

FROM FARM TO FRIDGE: REDUCING BACTERIAL SPORES AND
IMPROVING TEMPERATURE CONTROL TO ENHANCE DAIRY QUALITY

A Thesis

Presented to the Faculty of the Graduate School

of Cornell University

In Partial Fulfillment of the Requirements for the Degree of

Master of Science

by

Zoe Dora Wasserlauf-Pepper

December 2024

© 2024 Zoe Dora Wasserlauf-Pepper

ABSTRACT

Bacterial spores that survive pasteurization and cause defects late in shelf-life can be reduced in raw milk at the farm level using different management practices. For finished dairy products with unknown initial microbial levels, preventing temperature abuse is critical in avoiding premature outgrowth of bacteria that prefer to grow above 6°C. First, we compared the level of spores in bulk tank raw milk and whole milk powder before and after singeing the udder hair on four organic dairy herds, where mesophilic and thermophilic spores in the whole milk powder from pre- to post-intervention were significantly reduced. Secondly, we collected time-temperature data from finished dairy products from three e-commerce distribution pathways. Overall, high temperature variability was seen across all e-commerce channels, demonstrating the need for improved temperature control during e-commerce distribution. Ultimately, practical solutions to controlling factors affecting dairy quality from farm-to-fridge are needed to provide consumers with high quality dairy.

BIOGRAPHICAL SKETCH

Zoe Dora Wasserlauf-Pepper was born and raised in Barryville, NY and Beach Lake, PA. After graduating from Honesdale High School, she attended the University of Florida in Gainesville, FL, where she earned her Bachelor of Science in Microbiology and Cell Science with a minor in Food Science. During her undergraduate studies, she gained a passion for food microbiology through various coursework and her family's longstanding relationship with food and agriculture. After graduating, she began working for Cornell University's Milk Quality Improvement Program under Dr. Nicole Martin as a laboratory technician, where she learned how to apply her degree to dairy quality and spoilage. Being a technician gave her the opportunity to work on research aimed at improving dairy quality through both raw milk and finished dairy products, as well as allowing her to continue her studies through the Employee Degree Program. In her free time, Zoe enjoys relaxing with her husband, three cats, and one dog, piecing together jigsaw puzzles, playing the tenor saxophone, and playing on outdated video game platforms.

For Elbow, Ramen, Pierogi, and Mona Wasserlauf-Pepper

None of you know will know how to read this,

but I couldn't have done it without you

ACKNOWLEDGMENTS

I would like to thank my academic committee, Dr. Nicole Martin, committee chair, and Dr. Randy Worobo, committee member, for giving me the chance to further develop my skills as a scientist and for your guidance and feedback throughout the duration of my time as a student. I would also like to thank Dr. Martin Wiedmann, who advised me for most of my time as a student and worked with me on becoming a better critical thinker.

I would again like to thank Dr. Nicole Martin, who hired me and gave me the opportunity to work in the Milk Quality Improvement Program when I had no idea what direction I wanted my career to go. Your passion for all things dairy is contagious, and it has turned me into a firm believer in MQIP's research. Thank you for being so kind and assuring to me as a new lab technician starting right before a global pandemic, and patiently helping me develop my skills over the past few years. Your office has been a safe space for me to write, express my problems, learn, and ask all of the silly questions I would normally be too afraid to say.

I cannot thank the members of the Food Safety Lab and Milk Quality Improvement enough for welcoming me into the lab and making this experience into something I will never forget. It takes a village, and you're the best village anyone can ask for. Thank you to the media room team for making endless bottles and plates of media and still being so kind to us (me) when putting in large or urgent requests, and to all of the MQIP undergrads for the incredible amount of help you provide the lab. Thank you to Rachel Weachock, for training me on just about everything I do in the lab, always offering to help when I'm drowning in work, and being my first friend in Ithaca. Thank you to Sam Reichler, for ordering all of the lab's materials, knowing the lab top to bottom, and being a great neighbor. Thank you to Caroline Motzer, Mikayla Henry, Renee Lee, Sam Bolten, and YeonJin Jung, for a great girl gang summer, and

to office 316 for all of the great yapping and for providing peer pressure to focus when I needed it most.

Lastly, I would like to thank the family and friends who have always encouraged and supported me. Thank you to Courtney and Rebecca, The Aether, The Fantastics, and my UF roommates. Thank you to my incredible husband, Jakob, for being the best partner, friend, roommate, and co-parent I could ever ask for. Even though we got married, rescued another kitten, turtle, and our first puppy, had a few car accidents, spent nearly five months fixing my hip, and started a business since I started graduate school, you always prioritized my education when I needed support. Also, thank you for knowing me since middle school, and still choosing to love me anyway. Thank you to my mom for being so amazing and for giving me a life filled with gingerbread, weird movies, and many, many traditions. To my step-parents, for always treating me as your own and being proud of me, and for doubling the number of incredible parental figures I get to look up to. And thank you to my dad, for passing down your sweet tooth and work hard/play hard mentality to me. You knew I would work in the food sector before I did; I know you would be proud to see where I am now, and smug to know that you were right.

TABLE OF CONTENTS

ABSTRACT	iii
BIOGRAPHICAL SKETCH	iv
DEDICATION	v
ACKNOWLEDGEMENTS	vi
TABLE OF CONTENTS	viii
LIST OF FIGURES	ix
LIST OF TABLES	x
CHAPTER 1: INTRODUCTION	1
CHAPTER 2: SPORE LEVELS IN BULK TANK ORGANIC RAW MILK AND WHOLE MILK POWDER ARE REDUCED BY UDDER HAIR SINGEING	6
CHAPTER 3: VARIABILITY OF TEMPERATURE EXPOSURE OF DAIRY PRODUCTS DURING REFRIGERATED E-COMMERCE DISTRIBUTION AND ITS IMPACT ON DAIRY QUALITY	24
CHAPTER 4: CONCLUSIONS	62

LIST OF FIGURES

Figure 2.1	Box-and-whisker plot showing the mesophilic spore count (MSC) and thermophilic spore count (TSC) from whole milk powder manufactured from pre-intervention versus post-intervention bulk tank organic raw milk	16
Figure 3.1	Density plot of the arrival temperatures of fluid milk and Greek yogurt after third-party shopping and delivery	41
Supplemental Figure 3.1	Line graphs representing the time-temperature distributions of 1.89 L fluid milk containers held in 3 distinct locations for 33 direct-to-consumer refrigerated trucks.	61

LIST OF TABLES

Table 2.1	Characteristics of organic dairy farms (n=4) enrolled in udder hair singeing intervention study	10
Table 2.2	Mean log concentration of microbial parameters in organic bulk tank raw milk prior to and after udder hair singeing intervention applied on four certified organic dairy farms	15
Table 3.1	Conditions for model shelf-life validation study in commercially processed 1.89 L containers of milk	34
Table 3.2	Summary of temperature variation across three distribution channels	39
Table 3.3	Total bacteria count in commercially processed fluid milk containers throughout shelf-life after experimentally simulated e-commerce conditions categorized by if the sample showed evidence of post-pasteurization contamination or not	44
Table 3.4	Percentage of milk containers in a 100-container lot spoiled at day 21 of shelf-life as predicted by a Monte Carlo simulation for 3 different facility types after e-commerce distribution	46

CHAPTER 1

INTRODUCTION

Two primary factors that lead to dairy product spoilage and reduced shelf-life are bacterial contaminants present in raw and pasteurized products, and how dairy products are handled during distribution. Dairy product quality can be affected at each stage along the distribution chain, and it is critical to keep each of these steps in mind when implementing strategies for the extension of product shelf-life.

Bacterial spores can enter dairy products through a number of means, primarily into the raw milk supply through environmental sources such as bedding, soil, and manure (Martin et al., 2024), and then move on to persist through conventional high-temperature short-time (minimum 72°C for 15 seconds) pasteurization as an endospore and then germinating in finished products. Sporeforming bacteria, such as psychrotolerant *Bacillus* and *Paenibacillus* spp., are present in fluid milk in low concentrations immediately post-pasteurization but can grow to concentrations that cause spoilage later in shelf-life (e.g., 21 days post-pasteurization) (Huck et al., 2007, Ranieri and Boor, 2009). Many organic dairy processors reduce end-product microbial spoilage potential by ultra-pasteurizing (minimum 138°C for 2 seconds) their incoming raw milk, so that organic milk can travel farther to meet customer demands and to extend product shelf-life at the consumer level (Diamond, 2013). However, unlike high-temperature short-time pasteurization, ultra-pasteurization can impart a cooked flavor onto fluid milk, which may be unpleasant to some consumers (Jo et al., 2018). Organic dairy processors looking to make high quality fluid milk and milk products may look to reducing the incoming spore loads in their raw milk supply to extend product shelf-life without the use of more extreme heat treatments.

Organic dairy farms are unable to implement certain strategies for the intervention of microbial contamination into the raw supply while maintaining their

organic status. There are limited studies on the prevalence of different groups of bacterial spores in organic raw milk compared to conventional, however, a recent study by Qian et al. (Cornell University, Ithaca, NY, personal communication) evaluated the level of bacterial spores found in organic bulk tank raw milk and associated these levels with management practices used on the farm. A key practice identified to be associated with lower spore levels was the routine removal of udder hair from the milking herd. This practice can be quickly added to herd management routines, taking upwards of 15 seconds per individual cow (Strait, 2020), and could provide farmers with an opportunity for low-cost spore management.

E-commerce has been on the rise in recent years, propelled even higher since the COVID-19 outbreak (Shen et al., 2022, Tudor, 2022, US Census Bureau, 2024). In September 2020 to March 2021, 30-40% of U.S. shoppers reported using e-commerce for grocery purchases (Ellison et al., 2022). E-commerce use has only grown since then, and in 2023, e-commerce took a 15.4% market share of all U.S. commerce and approximately \$1.02 trillion was spent on grocery and meal delivery globally (Statista, 2024, US Census Bureau, 2024). Distinct e-grocery business models are used in the distribution of goods, such as perishable refrigerated products, to consumers, including i) direct-to-consumer (e.g., dairy processors who routinely deliver milk to consumer homes), ii) business-to-consumer (e.g., platforms where orders are placed and a third-party distribution warehouse fulfills and ships the orders through overnight delivery), and iii) business-to-business-to-consumer (e.g., mobile applications where orders are placed to a local grocery store and an independent, third-party employee fulfills and delivers the order to the consumer).

Consumers using e-commerce platforms for grocery fulfillment do not limit themselves to only purchasing nonperishable products; for example, 20% of U.S. online grocery shoppers reported purchasing dairy products between October 2023

and September 2024 (Statista, 2024). E-commerce businesses add or replace steps to the overall distribution chain for milk and other dairy products, and the lack of consistent temperature monitoring systems, or lack of any monitoring system at all, used across these distribution pathways can create potential exposure to temperatures above regulatory limits (FDA, 2023). Previous research on how short-term temperature abuse in milk and other dairy products impacts shelf-life is limited (Lin et al., 2016, Pinto et al., 2024) and, with the expanding use of e-commerce for refrigerated products, presents a need to understand what the potential for temperature abuse for milk and other dairy products is, and how that exposure at the beginning of shelf-life impacts their overall microbial quality.

In order to approach the potential impacts from these gaps in current knowledge, this thesis aims to take a farm-to-fridge approach to the improvement of dairy products. Chapter 2 assesses the bacterial spore load in bulk tank raw milk and manufactured whole milk dairy powder before and after udder hair removal by hair singeing on 4 organic dairy herds. Chapter 3 follows dairy products moving through 3 e-commerce distribution pathways to determine potential time-temperature exposure during transportation, and then simulates that time-temperature distribution in fluid milk to analyze if short-term temperature exposure during e-commerce pathways reduces product shelf-life.

REFERENCES

- Diamond, A. 2013. Treadmill acceleration and deceleration: Conflicting dynamics within the organic milk commodity chain. *Organ Environ* 26(3):298-317. <https://doi.org/10.1177/1086026613485667>.
- Ellison, B., M. Ocepek, and M. Kalaitzandonakes. 2022. U.S. household food acquisition behaviors during the COVID-19 pandemic. *PLoS One* 17(7):e0271522. <https://doi.org/10.1371/journal.pone.0271522>.
- Huck, J. R., B. H. Hammond, S. C. Murphy, N. H. Woodcock, and K. J. Boor. 2007. Tracking spore-forming bacterial contaminants in fluid milk-processing systems. *J. Dairy Sci.* 90(10):4872-4883. <https://doi.org/10.3168/jds.2007-0196>.
- Jo, Y., D. M. Benoist, D. M. Barbano, and M. A. Drake. 2018. Flavor and flavor chemistry differences among milks processed by high-temperature, short-time pasteurization or ultra-pasteurization. *J. Dairy Sci.* 101(5):3812-3828. [10.3168/jds.2017-14071](https://doi.org/10.3168/jds.2017-14071).
- Lin, H., M. Shavezipur, A. Yousef, and F. Maleky. 2016. Prediction of growth of *Pseudomonas fluorescens* in milk during storage under fluctuating temperature. *J. Dairy Sci.* 99(3):1822-1830. <https://doi.org/10.3168/jds.2015-10179>.
- Martin, N. H., F. M. Quintana-Pérez, and R. L. Evanowski. 2024. Sources, transmission, and tracking of sporeforming bacterial contaminants in dairy systems. *JDS Commun.* 5(2):172-177. <https://doi.org/10.3168/jdsc.2023-0428>.
- Pinto, G., G. A. Reyes, P. Corea, M. Pflugh Prescott, and M. J. Stasiewicz. 2024. Time and temperature abuse of milk in conditions representing a school cafeteria share table does not meaningfully reduce microbial quality. *J. Dairy Sci.* 107(5):2733-2747. <https://doi.org/10.3168/jds.2023-24175>.

