ABSTRACT

Share tables are tables or stations in school cafeterias where students can return unopened foods and beverages, providing an opportunity to access these items at no cost. Currently, research suggests that milk is among the most wasted items in breakfast and lunch programs in the United States. Share tables present a simple solution for reducing milk waste, but research is needed to understand the microbial spoilage potential of milk in STs. To this end, uninoculated milk cartons and milk cartons inoculated with 2–3 log10 (cfu/ml) Pseudomonas poae, a fast-growing psychrotroph, were exposed to ambient temperature during winter (mean temperature = 20.3°C) and summer (23.1°C) for 125 min; repeated over 5 d (the length of a school week). Microbial counts in the inoculated milk cartons increased linearly, exceeding the spoilage threshold of 6.0 log10 (cfu/ml) after Day 3 and after Day 4 in the winter and summer season trials, respectively. In the winter trial, the microbial counts for uninoculated milk cartons never exceeded the lower limit of detection, 2.31 log10 (cfu/ml), and in the summer trials, microbial counts never reached the spoilage threshold, indicating that initial contamination is a driving factor of microbial milk spoilage. Regardless of sharing status or seasonality, the greatest changes in counts for inoculated milk cartons occurred during overnight refrigeration, ranging from 0.56 to 1.4 log10 (cfu/ml), while during the share table ranged from no observable change up to 0.29 log10 (cfu/ml), emphasizing that school nutrition personnel should focus efforts on tightly controlling refrigeration temperatures and returning milk to refrigeration as soon as possible. A previously developed model for school cafeteria share tables was adapted to understand the typical residence time of milk in a simulated cafeteria with an ambient temperature share table for the summer and winter seasons over 1,000 weeks. Milk was predicted to have a very short mean residence time (85 min) regardless of sharing status or season, with 99.8% of milk consumed, discarded, or donated within the first 2 d. As a result, only 3 out of 451,410 and 6 out of 451,410 simulated milks spoiled in the winter and summer seasons, respectively. The data generated here can be used to inform science-based decision-making for including milk in share tables, and/or applied to any system where one might have to accept short-term unrefrigerated storage of milk to meet a waste reduction and/or food security goal.

Keywords: milk spoilage, food recovery, food waste, share tables

INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO) reported that 931 million metric tons of food waste was generated in 2019, 26% of which came from food service (Forbes et al., 2021). While reducing all food waste from agriculture to consumer homes is a priority, reducing waste in food service operations like school nutrition programs is particularly important because of the direct loss of nutrition for children. Of note, a report from the World Wildlife Fund’s Food Waste Warriors (FWW) program found that the average amount of food waste was 39.2 pounds per student per year in schools across the United States, with 58% of the total waste resulting from nutritious foods like fruits, vegetables, and milk (World Wildlife Fund, 2019). The United States Department of Agriculture (USDA) Child Nutrition Programs play a critical role in securing children’s access to nutritious foods. Studies have suggested that 47% or more of a child’s total daily energy intake can come from participation in the School Breakfast Program (SBP), the National School Lunch Program (NSLP), and/or the Special Milk Program (SMP) (Cullen and Chen, 2017).

While offering nutritious foods to students promotes access to important nutrients for development, the USDA is aware that these programs have sustainability challenges. Currently, research suggests that milk
is among the most wasted items in SBPs and NSLPs (Smith and Cunningham-Sabo, 2014, Blondin et al., 2017, Fox and Gearan, 2019, World Wildlife Fund, 2019). A summary of findings from the School Nutrition and Meal Cost Study, a nationwide study funded by the USDA, reported that milk ranked as the second highest plate waste item in NSLP lunches, with 29% discarded or not consumed (Fox and Gearan, 2019). Likewise, a study conducted to quantify the amount of fluid milk waste in SBPs in 6 schools in the United States found that 45% of the milk offered to students was wasted; meaning it was either offered, but not selected and then discarded, or selected by the student, partially or not consumed, and then discarded (Blondin et al., 2017). The FWW report found that the average amount of milk waste per student per year was 28.7 cartons, and was even higher when stratified for elementary schools, at 37.6 cartons (World Wildlife Fund, 2019).

To that end, the USDA has developed multiple strategies to help schools combat food waste, one of which is implementing share tables (STs). STs are tables or stations where students can return unopened foods and beverages that they do not consume during a meal service (United States Department of Agriculture, 2016), providing students with an opportunity to access items, like milk, at no cost. STs are already being successfully piloted across the United States and helping to reduce food waste. According to the World Wildlife Fund (2019) report, implementing share tables in school cafeterias helped contribute toward an average 12.4% reduction in milk waste by the end of the pilot. However, to implement a ST in a school it must align with state and/or local regulatory requirements, as restrictions may be placed on the types of food or beverage items, e.g., milk, which are eligible to be reser- *viced (United States Department of Agriculture, 2016). A qualitative policy analysis in the United States indicated that around half of the states have a state-level share table policy document, but few have guidance on practices for monitoring and corrective actions, among others (Prescott et al., 2020a). School nutrition personnel have expressed confusion, and even fear over whether the sharing or reserving of milk from a ST is appropriate from a policy, food safety, and food quality standpoint (World Wildlife Fund, 2019, Prescott et al., 2020b, Prescott et al., 2020a, Zagorski et al., 2021). Specifically, Prescott et al. (2020b) identified concerns over leaving milk out for even as little as an hour as a barrier to meeting food recovery goals.

