



Monte Carlo simulation model predicts bactofugation can extend shelf-life of pasteurized fluid milk, even when raw milk with low spore counts is used as the incoming ingredient

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ABSTRACT

Bacterial spores from raw milk that survive the pasteurization process are responsible for half of all the spoilage of fluid milk. Bactofugation has received more attention as a nonthermal method that can reduce the presence of bacterial spores in milk and with it the spoilage of fluid milk. The objective of this work was to determine the effectiveness of bactofugation in removing spores from raw milk and estimate the effect the spore removal could have on shelf-life of fluid milk. The study was conducted in a commercial fluid milk processing facility where warm spore removal was performed using one-phase bactofuge followed by warm cream separation and high temperature, short time pasteurization. Samples from different stages of fluid milk processing with and without the use of bactofuge were tested for total plate count, mesophilic spore count, psychrotolerant spore count (PSC), and somatic cell count. Results were evaluated to determine the count reductions during different stages of fluid milk processing and compare counts in fluid milk processed with and without bactofugation. Bactofugation on average reduced the total plate count by 1.81 ± 0.72 log cfu/mL, mesophilic spore count by 1.08 ± 0.71 log cfu/mL, PSC by 0.86 ± 0.59 log cfu/mL, and somatic cell count by $135,881 \pm 43,942$ cells/mL. Psychrotolerant spore count in final pasteurized skim milk processed with and without bactofugation was used to predict the shelf-life of the pasteurized skim milk using the Monte Carlo simulation model. Although PSC in the initial raw milk was already low (-0.63 ± 0.47 log cfu/mL), the predicted values from the simulation model showed that bactofugation would extend the shelf-life of pasteurized skim milk by approximately 2 d. The results of this study will directly help fluid milk processors evaluate

the benefits of using bactofugation as an intervention in their plants, and also demonstrate the benefits of using mathematical modeling in decision making.

Key words: bactofugation, spore removal, fluid milk, shelf-life, mathematical modeling

INTRODUCTION

Transfer of bacterial spores from raw milk to pasteurized fluid milk and other dairy products is responsible for the spoilage of a large proportion of these products, and it is associated with large economic loss in the dairy industry. Spores are metabolically dormant forms of bacterial cells that sporeforming bacteria such as *Bacillus* spp., *Paenibacillus* spp., or *Clostridium* spp. convert to under conditions of environmental stress. Presence of spores in the final product and their subsequent germination and growth can lead to quality issues and decreased shelf-life in pasteurized fluid milk, cheese, and dairy powders (Burgess et al., 2010; Lücking et al., 2013). Part of the reason for the persistence of this problem is the fact that spores are highly resistant to pasteurization, desiccation, and cleaning chemicals used in the dairy processing environment (Ryu and Beuchat, 2005; Scheldeman et al., 2006; Setlow, 2006).

Due to the ubiquity of spores in the farm environment, numerous studies have focused on the identification and control of raw milk contamination with bacterial spores at the farm level. Although improving farm practices can reduce both the frequency and level of contamination of raw milk with spores, this problem cannot be completely avoided (Scheldeman et al., 2005; Masiello et al., 2014; Miller et al., 2015). Therefore, dairy processors are also looking into interventions that can either inactivate or remove spores from the raw milk. Ultra-high temperature processing (for example, 138–140°C for 2–3 s) is effective at destroying spores; however, this treatment changes the sensory characteristics of milk (Chapman and Boor, 2001; Chavan et al., 2011). Additionally, this method is not suitable for cheese making, as temperatures used in UHT treatment alter the coagulation properties of milk.

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Alternatively, nonthermal processing methods such as bactofugation and microfiltration can be used to physically remove spores from milk without altering the product characteristics (Stack and Sillen, 1998; Gésan-Guiziou, 2010; Griep et al., 2018). Bactofugation is a centrifugation process designed to decrease the microbial load of milk by removing both vegetative bacterial cells and bacterial spores, based on the difference in density between bacterial cells or spores and milk (Walstra et al., 1999; Gésan-Guiziou, 2010). Bactofugation was reported to have no negative effect on the sensory properties of fluid milk and to have reduced the total bacterial counts in raw milk by up to 92% and bacterial spores by up to 98% (Stack and Sillen, 1998; Gésan-Guiziou, 2010; Jovanovska et al., 2017; Juraga et al., 2021). The bacterial spores removed by bactofugation include spores of psychrotolerant (i.e., cold-tolerant) bacteria which can germinate and grow at refrigeration temperatures and spoil fluid milk through different mechanisms including degradation of milk components (Ivy et al., 2012; Ribeiro-Júnior et al., 2019; Ribeiro-Júnior et al., 2020).

