ABSTRACT

LEE, ANDY P. The role of processing parameters and fat content on sensory perception and consumer acceptance of fluid milk. (Under the direction of Dr. MaryAnne Drake).

Per capita consumption of fluid milk has declined over the last 40 years despite increases in production. To compete with other beverages such as soft drinks, juices, and plant-based milks, higher quality fluid milks must be produced through a deeper understanding of the factors that impact quality, shelf life, and sensory characteristics. Previous research has discussed the role of processing parameters such as raw milk hold time and quality, and pasteurization temperature and hold time; however, these studies have not examined the role of raw milk cooling rate or the influence of ultra-pasteurization by different techniques on fluid milk quality. The objective of this thesis was to evaluate the role of the aforementioned factors on the sensory perception and consumer acceptance of fluid milks. This research will enable the dairy industry to further improve the quality and sale of fluid milks.

In the first study, the impact of raw milk cooling rate with regard to sensory perception and shelf life was addressed. Three milkings of warm raw milk were collected from the NCSU dairy farm then brought to the NCSU pilot plant facilities and cooled. Cooling was conducted by a plate heat exchanger to quickly cool the raw milk or slowly by jacketed bulk tank cooling. The raw milks were separated and the skim portions for each cooling treatment were pasteurized at 73° or 78°C. Difference tests and a consumer acceptance test were conducted with the pasteurized milks to determine if consumers could differentiate between cooling treatments or between pasteurization temperatures (four pairs total). Milks were also examined by descriptive analysis and microbial testing over shelf life.

Consumers could not differentiate between any pairs and did not show differences in liking for any milk treatment. Descriptive analysis did reveal differences between milks pasteurized at different temperatures up to day 7, but no differences were noted between cooling treatments at any time point. Day of sensory failure was not different between milks; all milks reached sensory failure at 44-53 days after processing on average. Neither raw milk cooling rate nor pasteurization temperature had an effect on the sensory perception or the shelf life of pasteurized fluid milks. These results indicate that other factor(s) during processing have greater impacts on the shelf life, quality, and sensory perception of fluid milk.

The objective of the second study was to identify differences that may exist between ultra-pasteurized milks processed by indirect or direct steam injection. Raw skim and 2% fat milks were processed by HTST pasteurization or ultra-pasteurization. Milks were examined by descriptive, microbial, furosine, and serum protein denaturation analyses. Consumer acceptance testing was also conducted on the milks at 10 days after processing with self-reported skim and 2% fat milk adult and child consumers. Ultra-pasteurized milks were characterized by greater cooked flavor and aroma than HTST milks. The direct steam injection milks were also defined by a distinct sulfur/eggy flavor and aroma. All aromatic flavor intensities decreased across the 14 day storage period. Furosine concentrations and serum protein denaturation were greatest for the indirect ultra-pasteurized treatments followed by direct steam injection and lastly the HTST milks, indicating descending severity of heat treatment. Fat content did not affect the values for furosine and serum protein denaturation. Both adult and child consumers for both fat contents preferred the HTST milks over either ultra-pasteurized milks, which were at parity for liking. These results will be

important for processors who must consider the effect on quality when producing milks by different pasteurization methods.

The role of processing parameters and fat content on sensory perception and consumer acceptance of fluid milk

by
Andy P. Lee

A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Food Science

Raleigh, North Carolina

2016

APPROVED BY:

_____	_____
Dr. Maryanne Drake Committee Chair	Dr. E. Allen Foegeding
_____	
Dr. Brian Farkas	

DEDICATION

This thesis is dedicated to my family, friends, and coworkers who have supported me through this journey. Thank you.

BIOGRAPHY

Andy Lee was born in North Bergen, NJ on October 31, 1990 to Yong and Sung Lee. He moved to North Carolina shortly after his sister Jane was born and has been a resident since. He entered North Carolina State University as a Chemical Engineering major in Fall 2009. In early 2011, he was invited to work part-time for a food science graduate lab. Here, he found a new opportunity to pursue his passions by adding Food Science as a second major. Shortly after graduating in May 2014, Andy was accepted and entered into the Masters in Food Science program under the direction of Dr. MaryAnne Drake, who he had worked for as an undergraduate.