Per the US Food and Drug Administration (“Model”) Food Code, pasteurized milk is considered a Time/ Temperature Controlled for Safety (TCS) food, requiring time/temperature for safety to limit pathogenic microorganism growth or toxin formation (United States Food and Drug Administration, 2023a). While pasteurization is sufficient to inactivate both pathogenic and non-spore-forming spoilage organisms in milk, post-pasteurization contamination (PPC) in the processing environment with pathogenic organisms like *Listeria monocytogenes* and spoilage organisms like *Pseudomonas* spp. is possible (Boor et al., 2017). Research has consistently demonstrated that *Pseudomonas* spp. are the most common bacteria responsible for PPC in milk (Cousin, 1982, Martin et al., 2018, Reichler et al., 2018, Lau et al., 2022, Ding et al., 2023), and they are particularly problematic due to their rapid generation times at or above refrigeration temperatures, and their associated ability to produce lipases and proteases at these temperatures (Sørhaug and Stepaniak, 1997, Meng et al., 2017, Narvhus et al., 2021). Production of lipases and proteases by PPC organisms can negatively impact the quality characteristics (flavor, odor, body, etc.) and reduce the shelf life of pasteurized milk by causing premature spoilage. Previously, the growth of *Pseudomonas* spp. above 6.0 log$_{10}$(cfu/ml) in milk has been negatively associated with organoleptic properties of milk (Punch et al., 1965, Cousin, 1982, Ternström et al., 1993, Champagne et al., 1994). Research indicates that *Pseudomonas* spp. are capable of exceeding this level in milk, and has been detected at levels up to 8.23 log$_{10}$(cfu/ml) within 14 d after pasteurization (Ranieri and Boor, 2009). Temperature control may be lost by allowing students to share milk, which may accelerate the rate of microbial spoilage if these organisms are present. Therefore, it is important to understand the effect that leaving milk without temperature control has on microbial spoilage potential.

The aims of this study were to (i) monitor the growth of *Pseudomonas poae* – a known, fast-growing psychrotroph– in milk across 5 d of simulated meal services with a non-temperature-controlled ST during 2 different seasons and (ii) investigate whether overnight refrigeration between meal services or the ST had the greatest impact on *P. poae* growth. An additional aim was to adapt a previously published model to predict the realistic residence time of milk in the same cafeteria system with a ST to understand if milk would spoil in the predicted residence time, and if a spoiled milk carton would be likely to reach a student. Meeting these objectives will address current stakeholder concerns and can inform science-based decision-making for including milk in school cafeteria STs, and could also be applied to any system where one might have to accept short-term unrefrigerated storage of milk to meet a waste reduction and/or food security goal.
MATERIALS AND METHODS

Developing an Experimental Worst-Case for Microbial Spoilage Share Table (ST)

To address a realistic, worst-case for microbial spoilage share table (ST) scenario, a challenge study was developed to understand changes to microbial quality as an effect of placing milk cartons contaminated with a strain of *Pseudomonas* (previously isolated from fluid milk) in ambient air temperature for 125 min each day and repeating this with the same milk cartons over 5 d. One trial ST was completed in December 2022, further referred to as the winter season, where the ambient air temperature was 20.3°C ± 1.2°C. Two additional trial share tables were completed in August 2023, further referred to as the summer season (e.g., Summer A and Summer B), where the ambient air temperature was 23.1°C ± 0.24°C. Repeating the share table conditions when ambient indoor temperature increases during the summer season was important, as it was hypothesized that higher ambient temperatures could lead to premature spoilage as compared with the winter season. All milk cartons were stored in stable refrigeration (4.2°C ± 0.9°C) before and after the ST on a given day, further referred to as overnight refrigeration.

Determining a Representative Lunch Service and Break Between Lunch Services Length

To correctly represent the length of a typical school cafeteria service, 2 main approaches were taken: (i) evaluation of the School Nutrition Association (SNA) 2018 report to determine real school lunch service lengths and (ii) collection of school bell schedules data from school websites across Illinois. The SNA report found that there was none to relatively small variability in median lunch period length within elementary, middle, and high schools of different district sizes and across different regions of the United States (School Nutrition Association (SNA)), 2018 #89). The SNA report did not contain information about lunch breaks, so additional data were analyzed from a publicly available list of school contact information and websites from the Illinois State Board of Education (Illinois State Board of Education (ISBE), 2022). The list contained information for 4,852 public schools and 1033 non-public schools in the state of Illinois. Data were collected from school websites in a convenience process until 2 searchers were unable to identify unique schedules.

The length of lunch services and breaks were recorded for each bell schedule, and the average lunch period and break length were calculated for each bell schedule for further analysis. When combining all school categories, the average lunch period lengths were 33.9, 30.0, 25.0, and 48.0 min, respectively. The average, median, 10th percentile, and 90th percentile for combined school break time between lunch periods were 11.6, 8.0, 0.0, and 23.0 min. To represent the worst-case for microbial spoilage ST scenario, the length of the lunch services was defined as 50 min (rounded up from 48 min from the observed data), and the length of the break between lunch services as 25 min (rounded up from 23 min from the observed data). A second lunch service was repeated after the break for a total of 125 min per day.

Bacterial strain selection and inoculum preparation

We selected *Pseudomonas poae*, a known, fast-growing psychrotroph as the bacterial strain to represent the worst case for microbial spoilage. While other non-spore-forming spoilage organisms, such as those in the *Enterobacteriaceae* family (coliforms, etc.), have been isolated from pasteurized fluid milk, research has shown that they are less frequently isolated, and that the majority grow at slower rates than *Pseudomonas* spp. (Masiello et al., 2016, Martin et al., 2018, Lau et al., 2022). An isolate of *P. poae*, which was previously isolated from pasteurized fluid milk (Reichler et al., 2018, Lau et al., 2022) by the Cornell University Milk Quality Improvement Program (MQIP; Ithaca, NY) was acquired. The isolate was cultured in Brain Heart Infusion Broth (Sigma Aldrich, St. Louis, MO) and stored at −80°C in 75% culture and 25% 12.5% glycerol (vol/vol). For each trial, cultures were revived onto Standard Methods Agar (SMA; Hardy Diagnostics, Santa Maria, CA) and put in static incubation at 32°C for 48 h. The inoculum for the milk cartons was prepared from an overnight (18-h) subculture, in alignment with the methods outlined by Van Tassell et al. (2012). The *P. poae* overnight culture population was confirmed by enumeration on SMA. Serial dilutions of the resuspended culture were performed to give an approximate inoculum of $10^4$-10$^6$ cfu/ml.