Although this technology has received increasing interest in recent years, few data are publicly available on the effectiveness of bactofugation in removing psychrotolerant sporeformers which are responsible for half of all fluid milk spoilage (Alles et al., 2018; Reichler et al., 2018). Data that are publicly available might also be considered by some to be outdated and not fully representative of high microbiological quality of large share of raw milk produced today. Additionally, the available information on bactofugation has never been evaluated to determine the effectiveness of this technology to reduce spoilage of pasteurized fluid milk, due to outgrowth of psychrotolerant sporeforming bacteria. To fill these knowledge gaps, the current study evaluated bactofugation in a commercial fluid milk processing facility and directly compared the microbiological quality of fluid milk processed with and without the use of the bactofugation. Data generated during this study were further evaluated using a Monte Carlo simulation model to predict the effect the observed reductions of spores of psychrotolerant sporeformers in raw milk have on the shelf-life of the final pasteurized fluid milk.

The Monte Carlo simulation model is a useful mathematical tool that is commonly used to predict outcomes of complex systems such as microbial growth in foods. As a stochastic model, it simultaneously considers all variables of the system to predict all possible outcomes of the system. A number of different Monte Carlo simulation models have been developed to predict spoilage of different dairy products, including cheese (Qian et

al., 2022), yogurt (Buehler et al., 2018a), and fluid milk (Buehler et al., 2018b; Lau et al., 2022). The framework of each model allows for sensitivity analysis to be performed to determine the relative importance of each model variable, and it also allows for testing of different “what-if” scenarios to evaluate the effectiveness of different intervention strategies. The characteristics of Monte Carlo simulation models, including ability to test different “what-if” scenarios, make these models a valuable tool in decision making.

The results of this study provide fluid milk processors with objective estimations of the benefits of bactofugation for fluid milk quality (i.e., expected additional number of days of shelf-life), as well as demonstrate the benefits of using mathematical modeling in decision making, both of which can assist them in making informed cost-benefit analysis of implementing bactofugation as an intervention (Enayaty-Ahangar et al., 2021).

MATERIALS AND METHODS

Processing

The study was conducted on cow milk in a commercial fluid milk processing plant in New York State during regular daily production. Because no live human or animal subjects were used in this study, institutional review board or institutional animal care and use committee approval was not required.

The milk processing flow was as follows: raw milk first passed through a one-phase bactofuge (operating speed 4,250 rpm; Tetra Pak), followed by cream separation on a centrifugal self-cleaning separator (operating speed 4,700 rpm; GEA Process Engineering Inc.) with solids ejection set at every 30 min (Figure 1). Both bactofugation and cream separation were performed on warm milk (60 to 65°C) at a flow rate of 23,400 L/h. After cream separation, the skim milk was pasteurized by passing through a standard HTST heat exchanger (78°C/30s). The duration of each processing run was between 10 and 13 h, during which time between 234,000 and 304,000 L of fluid milk were processed. After each processing run, the entire processing line was cleaned in place using consecutive alkaline and acid wash cycles separated by rinsing cycles. Some parts of the processing line (e.g., vacuum breaker, vents, and caps) were disassembled and manually cleaned using chlorinated alkaline cleaning solution. The entire processing line including the reassembled parts that were manually cleaned were sanitized before each processing run using peroxyacid-based sanitizer.

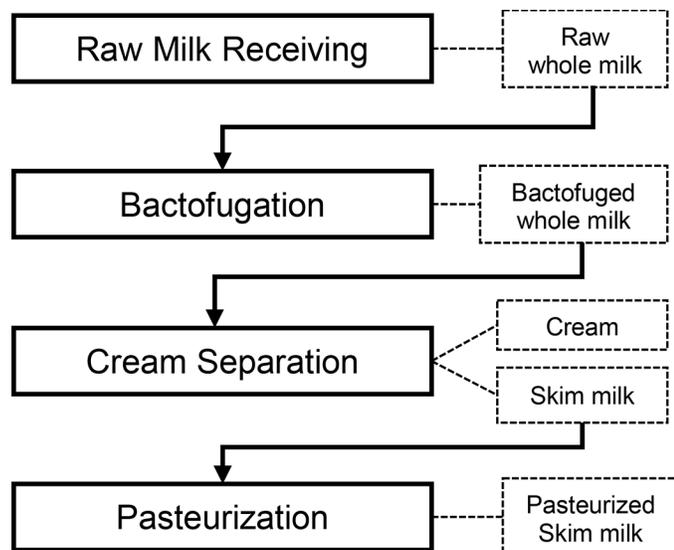


Figure 1. Flow diagram of the fluid milk processing line. Dashed boxes indicate collection points and collected streams.