- Ranieri, M. L. and K. J. Boor. 2009. Short communication: Bacterial ecology of high-temperature, short-time pasteurized milk processed in the United States. *J. Dairy Sci.* 92(10):4833-4840. 10.3168/jds.2009-2181.
- Shen, H., F. Namdarpour, and J. Lin. 2022. Investigation of online grocery shopping and delivery preference before, during, and after COVID-19. *Transp. Res. Interdiscip. Perspect.* 14(100580). <https://doi.org/10.1016/j.trip.2022.100580>.
- Statista. 2024. E-commerce in the United States - statistics & facts. Available at: <https://www.statista.com/topics/2443/us-ecommerce/#topicOverview>. Accessed 20 Oct 2024.
- Strait, G. 2020. Removal of Udder Hair Using a Simple Singe Procedure. Available at: <https://extension.psu.edu/removal-of-udder-hair-using-a-simple-singe-procedure#:~:text=Singeing%20off%20the%20hair%20is,wear%20a%20flame%2Dresistant%20glove>. Accessed 3 October 2024.
- Tudor, C. 2022. Integrated Framework to Assess the Extent of the Pandemic Impact on the Size and Structure of the E-Commerce Retail Sales Sector and Forecast Retail Trade E-Commerce. *Electronics* 11(19):3194. <https://doi.org/10.3390/electronics11193194>.
- US Census Bureau. 2024. E-commerce as percentage of total retail sales in the United States from 2000 to 2023 [Graph]. Available at: <https://www.statista.com/statistics/185351/share-of-e-commerce-in-total-value-of-us-retail-wholesale-trade-sales/>. Accessed 23 Oct 2024.

CHAPTER 2
SPORE LEVELS IN BULK TANK ORGANIC RAW MILK AND WHOLE MILK
POWDER ARE REDUCED BY UDDER HAIR SINGEING*

*Zoe D. Wasserlauf-Pepper, Rachel L. Weachock, Christina M. Geary, Martin
Wiedmann, and Nicole H. Martin

Prepared for submission to Journal of Dairy Science Communications

ABSTRACT

Routine removal of udder hair through singeing or clipping is a farm management practice that is often used with the intention of reducing somatic cell count and mastitis caused by environmental pathogens. Udder hair removal was also recently identified as a factor of importance for the level of bacterial spores in certified organic bulk tank raw milk. Sporeforming bacteria form resistant endospores, or spores, that allow the organisms to survive harsh environmental conditions including high temperatures, low moisture, exposure to chemicals, and other conditions that would otherwise be lethal to the vegetative cell. Due to this resistance, spores that are present in raw milk can survive processing hurdles used in dairy product manufacturing, ultimately causing spoilage and quality deterioration in dairy products including fluid milk, cheese, and products using dairy powders as ingredients. Therefore, the aim of this study was to determine if udder hair removal through singeing reduces the concentration of bacterial spores in certified organic bulk tank raw milk. Four organic dairy farms that had not routinely removed udder hair were recruited, and bulk tank samples were collected prior to and after a singeing intervention on all lactating dairy cows contributing to the bulk tank. Raw milk samples as well as whole milk powder manufactured from the raw milk collected before and after the intervention were assessed for different groups of dairy relevant bacterial spores. A numerical reduction from pre- to post-intervention was observed in the mean raw milk mesophilic spore count, thermophilic spore count, and butyric acid bacteria most probable number, while a numerical increase was observed in the mean raw milk total bacteria count and psychrotolerant spore most probable number, although none of these changes were significant. The mean mesophilic spore count and thermophilic spore count in whole milk powder manufactured from pre- and post-intervention raw milk was, however, significantly reduced from 2.46 to 1.58 log₁₀

cfu/g and 1.44 to 1.22 log₁₀ cfu/g, respectively. The results of our study indicate that udder hair removal may aid in reducing key populations of spores found in organic raw milk and resulting dairy powders manufactured from that raw milk, although the small sample size in our study likely impacted the significance of our results. Future studies should further investigate the role of udder hair removal on a larger set of organic farms, as well as the downstream impact on spore levels in other manufactured dairy products.

INTRODUCTION

Organic food product sales continue to increase in the United States, with an estimated \$69.7 billion in sales in 2023, with milk and cream sales accounting for a \$4.2 billion share (Organic Trade Association, 2024). The quality of dairy products is critical to consumer acceptance, and it is of particular importance for the organic dairy industry to ensure the highest quality products possible as their distribution channels are often longer than corresponding conventional products due to the reduced processing capacity for organic dairy (Diamond, 2013). Further, organic dairy producers face certain challenges for producing high quality raw milk, namely the restriction on the use of antibiotics, limitations on cleaning and sanitation chemicals allowed for use in the milking system, and increased time of animals on pasture where they become exposed to bacteria present in soil, water, and other natural environments.

A key microbial parameter that impacts the quality of raw milk is the presence and level of bacterial spores. Sporeforming organisms are unique in that they form resistant spores that withstand harsh environments, including pasteurization, with the possibility of causing quality defects and limiting shelf-life of processed dairy products. For example, psychrotolerant sporeforming bacteria, predominantly

Paenibacillus species, that grow at refrigeration temperatures, can reach concentrations where consumers may detect off-odors or flavors (Huck et al., 2007, Masiello et al., 2014, Masiello et al., 2017). Mesophilic and thermophilic sporeformers (e.g., *Anoxybacillus* and *Geobacillus*) are frequently monitored in dry dairy powders (e.g., skim milk powder, whey protein concentrate, etc.) where they may be subjected to stringent specifications, or cause spoilage in products where these powders are used as ingredients (Chen et al., 2004, Watterson et al., 2014, Sadiq et al., 2016). Lastly, anaerobic sporeforming bacteria that produce butyric acid, such as *Clostridium tyrobutyricum*, can cause late blowing in aged cheeses (Klijn et al., 1995, Podrzaj et al., 2022).

There are many factors that can affect spore levels in bulk tank raw milk, including management practices (e.g., bedding type, frequency of cleaning the bulk tank area) and udder and teat hygiene (Masiello et al., 2017, Martin et al., 2019). Research on bacterial spores in raw milk from organic dairy farms in the US is limited compared to studies on conventional dairy farms. However, a recent study was conducted on 102 certified organic dairy farms over 1 year across 11 states, to determine both the prevalence of key dairy associated spores in organic bulk tank raw milk and farm factors associated with spore presence and levels (C. Qian, Cornell University, Ithaca, NY, personal communication). One farm management practice identified to be important to the level of all spore types evaluated was routinely removing udder hair through clipping and/or singeing.

Therefore, the goal of this study was to evaluate the effectiveness of udder hair removal to reduce spores in organic bulk tank raw milk (**BTM**) as a means to provide dairy farmers with a practical, rapid, and non-resource intensive method to reduce spores in raw milk. In this preliminary study, we evaluated the concentration of bacterial spores in BTM for 1 week prior to and 1 week after udder hair singeing on 4

organic dairy farms, as well as in whole milk powder manufactured from the milk collected pre- and post-intervention. This study aims to serve as a proof-of-concept to identify a practical intervention strategy to reduce spores in organic bulk tank raw milk, which should ultimately be reproduced with a larger set of organic dairy farms.

MATERIALS AND METHODS

Farm selection

Certified organic dairy farms (A, B, C, and D) located in the Northeast US were recruited from a cohort of 102 certified organic dairy farms that were enrolled in a previous nationwide study concerning bacterial spore levels in organic raw milk (C. Qian, Cornell University, Ithaca, NY, personal communication) (Table 2.1). Only farms that had not previously routinely removed udder hair were considered for enrollment in the current study. The 4 farms varied by number of lactating cows, milking location, and number of years certified organic.

Table 2.1. Characteristics of organic dairy farms (n=4) enrolled in udder hair singeing intervention study

Farm ID	Number of milking cows	Housing style	Bedding	Milking location	Pre/post dip	No. years certified organic
A	41	Stanchions / tie stalls	Straw	Stanchions / tie stalls	Iodine based	15
B	92	Stanchions / tie stalls	Straw	Stanchions / tie stalls	Iodine based	26
C	35	Stanchions / tie stalls	Straw	Stanchions / tie stalls	Iodine based	26
D	96	Bedded pack	Sawdust	Swing parlor	Iodine based	16

Udder hair singeing intervention

The udder singeing procedure was approved by the Cornell University Institutional Animal Care and Use Committee (#2023-0259) and the experiment was conducted in April 2024. Producers were compensated for participation in this project. All lactating cows whose milk was contributing to the farm's bulk tank during the experimental period were included in the study (Table 2.1). The singeing procedure was performed as outlined by Strait (2020) with some modification as described here. To perform the udder singeing, a commercial singer (Udder Singe, Armor Animal Health, Beaver Dam, Wisconsin) was connected to a portable propane gas source. All eligible cows were flamed immediately after milking either in their tie stalls or in a post-parlor holding area. A yellow flame was used on the udder hair and singeing took between 5 – 15 seconds, depending on the amount of hair present on the udder. All singeing occurred in a well-ventilated area and both a fire extinguisher and wet towels were kept within reach while the flame was turned on to douse any flare ups. For consistent technique, singeing was performed by one trained technician.

Sample collection

Bulk tank raw milk samples (~250 mL) were collected by milk truck drivers prior to every milk pickup for 1 week prior to and 1 week after the singeing intervention on each of the 4 farms, with 4 samples collected from each farm before and 4 samples collected from each farm after the intervention was applied. Samples were immediately frozen at -20°C after collection and then were shipped on ice to the Cornell Milk Quality Improvement Program laboratory (Ithaca, NY). In a previous study, temporary freezing of raw milk samples was shown to have no impact on levels or populations of thermotolerant bacteria found in raw milk (Lee et al., 2024). In addition to the 250 mL bulk tank samples, 10 L BTM was collected from each farm from the

milking immediately prior to and the milking immediately after the intervention (typically within 12 h prior to and after the intervention). The 10 L pre- and post-intervention samples collected from the 4 farms were commingled in a 151 L stainless steel kettle prior to manufacturing into separate pre- and post-intervention whole milk powder (**WMP**) for further testing as described below.

Manufacturing of whole milk powder

The pre-intervention and post-intervention commingled bulk tank raw milk were held < 4°C until processing. Pre- and post-intervention milk was spray dried in a pilot scale spray drier (Niro, Copenhagen, Denmark) using an inlet temperature of 200°C and outlet temperature of 95°C. The finished product was aseptically transferred into a sterile plastic sampling bag and held at room temperature until testing. The spray drying unit was sanitized between processing pre- and post-intervention milk.

Microbiological analysis

Frozen milk samples were thawed in a 6°C incubator overnight before testing. Sample vials were shaken 25 times in 7 seconds according to Martin et al. (2024) prior to aliquoting milk for each test. An Aerobic Plate Count (**APC**) was performed by spiral plating 50 µL of raw milk (Neu-tec Eddy Jet 2, Farmingdale, NY) onto Standard Methods Agar (**SMA**) followed by incubation at 32°C for 48 h and then colonies were enumerated using a SphereFlash automated plate counter (Neu-tec, Farmingdale, NY). A 100 mL portion of raw milk was spore pasteurized (**SP**) at 80°C for 12 min in a shaking water bath and then immediately placed in an ice bath (Boor and Martin, 2024). Once the SP milk was cooled to 6°C or below, samples were pour plated by pipetting 1 mL SP milk into 20 empty Petri dish plates and then pouring 12-15 mL

molten, tempered SMA into each plate and gently mixing. Once agar was solidified, 10 plates were incubated at 32°C for 48 h (Mesophilic Spore Count; **MSC**) and 10 plates at 55°C for 48 h (Thermophilic Spore Count; **TSC**). Colonies were enumerated using an automated plate count reader (Q-count, Advanced Instruments, Norwood, MA). The level of anaerobic butyric acid-bacteria (**BAB**) was estimated using a 20-tube most probable number (**MPN**) method as previously described (Shi et al., 2022). Briefly, 5 mL raw milk was inoculated into 10 glass tubes filled with 9 mL Bryant and Burkey broth (**BB**) and 500 µL raw milk was similarly inoculated into 10 tubes containing 9.5 mL BB. All tubes were capped with 2 cm Paraffin wax plugs and heat treated at 75°C for 15 min. Tubes were stored at 35°C for 6 days at which point they were evaluated for presence of gas production, which was determined by the displacement of the wax plug. All tubes showing no displacement of the wax plug were scored as negative for gas production. The concentration of psychrotolerant spores was also estimated using a 15-tube MPN method, where 5 tubes of 10 mL SP milk, 5 tubes of 1 mL SP milk in 9 mL Skim Milk Broth (**SMB**), and 5 tubes of 100 µL SP milk in 9.9 mL SMB were incubated for 21 days at 6°C (Masiello et al., 2014). After incubation, tubes were vortexed, and a 10 µL aliquot of each tube was struck out onto SMA, incubated at 32°C for 48 h, and then checked for bacterial growth. Plates exhibiting growth were scored as positive and plates with no bacterial growth were scored as negative. An MPN table was subsequently used to determine final MPN/L concentration.

To determine spore concentrations in the WMP manufactured from pre- and post-intervention milk, 33 g powder samples were taken in triplicate from distinct sections of each of the 2 powder samples using a sterile scoop and placed in 627 mL Whirl-Pak filter bags (Nasco, Fort Atkinson, WI) with 297 mL phosphate buffered saline. Whirl-Pak bags were stomached for 1 min at 260 rpm. A 25 mL portion was

transferred into a sterile glass screw capped tube and was heated at 80°C for 12 min in a shaking water bath and then immediately placed on ice. SP reconstituted powder samples were pour plated and incubated for MSC and TSC as described above.

Data management and statistical analysis. Data were \log_{10} transformed prior to statistical analysis. All data and code used can be found in a publicly available data repository (<https://github.com/FSL-MQIP/OREI-Interventions-2024>). The Wilcoxon signed rank exact test was used to compare the bacterial spore concentrations for each raw milk test before and after udder singeing paired by farms. WMP results from this study could not be paired by farm because the raw milk from each farm was commingled prior to manufacturing, and therefore the Wilcoxon rank sum exact test was used to compare the pre- and post-intervention product. All statistical analyses were performed using the stats package in R version 4.3.1 (R Core Team, 2023).