Milk Carton Preparation and Selection Process

For each season, a 40-count case of 236 mL, pasteurized milk cartons (1%; Country Fresh) was purchased through a café at the University of Illinois at Urbana-Champaign and stored at 4.2°C ± 0.9°C upon receipt. Milk came from the same manufacturing site, as indicated by the state code and plant number, printed on each carton. On the first day of receipt, 30 cartons were selected to determine the initial microbial load. This “screening” process was important for selecting milk that carried a low microbial load, as the goal was...
to simulate a realistic, worst-case PPC event by adding the *P. poae* inoculum to milk before the start of the ST. Here, the date on each milk carton was also recorded.

Each carton was randomly assigned a number 1–30, perforated with a sterile pair of scissors at the top of the milk carton, and sealed with an adhesive aluminum film (Diversified Biotech, Dedham, MA). The milk cartons were mixed on a benchtop orbital shaker (Bellco Biotechnology, Vineland, NJ) for 1 min to ensure contents were well-mixed before aliquoting a sample. For milk sample collection, 1 mL was aseptically pipetted from each carton, and a carton was immediately resealed with a new piece of adhesive aluminum, mixed again, and returned to refrigeration (4.2°C ± 0.9°C) for 24 h. The concentration of *P. poae* cells was expected to increase overnight, as it was previously observed that *P. poae* is a fast-growing psychrotroph, with a lag time of less than 4 h in skim milk broth incubated at 6°C (Lau et al., 2022).

**Temperature Monitoring of Milk Cartons, Refrigeration, and Share Table**

Temperature was monitored in 1-min intervals using TR7 Series temperature data loggers with probes (T&D Corporation, Matsumoto, Japan). Two loggers were set to (i) record the ambient temperature of the refrigerator continuously and (ii) record the ambient temperature of the ST each day. Two additional loggers were set to record the internal milk temperature of 2 milk cartons: (i) continuously recorded for one milk carton left in refrigeration, and (ii) milk carton that was moved each day with the ST replicates.

**Experimental Worst Case for Microbial Spoilage Share Table Condition**

The worst-case for microbial spoilage ST condition, further referred to simply as the ST, was defined as ambient air temperature (no temperature control) during 2, 50-min lunch services with a 25-min break (no temperature control) in between each lunch service. Milk cartons were left at ambient air temperature for the entire 125 min, which represents the first student residence time. The process, as shown in Figure 1, was set up such that the ST was started at the same time each day and repeated over 5 d using the same milks each day (Days 1–5).

At the start of the ST (*t* = 0 min) on Day 1, 1 mL samples were drawn from each ST replicate (*n* = 6) following the same sampling protocol as the initial screening of milk cartons. At the same time, a sample from each refrigerated control was also drawn. The appropriate dilutions were made, and each sample was inoculated on SMA and Crystal-Violet Tetrazolium Agar (CVTA; HiMedia, India) by spiral plating method, and placed in static incubation at 32°C for 48 h, in alignment with the practices outlined in *Standard Methods for the Examination of Dairy Products* (Duncan et al., 2004, Laird et al., 2004). In the winter trials, CVTA was chosen as an additional media to monitor the growth of *P. poae*, as it is has been previously used for the selective enumeration of *Pseudomonas* spp. from milk (Van Tassell et al., 2012). However, it was decided
in the summer trials to only plate onto SMA, as there were no significant differences between microbial counts observed on SMA and CVTA in the winter for each milk carton and collection time ($P = 1.00$, see supplemental Figure S3). Though, there was an additional plating for inoculated milk samples onto CVTA at the start of Day 1 during the summer trials to confirm that the inoculation was successful by observing red colonies, which indicate the presence of Gram-negative organisms. Each day, sampling milk was repeated at the end of the second lunch service ($t = 125$ min), and the ST replicates were moved immediately back to the refrigeration after sampling. Each sample was plated and incubated similarly to the collection at the start of the first service. After 48 h, plates were enumerated, and colony counts per plated volume were recorded for each media type. For a given time point, the log$_{10}$ cell concentration (log$_{10}$(cfu/ml)) was calculated for each dilution and the mean values were calculated. The process was repeated for Days 2–5, using the same milk cartons throughout the entirety of the experiment.

**Calculating the change in microbial counts in the share table, overnight refrigeration, and for refrigerated controls**

To calculate the change in microbial counts for ST replicates during the share table, the mean concentration of cells from the start of the ST on a given day ($t = 0$ min) was subtracted from the end of the ST ($t = 125$ min) on that same day. For the change in ST replicates during overnight refrigeration (~22 h) the concentration of cells from the end of the ST ($t = 125$ min) on the day prior was subtracted from the start of the ST ($t = 0$ min) on a given day. The change in counts for the refrigerated controls (~24 h) was calculated from the concentration of cells from the start of the ST ($t = 0$ min) on the day prior, was subtracted from the start of the ST ($t = 0$ min) on a given day.

**Assessing Microbial Quality**

Literature suggests that 6.0–8.0 log$_{10}$(cfu/ml), is the range, depending on the microorganism and its metabolic state (Punch et al., 1965, Cousin, 1982, Schröder et al., 1982, Ternström et al., 1993, Champagne et al., 1994), that has been negatively associated with pasteurized milk quality, e.g., flavor, odor, and body defects. Therefore, we selected 6.0 log$_{10}$(cfu/ml), as the spoilage threshold. The authors recognize that another way that spoilage is often defined in literature is as exceedance of the Pasteurized Milk Ordinance (PMO) standard of 20,000 cfu/ml [4.3 log$_{10}$(cfu/ml)] (United States Food and Drug Administration, 2019). This lower threshold is more appropriate for studies focused on milk processing where meeting the grade “A”
PMO standard applies, but it is less relevant to this study which is focused on the endpoint of consumer perception of acceptable milk quality.