Sampling

The study consisted of 2 experiments: (1) the main experiment, with the bactofuge installed in the fluid milk processing line before cream separation, and (2) a control experiment, with the bactofuge bypassed and whole milk pumped directly to the cream separator. Both experiments were repeated 3 times; each repetition was performed on a separate processing day using separate fresh raw milk collected on the same day. During each repetition of the experiment, we collected triplicate samples of raw whole milk from the balance tank, bactofuged whole milk, raw skim milk, raw cream, and pasteurized skim milk; the control experiment did not generate bactofuged whole milk. All samples were collected within the first 90 min of the processing run. During the main experiment another complete set of triplicate samples was also taken at the end of the run (after 10–13 h of processing), to verify if differences in effectiveness of the spore removal occurred during the run. Sampling was done using either an aseptic sampling port with a sterile sampling bag (QualiTru Sampling Systems) or pre-sterilized sampling dippers (Cole Parmer). All samples were immediately cooled to less than 4°C using an ice-bath and stored below 4°C until analyses.

Sample Analyses

The first of the triplicate samples from each collection point was analyzed onsite for total plate count (TPC) within 6 h of collection; the first samples of raw

whole milk and raw skim milk were also analyzed onsite for total composition. The second sample was transported refrigerated (below 4°C) to the Milk Quality Improvement Program laboratory (Cornell University) and analyzed for the presence of spores within 40 h of collection; both mesophilic spore count (MSC) and psychrotolerant spore count (PSC) were determined. The last of the triplicate samples was transported, refrigerated (below 4°C), to a commercial laboratory (Dairy One) and analyzed for SCC and total protein content within 40 h of collection. Spore germination and lag phase of growth for major groups of sporeformers responsible for spillage of fluid milk was determined to be between 3.1 and 46.2 d at 4°C (Buehler et al., 2018b); assumption was made based on this information that the MSC, PSC, and chemical composition of milk samples would not change within 40 h of sample collection.

Microbiological Analyses

Total plate count was determined by performing initial 10-fold dilutions (i.e., 1:10 dilution) of each sample using 0.1% peptone water (Becton, Dickinson and Company) and pour plating appropriate dilutions in duplicate on standard plate count agar (Difco, Becton, Dickinson and Company); the plates were incubated for 48 h at 32°C. For spore count analyses, a spore pasteurization step was conducted, which allowed inactivation of vegetative cells and germination of existing spores. The methodology consisted of heating 10 mL of sample in a water bath at 80°C for 12 min and cooling it to 6°C before enumeration. Mesophilic spore count was determined by pour plating appropriate 10-fold dilutions in duplicate on brain heart infusion agar (Difco, Becton, Dickinson and Company); the plates were incubated for 48 h at 32°C. Due to anticipated low PSC in tested samples, a 3-tube most probable number technique was used for enumeration (Davidson et al., 2004). Briefly, 10 mL of spore pasteurized sample was aliquoted into 3 separate sterile tubes (no dilution), 1 mL of spore pasteurized sample was aliquoted into 3 sterile tubes with each containing 9 mL of sterile skim milk broth (1:10 dilution), and finally, 0.1 mL of spore pasteurized sample was aliquoted into 3 sterile tubes containing 9.9 mL of sterile skim milk broth (1:100 dilution). The 9 tubes were vortexed and incubated at 6°C for 21 d before spread plating 100 µL from each tube on standard plate count agar. Plates were evaluated for presence or absence of growth after 48 h of incubation at 32°C. The most probable number data were interpreted using a 3-tube most probable number table (Davidson et al., 2004).

Other Analyses

All milk samples including cream were also tested for SCC and total protein content to determine the effect of bactofugation on these 2 important factors that can influence the yield, profitability, and quality of milk and milk products. Somatic cell count was determined using direct microscopic count (Fitts and Laird, 2004). Cream samples were analyzed for total protein using AOAC Official Method 991.20 (AOAC International, 1998). The protein content of all other samples was determined using a Milkoscan Minor (Foss).

Statistical Analysis

The data were collected and managed in Excel (Microsoft Corp.) and analyzed using R statistical software together with the R stats package (version 3.6.2.; R Project). All microbial counts and SCC were log-transformed for analysis. The counts and protein content from samples of different product streams within each of the 2 experiments were analyzed for significant differences ($P \leq 0.05$) using one-way ANOVA and Tukey's honest significant difference test. The microbial counts in skim, cream, and pasteurized skim milk were individually tested, using Student's *t*-test, for significant difference ($P \leq 0.05$) between product streams obtained with and without the use of bactofuge.

Computer Model Simulations

Data on PSC in final pasteurized milk obtained with and without the use of bactofugation were used in a Monte Carlo simulation model to predict fluid milk spoilage due to outgrowth of psychrotolerant gram-positive sporeformers. For the purpose of this study, spoilage of fluid milk was defined as microbial counts above the legal limit of 20,000 cfu/mL (4.3 log cfu/mL) set by the Pasteurized Milk Ordinance (FDA, 2019). The simulation model, previously published by Buehler et al. (2018b), was based on 14 most common subtypes of psychrotolerant sporeforming bacteria found in raw and pasteurized milk. The model is designed to simulate the growth of individual subtypes in HTST pasteurized milk that is bottled in half-gallon plastic containers and stored at refrigeration temperature of 6°C. Two separate model simulations were performed; (1) using PSC results from the main experiment with the use of bactofuge; (2) using PSC results from the control experiment where bactofuge was bypassed. Each of the 2 simulations consisted of 6,000 iterations, where each iteration represented a single half-gallon of pasteurized milk tracked over 28 d of refrigerated storage at 6°C.