RESULTS

Spores in organic whole milk powder can be reduced with udder hair singeing, but not in bulk tank raw milk

Overall, we observed a numerical reduction from 0.85 to 0.60 \log_{10} cfu/mL for MSC, 0.59 to 0.53 \log_{10} cfu/mL for TSC, and 2.25 to 2.18 \log_{10} MPN/L for BAB from pre- to post-intervention, however, these results were not statistically significant ($p = 0.125, 0.438$ and 0.438 , respectively) (Table 2.2). Conversely, we observed a slight numerical increase from 3.74 to 3.80 \log_{10} cfu/mL in the APC and 1.22 to 1.30 \log_{10} MPN/L in the PSC MPN ($p = 0.438$ and 0.563 , respectively) (Table 2.2). We observed trends within the data from each of the 4 farms in the study (A, B, C, D), that provide further context to our data. For example, in milk samples collected from farms A, B, and C, we observed a decrease in mean \log_{10} APC (ranging from 0.08-0.27 \log_{10}

cfu/mL) and an increase of 0.68 log₁₀ cfu/mL in farm D from pre- to post-intervention. Similarly, mean log₁₀ MSC from milk samples collected from farms B, C, and D was reduced (ranging from 0.14-0.70 log₁₀ cfu/mL), while an increase was observed in milk from farm A by 0.02 log₁₀ cfu/mL from pre- to post-intervention. We observed a decrease in mean log₁₀ TSC in milk collected from farms A and B (ranging from 0.10-0.34 log₁₀ cfu/mL) and an increase in mean TSC from milk collected from farms C and D (ranging from 0.01-0.21 log₁₀ cfu/mL). Mean BAB-MPN log₁₀ MPN/L from pre- to post-intervention was reduced in milk from farms B and D (ranging from 0.02-0.51 log₁₀ MPN/L) and increased in milk from farms A and C (ranging from 0.02-0.22 log₁₀ MPN/L), while mean PSC-MPN log₁₀ MPN/L was reduced in milk from pre- to post-intervention from farms A and D (ranging from 0.07-0.16 log₁₀ MPN/L) and increased in milk collected from farms B and C (ranging from 0.04-0.53 log₁₀ MPN/L).

Table 2.2. Mean log concentration of microbial parameters in organic bulk tank raw milk prior to (pre-) and after (post-) udder hair singeing intervention applied on four certified organic dairy farms

	Mean ¹		P-value ²
	Pre-intervention	Post-intervention	
Aerobic Plate Count (APC)	3.74	3.80	0.438
Mesophilic Spore Count (MSC)	0.85	0.60	0.125
Thermophilic Spore Count (TSC)	0.59	0.53	0.438
Butyric Acid Bacteria - Most Probable Number (BAB-MPN)	2.25	2.18	0.438
Psychrotolerant Spore Count - Most Probable Number (PSC-MPN)	1.22	1.30	0.563

¹log₁₀ CFU/ml for APC, MSC, TSC; log₁₀ MPN/L for BAB MPN, PSC MPN

²P-values calculated using a paired Wilcoxon signed rank exact test

In addition to evaluating the impact of udder hair removal on spores in bulk tank raw milk, we also determined MSC and TSC in WMP manufactured from pre- and post-intervention milk to determine the impact of the intervention on downstream dairy products. The mean MSC \log_{10} cfu/g in WMP manufactured from milk collected prior to the singeing intervention was 2.46, while the mean MSC \log_{10} cfu/g manufactured from milk post-intervention was 1.58 ($p = 0.05$; Figure 2.1). Similarly, we observed a decrease in mean TSC \log_{10} cfu/g in post-intervention WMP to 1.22 from 1.44 in WMP manufactured from pre-intervention milk ($p = 0.05$; Figure 2.1). The spore counts in this study were consistent across the discrete samples taken from the manufactured product, with standard deviations of 0.04 \log_{10} cfu/g for MSC in both pre- and post-intervention WMP, and 0.04 and 0.12 for TSC in pre- and post-intervention WMP, respectively (Figure 2.1).

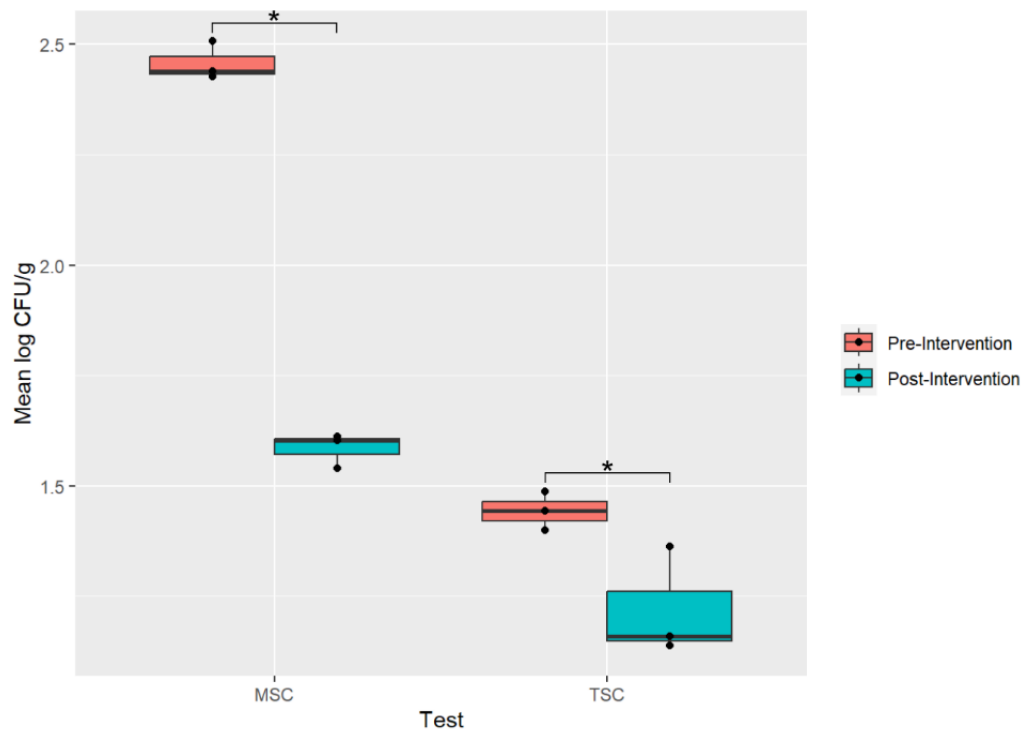


Figure 2.1. Box-and-whisker plot showing the mesophilic spore count (MSC) and thermophilic spore count (TSC) from 3 distinct 33 g samples within 1 bag of whole milk powder manufactured from pre-intervention (pink) versus 1 bag of post-intervention (blue) bulk tank organic raw milk commingled from four certified organic producers. The lower and upper horizontal edges of the box indicate the first and third quartiles, respectively, and the whiskers extend to 1.5 times the interquartile range. Black dots overlaying the boxes represent individual data points. Asterisks indicate statistical significance using the Wilcoxon rank sum exact test (* $p \leq 0.05$).

DISCUSSION

Improving bulk tank raw milk through on-farm interventions

Previous research has investigated intervention strategies for reducing spores in conventional bulk tank raw milk. For example, spore reductions observed in our current study are similar to those reported by Evanowski et al. (2020), that showed mean MSC and TSC were reduced from 0.3 to 0.2 \log_{10} cfu/ml, and 0.4 to 0.2 \log_{10} cfu/mL, respectively in bulk tank raw milk through a combination of on-farm employee training regarding teat-end cleanliness and procedural changes made to the cleaning and drying of laundered towels used during milking preparation on conventional NY dairy farms. While spore reductions in our study and others are generally quite small, this is likely due to the exceptionally low initial level of spores in bulk tank raw milk. However, our study provides preliminary evidence that utilizing simple strategies such as udder hair removal may result in reduced spore levels in bulk tank raw milk.

In addition to achieving lower spore concentrations in raw milk, udder hair removal has been recommended to minimize the spread of mastitis within dairy herds (Edmondson, 2012, Stefan and Baraitareanu, 2024). Previous studies involving udder hair singeing primarily focus on its predicted association with somatic cell count (Dufour et al., 2011). For example, in a study evaluating farm management associations with bulk tank somatic cell count (BTSCC) on 130 conventional dairy

farms in Colombia, the combination of udder hair singeing with the use of pre-dip yielded significantly lower BTSCC compared to farms that did not adhere to those practices (Reyes et al., 2017). Practices that offer multiple benefits (i.e., lower bulk tank spore levels as well as reduced BTSCC) may be more likely to be adopted by dairy producers, however, a recent study by Qian et al. (Cornell University, Ithaca, NY, personal communication) indicated that only 36% of certified organic dairy producers in the US include udder hair removal in their management practices. This finding suggests that there are opportunities to reach certain segments of the dairy production community with information on improving milk quality through udder hair removal.

Strict spore tolerances in dairy powders may benefit from higher quality raw milk

Mesophilic and thermophilic spores in raw milk contribute to the overall level of spores in dry dairy products as they survive processing conditions and are concentrated in the final product. Spore levels in dry dairy products are often closely monitored, and customers may set stringent specifications, specifically for thermophilic spores (Watterson et al., 2014). For example, limits of as low as 500 cfu/g ($2.7 \log_{10}$ cfu/g) MSC and TSC have been reported for WMP and skim milk powder (Bienvenue, 2013). Although the manufactured WMP from pre-intervention milk manufactured in our study did not exceed that limit, dairy powder processing facilities with difficulty adhering to their specific tolerance levels may set spore limits for incoming raw milk, encouraging the implementation of management practices linked to lower spore levels in BTM.

CONCLUSION

Our study provides a preliminary investigation of the impact of udder hair removal on spore levels in organic bulk tank raw milk, as well as in downstream dairy products manufactured from raw milk. The results of this study are promising as a potential spore reduction intervention; however, this study does have several limitations that should be addressed in future research. Namely, the sample size (n=4 farms) and the small geographical area used in the study were major limitations and was the likely reason that we did not identify a statistically significant reduction in spores. Further, our data lacked normality, again, likely due to the small number of experimental units, which necessitated the use of non-parametric statistical analyses, which further affected our ability to detect significant changes in spore concentration. Despite these limitations, we believe the outcome of this study demonstrates that udder hair removal by singeing is a rapid, easy, and low-resource practice that should be further studied in relation to the impact on milk quality parameters, specifically bulk tank spore levels and downstream dairy products.

ACKNOWLEDGEMENTS

This study was funded by United States Department of Agriculture (Washington, DC) Organic Research and Extension Initiative (USDA OREI) grant no. 2019-51300-30242. The authors thank the organic dairy farms who participated in the udder hair removal interventions and the Cornell Statistical Consulting Unit (Ithaca, NY) for their guidance on statistical analyses. The authors would also like to thank the members of the Milk Quality Improvement Program at Cornell University (Ithaca, NY) for their help throughout the study.

REFERENCES

- Bienvenue, A. 2013. Opportunities for low-spore milk powder in a global marketplace. in Proc. US Dairy Industry Spore Seminar. San Francisco, CA.
- Boor, K. J. and N. H. Martin. 2024. Tests for Groups of Microorganisms. Pages 341-388 in Standard Methods for the Examination of Dairy Products. Vol. 18. American Public Health Association, Washington, D.C.
- Chen, L., T. Coolbear, and R. M. Daniel. 2004. Characteristics of proteinases and lipases produced by seven *Bacillus* sp. isolated from milk powder production lines. *Int. Dairy J.* 14(6):495-504.
<https://doi.org/10.1016/j.idairyj.2003.https://doi.org/10.006>.
- Diamond, A. 2013. Treadmill acceleration and deceleration: Conflicting dynamics within the organic milk commodity chain. *Organ Environ* 26(3):298-317.
<https://doi.org/10.1177/1086026613485667>.
- Dufour, S., A. Fréchette, H. W. Barkema, A. Mussell, and D. T. Scholl. 2011. Invited review: Effect of udder health management practices on herd somatic cell count. *J. Dairy Sci.* 94(2):563-579. <https://doi.org/10.3168/jds.2010-3715>.
- Edmondson, P. 2012. Mastitis control in robotic milking systems. *In Practice* 34(5):260-268. <https://doi.org/10.1136/inp.e2660>.
- Evanowski, R. L., D. J. Kent, M. Wiedmann, and N. H. Martin. 2020. Milking time hygiene interventions on dairy farms reduce spore counts in raw milk. *J. Dairy Sci.* 103(5):4088-4099. <https://doi.org/10.3168/jds.2019-17499>.
- Huck, J. R., B. H. Hammond, S. C. Murphy, N. H. Woodcock, and K. J. Boor. 2007. Tracking spore-forming bacterial contaminants in fluid milk-processing systems. *J. Dairy Sci.* 90(10):4872-4883. <https://doi.org/10.3168/jds.2007-0196>.