Data Analysis

Raw experimental data were collected and organized in Microsoft Excel [Microsoft Excel for Microsoft 365 MSO (Version 2304 Build 16327.20248) 32-bit; Microsoft Corporation]. Data analysis was performed in Microsoft Excel and data manipulation, statistical analysis, and plotting was performed in R and RStudio (version 4.2.2; The R Project for Statistical Computing; Vienna, Austria) (R Core Team, 2022). For each milk category (uninoculated and inoculated) an ANOVA with Tukey’s Honest Significant Difference test was performed. First, the interaction between seasonality of the trial (Summer, Summer A, Summer B, Winter) and the storage type (overnight refrigeration, share table, and refrigerated control) were assessed. Following this, the interaction between storage type, seasonality of the trial, and day (1–5) were assessed. Raw data and code are available at: https://github.com/foodsafetylab/Pinto-2023-Milk.

Simulation to assess milk spoilage in a school cafeteria system

A previously published quantitative microbial risk assessment (QMRA) (Reyes et al., 2022) was adapted to simulate and track milk residence time in the school cafeteria system. The worst-case spoilage scenario condition was simulated to assess the likelihood of milk spoiling. This scenario consisted of simulating 1 week of service (5 d), where each day consisted of 2, 50-min services, with a 25 min break in between services (125 min total), after each day a 21 h. 55 min overnight storage was simulated.

Overview of the process model

The process model simulates a student interacting with milk as they go through a lunch service. The modules for the process model are (i) selection, (ii) sit-down, and (iii) share table. In the selection module, the likelihood of a student selecting milk from the service line is simulated, if the milk is selected it moves to the sit-down step, if it is not selected it remains in the service line for further interaction. The sit-down module simulates the likelihood that a student may or may not consume the milk. If the milk is consumed, the milk’s residence time in the system is recorded. If the student decides not to consume the milk, then the milk can be discarded or shared. The share table module simulates the student interaction with the share table and milk on the share table. Here the student may share leftover milk if they decide not to consume it during the sit-down step, students can also approach the share table and pick up already shared milk if available. Subsequent sit-down steps occur after visiting and selecting food from the share table, where the student may consume, discard, or re-share the item.

At the start of each week, the model fully stocks the service line with milk (n = 50). As the milk cartons are selected from the service line, the model restocks the service line with milk cartons (set of n = 20), and older milk is put on top following First-in First-out (FIFO) protocols. However, to simulate worse case conditions all milk cartons served in a day are assumed to be out of refrigeration since the beginning of the day of service. During service and breaks the milk cartons sit at ambient temperature (22.2°C ± 0.5°C), during overnight storage the milk cartons sit at refrigeration temperatures (4.4°C ± 0.2°C) The residence time of each milk carton is recorded after (i) they are consumed, discarded, or donated, (ii) at the end of every service, (iii) at the end of each break, and (iv) after overnight storage. An overview of the steps related to the process model is in Fig. S1.

Milk Spoilage Model

The milk spoilage model was adapted from Lau et al. (Lau et al., 2022). This model predicts the growth of psychrotolerant gram-negative organisms as a factor of time and temperature. For this model, we used the Buchanan 3-phase linear model (Buchanan et al., 1997) with growth parameters requested and provided by Lau et al. for Pseudomonas poae. (Lag time = 0 h, µmax = 0.084 (ln cfu/ml hr−1), and Nmax = 8.14 (log10 cfu/mL)).

The Buchanan 3-phase linear model was chosen as the primary model because it can be used with non-isothermal temperature profiles. For this model time intervals were set at 1 min, and the growth rate was adjusted at every interval. Since the lag phase for Pseudomonas poae is 0 h, no lag phase was calculated.

The growth during the exponential phase was also adjusted dynamically for the specific temperature at a time interval. Total growth for a specific time was obtained by adding up the growth at each interval:

\[ \text{Growth}_{\text{Total}} = \sum_{i=1}^{T_{\text{time}}} \text{Growth } _{\text{Interval}} (\log \text{ CFU } / \text{mL}), \]

(2)
Where $Growth_{Interval}$ is the total growth during the 1-min interval; $\mu_{max}$ is the adjusted maximum growth rate at the specific temperature; time is the length of the time interval in hours (0.016); $AF$ is an adjustment factor when $AF = 1$ means no adjustment. $Growth_{Total}$ is the total growth over multiple time intervals. $Time_{R}$ is the total residence time of the milk in the cafeteria system.

The temperature profiles obtained from the spoilage studies were used to model the growth of $P. poae$ over the proposed 5-d period. The growth rates obtained from the adapted growth model and the growth rate obtained from the spoilage experimental data collected in the present study were compared. The adapted model with an adjustment factor of 1 underpredicted growth compared with the spoilage experimental data. Therefore, an adjustment factor of 1.34 (obtained by getting the quotient between the observed average growth rate and the predicted average growth rate with $AF = 1$) was applied to the growth model to predict milk spoilage more conservatively, i.e., milk would be more likely to spoil, Fig. S2.

The change in the $Pseudomonas poae$ population from the temperature profiles for each season was adapted to each milk as a function of the residence time (Fig S2). The new $P. poae$ population for each milk was recorded. The spoilage threshold, $6 \log_{10}(\text{cfu/ml})$, was likewise used to assess milk spoilage.