Both simulations were performed using R statistical software (version 3.6.2.; R Project).

RESULTS AND DISCUSSION

Bactofugation Maintained Constant Effectiveness Throughout the Processing Run

During the main experiment, where raw milk was subjected to bactofugation before cream separation, we collected a full set of samples during both the early and late phases of milk processing. The TPC of the raw milk processed during the early phase (first 90 min of processing) on the 3 experimental days ranged between 3.60 and 4.02 log cfu/mL, and MSC between 0.60 and 1.50 log cfu/mL. The raw milk processed during the late phase (after 10 to 13 h of processing) on the 3 processing days had TPC between 3.65 and 5.51 log cfu/mL, and MSC between 0.60 and 1.24 log cfu/mL. The counts for raw milk after bactofugation ranged between 1.73 and 2.27 log cfu/mL (TPC, early processing phase), 2.22 and 2.48 log cfu/mL (TPC, late processing phase), < -0.90 and 0.18 log cfu/mL (MSC, early phase), < -0.90 and 0.48 log cfu/mL (MSC, late phase). The results for samples collected during the 2 phases of processing showed no significant differences between them ($P > 0.200$), based on these results we considered the performance of the bactofuge to be unchanged during the processing run. Maintaining consistent performance over the entire processing run is important for the processor because it can ensure consistent quality of the final product throughout entire processing run. For further analysis all the results from each product stream and experiment were combined; (1) for the main experiment with the use of bactofuge results were represented as averages of 6 individual data points, while (2) for the control experiment without the use of bactofugation results were represented as averages of 3 individual replicates (Table 1; Figures 2 and 3).

Bactofugation Was Effective at Removing Bacteria from Raw Whole Milk

Bactofugation effectively removed bacteria from raw whole milk, with an average TPC reduction of 1.81 ± 0.72 log cfu/mL (Figure 2). Over the 6 paired data points, reduction by bactofugation ranged between 1.33 to 3.03 log cfu/mL, or 95.3% to 99.9% of the initial bacterial load in the raw whole milk. These results are showing slightly higher efficiency of bactofugation compared with previous reports available in the literature that mention efficiencies of up to 92% (Stack and Silen, 1998; Gésan-Guiziou, 2010; Juraga et al., 2021).

Table 1. Mesophilic (MSC) and psychrotolerant (PSC) spore count in various product streams obtained with and without bactofugation¹

Product stream	Temperature at collection (°C)				MSC (log cfu/mL)		PSC (log MPN/mL)	
	With bactofuge		Without bactofuge		With bactofuge	Without bactofuge	With bactofuge	Without bactofuge
	Raw whole milk	2.2 ± 1.8	1.4 ± 1.5	0.92 ± 0.39 ^a	1.30 ± 0.35 ^a	-0.63 ± 0.47 ^x	-0.88 ± 0.49 ^{xx}	
Bactofuged raw whole milk	60.3 ± 2.6	N/A ²	-0.16 ± 0.59 ^{bc}	N/A	-1.49 ± 0.35 ^{xy}	N/A		
Raw skim milk	63.5 ± 1.1	64.0 ± 1.0	-0.47 ± 0.67 ^c	0.93 ± 0.40 ^{ab}	-2.12 ± 0.00 ^{yz}	-1.24 ± 0.81 ^{yxz}		
Raw cream	63.5 ± 1.5	63.5 ± 1.4	0.26 ± 0.20 ^{abc}	0.53 ± 0.46 ^{abc}	-1.00 ± 0.70 ^{sw}	-1.27 ± 0.77 ^{xyz}		
Pasteurized milk	2.1 ± 0.2	1.9 ± 0.1	-0.49 ± 0.65 ^c	0.99 ± 0.34 ^{ab}	-1.90 ± 0.35 ^{wxy}	-0.97 ± 0.42 ^{xyz}		

^{a-c}Means with different superscripts within MSC are significantly different from each other ($P < 0.05$).

^{x-z}Means with different superscripts within PSC are significantly different from each other ($P < 0.05$).

¹Counts below the detection limit were treated as 25% of the detection limit value (e.g., PSC under the detection limit of 0.03 cfu/mL were counted as 0.0075 cfu/mL, and MSC under 0.5 cfu/mL limit of detection were counted as 0.125 cfu/mL); MPN = most probable number.

²N/A = not applicable.

This higher efficiency of bacterial removal is likely the result of the higher raw milk temperatures ($60.3 \pm 2.6^\circ\text{C}$; Table 1) used during bactofugation in the current study, which might have both a (1) direct effect, by inactivating some of the heat sensitive vegetative bacterial cells, and (2) indirect effect, by reducing the viscosity of the milk (Stack and Sillen, 1998; Ribeiro-Júnior et al., 2020). Although higher temperatures used for bactofugation are known to have a positive effect on bacterial removal, physicochemical changes in milk such as serum protein denaturation and even coagulation might negatively affect the process if temperatures are increased above 60°C (Gésan-Guiziou, 2010).

