step gram stain kit (Becton, Dickinson and Co., Sparks, MD) and subsequently frozen at −80°C in 15% glycerol.