**RESULTS & DISCUSSION**

Microbial growth above the spoilage threshold was only detectable in inoculated milk, not uninoculated milk, from share table challenge studies for both seasons. Microbial counts ($\log_{10}(\text{cfu/ml})$) on nonselective (SMA) media for uninoculated milk, milk inoculated with $P. poae$ (starting concentration of $2-3 \log_{10}(\text{cfu/ml})$), and refrigerated controls at the start and end of the lunch services each day are shown for each trial (2 during summer: Summer A and B; 1 during winter) in Figure 2; where each point represents the mean value of all milk cartons at a given sampling time and error bars represent the minimum and maximum values. In the winter, microbial counts in the inoculated milks exceeded the spoilage threshold, $6.0 \log_{10}(\text{cfu/ml})$, during overnight refrigeration between Day 3 and Day 4 (first detected all at the start on Day 4), ranging from $6.16 - 6.32 \log_{10}(\text{cfu/ml})$ at the start of Day 4 and maximally reaching $7.10 - 7.27 \log_{10}(\text{cfu/ml})$ by the end of Day 5. In the summer trials, microbial counts in the inoculated milks (Figure 2) exceeded the spoilage threshold, $6.0 \log_{10}(\text{cfu/ml})$, during overnight refrigeration between Day 4 and Day 5 (first detected all at the start of Day 5) for both trials. For Summer A, counts ranged from $6.56 - 6.79 \log_{10}(\text{cfu/ml})$ at the start of Day 5 and maximally reached $6.62 - 6.89 \log_{10}(\text{cfu/ml})$ by the end of Day 5. For Summer B, counts ranged from $6.23 - 6.59 \log_{10}(\text{cfu/ml})$ at the start of Day 5 and maximally reached $6.66 - 6.75 \log_{10}(\text{cfu/ml})$ by the end of Day 5. In the winter trial, counts in the inoculated refrigerated control exceeded the spoilage threshold during overnight refrigeration between Day 4 and Day 5 (first detected for all at the start of Day 5), maximally reaching $6.19 - 6.48 \log_{10}(\text{cfu/ml})$, but did not exceed the spoilage threshold in either summer trial, maximally reaching only $5.31 \log_{10}(\text{cfu/ml})$ for both (data available online at: https://github.com/foodsafetylab/Pinto-2023-Milk).

The results presented here show similar trends to other published studies. Lin et al. (2016) found that $Pseudomonas fluorescens$ strains in pasteurized milk reached or exceeded $6.0 \log_{10}(\text{cfu/ml})$ after 90 h at constant refrigeration ($4^\circ C$), which was similar to our findings in the present study for the inoculated refrigerated control that remained in refrigeration ($4.2^\circ C$), as spoilage was detected at 96 h in the winter season trial (Day 5). The data from Lin et al. (2016) also showed an exceedance of $6.0 \log_{10}(\text{cfu/ml})$ at 55 h, but the milk was exposed to $29^\circ C$ for 4 h once per day over 2 d. In our study, the temperature fluctuations during the ST were of shorter length ($2$ h and $5$ min per day) and at a lower ambient temperature in the winter and summer trials ($20.3^\circ C$ and $23.1^\circ C$ during winter and summer, respectively, in Urbana, IL), which may explain why the exceedance of the spoilage threshold in inoculated milks was observed later (detected at 72 h) for inoculated milk for all trials than what was reported in literature (55 h).

In contrast to the consistent increases observed in inoculated milk, microbial counts were never detected above the lower LOD, $2.31 \log_{10}(\text{cfu/ml})$, in uninoculated milk nor in the uninoculated refrigerated control in the winter (Figure 2). In the summer, microbial counts increased in uninoculated milk, maximally reaching $5.0 \log_{10}(\text{cfu/ml})$, and in the uninoculated refrigerated control, maximally reaching $3.1 \log_{10}(\text{cfu/ml})$. Importantly, the spoilage threshold was never exceeded. It was observed that the milk received in the summer season was 9 d from the date on label, whereas in the winter the milk received was 14 d from the date on label. Given the lower LOD of $2.31 \log_{10}(\text{cfu/ml})$ and the milk being received in the summer with a shorter time to the date on the label, it is possible that levels were very close to the lower LOD on the day which the milk was screened. Here, the milk was received from the same manufacturing site, but, importantly, the date coding system may differ across milk vendor manufacturing sites which could mean different interpretations for quality. Of note, there is no required time interval between milk receiving and the date (likely due to non-
uniform date coding), but the USDA recommends that schools interpret the date as the milk expiration date (Richardson and Hall-Campbell, 2020).

It is also important to note that, while there was growth observed in the uninoculated milk cartons during the summer season, the counts per day were consistently 1.0–2.0 $\log_{10}(\text{cfu/ml})$ lower than that of what was observed for the inoculated milk cartons. All of this suggests that daily, mild temperature abuse does not meaningfully impact microbial milk spoilage when the initial microbial counts are low. For a school, this could translate to the addition of a ST being unlikely to cause spoilage when initial milk quality is high (e.g., no detectable microbial counts), relative to normal practices in school cafeterias. Additionally, this may shed light on the importance of how quickly school nutrition personnel should move milk into refrigeration upon receiving it at the school. Due to the COVID-19 pandemic, labor shortages have become commonplace across the United States, including in K-12 schools (Cohen et al., 2022, Zuercher et al., 2022). Because labor shortages might impact the maintenance of the cold chain, our data also

**Figure 2.** Mean counts ($\log_{10}(\text{cfu/ml})$) on nonselective media (Standard Methods Agar; SMA) for inoculated milk cartons and uninoculated milk cartons at the beginning and end of an ambient temperature share table in summer (23.1°C ± 0.24°C) and winter (20.3°C ± 1.2°C) over 5 d, and for refrigerated controls. For inoculated milk cartons, Summer A and B represent individual trials during the summer season, and Winter represents an individual trial during the winter season, e.g., for each trial milks were inoculated with a biological replicate of the *P. poae* culture stock. Each point represents a mean value for 3–4 milk cartons and error bars represent minimum and maximum counts observed across all milk cartons for a given time point. Counts below the SPLC LOD of 2.31 $\log_{10}(\text{cfu/ml})$ (dashed line) were plotted at 2.31 $\log_{10}(\text{cfu/ml})$, and the spoilage threshold is drawn at 6.0 $\log_{10}(\text{cfu/ml})$ (solid line).
demonstrates that when milk cannot be moved into a refrigerator immediately, it is also unlikely to cause spoilage when milk quality is high.