Considering that the Pasteurized Milk Ordinance limit for bacterial load in raw milk ($5.00\text{--}5.48$ log cfu/mL) is based on expected maximum capacity of standard pasteurization (FDA, 2019), the average bacterial removal observed during this study (1.81 ± 0.72 log cfu/mL) can ensure bacterial loads in raw milk are within the capacity of pasteurization. Although high pre-pasteurization bacterial loads are less likely to be an issue in countries with well-developed dairy industries and regulations, high bacterial loads in raw milk are still an issue in countries that lack effective cooling practices during collection, storage, and transportation of raw milk (Martin et al., 2018).

Bactofugation Reduced Bacterial Spores in Pasteurized Skim Milk

The production flow that included bactofugation of raw whole milk significantly reduced the spore load (MSC) in the final pasteurized skim milk ($P < 0.003$); when bactofugation was not used, no statistically significant reduction of MSC in milk was observed ($P > 0.998$). Bactofugation reduced the MSC from 0.92 ± 0.39 log cfu/mL to -0.16 ± 0.59 log cfu/mL (91.7% of the initial count), and after this initial significant reduction ($P = 0.032$) the concentration of MSC likely remained comparable to skim milk after cream separation ($P = 0.982$) and pasteurization ($P = 1.000$). Mesophilic spore count includes both psychrotolerant sporeformers and sporeformers that are not able to grow at refrigeration temperature; although the psychrotolerant sporeformers can grow at refrigeration temperatures, their optimal growth temperatures are typically around 21°C or higher (Trmčić et al., 2015). Psychrotolerant sporeformers, as well as non-psychrotolerant sporeformers that are not able to grow at refrigeration temperature, can cause rapid spoilage of fluid milk if fluid milk is exposed to temperature abuse. Because of this it is important that bactofugation can remove both psychrotolerant and non-psychrotolerant sporeformers from milk. Bactofugation reduced the PSC from -0.63

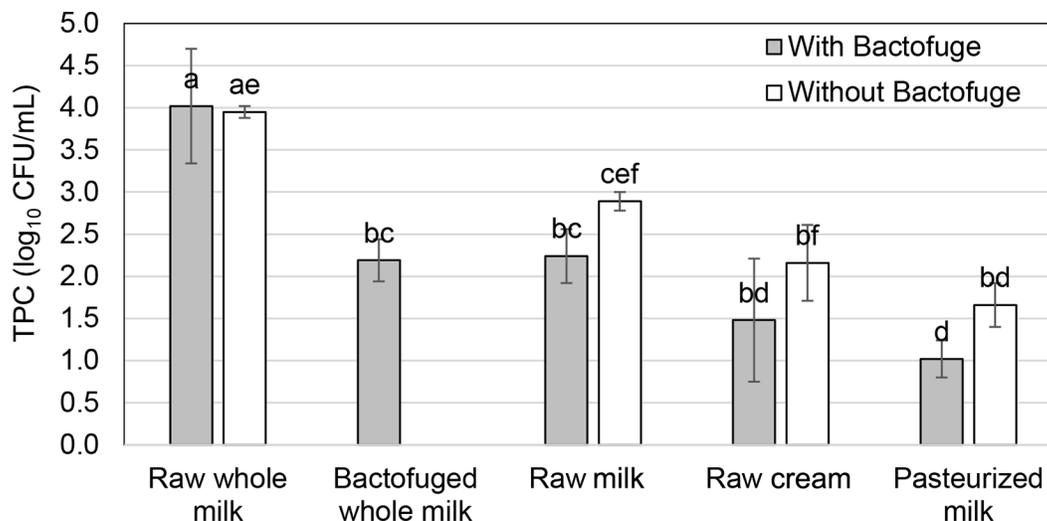


Figure 2. Total plate counts (TPC) in various product streams, with and without bactofugation. Means with different letters are significantly different from each other ($P < 0.05$). Error bars represent SD.

± 0.47 log cfu/mL to -1.49 ± 0.35 log cfu/mL; this reduction was not statistically significant by itself ($P = 0.099$), but in combination with cream separation resulted in significant reduction ($P = 0.0003$). The high variability of the data (indicated by the relatively large standard deviation) is likely due to the low PSC in the incoming raw milk. Future studies should focus on confirming effectiveness of bactofugation as intervention for reducing PSC in raw milk and should increase the number of raw milk samples tested before and after bactofugation to achieve appropriate statistical power (i.e., 80%).