- Klijn, N., F. F. Nieuwenhof, J. D. Hoolwerf, C. B. Van Der Waals, and A. H. Weerkamp. 1995. Identification of *Clostridium tyrobutyricum* as the causative agent of late blowing in cheese by species-specific PCR amplification. *Appl. Environ. Microbiol.* 61(8):2919-2924. <https://doi.org/10.1128/aem.61.8.2919-2924.1995>.
- Lee, R. T., R. L. Evanowski, H. E. Greenbaum, D. A. Pawloski, M. Wiedmann, and N. H. Martin. 2024. Troubleshooting high laboratory pasteurization counts in organic raw milk requires characterization of dominant thermotolerant bacteria, which includes nonsporeformers as well as sporeformers. *J. Dairy Sci.* 107(6):3478-3491. <https://doi.org/10.3168/jds.2023-24330>.
- Martin, N. H., D. J. Kent, R. L. Evanowski, T. J. Zuber Hrobruchak, and M. Wiedmann. 2019. Bacterial spore levels in bulk tank raw milk are influenced by environmental and cow hygiene factors. *J. Dairy Sci.* 102(11):9689-9701. <https://doi.org/10.3168/jds.2019-16304>.
- Martin, N. H., S. C. Murphy, R. Jechorek, R. S. Salter, F. Weber, and W. W. Northeimer. 2024. Microbiological Count Methods. Pages 193-263 in *Standard Methods for the Examination of Dairy Products*. Vol. 18. American Public Health Association, Washington, D.C.
- Masiello, S. N., D. Kent, N. H. Martin, Y. H. Schukken, M. Wiedmann, and K. J. Boor. 2017. Longitudinal assessment of dairy farm management practices associated with the presence of psychrotolerant Bacillales spores in bulk tank milk on 10 New York State dairy farms. *J. Dairy Sci.* 100(11):8783-8795. <https://doi.org/10.3168/jds.2017-13139>.
- Masiello, S. N., N. H. Martin, R. D. Watters, D. M. Galton, Y. H. Schukken, M. Wiedmann, and K. J. Boor. 2014. Identification of dairy farm management practices associated with the presence of psychrotolerant sporeformers in bulk

- tank milk. *J. Dairy Sci.* 97(7):4083-4096. <https://doi.org/10.3168/jds.2014-7938>.
- Organic Trade Association. 2024. 2024 Organic Industry Survey. Available at: <https://ota.com/market-analysis/organic-industry-survey/2024-organic-industry-survey>. Accessed 3 October 2024.
- Podrzaj, L., J. Burtscher, and K. J. Domig. 2022. Comparative genomics provides insights into genetic diversity of *Clostridium tyrobutyricum* and potential implications for late blowing defects in cheese. *Front. Microbiol.* 13(<https://doi.org/10.3389/fmicb.2022.889551>).
- R Core Team. 2023. R: A Language and Environment for Statistical Computing. Available at: <https://www.R-project.org/>.
- Reyes, J., J. Sanchez, H. Stryhn, T. Ortiz, M. Olivera, and G. P. Keefe. 2017. Influence of milking method, disinfection and herd management practices on bulk tank milk somatic cell counts in tropical dairy herds in Colombia. *Vet. J.* 220(34-39). <https://doi.org/10.1016/j.tvjl.2016.12.011>.
- Sadiq, F. A., Y. Li, T. Liu, S. Flint, G. Zhang, L. Yuan, Z. Pei, and G. He. 2016. The heat resistance and spoilage potential of aerobic mesophilic and thermophilic spore forming bacteria isolated from Chinese milk powders. *Int. J. Food Microbiol.* 238(193-201). <https://doi.org/10.1016/j.ijfoodmicro.2016.09.009>.
- Stefan, G. and S. Baraitareanu. 2024. Approaches of Milking Biosecurity and Milking Parlour Hygiene in Dairy Farms. IntechOpen. <https://doi.org/10.5772/intechopen.113084>.
- Strait, G. 2020. Removal of Udder Hair Using a Simple Singe Procedure. Available at: <https://extension.psu.edu/removal-of-udder-hair-using-a-simple-singe-procedure#:~:text=Singeing%20off%20the%20hair%20is,wear%20a%20flame%2Dresistant%20glove>. Accessed 3 October 2024.

Watterson, M. J., D. J. Kent, K. J. Boor, M. Wiedmann, and N. H. Martin. 2014.

Evaluation of dairy powder products implicates thermophilic sporeformers as the primary organisms of interest. *J. Dairy Sci.* 97(4):2487-2497.

<https://doi.org/10.3168/jds.2013-7363>.

CHAPTER 3
VARIABILITY OF TEMPERATURE EXPOSURE OF DAIRY PRODUCTS
DURING REFRIGERATED E-COMMERCE DISTRIBUTION AND ITS IMPACT
ON DAIRY QUALITY*

*Zoe D. Wasserlauf-Pepper, Aljosa Trmcic, Martin Wiedmann, and Nicole H. Martin
Prepared for submission to Journal of Dairy Science

ABSTRACT

The rise of e-commerce food distribution has grown drastically in recent years, a trend that was accelerated by the COVID-19 pandemic. The range of perishable products purchased through e-commerce as well as the distance over which these products will travel to consumers has also increased considerably. Perishable goods are commonly available through various e-commerce channels (e.g., third-party grocery shopping and delivery, direct processor to consumer delivery, and overnight shipment via centralized distribution centers). New or expanded distribution methods have surfaced to accommodate the increasing demand of grocery delivery, which can introduce the potential for temperature abuse of these perishable goods, possibly leading to premature spoilage. To determine the impact of e-commerce distribution on the microbial quality of dairy products, we assessed the variability of simulated and real e-commerce time-temperature profiles of fluid milk and Greek yogurt. We assessed 3 unique transportation channels associated with e-commerce distribution, including, (1) direct-to-consumer, (2) distributor or business-to-consumer, and (3) business-to-business-to-consumer. Our results showed that product temperature at the time of delivery ranged from 0.2 to 10.1°C for the direct-to-consumer pathway, -0.9 to 19.2°C for the business-to-consumer pathway, and 3.1 to 18.3°C during the simulated business-to-business-to-consumer pathway. To further identify how real dairy products ordered through business-to-business-to-consumer (e.g., grocery delivery to consumers from retail stores through a third-party) channels, containers of 1.89 L milk and 157 mL Greek yogurt were delivered to customers from local retail chains. Average milk and yogurt arrival temperatures were 7.2°C and 7.3°C, respectively, and ranged from 3.1 to 10.5°C and 3.4 to 12.7°C, respectively. The temperature profiles measured during 1.89 L milk transportation were incorporated into a previously developed computational model that predicts milk spoilage based on

initial concentration of primary groups of bacterial contaminants and other relevant conditions (e.g., storage temperature). The results of our model indicate that there are minimal changes to expected microbial spoilage of fluid milk at the end of shelf-life when distributed through e-commerce pathways, however, our models only predict microbially growth and not sensorial quality which has a larger impact on consumer acceptance. Overall, our study provides important information on the exposure of dairy products to various temperatures during e-commerce distribution, which can be used to further develop strategies for controlling and monitoring the cold chain.

INTRODUCTION

The landscape of consumer purchasing habits has greatly evolved since the COVID-19 pandemic. While purchasing household goods, including food, through e-commerce is not new and has been on the rise over the past decades, a definitive boom in 2020 has accelerated the upward trend for the demand of goods through e-commerce (Shen et al., 2022, Tudor, 2022, US Census Bureau, 2024). E-commerce rose in popularity, increasing from comprising of 10.7% of all U.S. retail sales in 2019 to 15.4% in 2023 (US Census Bureau, 2024), creating demands for delivery drivers, independent shoppers, and order fillers at distribution centers both within the U.S. as well as globally (Benner et al., 2020, Collins, 2021, Bryson, 2023, Ridhwan et al., 2023). Ellison et al. (2022) reported that of grocery purchases made in the U.S. in September 2020, December 2020, and March 2021, 30-40% of consumers reported both grocery shopping offline as well as online. Since the height of the pandemic, some commerce trends were predicted to revert back to levels consistent with pre-pandemic consumer habits, however, certain demographics (e.g., Millennial and Generation Z, people without personal transportation, higher income individuals) were reported to likely continue to utilize e-commerce at a higher rate (Hossain et al.,

2022). Despite these predictions, e-commerce has continued to grow globally, with an estimated \$1.02 trillion spent on grocery and meal delivery in 2023 globally (Statista, 2024).

E-commerce has continued to grow to adapt to the demands of business-to-business, business-to-consumer, and direct-to-consumer sales strategies for manufacturers to try and create their own niches to accommodating the demands of customers for refrigerated and frozen goods typically purchased at the retail level. For example, direct-to-consumer delivery from dairy producers and processors straight to the doorsteps of consumers has evolved since 19th century when the first “milkman” deliveries started in the U.S. This method of delivery has rapidly declined since more households gained access to refrigerators and personal vehicles, with consumers choosing to buy milk from groceries stores as the most popular outcome (Dunn, 2023). Decades later, the COVID-19 pandemic has shifted consumer purchasing habits away from community grocery stores in favor of direct-to-consumer doorstep delivery to save personal time as well as decrease social interaction (Olsson et al., 2022, Shen et al., 2022). Shifting consumer priorities brought back the direct-to-consumer dairy delivery in some locations and has also given more opportunities for e-grocery business-to-business-to-consumer models where consumers can use websites and mobile applications to buy chilled goods from local retail stores and have a third-party person employed through these websites complete the shopping and delivery for them.

One limitation of e-commerce distribution is the lack of maintaining a consistent cold chain in some of the delivery methods. Refrigerated transportation, such as complex systems like refrigerated vehicles and unit load devices in air cargo, or simple systems such as a contract shopper delivering grocery bags from the local grocery store, can be used for last-mile delivery of grocery items. Currently, refrigerated vehicles commonly have a simple temperature sensor located at the hottest

point in the vehicle (typically by the loading doors), creating a feedback loop if the temperature reaches above a predetermined threshold (James et al., 2006). However, in refrigerated trucks tightly packed with multiple layers of goods (e.g., stacked milk crates), the bottom layer of crates closest to the cooling device, often located at the top of the cargo unit in the front of the truck, can experience temperature abuse due to inadequate airflow (So et al., 2021). In contract shopping, independent employees of third-party grocery e-commerce platforms are largely unregulated due to the lack of supervision on the shopping and delivery process. The potential for temperature abuse among this type of business model is largely understudied and provides an opportunity to determine how these practices potentially affect the products after they arrive to the end-consumer.

Between October 2023 and September 2024, 20% of online grocery shoppers reported purchasing dairy products online in the U.S. (Statista, 2024). Purchasing refrigerated and frozen goods for same-day or overnight shipping is increasingly popular with the availability of websites and mobile applications for consumer use in both urban and rural communities. Refrigerated products, including dairy products, pose challenges compared to non-perishable goods, such as maintaining required refrigeration temperatures (e.g., $< 45^{\circ}\text{F}$ (7.2°C) for fluid milk) set by the Pasteurized Milk Ordinance (**PMO**) as well as transporting goods in a timely manner to decrease the amount of shelf-life time wasted prior to arriving at consumer level (FDA, 2023). Fluid milk and other finished dairy products are available through same-day delivery through e-grocery applications that deliver goods from retail stores to consumers through an intermediate party. Non-fluid dairy products, such as yogurt, cheese, and ice cream, are also frequently available for shipping directly from the manufacturer or from a distribution warehouse to achieve nationwide delivery.

The potential impact of expanded e-grocery systems on the quality of perishable refrigerated goods, such as in dairy products, should be evaluated to determine if additional temperature monitoring systems may be necessary along the distribution channels of interest. Therefore, the objective of this study is to evaluate time and temperature profiles throughout 3 e-commerce distribution chains for fluid milk as well as other finished dairy products, and to use that data to determine the impact of exposure on dairy product quality. Ultimately, this study provides dairy industry stakeholders, including processors and distributors, with knowledge and strategies to address temperature variability in e-commerce distribution pathways.

MATERIALS AND METHODS

Time-temperature distributions of dairy products through various e-commerce distribution channels

We identified 3 channels as pertinent e-commerce distribution methods for the purpose of this study, i) direct-to-consumer, ii) business-to-consumer, and iii) business-to-business-to-consumer. The first distribution channel, direct-to-consumer, includes dairy processors who offer home delivery via refrigerated truck directly from the facility to the consumer's location. In the second distribution channel, business-to-consumer, products originating from a distribution center or warehouse are express shipped in insulated packaging with passive cooling (e.g., dry ice or ice packs) directly to consumers. The final distribution channel investigated in this study was business-to-business-to-consumer, which involves purchasing groceries from a retailer that are then shopped for and delivered by independent contract shoppers to the consumer.

Temperature data from the direct-to-consumer channel was collected from 2 fluid milk processors who offer online ordering and home delivery directly to customer households. The first processor has 2 refrigerated trucks that deliver 3 days

per week and the second has 2 refrigerated trucks that deliver approximately 5 days per week. Product temperatures were recorded during the duration of delivery for both processors across different months (i.e., June and July 2022 for the first processor, and April and September 2022 for the second) to capture a variety of outdoor ambient temperatures, for a total of 12 days of delivery for the first processor and 21 days for the second. Submersible temperature dataloggers (OMEGA, Norwalk, CT) were placed into three clearly marked 1.89 L containers of milk from each plant's refrigerated storage before being placed in a milk crate designated for delivery service. Plant employees were instructed to strategically place each marked milk crate inside the trucks on each day of delivery to capture the temperature in the front, middle, and back of the refrigerated unit, as well as to note the time that each product delivery occurred. Upon return to the processing facility, each marked container of milk container of milk was held in the warehouse until the next day of delivery. At the end of the experimental period, data loggers were retrieved from each container and returned to the MQIP laboratory, where data was downloaded and saved for further analysis.

To assess the arrival temperature of dairy products from centralized business-to-consumer distribution centers to consumer doorsteps, we recruited a dairy product distribution company to place submersible data loggers into 177 mL cultured dairy products prior to shipping the products in standard packaging with standard passive cooling to 2 consumer participants. The two consumer participants receiving the dairy products with data loggers were located approximately 240 km and 390 km away from the distribution center. Both customers received 3 overnight shipments during March 2022 and 3 overnight shipments during September 2022. The time at which the dataloggers were inserted into the product and the package shipment time were

recorded by the warehouse personnel and package arrival times were recorded by the consumer participants.