ACKNOWLEDGMENTS

I would like to thank my parents first and foremost for their constant support throughout my education. I hope I have made you proud 엄마 and 아빠. I would also like to thank my sister Jane, who I grew closer to by staying nearby. It's been great watching you grow up and start many firsts. Next, I would like to thank my friends (Scott, Jeff, Abbi, Erin, Kyle, Sarah, Hannah, Meg, Kelsey, and anyone else I missed) for their efforts in keeping me sane through graduate school. Being around you all gave me the energy to keep moving forward. To all my co-workers, both past and present, thank you for teaching me and supporting me on this journey. I have seen generations come and go in the lab; now, it's my turn to pass on the torch. I believe in each of you that remain and hope you will keep this dysfunctional, yet somehow functioning, lab family going.

Lastly, I would like to thank Dr. Drake for her continuous support and guidance through the years. Although there were some rough patches along the way, we made it through and we were better for it. I wish you luck as the older generation moves on and the new generations come up.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1:

LITERATURE REVIEW

Introduction

Milk is, has been, and will continue to be an important food for man. Depending on the geographic location, sources may include cow, goat, sheep, yak, buffalo, camel, and horse (MacDonald, 2010). Regardless of source, milk serves as a nourishing food from infancy through adulthood as a fluid product, in cultured or fermented forms as with cheese or yogurt, or in ingredient applications such as with milk and whey protein powders. In its fluid form, milk provides a good source of protein, minerals, and vitamins.

Milk is defined in 21 CFR 131.110 as "the lacteal secretion, practically free from colostrum, obtained by the milking of one or more healthy cows" (FDA, 2015b). It is comprised mostly of water with lactose (4.6-4.9% w/w), fat (3.5-4.0% w/w), protein (2.9-3.4% w/w), and ash (<1% w/w) (Weimer, 2001; Varnam and Sutherland, 2001; Walstra et al., 2006). The proteins are divided into two main categories, casein and whey proteins. Casein proteins include alpha$_{s1}$, alpha$_{s2}$, beta, gamma, and kappa-caseins. These comprise over 80% of the protein in milk. The remaining protein comes from serum or whey proteins which consist of beta-lactoglobulin, alpha-lactalbumin, serum albumin, and immunoglobulins (Varnam and Sutherland, 2001). Various processes can be used to separate and isolate these various proteins. In Cheddar cheese manufacture, caseins aggregate when a starter culture or acidic addition lowers the pH, allowing a milk coagulant/rennet enzyme to form curds with or without the presence of calcium chloride (Farkye, 2004). The cheese manufacture process can continue while the whey proteins, which have isoelectric points between pH 4-5 and remain soluble, can be further concentrated to create whey protein ingredients. Additional processing by micro-, ultra-, or nanofiltration can be used to concentrate the whey proteins for use as a value-added ingredient (Walstra et al., 2006).

These same technologies can be used on milk, without the prior step of the cheese making process, to create micellar casein concentrate, serum protein concentrate, and milk protein concentrates and isolates (Brans et al., 2004).

The Pasteurized Milk Ordinance (PMO) is the standard guide for Grade "A" fluid milk in the United States and includes regulations for processing, packaging, and marketing for not only milk, but also other products such as buttermilk, whey, condensed or dry milk and their associated products. Grade "A" milk and milk products are those that meet the "chemical, physical, bacteriological and temperature standards and the sanitization requirements" of Section 7 of the PMO (FDA, 2013). The PMO is considered the basic standard for the Cooperative State-USPHS/FDA Program for the Certification of Interstate Milk Shippers; however, state regulations for milk can be more stringent than the PMO (FDA, 2013; 1 NYCRR Part 2). According to 21 CFR 1240.61, milk and milk products shipped over state lines must be pasteurized by the guidelines set in paragraph b and c (which are the same as those found in the PMO), or by any other FDA-approved pasteurization process (FDA, 2015a).