Pasteurization had a minimal effect on the reduction of MSC and PSC in skim milk (Table 1). The difference in MSC and PSC between skim milk before and after pasteurization was nonsignificant for both skim milk that was processed with or without bactofugation ($P = 1.000$ and $P \geq 0.996$ for MSC and PSC, respectively). These results were to be expected because bacterial spores can withstand much higher heat treatments than pasteurization (Lindsay et al., 2021). These results show that interventions that physically remove the spores are most beneficial when standard HTST or vat pasteurization is used for processing of fluid milk.

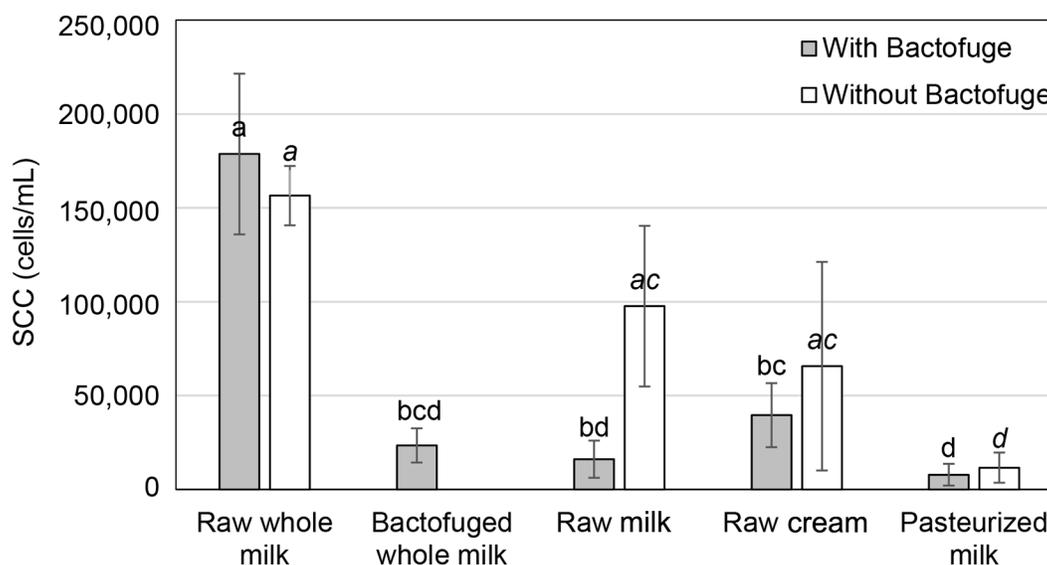


Figure 3. The SCC in various product streams, with and without bactofugation. Means with different letters are significantly different from each other ($P < 0.05$). Error bars represent SD.

Our results indicate that bactofugation is effective at removing spores of mesophilic sporeformers and helps reduce spores of psychrotolerant sporeformers. Based on our results we are expecting that bactofugation would be beneficial even when spore loads in raw milk are relatively low; however, further studies are needed to confirm effectiveness under different conditions including different dairy processing plants, processing periods, and raw milk characteristics. Effectiveness of interventions when processing incoming raw milk with low spore loads is important because most of the raw milk processed by the contemporary dairy plants contains low bacterial and spore counts due to high standards and advancements in milk production and dairy farm management (Masiello et al., 2014, 2017; Martin et al., 2019).

Reductions of Psychrotolerant Sporeformers by Bactofugation Are Predicted to Increase Shelf-Life of Pasteurized Skim Milk

The concentrations of psychrotolerant sporeformers determined in pasteurized skim milk during our experiments were used to model growth in milk during refrigerated storage (6°C) using Monte Carlo simulation models. The median concentration of psychrotolerant sporeformers in pasteurized skim milk processed with the bactofuge was predicted to be -1.55 log cfu/mL on d 4 of refrigerated storage and increase to 4.69 log cfu/mL by d 24 (Figure 4A). The predicted median concentrations in pasteurized skim milk obtained without the use of bactofuge were -0.82 and 5.50 log cfu/mL, respectively, on d 4 and 24 of refrigerated storage. For the purpose of this study, we considered milk to be spoiled when microbial counts reached the limit of 20,000 cfu/mL (4.3 log cfu/mL) which is the legal limit set by the FDA (2019). Based on the values predicted by the model, we can expect that 50% of the half-gallon milk containers would reach spoilage on d 19.6 if a bactofuge was not used and on d 22.6 if a bactofuge was used in the processing flow. If the amount of allowed spoiled product is arbitrarily set at 20% of half-gallon containers produced, this limit is predicted to be reached on d 17.8 if a bactofuge is not used and d 19.6 if a bactofuge is used (Figure 4). According to expert opinion provided by one large US fluid milk processor (who requested to remain anonymous), extending shelf-life of fluid milk by 1 to 2 d would already reduce the cost of processing and transport by reducing the number of changeovers and number of trucks needed for delivery, as well as increasing the time and distance the product could be transported.