Importantly, these findings demonstrate that the incoming microbial quality of milk is a key driver of pasteurized milk spoilage. We demonstrate here that repeated, 125-min of temperature abuse over 5 d does not result in spoilage in milk with nondetectable microbial counts initially (below 2.31 log10 (cfu/ml)). And though the realistic, worst-case contaminated milk (containing 2–3 log10(cfu/ml) fast-growing psychrotroph on Day 1 of the share table) exceeded the spoilage threshold after 4–5 d regardless of sharing status (except for the refrigerated controls during the summer trials), this may not be representative of typical contamination levels in pasteurized fluid milk. For example, Martin et al. (2012) collected data from milk plants with varying performance over 10 years and showed that the yearly average standard plate count for milk 14 d post-pasteurization remained below 6 log10(cfu/ml) in plants that consistently produced “good” quality milk, while plants with consistent quality challenges averaged above 6 log10(cfu/ml) 14 d post-processing. While standard plate count exceeding 6.0 log10(cfu/ml) does not necessarily indicate 6.0 log10(cfu/ml) of Gram-negative psychrotrophic spoilage bacterial growth, this nonetheless underscores the importance of limiting school milk procurement to quality vendors. The USDA has clearly defined requirements for becoming a vendor, which can include audits and inspections, and more specific requirements for fluid milk (United States Department of Agriculture). This comes in addition to the requirement for all grade “A” Pasteurized Milk (regardless of where milk is being supplied), which stipulates that the pasteurized fluid milk may not exceed the limit of 20,000 cfu/ml, as outlined in the Pasteurized Milk Ordinance (United States Department of Agriculture, 2018, United States Food and Drug Administration, 2019).

Changes in microbial milk quality are smaller during share table storage than during refrigeration

The changes in bacterial counts (log10(cfu/ml)) in inoculated and uninoculated milks during the share table and in overnight refrigeration, as well as for the refrigerated controls for all trials are shown in Figure 3. Regardless of seasonality of trials and inoculation status, the increase in counts on a given day from the start to the end of services (the ST) was small. For inoculated milks, the overall mean changes during the ST were 0.15, 0.18, and 0.19 log10(cfu/ml) for winter, Summer A, and Summer B trials, respectively, ranging from −0.050 to 0.29 log10(cfu/ml) on individual days.

For uninoculated milks, the overall mean changes during the ST were 0.0 and 0.14 log10(cfu/ml) for winter and summer trials, respectively, ranging from 0.0 to 0.35 log10(cfu/ml) on individual days. The few cases where a negative change (a decrease) was observed during the share table were likely due to inherent error in plate counting obscuring small real changes. In contrast, the increase in counts from the end of one day to the start of the following day (“overnight refrigeration”) was always larger than what was observed during the share table, ranging from 0.71 to 1.2 log10(cfu/ml) in the winter and 0.56–1.4 log10(cfu/ml) in the summer trials for inoculated milks, and from no change in the winter and 0.07–0.67 log10(cfu/ml) in the summer trials for uninoculated milks.

During the winter trial, no changes were observed in uninoculated milks nor in the uninoculated refrigerated control because counts never exceeded the lower LOD throughout the 5 d. The overall mean change in counts for inoculated milks during overnight refrigeration was 1.1 ± 0.22 log10(cfu/ml) (mean ± standard deviation) per day while during the share table was only 0.15 ± 0.11 log10(cfu/ml), and for the inoculated refrigerated control was 0.96 ± 0.12 log10(cfu/ml) (all values reported in Supplemental Materials Table S1). There was also a relative decrease in the average change in counts in inoculated milk on Day 4 overnight which is due to P. poae approaching its population max.

During the summer trials, the overall mean change in counts for uninoculated milks during overnight refrigeration was 0.45 ± 0.26 log10(cfu/ml) per day, while during the share table was only 0.14 ± 0.15 log10(cfu/ml) per day. The overall mean change in counts for the uninoculated refrigerated control in the summer season was 0.38 log10(cfu/ml). As explained, the observation of growth in uninoculated milks during the summer trials could be due to the milk cartons being 5 d closer to the date on the label compared with winter (and therefore closer to the lower LOD). The change in counts observed in uninoculated milk cartons during the summer trials and lack thereof during the winter trial ultimately caused all overnight shifts during the summer trials to be significantly different (P < 0.001 for all) than the overnight shifts during the winter trial, with the one exception being the overnight shift between Day 1 and 2 in the summer trials. The overall mean change in counts per day for inoculated milks during overnight refrigeration was 1.1 ± 0.28 log10(cfu/ml) and 0.99 ± 0.36 log10(cfu/ml), while during the share table was only 0.18 ± 0.15 log10(cfu/ml), and 0.19 ± 0.15 log10(cfu/ml) per day for Summer A and B, respectively. For the inoculated refrigerated control, the overall mean change in counts was 0.96 ± 0.12 log10(cfu/ml). There appeared to be a longer lag phase for P. poae in the...
summer trials, so all data from inoculated milks during Day 1 of the share table and overnight between Day 1 and 2 were omitted from the calculation of overall mean change in counts. Similarly to the winter trial, there was a relative decrease in the average change for each overnight storage on Day 4.