Finally, to assess the temperature of dairy products distributed via third-party grocery delivery business-to-business-to-customer pathways (e.g., Instacart), project staff experimentally simulated grocery shopping and delivery on 3 separate occasions. In order to determine relevant parameters for the experiment, we first determined the minimum, maximum, and average shopping and delivery times by surveying an expert panel of independent contract employees (n=3) about their experiences when completing a typical order (including shopping for multiple customers at once) and how they insulate the refrigerated products they purchase on behalf of the customers. Based on expert panel responses, we determined that a typical shopping experience takes approximately 1 h and delivery takes approximately 1.5 h. The use of insulated bags with or without passive cooling (i.e., frozen ice packs) was inconsistent, so this experiment was repeated in triplicate for 1.89 L fluid milk containers i) without any insulation (i.e., containers were exposed to open air conditions both in the store and during delivery), ii) with insulation in a foam-lined resealable tote bag, and iii) with both an insulated tote bag and frozen ice packs. The shopping and delivery processes were simulated by project staff whereby for each of 3 replicates, shopping was conducted at a local retail market, where 3 separate milk containers (1.89 L) were collected, a data logger was placed immediately into each container, and the containers were held under conditions described for each of the insulation methods above. Products in each of the 3 insulation treatments were kept in the shopping cart in the retail market for 1 h, followed by a simulated delivery in a vehicle for 1.5 h. These experiments were conducted during a period of high ambient temperatures (i.e., mean ambient temperature 24.0°C) to assess how internal container temperatures change under more extreme conditions.

Arrival temperature of dairy products delivered in urban, suburban, and rural locations

Fluid milk and Greek yogurt containers were ordered and delivered through a third-party shopping application that promotes grocery deliveries within a 2 h time window to evaluate the arrival temperatures of refrigerated dairy products through business-to-business-to-consumer pathways. A 1.89 L container of 2% fat fluid milk and a single-serve (157 mL) container of fat-free plain Greek yogurt were ordered on 5 separate occasions over the course of 2 weeks and delivered to 6 participating households. Household location varied based on population density (rural, n=1; suburban, n=2; urban, n=3), as well as distance from the grocery store where delivery was scheduled from (rural, 25.7 km; suburban, 2.4 – 7.7 km; urban, 3.4 – 6.0 km).

Each participating household was provided with two submersible temperature loggers (OMEGA, Norwalk, CT) and were instructed to refrigerate them for a minimum of 24 h prior to the scheduled grocery delivery. The participants were also instructed to place a temperature logger in both the milk and yogurt containers immediately after delivery by quickly dropping the milk datalogger into the container and closing the lid, and the yogurt datalogger by partially opening the aluminum lid, fully submersing the datalogger into the yogurt, and taping the lid onto the top of the container. Participants were told to record when the products were ordered, delivered, and when the dataloggers were inserted into the containers. Product temperatures were recorded until the next delivery date, where the dataloggers were removed from the containers, rinsed, and placed in the refrigerator for 24 h prior to the subsequent delivery. Following data collection, dataloggers were shipped back to the Cornell Milk Quality Improvement Program (**MQIP**, Ithaca, NY) for temperature data extraction.

Shelf-life of naturally contaminated fluid milk after experimentally simulated e-commerce conditions

Using the temperature distribution data collected for 1.89 L containers of milk representing the direct-to-consumer and the business-to-business-to-consumer e-commerce channels previously discussed, 3 experimental simulations were conducted on commercially processed fluid milk, representing i) the lowest temperature and shortest time (Condition 1), ii) mean time and temperature (Condition 2), and iii) highest temperature and longest time (Condition 3) observed scenarios for each of the 2 channels (i.e., direct-to-consumer and business-to-business-to-consumer), as well as a control held at 6°C (Table 3.1). Containers of 2% milk (1.89 L) were collected from 2 separate dairy processing facilities and were transported on the same day (i.e., d 0 of shelf-life) to the MQIP laboratory. Samples were maintained at 1°C during transport and a temperature control was monitored using a miniature submersible data logger (OMEGA, Norwalk, CT). Samples were stored at 1°C between arrival and e-commerce experimental simulations. Replicate samples were collected on 3 separate processing days for each of the 6 e-commerce conditions described above plus 2 control groups for a total of 48 containers evaluated. Milk containers were placed into incubators at the temperatures described in Table 3.1, with containers spaced out to ensure adequate air flow for direct-to-consumer conditions. Conditions for the business-to-business-to-consumer channel were met by replicating the conditions described in Table 3.1 using commercially available insulated tote bags in Conditions 1 and 2 and 4 ice packs per bag in Condition 1, with ambient air temperature maintaining approximately 24°C. Following the temperature treatments, all containers were held at 6°C for 21 days to simulate consumer refrigerator conditions. Ambient air temperature within the incubators, as well as the internal milk temperature, was monitored for the duration of storage.

Table 3.1: Conditions for model shelf-life validation study in commercially processed 1.89 L containers of milk

Distribution channel	Temperature/time conditions ¹			
	Condition 1	Condition 2	Condition 3	Control
Direct-to-consumer	1°C / 5 h	4°C / 8 h	6°C / 3 h 12°C / 7 h	6°C / 10 h
Business-to-business-to-consumer	Insulated bag & ice packs / 2.5 h	Insulated bag / 2.5 h	No insulation / 2.5 h	6°C / 2.5 h

¹Temperature conditions reflect the ambient air temperature in direct-to-consumer, and insulation conditions in a 24°C climate-controlled area in business-to-business-to-consumer conditions

Following the e-commerce experimental simulation described above, microbial shelf-life analysis was conducted on days 1, 7, 14, and 21 after pasteurization. Milk containers were removed from the 6°C incubators and inverted 25 times before removing 1 mL from each container for analysis (Martin et al., 2024). Briefly, 50 µL aliquots were spiral plated (Neu-tec Eddy Jet 2, Farmingdale, NY) in duplicate on standard methods agar (**SMA**) for a total bacteria count and on Crystal Violet Tetrazolium Agar (**CVTA**) for a total Gram-negative bacteria count. Following plating, SMA and CVTA plates were incubated at 32°C for 48 h and 21°C for 48 h prior to enumeration, respectively, and colonies were enumerated using the SphereFlash automatic counting software (Neu-tec, Farmingdale, NY).

On d 21 post-pasteurization, sensory panelists (n=6) trained in milk defect judging as outlined by Carey et al. (2020) were recruited to evaluate a single blinded 30 mL sample from each milk container at room temperature (~21°C). Briefly, panelists were recruited from a cohort of Cornell University community members who passed both a basic taste pre-screening as well as training on milk-specific defects (e.g., oxidation, rancidity, farm-associated flavors). Panelists were given a standardized judging guide to score each milk sample on a scale from 0 to 10. Flavor and odor comments were assigned to a sample if a consensus of n=2 was reached.

Samples were graded as excellent (9.0-10.0), good (8.0-8.9), fair (7.0-7.9), poor (6.0-6.9), or unacceptable (< 6.0).

Monte Carlo simulation

To assess the overall impact of e-commerce distribution on microbial growth in fluid milk over shelf-life, we modified an existing Monte Carlo simulation model (J. Su, Cornell University, Ithaca, NY, personal communication) that simulates the growth of Gram-negative bacteria introduced by post-pasteurization contamination (**PPC**) and psychrotolerant sporeforming bacteria in milk containers transported along a 5-stage supply chain. Given the percentage of milk products contaminated with PPC and sporeforming bacteria, the model can predict the proportion of milk containers expected to have $> 6 \log_{10}$ cfu/mL (i.e., the approximate threshold that consumers typically perceive off-flavors and odors) at different days of shelf-life (Law, 1979, Martin et al., 2018). The temperature stages included in the base model represent i) storage at the processing facility, ii) transportation from the facility to retail, iii) storage at retail, iv) transportation from retail to consumer, and v) storage at consumer. We incorporated the time-temperature distribution data collected from e-commerce pathways using fluid milk containers (i.e., direct-to-consumer and business-to-business-to-consumer) into the base model to understand if temperature exposure introduced by e-commerce affects the proportion of expected spoiled containers. The temperature data collected from the direct-to-consumer channel eliminated stages ii – iv, replacing them with the temperature data collected from the 2 processing facilities that deliver milk to customers. The business-to-business-to-consumer channel data from all insulation methods used as described above replaced stage iv. Model output was presented for fluid milk processing facilities with varying frequency of PPC; facilities with 0 – 33% PPC were classified as long shelf-life, 34 – 66% as medium

shelf-life, and 67 – 100% as short shelf-life, as our previous studies have established that Gram-negative contaminants that cause PPC are responsible for a considerable proportion of premature fluid milk spoilage (Ralyea et al., 1998, Dogan and Boor, 2003, Reichler et al., 2018).

Statistical analysis and data preservation

All data were stored in Microsoft Excel. Statistical analyses were performed using R version 4.4.1 (R Core Team, 2024). R code and raw data are available in a publicly accessible repository (https://github.com/FSL-MQIP/2024_E-commerce). Bacterial concentration data were \log_{10} transformed prior to analysis. All graphical figures were made using ggplot2 (Wickham, 2016) and descriptive statistics were performed using the stats package (R Core Team, 2024). The arrival temperatures for fluid milk and Greek yogurt after third-party shopping and delivery through the business-to-business-to-consumer channel were analyzed using nonparametric alternatives to the t-test (i.e., Wilcoxon rank sum exact test) and ANOVA (i.e., Kruskal-Wallis exact test) using rstatix (Kassambra, 2023).

RESULTS

Three e-commerce distribution channels differ in time-temperature distributions

Across all of the direct-to-consumer delivery routes tracked (n=33), the time between departure from the respective processing facilities and completing product delivery took an average of 7.7 ± 2 h (range 4.4 to 14.4 h) and the overall mean temperature recorded was 4.5°C and ranged between 0.2 and 10.5°C (Table 3.2). Mean temperatures by location in the truck over all routes was found to be 4.8°C for the back of the trucks, closest to the truck door opening for loading and unloading product, 4.3°C in the middle of the trucks, and 4.4°C for the front of the trucks,

farthest from the loading doors. However, several trends were observed in the temperature of product between locations (i.e., front, middle, and back) within each truck, with the standard deviations between the 3 truck locations ranging between 0.12 (front, or location furthest from the door) and 1.92°C (back, or location closest to the door). In 9 delivery routes, temperature data from 1 of the 3 logger locations was not recovered. The dataloggers in the location closest to the truck door (i.e., back) showed higher temperatures for the duration of 5 routes, while the temperatures during the other routes either remained consistent with low variation among the 3 locations (n=6) or all locations showed similar temperature increases throughout transportation (n=12) (Supplemental Figure 3.1).

Due to datalogger and package receiving errors, the temperature data from the consumer 240 km from the warehouse was recovered for 2 of 3 March and September 2022 packages each, while the temperature data from the consumer 390 km from the warehouse was recovered for all March and 2 of 3 September 2022 packages. Overall, the time from shipment to delivery for the business-to-consumer distribution (n=9) took between 20.4 h and 23.5 h. All packages arrived with the same packaging, consisting of an outer cardboard box, an inner extended polystyrene container, crumpled paper to hold the product in place, ice packs, and a single container of 177 mL cultured dairy product. Notably, the ice packs in one of the packages were not frozen at the time of arrival; the cultured dairy product temperature was initially 5.7°C at the time of shipping, cooled to 3.2°C after the first ~10.5 h of transport, and increased to 19.2°C over the following ~11.5 h. Excluding this outlier, product temperatures ranged between -0.9 and 6.1°C (mean $2.4 \pm 1.4^\circ\text{C}$) during the duration of shipping and decreased by an average of 0.7°C from departure to arrival time (Table 3.2).

Throughout the business-to-business-to-consumer experimental simulation, mean ambient temperature during shopping was 22.2°C and delivery was 25.1°C. Immediately after removing the milk containers from the retail shelf, the mean internal milk temperature was 3.8°C (n=9, excluding the first 0.1 h of temperature readings for datalogger acclimation). Mean internal milk temperature for 1.89 L containers without any insulation method, containers held in insulated tote bags, and containers held in insulated bags in combination with ice packs after the 0.9 h shopping simulation were 8.5, 5.7, and 3.5°C, respectively (Table 3.2). Finally, following the 1.5 h experimentally simulated delivery, the internal milk temperatures for the containers without any insulation method, containers held in insulated tote bags, and containers held in insulated bags in combination with ice packs were 14.3, 8.5, and 4.4°C, respectively (Table 3.2).

Table 3.2: Summary of temperature variation across three distribution channels

Distribution channel	Time (h) ¹		Temperature (°C) ¹	
Direct-to-consumer	7.7 ± 2.0; [4.4, 14.4]		4.5 ± 1.0; [0.2, 10.5]	
Business-to-consumer	21.9 ± 1.0; [20.4, 23.5]		3.3 ± 3.7; [-0.9, 19.2]	
Business-to-business-to-consumer	Shopping ²	Delivery	Shopping	Delivery
No insulation	0.9	1.5	6.3 ± 1.3; [4.0, 8.7]	11.7 ± 1.6; [8.9, 14.8]
Insulated grocery bag	0.9	1.5	5.0 ± 0.8; [3.6, 6.6]	7.3 ± 0.8; [5.6, 9.0]
Insulated bag & ice packs	0.9	1.5	3.3 ± 0.1; [3.1, 4.0]	4.2 ± 0.6; [3.5, 5.6]

¹Mean ± standard deviation; [range]

²All business-to-business-to-consumer third-party grocery shopping took 1 h, excluding the first 0.1 h of shopping for datalogger temperature acclimation

Time and temperature distributions through third-party delivery

In order to assess arrival temperatures of dairy products through commercial e-commerce distribution, we ordered 1.89 L 2% plastic fluid milk containers and 157 mL plain Greek yogurt on 5 separate days through a third-party grocery shopping application for delivery to 6 separate study participants in different geographic locations (urban, suburban, and rural). Each study participant received each of the 5 orders of 1.89 L fluid milk and 157 mL Greek yogurt, with the exception of one urban participant that was only able to receive 4 milk and 0 yogurt samples. Therefore, data were collected for n=29 and n=25 total milk and yogurt containers, respectively. The amount of time between the order placement and when the participants were notified that delivery was complete varied considerably, with the rural (n=1; 25.7 km from the retail location), suburban (n=2; 2.4 – 7.7 km from the retail location), and urban (n=3; 3.4 – 6.0 km from the retail location) locations ranging from 0.6 – 1.4 h, 0.3 – 1.1 h, and 0.4 – 4.1 h, respectively. Mean arrival temperatures of 1.89 L milk and 157 mL Greek yogurt for the rural deliveries was $8.2 \pm 1.3^{\circ}\text{C}$ (n=5) and 9.9 ± 2.3 (n=5), while the mean arrival temperatures for the suburban deliveries were $7.2 \pm 1.2^{\circ}\text{C}$ (n=10) and $7.5 \pm 1.3^{\circ}\text{C}$ (n=10), and finally the mean arrival temperatures for the urban deliveries were $6.9 \pm 2.3^{\circ}\text{C}$ (n=14) and $5.8 \pm 2.1^{\circ}\text{C}$ (n=10) (Figure 3.1). However, it is notable that for both the urban and rural deliveries, the distribution of arrival temperatures is bimodal, with products arriving below the PMO limit of 7.2°C (FDA, 2023), or products arriving well above that limit (Figure 3.1), while the distribution of temperatures of products delivered to suburban participants was unimodal.