The predicted extension of fluid milk shelf-life by 1.8 to 3.0 d indicates that bactofugation can be expected

to be an effective intervention for reducing the spoilage of fluid milk by psychrotolerant sporeformers. However, psychrotolerant sporeformers are responsible for only half of the total quantity of fluid milk spoiled; the other half is spoiled due to post-pasteurization contamination and outgrowth of psychrotolerant gram-negative bacteria, such as *Pseudomonas* spp. (Alles et al., 2018; Reichler et al., 2018). Because bactofugation is performed before the final pasteurization step, it will not contribute to reduction of spoilage due to outgrowth of psychrotolerant gram-negative bacteria introduced through post-pasteurization contamination.

Bactofugation Reduces the Number of Somatic Cells in Milk

Bactofugation of raw whole milk resulted in significant reduction of SCC ($P < 0.0001$). The initial mean SCC of $178,667 \pm 42,786$ cells/mL was reduced to $22,800 \pm 10,016$ cells/mL after bactofugation and remained at a comparable level in both skim milk after cream separation and in final pasteurized skim milk; each reduced the mean SCC by 6,633 and 8,250 cells/mL, respectively. (Figure 3). During the control experiment (no bactofuge), the mean SCC was reduced by 58,833 cells/mL during cream separation, whereas a significant reduction of 96,500 cells/mL was observed during pasteurization of skim milk ($P = 0.0002$). Even though somatic cells are not affected by heat treatment, the shear forces created during HTST pasteurization and other milk processing steps can cause physical lysis of these cells and thus a reduction in SCC (Elwell and Barbano, 2006). The lysed somatic cells introduce proteolytic and lipolytic enzymes into the final pasteurized milk, which can cause a decrease in sensory quality independent of any bacterial spoilage (Ma et al., 2000). Therefore, because bactofugation can physically remove somatic cells from the raw milk, it can also limit the spoilage of the final product by the enzymes released from the lysed somatic cells, in addition to reducing the bacterial spoilage.

Protein Loss During Bactofugation Is Less Than 2%

Raw whole milk used in the main experiment contained $3.43 \pm 0.02\%$ total protein, which was reduced to $3.37 \pm 0.04\%$ after bactofugation. Some protein loss during bactofugation was expected and was previously reported (Kosikowski and Fox, 1968). This happens because casein micelles have a higher density than milk; it is closer to the density of bacterial cells than the density of milk (Gésan-Guiziou, 2010; de Kruif et al., 2012). The 1.7% protein loss observed during bactofugation is smaller than the natural variation of protein