Louis Pasteur was the first to patent the pasteurization process and others, such as Franz von Soxhlet, applied this process to prolong the storage time of milk in the 1800s (Westhoff, 1978). Later, pasteurization was applied to destroy pathogens, especially tuberculosis-causing *Mycobacterium tuberculosis* and *bovis*. The heating of milk by low temperatures (between 60-70°C) and long times (10-45 minutes) gained popularity in the late 1800s to early 1900s in the US (Westhoff, 1978). Prior to the modern PMO, the *Standard Milk Ordinance* was developed in 1924 as the first model regulation which was then followed by the *Code* in 1927 to provide more thorough details for compliance. The

Ordinance and Code were replaced by the PMO in 1965 (FDA, 2013). With this guidance, states began establishing laws and regulations for milk pasteurization with Michigan being the first in 1947 to legislate a law (Steele, 2000). Over the next several decades, additional technologies arose including those for high temperature, short time (**HTST**) pasteurization, ultra-high temperature (**UHT**) pasteurization, and ultra-pasteurization (**UP**) (Steele, 2000; Westhoff, 1978). Today, HTST pasteurization is by far the most common in the US, but milks processed by other techniques can also be found. These include intermediate heat or non-thermal treatments (e.g. processing by microfiltration or bactofugation) to create extended shelf life (**ESL)** milks.

Global sales reflect the importance of milk with over 200 million metric tons (441 billion pounds) consumed globally in 2015 (Euromonitor, 2016). In the U.S., approximately 23 million metric tons (50.7 billion pounds) were sold in 2014 (USDA, 2015). Despite growth in production, there has been a steady decline in the per capita milk consumption. Whole milk consumption has decreased while lower fat milk consumption has increased, but overall per capita consumption is down from 112.0 kg of fluid milk in 1975 to 72.1 kg in 2014 (USDA ERS, 2015). Consumption of alternative beverages such as soft drinks, juices, and other sugar sweetened beverages has been associated with the decrease in milk consumption (Popkin, 2010). The reduced consumption of breakfast in the US has also contributed to lower milk consumption (Song et al., 2006). Milk pasteurization may be another factor in the reduced consumption as the various processing methods can affect the flavor and quality of milk. The more severe thermal treatment of ultra-pasteurization techniques provides milk with at least 60 days shelf life compared to typical HTST milks at 10 to 21 days (Boor and Nakimbugwe, 1998), but impacts the sensory profile and consumer

acceptance of the milk (Gandy et al., 2008; Chapman and Boor, 2001). The objective of this review is to highlight key understandings of how processing parameters affect the quality of milk, especially with regard to sensory perception, and to identify gaps in the knowledge that need to be filled.

Raw Milk

Raw milk was a common source of disease outbreaks before the advent of pasteurization. Brucellosis and tuberculosis were the prevailing pathogens of concern before improvements were made for milk handling and processing (Muir, 1996a). Now, milk-borne disease is rarely encountered due to efforts made by organizations promoting milk sanitation and safety (FDA, 2013). Despite marked improvements in milk safety, a large variety of pathogenic microorganisms can still be cause for concern and require constant vigilance. These organisms include *Listeria monocytogenes*, *Salmonella* spp., *Coxiella burnetii*, *Campylobacter jejuni*, and more (Claeys et al., 2013). As the base for fluid, cultured, fermented, and other dairy-derived products and ingredients, it is essential to understand the factors that affect raw milk quality. Along with product safety, sensory characteristics and physical properties all determine quality (Muir, 1996a; Boor, 2001). Climate, season, feed, and the physical state of the animal can affect milk biosynthesis, altering the chemical composition, microbiota, and somatic cell count, all of which may influence raw milk quality (Farkye, 2004). Other factors include the environment, milking equipment, and the pre- and post-treatment of the udders (Hayes and Boor, 2001). Lastly, the processing conditions of the milk, how it is pumped, cooled, and stored, can affect quality.

On-farm Practices

Raw milk safety and quality control begins with on-farm practices. Spore-formers like *Bacilluss* spp. are a limiting factor on the shelf life of pasteurized milk and can be found ubiquitously at the farm (Meer et al., 1991). These organisms can have proteolytic, lipolytic, or toxinogenic activity that can affect both the raw milk and subsequent pasteurized milk (De Jonghe et al., 2010). Spores can enter into milk a number of ways including by feces, feed contamination, and soil. A two year study by Elmoslemany et al. (2010) reported that microbial counts were associated with the amount of soil on teats before milking, preparation of teats/udders before milking, method of bulk tank cleaning, and presence of a plate cooler (Elmoslemany et al., 2010). Masiello et al. (2014) also reported that farms that had > 25% cows with soiled teats/udders were 3.15 times more likely to have high spore counts of ≥3 log cfu/mL (Masiello et al., 2014). Additionally, herd size, bedding material type, and milking routine (i.e. forestripping, dry massaging, use of a postmilking disinfectant spray) were found to influence the levels of mesophilic and thermophilic spores (Miller et al., 2015). Vissers et al. (2007) estimated that 60% reduction of *B. cereus* spores could be achieved by reducing spore counts in feed for housed cows. A 99% reduction could be achieved for grazing cattle by minimizing soil contamination of teats (Vissers et al., 2007).