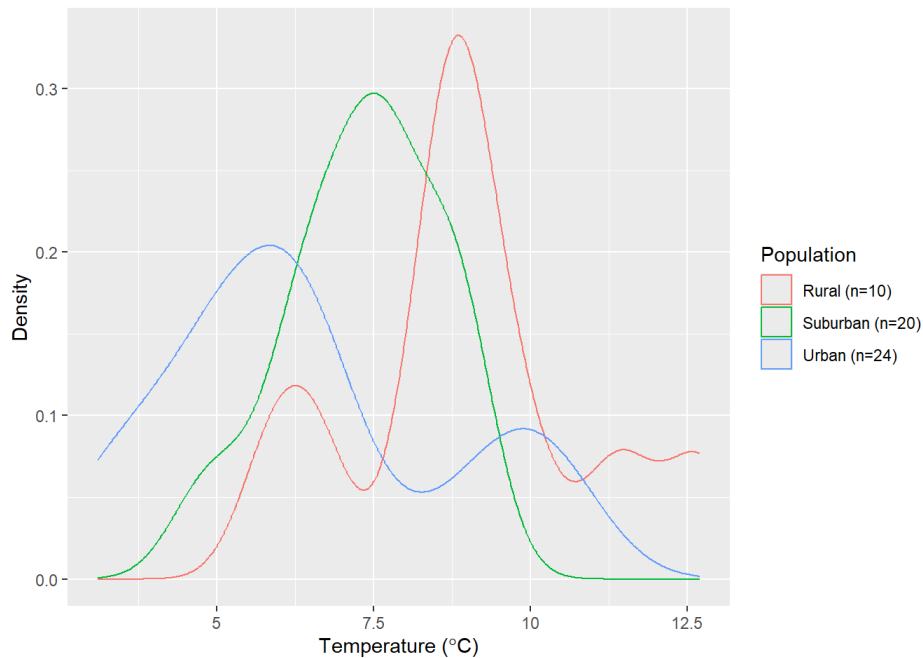


Figure 3.1: Density plot of the arrival temperatures of fluid milk (1.89 L) and Greek yogurt (157 mL) after third-party shopping and delivery to rural (n=5 milk; n=5 yogurt), suburban (n=10 milk; n=10 yogurt), and urban (n=14 milk, n=10 yogurt) NY households.

To analyze the potential effects of the different parameters on arrival temperature, the nonparametric Wilcoxon rank sum and Kruskal-Wallis exact tests were performed using the product type (milk, yogurt) and the population density of the participating household (rural, suburban, urban), respectively. The combined shopping and delivery time was excluded from this analysis, due to unknown factors including when products were removed from their shelves during shopping, and if the contract shoppers used any insulating material when storing the products. The arrival temperatures did not significantly differ ($p = 0.0986$) between fluid milk and Greek yogurt, indicating that the container size and product type did not have an impact on the internal temperature. Arrival temperature, however, was significantly associated with the location population density of the participants ($p = 0.006$). Post-hoc analysis

using a Dunn's test of multiple comparisons revealed that only a significant difference was seen between rural and urban arrival temperatures ($p = 0.002$). No significant interaction was observed between the product type and location.

Dairy products held under e-commerce conditions within the first 10 hours of shelf-life did not have significantly lower microbial and sensory quality compared to products held under control conditions

The total bacterial growth and total Gram-negative growth were assessed on SMA and CVTA, respectively, on d 1, 7, 14, and 21 of fluid milk shelf-life following experimental simulation of 6 e-commerce conditions (Table 3.3). Overall, samples that showed evidence of PPC (i.e., Gram-negative bacteria growth reaching $> 6 \log_{10}$ cfu/mL) had higher mean bacterial concentrations than samples with no evidence of PPC, particularly at 14 and 21 days of shelf-life (Table 3.3). For example, on d 21, 29% (14/48) milk containers were positive for PPC with mean bacterial concentration in samples with PPC for each condition between 7.13 and 7.77 \log_{10} cfu/mL, compared to the mean bacterial concentration of samples without PPC for each condition which ranged between 2.22 and 5.69 \log_{10} cfu/mL (Table 3.3). While we did not analyze these results for significance between e-commerce conditions, due to the lack of normal distribution of samples with and without PPC, we observed that overall, bacterial concentration was similar on day 21 for all conditions tested, with the exception of the samples with no evidence of PPC held under the business-to-business-to-consumer Condition 2, which showed no change in bacterial concentration over the duration of the shelf-life (Table 3.3).

Among all milk samples ($n=48$) evaluated for sensory characteristics on d 21 following experimentally simulated conditions previously described (Table 3.1), the mean sensory score varied considerably by whether the product had evidence of PPC

(sensory score of 4.9) or not (sensory score of 7.8) (Table 3.3). Flavor and odor defects noted for samples with PPC were fruity fermented (n=5), acid (n=2), cooked (n=2), rancid (n=2), bitter (n=1), coagulated (n=1), and flat (n=1), while flavor and odor defects noted for samples without PPC were cooked (n=5), feed (n=4), astringent (n=2), flat (n=2), barny (n=1), lacks freshness (n=1), foreign chemical (n=1), lipid oxidized (n=1), and malty (n=1). However, we observed no trends in the sensory data for milk samples held under various e-commerce conditions (Table 3.3).

Table 3.3: Total bacteria count in commercially processed fluid milk containers (1.89 L) throughout shelf-life after experimentally simulated e-commerce conditions categorized by if the sample showed evidence of post-pasteurization contamination (PPC positive) or not (PPC negative)

	Mean log ₁₀ cfu/mL on SMA ³								Mean sensory score ⁴	
	Day 1 ⁵		Day 7		Day 14		Day 21		Day 21	
	PPC ^{6,7} negative	PPC positive	PPC negative	PPC positive	PPC negative	PPC positive	PPC negative	PPC positive	PPC negative	PPC positive
Direct-to-consumer ¹										
Condition 1	2.31	2.20	2.13	1.92	2.68	6.15	5.59	7.30	7.4	7.9
Condition 2	1.86	NA	2.26	NA	2.49	NA	4.55	NA	7.4	NA
Condition 3	2.02	2.02	2.33	3.99	2.63	7.21	5.06	7.46	7.9	5.8
Control	2.05	1.00	2.34	2.59	2.69	7.11	5.10	7.13	7.9	2.5
Business-to-business-to-consumer ²										
Condition 1	1.95	2.62	2.18	3.63	2.54	6.97	5.38	7.77	7.6	3.8
Condition 2	2.14	1.91	2.12	2.80	1.93	6.72	2.22	7.68	7.4	4.3
Condition 3	2.21	2.11	2.17	2.74	3.47	7.01	5.69	7.49	8.1	6.8
Control	2.01	2.58	2.18	4.74	2.19	6.92	4.06	7.39	8.3	3.7

¹Condition 1: 1°C for 5 h; Condition 2: 4°C for 8 h; Condition 3: 6°C for 3 h immediately followed by 12°C for 12 h. All products held at 6°C for the remainder of shelf-life following each experimentally simulated e-commerce condition

²Condition 1: insulated tote bag with ice packs for 2.4 h; Condition 2: insulated tote bag for 2.4 h; Condition 3: no insulation for 2.4 h. All conditions held at ambient temperature of 25°C

³SMA: standard methods agar

⁵Day of shelf-life is represented by the number of days since pasteurization (i.e., Day 0)

⁴Mean sensory scores were awarded on a 0-10 scale by n=6 trained sensory panelists; panelists graded samples as excellent (9.0-10.0), good (8.0-8.9), fair (7.0-7.9), poor (6.0-6.9), or unacceptable (< 6.0)

⁶PPC: post-pasteurization contamination

⁷Samples showing evidence of Gram-negative bacteria at any point in shelf-life were categorized as PPC positive. Number of samples for each condition categorized as PPC positive out of n=6 containers were; direct-to-consumer Condition 1 (n=1), direct-to-consumer Condition 2 (n=0; "NA"), direct-to-consumer Condition 3 (n=1), direct-to-consumer control (n=1) business-to-business-to-consumer Condition 1 (n=2), business-to-business-to-consumer Condition 2 (n=5), business-to-business-to-consumer Condition 3 (n=3), business-to-business-to-consumer control (n=1)

A fluid milk Monte Carlo spoilage model predicts that fluid milk exposed to e-commerce temperatures has limited impact on shelf-life compared to control

Here we modified an existing fluid milk spoilage predictive model to incorporate the temperature profiles from both the direct-to-consumer and business-to-business-to-consumer e-commerce channels. We ran 10,000 model simulations for milk containers originating from long, medium and short shelf-life processing facilities. These categories have been set based on the proportion of milk containers contaminated with PPC. The model for milk containers held under control conditions (i.e., using historical data for all stages of distribution and storage established by Su et al. (2024)) for the long, medium, and short shelf-life categories, predicted that 20%, 45%, and 75% of milk containers would exceed $6 \log_{10}$ cfu/mL by 21 days of shelf-life, respectively (Table 3.4). Compared to the shelf-life predictions for the control conditions, we observed very minimal changes with the incorporation of e-commerce temperature profiles (Table 3.4). The predicted containers exceeding $6 \log_{10}$ cfu/mL by 21 days of shelf-life for the direct-to-consumer channel was slightly higher (22% compared to 20% for the control) for the long shelf-life products, but slightly lower for medium (44% compared to 45% for the control) and short (72% compared to 75% for control) shelf-life products (Table 3.4). In contrast, the Monte Carlo model predicted that there would be a slight increase in proportion of containers exceeding $6 \log_{10}$ cfu/mL by 21 days of shelf-life for business-to-business-to-consumer channel for all 3 product shelf-life categories compared to the controls (increase of 1.5%, 1%, and 1.5%, respectively; Table 3.4).

Table 3.4: Percentage of milk containers in a 100-container lot spoiled (i.e., $> 6 \log_{10}$ cfu/mL) at day 21 of shelf-life as predicted by a Monte Carlo simulation (10,000 iterations) for three different facility types after e-commerce distribution

	Percentage of milk containers spoiled		
	Long shelf-life ¹	Medium shelf-life ¹	Short shelf-life ¹
Control	20.00 [15.00, 28.05] ²	45.0 [36.95, 55.00]	75.00 [67.00, 83.00]
Direct-to-consumer	22.00 [15.95, 30.00]	44.00 [34.95, 53.05]	72.00 [61.00, 79.05]
Business-to-business-to-consumer	21.50 [14.00, 30.00]	46.00 [36.95, 56.00]	76.50 [66.00, 86.05]

¹Long, medium, and short shelf-life facilities have 0 – 33%, 34 – 66%, and 67 – 100% fluid milk containers with post-pasteurization contamination, respectively

²Median [5th percentile, 95th percentile]

DISCUSSION

Here we describe variability in time and temperature distribution of 3 commonly applied e-commerce distribution pathways, and the impact of these conditions on dairy product shelf-life. Overall, we observed that dairy products are exposed to a large degree of temperature variability for short periods of time during e-commerce distribution, and these temperatures are impacted by i) location within refrigerated transportation vehicle, ii) method used to insulate the product during delivery, and iii) time to delivery (or distance from the store). However, our data also indicates that despite the exposure to temperature fluctuations during e-commerce distribution, there is little evidence that there is ultimately a considerable impact on the expected shelf-life of the product.

Dairy products may be exposed to a high degree of variability in time and temperature during e-commerce distribution

Grocery and meal delivery has been on the rise over the past decade, accelerated by the COVID-19 pandemic. In 2023, online grocery delivery was

estimated to generate \$630 billion in U.S. sales, and as of June 2024, 20% of online grocery shoppers (n=10,085) in the U.S. reported buying dairy products through this service (Statista, 2024). Overall, we observed temperature deviations across all e-commerce distribution channels studied here that could potentially impact dairy product quality. For example, the mean internal fluid milk temperature throughout same-day direct-to-consumer travel during this study was found to be 4.5°C, however, the difference between minimum and maximum temperature and time spent on delivery routes spanned 10.3°C and 10 h, respectively. Product temperature between locations within each truck did not vary as much as between trucks themselves, with average temperatures by location being 4.8°C for the back of the trucks, closest to the truck door opening for loading and unloading product, 4.3°C in the middle of the trucks, and 4.4°C for the front of the trucks, farthest from the loading doors. Evidence indicates that there are difficulties with maintaining refrigeration temperatures in perishable products closest to loading and unloading zones in these trucks, especially in climates with higher ambient temperatures (James et al., 2006).