For all trials, regardless of the seasonality of the trial, the overall mean changes during overnight refrigeration for both uninoculated and inoculated milks were found to be significantly higher than during the ST ($P < 0.05$ for all), which shows that the time spent in overnight storage – 22 h and 55 min vs. 2 h and 5 min in the ST – has a larger influence on microbial growth. Importantly, the changes during the share tables in inoculated milk across all 3 trials never differed significantly (see Supplemental Table S1). However, when comparing observations on overnight changes by day and trial for each inoculated and uninoculated milks, there were some exceptions identified by Tukey’s Honest Significant Difference test ($\alpha = 0.05$). For example, in the inoculated milks, on some days – typically on the first and last days of the ST – the mean shifts during the ST were not significantly different than the mean shift overnight (see Supplemental Table S1; no difference in means indicated by lower case letters). As mentioned, there appeared to be a relatively longer lag growth.
phase of *P. poae* in the summer than that of the winter season trial. In addition, smaller overnight changes were generally observed during the last overnight shift (between Day 4 and 5), which can be attributed to *P. poae* reaching the stationary growth phase. The basic behavior of bacteria (in this case *P. poae*) growing in a nutritive media (milk) in suitable temperatures for growth can explain the lack of significant difference on the front- and back-end of this protocol.

Further, the change for inoculated milks during overnight refrigeration, and the mean change observed in the refrigerated control, over 24 h, were generally not found to be significantly different, with a few exceptions that can also be explained by the longer lag growth phase in the summer trials, as previously described. With that said the general similarity between changes seen in overnight refrigeration for inoculated milks and changes in the refrigerated control further demonstrates that time has the greatest influence on bacterial counts. In fact, the cumulative change in counts for inoculated milks (and uninoculated milks) occurring during the share table over 5 d did not even equate to 1.0 log$_{10}$(cfu/ml) in any of the trials, whereas the daily average for overnight refrigeration for inoculated milks in all 3 trials ranged from 0.99 to 1.1 log$_{10}$(cfu/ml) (see Table S1). Therefore, tightly controlling the refrigeration temperature before and after cafeteria services would better preserve milk quality than instituting temperature control during the ST. This suggests one could best manage the risk of spoilage by focusing on refrigeration temperature, and not ST temperature, although local/state food service regulations for Time/Temperature Controlled For Safety Foods may not allow such a management strategy. Accordingly, quickly returning milk to well-controlled refrigeration will also minimize bacterial growth.

In general, bacterial growth is slower at lower temperatures, and controlling the growth of *Pseudomonas* spp. by means of temperature is already well-studied in the dairy industry. Previous research showed that 24 h of “suboptimal” (10°C) raw milk storage tank temperatures when compared with their “optimal” (6°C) resulted in a 2 log$_{10}$(cfu/ml) increase in the concentration of *Pseudomonas* spp. present (De Jonghe et al., 2011). Another study where pasteurized milk was held at 4°C and 6°C 21 d after processing showed that the milk stored at 6°C had a significantly greater bacterial count (+ 2.0 log) than the 4°C condition (Andrus et al., 2015). Further, Lau et al. (2022) built a model that simulated the effect of improved temperature control on microbial spoilage of milk by Gram-negative PPCs. When milk containers could only maximally reach 6°C (improved temperature control) vs. −1°C-15°C in the baseline system, the number of containers that exceeded 20,000 cfu/ml (PMO standard) 10 d after pasteurization was reduced from 56.18% to 44.36%. Additionally, their sensitivity analysis found that storage temperature was the most influential parameter for determining the number of containers that would exceed the PMO standard. This elucidates the importance of adequate refrigeration temperatures over longer periods to control psychrotrophic, Gram-negative spoilage organism growth. Research even suggests that the proteolytic activity of *Pseudomonas* isolates in raw milk decreased when stored at 2°C vs. 10°C (Meng et al., 2017). Refrigeration temperature monitoring is already a routine part of many school nutrition programs, so it is important to (i) continuously prioritize adherence to these practices and institute corrective actions when issues arise (e.g., temperature increases), and (ii) return milk to refrigeration as soon as possible to reduce the rate of bacterial growth.

It is, however, relevant to address the limitations of the present study relevant to microbial growth. First, the ambient temperature of the room could be higher during warmer months in schools that do not have a central air system, and/or in geographically warmer regions of the United States. For example, a previous study in Hawaii demonstrated that the air temperature of the room during the “hot” season was 28.8°C in naturally ventilated schools and 23.6°C in air-conditioned classrooms (Kwok, 1998). Warner ambient temperatures could potentially increase the amount of psychrotrophic bacterial growth during the share table than what was observed in the present study, which could result in the exceedance of the spoilage threshold sooner. In the present study, we aimed to address this by completing trials during the summer where the mean ambient temperature was 23.1°C and, importantly, did not observe any significant differences in day-to-day changes in microbial counts in summer and winter trials (when the mean ambient temperature was 20.3°C). Likewise, exploring additional refrigeration temperatures, such as 5°C (the maximum allowable refrigeration storage temperature), or less than 4.2°C (the mean temperature reported here) could further characterize spoilage potential of milk in a cafeteria system. Another limitation is how quickly milk would be realistically returned to refrigeration after cafeteria services. In the present study, milk cartons were immediately moved into refrigeration after 125 min to precisely understand changes during the ST and changes during refrigeration. Extending the amount of time spent in ambient temperature before returning to refrigeration (when cafeteria services conclude) could likewise result in additional microbial growth. This could also be relevant to schools that would have a longer cumulative time of lunch services and breaks together longer than
125 min. Nonetheless, milk will still spend most of its residence time in refrigeration, which is where we have demonstrated here that the largest changes occur. Additional studies would need to be performed to evaluate the impact of extending the amount of time spent at ambient temperature, warmer ambient temperature, and different refrigeration temperature profiles.