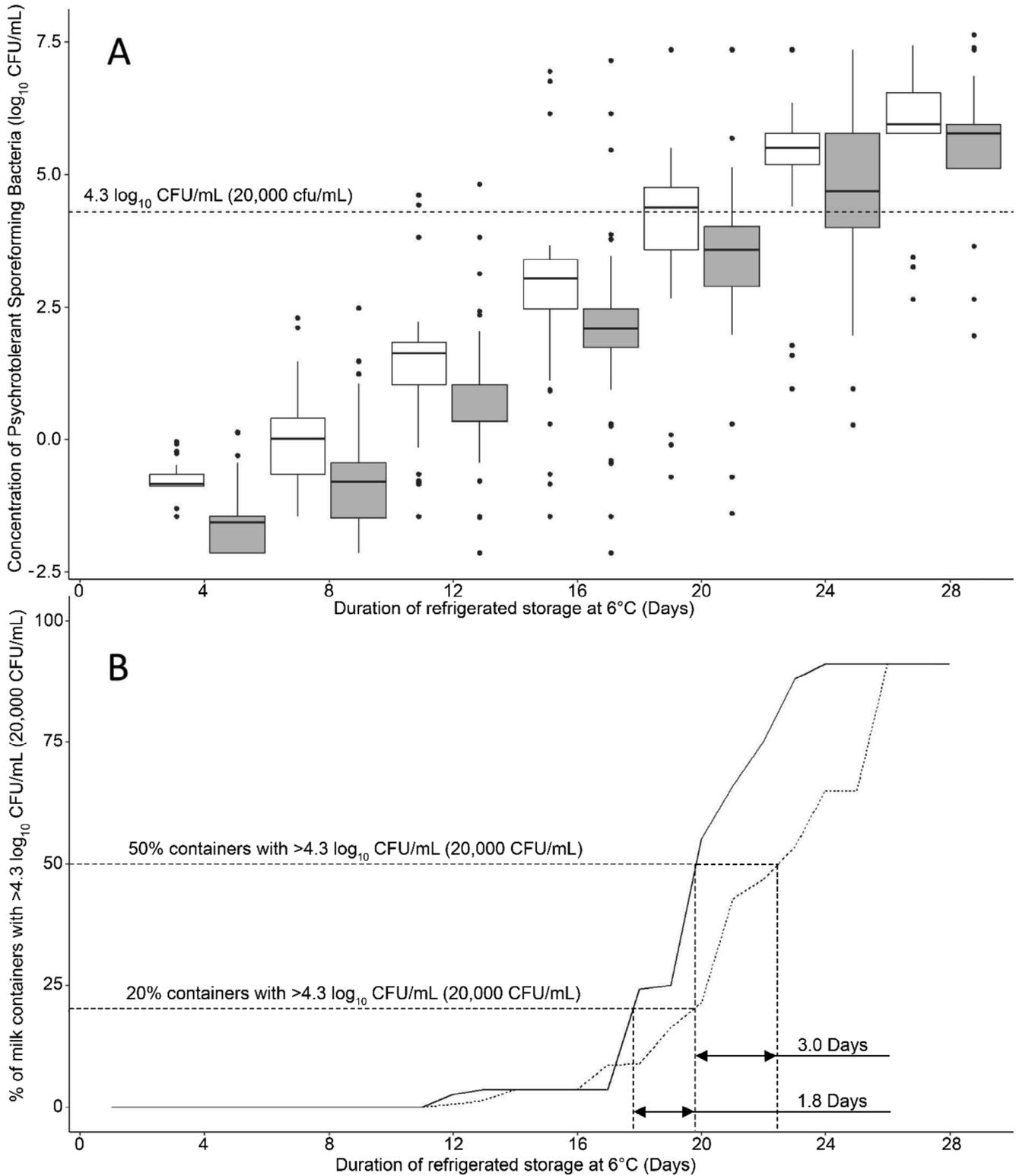


Figure 4. Growth of psychrotolerant sporeforming bacteria in pasteurized skim milk during refrigerated storage (6°C) predicted by Monte Carlo simulation model. (A) Gray boxes represent predicted values when bactofuge is used and white boxes represent predicted values when bactofuge is not used in the processing flow. Dashed line represents the Pasteurized Milk Ordinance limit of 20,000 cfu/mL, used as the limit at which fluid milk is considered spoiled. (B) Dotted line represents predicted values when bactofuge is used, and solid line represents predicted values when bactofuge is not used in the processing flow. Dashed lines represent the difference in storage days when 20 or 50% of milk containers reach the limit of 20,000 cfu/mL.

in raw milk (Silvestre et al., 2009) and is not expected to have an effect on the shelf-life of the final pasteurized fluid milk (Jovanovska et al., 2017; Juraga et al., 2021). The detected protein loss, that accounts for 0.06% of total milk volume, together with standard volume loss of 0.15% due to discharged bactofugate during single stage bactofugation is something a dairy processor might consider when evaluating the cost of implementing bactofugation as an intervention (Bylund, 1995).

Centrifugal Cream Separators Can Reduce the Bacterial Load in Raw Whole Milk

In the absence of a bactofuge, the centrifugal separation of milk can reduce the microbial load in raw skim milk, most likely because some of the microorganisms partition with the solids that are ejected during the separation process (Bylund, 1995, 2003; Kozub et al., 2020). In our study, the bactofuge reduced the TPC in raw whole milk from 4.00 ± 0.68 log cfu/mL to 2.19 ± 0.25 log cfu/mL. After this significant ($P < 0.0001$) initial reduction during bactofugation the TPC of raw whole milk likely remained comparable to the TPC of the skim milk after cream separation (2.22 ± 0.32 log cfu/mL; $P = 1.000$). Cream separation during the control experiment (no bactofuge) reduced the TPC from 3.95 ± 0.07 log cfu/mL to 2.89 ± 0.11 log cfu/mL. This observed reduction in TPC was not significant ($P = 0.109$); however, it contributed to the overall reduction and observed TPC in the final pasteurized skim milk. The results of our study indicate that although a centrifugal cream separator can reduce the microbial load in raw skim milk (and potentially to some degree reduce the need for bactofugation), it might not be able to reduce the microbial loads when the counts in raw whole milk are already low; for example, if the TPC is below 2.90 log cfu/mL. It should be noted that differences might be present in what bacteria are removed and what bacteria remain in the milk during bactofugation compared with regular centrifugal separation. Future studies should further evaluate the effectiveness of cream separation in reducing bacterial loads in milk, focus on determining what bacterial species and strains remain in the milk after cream separation and bactofugation, and provide a better understanding of the microbiology of bactofuged milk and its effect on the quality and shelf-life of the final product.

CONCLUSIONS

Bactofugation can be an effective intervention for reducing vegetative cells, spores, and SCC in raw milk, and thus can help extend the shelf-life of pasteurized fluid milk even when incoming raw milk contains low

spore counts. Though predicted extension of shelf-life is expected to have a positive effect on the final bottom line for the fluid milk processors, each processor is encouraged to evaluate this intervention by performing a cost-benefit analysis using data specific to their operation and conditions. This study used Monte Carlo simulation modeling to demonstrate how low-cost modern mathematical tools can be used to evaluate scenarios with potential high-cost implications. This data will help fluid milk processors evaluate the benefits of using bactofugation as an intervention in their processing plant and demonstrate the benefits of using mathematical modeling as a tool in decision making.

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