In addition to the impact of exposure on perishable goods to ambient temperatures during direct-to-consumer distribution, especially during periods of extreme heat, we identified that the method and consistency of insulation and packaging during business-to-consumer, and business-to-business-to-consumer distribution has a considerable impact on temperature deviation in dairy products traveling through e-commerce. In recent decades, advances have been made to efficiently move perishable food products from central distribution warehouses directly to consumers in packages utilizing passive cooling solutions (e.g., ice packs, insulating materials) within refrigerated transport units to maintain cost, convenience, and the ability to effectively protect the goods being moved (Ren et al., 2022). However, this study showed the potential for products to be shipped overnight without

proper cooling mechanisms as well as to be exposed to temperatures below 0°C. Individually packaged dairy products insulated with passive cooling devices in addition to a temperature-controlled holding unit are both necessary for adequate temperature control, however this can unintentionally cause freezing. Freeze-thaw cycles can create a watery appearance due to precipitation of milk solids and can similarly cause the separation of free whey in yogurt (Alvarez, 2023, Tribby and Teter, 2023). In the dataset (n=9) from 177 mL cultured dairy products shipped to customers overnight, 2 packages reached temperatures below 0°C. Coupled with the package in this study that arrived at ambient temperature, a case could be made that these products are not handled consistently during travel, and customers may have very different experiences with receiving their shipped goods, potentially impacting acceptance and willingness to buy through those channels in the future.

Finally, popular U.S. grocery delivery services typically ask their contract shoppers and delivery drivers to use insulated bags when handling temperature sensitive products and recommend ice packs when necessary; however, this practice is honor code based as there is no regulatory system, such as temperature loggers, in place to confirm whether or not this is happening. To decrease the use of gas and time, and increase the number of orders fulfilled, thereby increasing driver payout, it is also typical for drivers to take on multiple deliveries at once. These orders are often grouped by final destination and order size, to efficiently deliver the highest number of products at once. Therefore, grocery orders for customers in less densely populated areas may remain in delivery vehicles for a longer period of time compared to the time products are held in vehicles during personal shopping, increasing the potential for temperature abuse of refrigerated and frozen products.

Short-term exposure to temperature abuse may not be an important factor in expected fluid milk shelf-life

The long-term storage temperature of high-temperature short-time (HTST) milk has been shown to have a significant impact on the microbial load of the milk at d 14 and 21 post-pasteurization (Andrus et al., 2015, Lott et al., 2023), however, the results seen here suggest that short-term temperature abuse immediately after processing (i.e., during distribution) may have minimal effects on the microbial concentration at the end of shelf-life. Here we observed no clear trends in the bacterial concentrations of commercially available fluid milk with or without PPC after undergoing simulated e-commerce time and temperature conditions then held at constant temperature throughout shelf-life, which is supported by the predicted spoilage outcome in milk containers exposed to real e-commerce distributions using a Monte Carlo simulation. In one study by Pinto et al. (2024), school milk cartons inoculated with 2-3 log₁₀ cfu/mL *Pseudomonas poae* did not have significantly reduced shelf-life following 125 min (2.1 h) of room temperature conditions (20.3 – 23.1°C) repeated on days 2 through 6 of shelf-life compared to constant refrigeration at 4.2°C, similar to the length of time estimated for the shopping and delivery conditions used in this study. In another study evaluating longer periods of temperature abuse (29°C) for 4 or 6 h at 24 or 48 h post-inoculation with *P. fluorescens* at 3 log₁₀ cfu/mL and then held at 4°C for the duration of shelf-life, a larger shelf-life reduction was seen in samples with temperature abuse at 24 h compared to 48 h (Lin et al., 2016). These studies suggest that while temperature abuse early in shelf-life may impact the microbial growth during storage, short-term exposure to moderately elevated temperatures is unlikely to have a considerable impact on product shelf-life from a microbial perspective.

While we did not observe a significant impact of e-commerce conditions on the shelf-life of fluid milk in our experimental simulation or in the Monte Carlo simulation, we did observe a considerable impact of the presence of Gram-negative bacteria on the end of shelf-life bacterial concentration. The presence of Gram-negative bacteria in pasteurized milk is typically an indicator of post-pasteurization contamination (Dogan and Boor, 2003, Alles et al., 2018, Reichler et al., 2018). Gram-negative bacteria, such as *Pseudomonas* spp. typically grow faster and can outcompete Gram-positive bacteria that could be introduced either pre- or post-pasteurization (Ranieri and Boor, 2009). Gram-negative bacteria, in high concentrations, are associated with sensory defects such as coagulation and a fruity fermented flavor in milk contaminated with *P. fluorescens* (Reichler et al., 2018), and are commonly a limiting factor in determining HTST milk shelf-life (Ralyea et al., 1998, Dogan and Boor, 2003, Lau et al., 2022).

To determine the impact of e-commerce distribution on bacterial concentration at d 21 post-pasteurization, we simulated the number of fluid milk containers exceeding 6 log₁₀ cfu/mL by different processing facility categories, specifically, those that have high rates of PPC (short shelf-life), mid-range rates of PPC (medium shelf-life), and low rates of PPC (long shelf-life) (J. Su, Cornell University, Ithaca, NY, personal communication). In most cases, incorporating the direct-to-consumer and business-to-business-to-consumer time and temperature distributions collected in this study minimally impacted the predicted outcome, with 1 to 2 percentage point change in expected spoilage. Notably, direct-to-consumer transportation (mean 4.5°C for 7.7 h) reduced the predicted percentage of containers spoiled for processing facilities with medium and short shelf-life by 1 to 3 percentage points. This result is likely linked to the elimination of key steps in the distribution chain for the direct-to-consumer model, decreasing both the time of transport as well as the potential for temperature abuse

during transportation between facility and retail, at the retail locations, and between retail and consumer households. The direct-to-consumer channel, while highly variable, may provide those facilities with shorter shelf-lives a means to both provide consumers with products earlier in shelf-life as well as protect products from undergoing longer periods of unknown temperature abuse before the end of the distribution chain.

Strategies for controlling temperature variability during e-commerce distribution should be developed and validated

Despite evidence for minimal shelf-life implications from short-term temperature abuse in dairy products shown here, there are other areas of concern that stakeholders should consider when controlling temperature abuse in dairy products through e-commerce channels. Consumers expecting refrigerated items to be delivered at chilled temperatures may not accept those products if they are frozen or warmed to ambient temperatures. This could lead to unsatisfactory ratings and reviews of the manufacturer or distributor, as well as refund requests for items that will ultimately become food waste. Refrigerated products that arrive at the expected temperature but undergo temperature abuse prior to final delivery may also impart sensory defects, such as whey separation in yogurt due to freeze-thaw cycles or a watery appearance in whole milk from milk solid precipitation (Alvarez, 2023, Tribby and Teter, 2023).

New strategies for controlling the cold chain should be developed and validated specifically in e-commerce distribution channels to reduce the exposure of perishable dairy products to highly variable temperatures during e-commerce distribution. These strategies may include i) improved guidance and oversight for third-party shoppers, and ii) temperature monitoring or indicating devices. The current lack of consistent practices among third-party grocery shopping contract employees

represents a large area where perishable goods can be exposed to fluctuating temperatures. Third-party shopping and delivery companies give their contract shoppers guides on how to handle refrigerated and frozen grocery items, and often require or recommend insulated shopping bags to control the temperatures of these products. However, some companies may not directly provide these bags, therefore shoppers must purchase or provide their own, potentially reducing compliance. Contract shoppers are also often provided guides for food safety and handling, but there are currently no standards in place to ensure follow-through. Even so, there are incentives for drivers to take appropriate care of grocery items for their customers in the form of shopper ratings (typically on a 1- through 5-star scale) and tips for drivers from their customers. Another area of concern identified through the observations made in this study is the lack of best practices for refrigerated trucks used in direct-to-consumer distribution systems. Refrigerated trucks used by dairy processing facilities can vary in size, door location, and refrigeration capacity. Using smaller transportation vehicles (e.g., cargo vans) for local dairy processing facilities to offer an e-commerce platform for direct-to-consumer delivery can come with difficulties in managing thermal stability; smaller cargo units have doors that cover more external surface area and makes the unit overall more susceptible to ambient temperature exposure, and packing milk crates into refrigerated vehicles in such a way to increase the ability to reach more consumers in a single delivery route could indicate that adequate airflow is not met for some individual milk containers depending on the location of the cooling unit (Zhang et al., 2018, So et al., 2021).

In addition to improved practices, a combination of temperature monitoring solutions may be necessary to adequately track temperature abuse through e-commerce distribution. Current methods used to track temperatures measure the temperature output from the trucks themselves, not the temperatures of the products.

There are currently available solutions for closer tracking on the products themselves; for example, a study on German shipping logistics by Waldhans et al. (2024) evaluated current time-temperature tracking methods as well as implementing the use of Time-Temperature Indicators that evaluate color changes in products which can be inserted into individual packages within refrigeration transportation units and have temperature data sent to a cloud-based Android mobile application for real-time monitoring. These indicators can be strategically placed in several containers within refrigerated trucks to track within-truck variability. Similarly, Bluetooth-enabled temperature dataloggers may provide useful information for direct-to-consumer dairy processors offering home delivery by adding loggers to milk crates during daily delivery routes, as well as for business-to-business-to-consumer platforms to monitor the temperature within grocery bags containing refrigerated or frozen goods during third-party transportation, so that processors and consumers can monitor the temperature of their products during both in-store shopping as well as delivery.

CONCLUSION

Dairy product distribution through e-commerce pathways is expected to continue to grow, and the results of our study indicate that there are considerable opportunities for exposure to temperatures that exceed requirements for perishable products during transportation (e.g., maintaining cold chain $< 7.2^{\circ}\text{C}$). There are currently no widely accepted best practices for monitoring the cold chain throughout e-commerce distribution, thereby limiting the ability for processors and distributors to ensure consistent practices. While our data suggests that short-term temperature abuse at the beginning of shelf-life is unlikely to considerably impact the overall microbial shelf-life of perishable dairy products such as fluid milk, other factors including

consumer acceptance and sensory quality may be impacted and further study is warranted to reduce variability across these distribution channels.

ACKNOWLEDGEMENTS

This project was funded by the New York Dairy Promotion Advisory Board. The authors would like to thank the dairy processors, distributors, and consumers who participated throughout this project, the members of the Cornell Food Safety Laboratory and Cornell Milk Quality Improvement Program for their support with data collection and lab work, and the Cornell Statistical Consulting Unit for aiding with data analyses.

REFERENCES

- Alles, A. A., M. Wiedmann, and N. H. Martin. 2018. Rapid detection and characterization of postpasteurization contaminants in pasteurized fluid milk. *J. Dairy Sci.* 101(9):7746-7756. <https://doi.org/10.3168/jds.2017-14216>.
- Alvarez, V. B. 2023. Fluid Milk Products. Pages 79-142 in *The Sensory Evaluation of Dairy Products*. Springer International Publishing, https://doi.org/10.1007/978-3-031-30019-6_5.
- Andrus, A. D., B. Campbell, K. J. Boor, M. Wiedmann, and N. H. Martin. 2015. Short communication: Postpasteurization hold temperatures of 4 or 6°C, but not raw milk holding of 24 or 72 hours, affect bacterial outgrowth in pasteurized fluid milk. *J. Dairy Sci.* 98(11):7640-7643. <https://doi.org/10.3168/jds.2015-9531>.
- Benner, C., S. Mason, F. Carré, and C. Tilly. 2020. *Delivering Insecurity: E-commerce and the Future of Work in Food Retail*. Available at: <https://laborcenter.berkeley.edu/delivering-insecurity/>. Accessed 11 Nov 2024.
- Bryson, J. R. 2023. Post-Pandemic Impacts of COVID-19 on Urban Logistics. Pages 395-405 in *The Routledge Handbook of Urban Logistics*. Routledge, <https://doi.org/10.4324/9781003241478-35>.
- Carey, N. R., D. J. Kent, S. C. Murphy, K. J. Boor, M. Wiedmann, and N. H. Martin. 2020. A practical training program for fluid milk defect judging should focus on initial training of panelists. *J. Dairy Sci.* 103(7):6716-6726. <https://doi.org/10.3168/jds.2019-17336>.
- Collins, J. 2021. Amazon triples Southern California delivery hubs to get packages out faster. Available at: <https://www.ocregister.com/2021/03/26/amazon-triples-its-last-mile-delivery-network-in-southern-california/>. Accessed 11 Nov 2024.
- Dogan, B. and K. J. Boor. 2003. Genetic Diversity and Spoilage Potentials among *Pseudomonas* spp. Isolated from Fluid Milk Products and Dairy Processing

- Plants. Appl. Environ. Microbiol. 69(1):130-138.
<https://doi.org/10.1128/AEM.69.1.130-138.2003>.
- Dunn, J. 2023. The Rise And Fall Of The Milkman. Available at:
<https://www.thedailymeal.com/1142465/the-rise-and-fall-of-the-milkman/>.
Accessed 9 Nov 2024.
- Ellison, B., M. Ocepek, and M. Kalaitzandonakes. 2022. U.S. household food acquisition behaviors during the COVID-19 pandemic. PLoS One 17(7):e0271522. <https://doi.org/10.1371/journal.pone.0271522>.
- FDA. 2023. Standards for grade “A” milk and milk products. US Department of Health and Human Services, Public Health Service, Washington, DC
- Hossain, M. S., M. R. Fatmi, and C. E. Thirkell. 2022. How Will In-Person and Online Grocery Shopping and Meal Consumption Activities Evolve After COVID-19? Transp. Res. Rec. 036119812211191.
<https://doi.org/10.1177/03611981221119183>.
- James, S. J., C. James, and J. A. Evans. 2006. Modelling of food transportation systems – a review. Int. J. Refrig. 29(6):947-957.
<https://doi.org/10.1016/j.ijrefrig.2006.03.017>.
- Kassambra, A. 2023. rstatix: Pipe-Friendly Framework for Basic Statistical Tests.
<https://CRAN.R-project.org/package=rstatix>
- Lau, S., A. Trmcic, N. H. Martin, M. Wiedmann, and S. I. Murphy. 2022. Development of a Monte Carlo simulation model to predict pasteurized fluid milk spoilage due to post-pasteurization contamination with gram-negative bacteria. J. Dairy Sci. 105(3):1978-1998. <https://doi.org/10.3168/jds.2021-21316>.