Share table simulation shows milk will rarely be in the system long enough for spoilage to occur, even if the initial contamination is close to the spoilage threshold. A previously published ST process model for norovirus cross-contamination in apples was adapted to simulate milk cartons going through a ST system without temperature control (Reyes et al., 2022). The simulation results, Figure 4, show the 451,410 milk cartons that were consumed, discarded, or donated over 1,000 simulated weeks for each season. A milk carton could be shared or not shared by the student who selected it. The model indicated that of the milks that get consumed by a student (386,704/451,410; 85.7%) in this system, that 89.1% (344,745/386,704) are consumed on the first day they are serviced, an additional 10.6% (41,152/386,704) are consumed within 2 d of being serviced, 0.206% within 3 d (797/386,704), 0.00259% (10/386,704) within 4 d, and 0% are serviced on a 5th day (see Supplemental Materials, Table S3). The rest of the milks which go through the system are discarded (47,275/451,410; 10.5%) or donated (11,728/451,410; 2.6%) and follow a similar trend as the milks which are consumed. 89.7% of the milks discarded and 97.8% of the milks donated were done so on the first day of service, respectively, and none of the milk discarded or donated made it to a fifth day. The model was also able to estimate the median residence time for each season, which is shown in Table S2 (see Supplemental Materials). In both seasons, the median residence time for all milk cartons in the system (not stratified by sharing status), for milk cartons that were shared, and for milk cartons that were not shared was 85 min, 118 min, and 81 min, respectively. This importantly illustrates that most milk cartons that are put out on a service line or share table once will not be put out a second time in that same week, and even fewer make it past a second day.

Despite most milks having a low residence time in the modeled system, the initial level of P. poae at $t = 0$ min differed in each milk carton because it was drawn from a distribution of levels of psychrotrophic Gram-negative spoilers previously observed in real milk samples (Lau et al., 2022). While none of the milk entered the system above our defined spoilage threshold of 6.0 log$_{10}$(cfu/ml), some were close to this level initially. Even with that said, the results of the simulation displayed in Table S2 also show that the overall chance of milk spoiling, regardless of sharing status, is only 0.000665% (3/451,410) in the winter season when the mean ambient temperature of the room is 20.3°C, and 0.00133% (6/451,410) in the summer season when mean ambient temperature of the room is 23.1°C. In the simulated winter season, milk cartons that were shared spoiled 0.00292% (3/102,657) of the time and had a median population change of $0.240 \log_{10}$(cfu/ml), while milk cartons that were not shared never spoiled (0/348,753) and had a (slightly lower) median population change of 0.162 log$_{10}$(cfu/ml). In the simulated summer season, milk cartons that were shared spoiled 0.00584% (6/102,657) of the time and had a median population change of $0.252 \log_{10}$(cfu/ml), while milk cartons that were not shared never spoiled (0/348,753) and had a (slightly lower) median population change of 0.172 log$_{10}$(cfu/ml). For the milks which spoiled, the initial contamination levels were relatively high, which, as discussed may not reflect what would be present in milk received at a school. These findings are particularly relevant to stakeholder concerns about leaving milk out on a ST as a barrier to meeting food recovery goals (Prescott et al., 2020b), as the results of the model in the present study demonstrate that while spoilage of milk is possible, that this is a rare event because residence time in the system does not exceed one day for 89.4% (403,748/451,410) of milk serviced over the equivalent of approximately 19 years (1,000 simulated weeks). Further, despite higher ambient temperature in the summer season, the amount of milk cartons that exceed the spoilage threshold is comparable to the winter season, remaining low. To our knowledge, this is the first study that simulates the dynamics of sharing milk in a cafeteria and estimates the amount of spoiled milk.

Further, the simulation results illustrate how STs can increase opportunities for the consumption of milk and/or reduce milk waste. Our model predicted that over 1,000 simulated wk 102,657/451,410 (22.7%) milks were shared, which otherwise could have been discarded without a ST. For the milks that were shared, 80.1% (82,190/102,657) were consumed and 11.7% (11,997/102,657) were donated, while the smallest proportion of shared milks, 8.3% (8,470/102,657), were discarded. This all lends to suggest that STs could improve food recovery and relatively reduce food waste by up to 21% (94,187 shared milks that were consumed or donated/451,410 total milks). In future models, altering the assumptions around sharing behavior (probability of sharing food items, probability of picking food from the ST) may provide additional insight into the food waste reduction which is possible by implementing ST.

Some limitations to our model are that only a single school bell schedule was simulated and only 2 ambient temperatures for the ST. The present model could be
expanded in future work to explore different worst-case ambient temperatures (e.g., higher temperatures), as well as shorter and longer cumulative meal service lengths to understand the effects this could have on microbial growth.

**Figure 4.** *P. poae* population from the share table simulation (log₁₀ cfu/ml) compared with total residence time in the system (min) for ambient temperature share table during winter, where ambient room temperature was measured as 20.3°C ± 1.2°C (mean ± SD), and during summer, where ambient room temperature was measured as 23.1°C ± 0.24°C. Each point represents a milk carton. The dashed lines represent the start of the service each day. Most milk cartons will be consumed the same day they are serviced, and very few milk cartons will be in the system for 4 d. Milk cartons will rarely be in the system long enough to spoil in either season (3 and 6 spoiled out of 451,410 simulated in winter and summer, respectively).
CONCLUSION

We have demonstrated here that share tabless, even when lacking temperature control, can be implemented without causing a meaningful reduction in microbial milk quality and, importantly, provide an opportunity to increase milk consumption and reduce milk waste. Spoilage organism levels in milk may vary, even if the PMO standard is met. Therefore, it is important to focus on tightly controlling refrigeration temperatures and to return milk to refrigeration quickly to preventively slow the growth of spoilage organisms. Even so, our simulation results show that nearly all milk in the system will be consumed within 2 d of services and that consumption of spoiled milk is a rare occurrence. In the future, stakeholders can use this data to inform science-based decision-making on share table guidance and can be applied to any system where one might have to accept short-term unrefrigerated storage of milk to meet a waste reduction and/or food security goal.

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