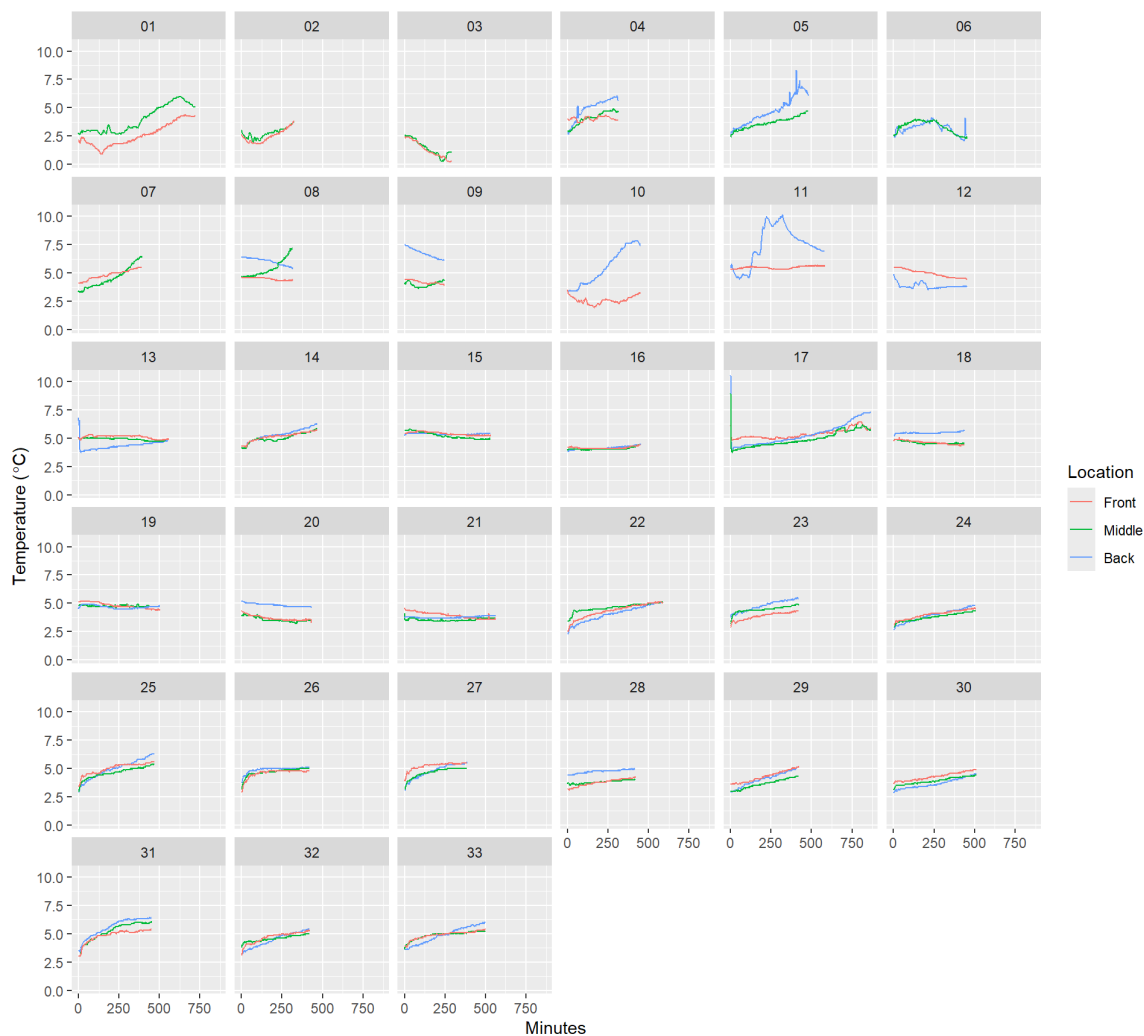
- Law, B. A. 1979. Enzymes of psychrotrophic bacteria and their effects on milk and milk products. *J. Dairy Res.* 46(3):573-588.
<https://doi.org/10.1017/S0022029900017611>.
- Lin, H., M. Shavezipur, A. Yousef, and F. Maleky. 2016. Prediction of growth of *Pseudomonas fluorescens* in milk during storage under fluctuating temperature. *J. Dairy Sci.* 99(3):1822-1830. <https://doi.org/10.3168/jds.2015-10179>.
- Lott, T. T., M. Wiedmann, and N. H. Martin. 2023. Shelf-life storage temperature has a considerably larger effect than high-temperature, short-time pasteurization temperature on the growth of spore-forming bacteria in fluid milk. *J. Dairy Sci.* 106(6):3838-3855. <https://doi.org/10.3168/jds.2022-22832>.
- Martin, N. H., K. J. Boor, and M. Wiedmann. 2018. Symposium review: Effect of post-pasteurization contamination on fluid milk quality. *J. Dairy Sci.* 101(1):861-870. <https://doi.org/10.3168/jds.2017-13339>.
- Martin, N. H., S. C. Murphy, R. Jechorek, R. S. Salter, F. Weber, and W. W. Northeimer. 2024. Microbiological Count Methods. Pages 193-263 in *Standard Methods for the Examination of Dairy Products*. Vol. 18. American Public Health Association, Washington, D.C.
- Olsson, J., M. C. Osman, D. Hellström, and Y. Vakulenko. 2022. Customer expectations of unattended grocery delivery services: mapping forms and determinants. *Int. J. Retail Dist. Manage.* 50(13):1-16.
<https://doi.org/10.1108/ijrdm-07-2020-0273>.
- Pinto, G., G. A. Reyes, P. Corea, M. Pflugh Prescott, and M. J. Stasiewicz. 2024. Time and temperature abuse of milk in conditions representing a school cafeteria share table does not meaningfully reduce microbial quality. *J. Dairy Sci.* 107(5):2733-2747. <https://doi.org/10.3168/jds.2023-24175>.

- R Core Team. 2024. R: A Language and Environment for Statistical Computing.
Available at: <https://www.R-project.org/>.
- Ralyea, R. D., M. Wiedmann, and K. J. Boor. 1998. Bacterial Tracking in a Dairy Production System Using Phenotypic and Ribotyping Methods. *J. Food Prot.* 61(10):1336-1340. <https://doi.org/10.4315/0362-028x-61.10.1336>.
- Ranieri, M. L. and K. J. Boor. 2009. Short communication: Bacterial ecology of high-temperature, short-time pasteurized milk processed in the United States. *J. Dairy Sci.* 92(10):4833-4840. <https://doi.org/10.3168/jds.2009-2181>.
- Reichler, S. J., A. Trmčić, N. H. Martin, K. J. Boor, and M. Wiedmann. 2018. *Pseudomonas fluorescens* group bacterial strains are responsible for repeat and sporadic postpasteurization contamination and reduced fluid milk shelf life. *J. Dairy Sci.* 101(9):7780-7800. <https://doi.org/10.3168/jds.2018-14438>.
- Ren, T., J. Ren, D. B. Matellini, and W. Ouyang. 2022. A Comprehensive Review of Modern Cold Chain Shipping Solutions. *Sustainability* 14(22):14746. <https://doi.org/10.3390/su142214746>.
- Ridhwan, M. M., A. Suryahadi, J. F. Rezki, and D. T. Andariesta. 2023. The impact of COVID-19 on the labour market and the role of E-commerce development in developing countries: Evidence from Indonesia. *J. Asia Pac. Econ.* 1-44. <https://doi.org/10.1080/13547860.2023.2195710>.
- Shen, H., F. Namdarpour, and J. Lin. 2022. Investigation of online grocery shopping and delivery preference before, during, and after COVID-19. *Transp. Res. Interdiscip. Perspect.* 14(100580). <https://doi.org/10.1016/j.trip.2022.100580>.
- So, J.-H., S.-Y. Joe, S.-H. Hwang, S. Jun, and S.-H. Lee. 2021. Analysis of the Temperature Distribution in a Refrigerated Truck Body Depending on the Box Loading Patterns. *Foods* 10(11):2560. <https://doi.org/10.3390/foods10112560>.

- Statista. 2024. E-commerce in the United States - statistics & facts. Available at:
<https://www.statista.com/topics/2443/us-ecommerce/#topicOverview>.
Accessed 20 Oct 2024.
- Su, J., T. Chandross-Cohen, C. Qian, L. Carroll, K. Kimble, M. Yount, M. Wiedmann, and J. Kovac. 2024. Assessment of the exposure to cytotoxic *Bacillus cereus* group genotypes through high-temperature, short-time milk consumption. *J. Dairy Sci.* 107(10):7631-7647. <https://doi.org/10.3168/jds.2024-24703>.
- Tribby, D. and V. Teter. 2023. Yogurt. Pages 199-234 in *The Sensory Evaluation of Dairy Products*. S. Clark, M. Drake, and K. Kaylegian, ed. Springer International Publishing, https://doi.org/10.1007/978-3-031-30019-6_8.
- Tudor, C. 2022. Integrated Framework to Assess the Extent of the Pandemic Impact on the Size and Structure of the E-commerce Retail Sales Sector and Forecast Retail Trade E-commerce. *Electronics* 11(19):3194.
<https://doi.org/10.3390/electronics11193194>.
- US Census Bureau. 2024. E-commerce as percentage of total retail sales in the United States from 2000 to 2023 [Graph]. Available at:
<https://www.statista.com/statistics/185351/share-of-e-commerce-in-total-value-of-us-retail-wholesale-trade-sales/>. Accessed 23 Oct 2024.
- Waldhans, C., A. Albrecht, R. Ibal, D. Wollenweber, S.-J. Sy, and J. Kreyenschmidt. 2024. Temperature Control and Data Exchange in Food Supply Chains: Current Situation and the Applicability of a Digitalized System of Time–Temperature-Indicators to Optimize Temperature Monitoring in Different Cold Chains. *J. Packag. Technol. Res.* 8(1):79-93. <https://doi.org/10.1007/s41783-024-00165-2>.
- Wickham, H. 2016. *ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York. <https://ggplot2.tidyverse.org>

Zhang, X., J. W. Han, J. P. Qian, Y. Z. Wang, L. Wang, and X. T. Yang. 2018.
Computational fluid dynamic study of thermal effects of open doors of
refrigerated vehicles. *J. Food Process Eng.* 41(3):e12662.
<https://doi.org/10.1111/jfpe.12662>.

SUPPLEMENTAL MATERIAL



Supplemental Figure 3.1. Line graphs representing the time-temperature distributions of 1.89 L fluid milk containers held in 3 distinct locations (i.e., back, or closest to the loading doors, middle, and front, or farthest from the loading doors) for 33 direct-to-consumer refrigerated trucks.

CHAPTER 4

CONCLUSIONS

Creating high quality dairy products requires a wholistic approach to the entire dairy system, where controls can be made at each stage of the distribution chain to ensure that consumers can enjoy long-lasting dairy products. There isn't one single way to reduce the spoilage potential in pasteurized products made from raw milk (e.g., fluid milk, cultured dairy foods, powders, etc.); instead, it is necessary to target multiple control strategies where improvements can be made in a practical and effective manner so that each stakeholder in the dairy industry, including farmers, processors, distributors, and retailers, can make informed improvements for how best manage the products that they are handling. Here, we can see that two means for improving the quality and handling of dairy products are through reducing the concentration of bacterial spores in bulk tank raw milk and reducing temperature abuse during cold-chain transportation in dairy products intended for e-commerce distribution.

Sporeforming bacteria, which can withstand high-temperature short-time pasteurization time-temperature conditions in their endospore form, are able to enter the raw milk supply at the farm through the environment, equipment, and from the cows themselves. As the demand for organic products continue to grow, organic dairy processors looking to create high quality products without the use of ultra-pasteurization may limit the incoming spore load in their raw milk prior to making products such as fluid milk, where psychrotolerant sporeforming bacteria can cause end of shelf-life defects, or dairy powders, where mesophilic and thermophilic sporeformers are concentrated during manufacturing and can cause off-odors and off-textures in the final product. Chapter 2 explored the effects of a low-cost, time efficient intervention on organic dairy herds by udder hair singeing on the level of

bacterial spores in bulk tank raw milk and manufactured whole milk powder and found that both mesophilic and thermophilic spore concentrations were significantly reduced in whole milk powder, however no significant reductions were found among spore types in bulk tank raw milk. Sample size was a limiting factor in this study, and future research should focus on expanding the number of herds tested, as well as exploring potential longitudinal effects from this practice.

The continuous rise in utilizing e-commerce for grocery delivery in recent years provokes questions on how refrigerated goods could potentially be temperature abused during e-commerce distribution, and if short-term temperature abuse during distribution reduces the predicted shelf-life outcome. In Chapter 3, we followed dairy products, namely fluid milk and single serve cultured dairy foods, through three commonly used e-commerce distribution pathways through both real and simulated conditions. We also simulated the extremes of those conditions in a laboratory setting using fluid milk containers to determine the concentration of bacteria at different time points throughout shelf-life. Interestingly, the dairy products involved in e-commerce distribution underwent a wide range of time-temperature conditions, showcasing a large potential for short-term temperature abuse of refrigerated dairy products during e-commerce delivery, although there is not a large predicted change in spoilage potential after these conditions. Using the findings from this study, we are able to incorporate e-commerce into a milk spoilage prediction model to better capture how dairy products move in a modern landscape.

Ultimately, our approach to improving dairy shelf-life should follow a farm-to-fridge approach where low-cost, efficient, and easy to adopt changes are made at all levels of the dairy continuum. Although the results from these studies are limited, this thesis aims to highlight different methods for the extension of dairy product microbial quality for the end consumer.