Transportation

With regard to raw milk transportation, the PMO states that milk trucks must be cleaned-in-place every 24 hours (FDA, 2013). A study by Darchuk et al. (2015) reported that continuous use during this 24 hour period did not negatively impact the quality of the raw milk. The study used additional water rinses or sanitizer treatment steps after each load or every 12 hours of transportation but found no significant improvement over continuous

hauling (Darchuk et al., 2015). The concern in the aforementioned study was with regard to biofilms which may form in raw milk tankers and which may produce heat-resistant enzymes that can affect the quality of pasteurized milk.

Cooling and Storage

In accordance with the Pasteurized Milk Ordinance (PMO), raw milk must be cooled to 10°C (50°F) or less within four or less hours from the start of the first milking. This milk must then be cooled to 7°C (45°F) or less within two hours after the end of milking. For batches commingled, the temperature of the bulk milk must not exceed 10°C (50°F) (FDA, 2013). Two technologies are approved and commonly employed for cooling milk. Vat cooling utilizes bulk tanks with cooling jackets to slowly cool milk in a batch process. In contrast, instantaneous cooling utilizes heat exchangers to quickly cool the milk in a continuous process before storage in an insulated or refrigerated bulk tank (Guul-Simonsen et al., 1996). Cooling and storage temperature have been shown to affect the microbial quality of raw milk as well as other quality factors including lipolysis.

The PMO requires a minimum cooling temperature of 7°C (50°F) for raw milk (FDA, 2013), but further cooling can be used to extend the storage life of raw milk. The maximum allowable microbial load in the PMO for raw milk is 100,000 cfu/ml for an individual producer and 300,000 cfu/ml for commingled milk. Gebre-Egziabher et al. (1985) found that with good sanitation and proper storage, raw milk could be held for up to three days at 4°C before quality was degraded. At 2°C, the time for the psychrotrophic bacteria count to exceed 10^6 cfu ml^{-1} was increased from 2.9 days at 6°C to 5 days when the initial count was below 10^4 cfu ml^{-1} (Griffiths et al., 1987). In another study by Guul-Simonsen et al. (1996), raw milk instantly cooled to 2.5°C by a heat exchanger had one to two days extra storage in

comparison to raw milk that was cooled to 4°C, which could be stored for up to three days. Instant cooling to 2.5°C by plate heat exchanger before bulk tank storage significantly retarded bacterial growth and decreased the rate of hydrolysis and lipolysis, which extended the time that the raw milk could be stored with good quality (Guul-Simonsen et al., 1996). Lastly, a recent study by O'Connell et al. (2016) found that storage of milk at 2 or 4°C for up to 96 hours did not significantly affect the microbial quality of the raw milk when fresh milk was added twice daily (O'Connell et al., 2016). The cooling of raw milk also affects the free fatty acid levels formed by pumping, which disrupts the milk fat globule membrane, allowing lipoprotein lipase activity to increase (Wiking et al. 2005; Dickow et al., 2011). The increased free fatty acids from lipolysis can cause rancid flavors in milk and lipolysis can also affect functionality with regard to foaming, creaming during separation, and butter manufacture (Deeth, 2006). Fortunately, lipoprotein lipase is heat labile, so is easily inactivated during HTST pasteurization.

Biofilm/Enzymes/SCC

Biofilms are composed of microbes and an "extracellular polymeric substance" matrix (Donlan, 2002). They are associated with both pathogenic and spoilage organisms in all processing environments (Donlan, 2002; Teh et al., 2014). For dairy processing, biofilms can lead to increased microbial load in the raw milk and/or release of heat-resistant enzymes that may inhibit the shelf life of pasteurized products (Marchand et al., 2012; Teh et al., 2014). Other enzyme-producing sources include psychrotrophic bacteria and somatic cells which can contribute enzymes which induce lipolysis or proteolysis (Hantsis-Zacharov and Halpern, 2007; Barbano and Santos, 2006).

The PMO clearly defines limits for bacteria and somatic cells at 100,000 cfu ml^{-1} from an individual producer or 300,000 cfu ml^{-1} for commingled milk and 750,000 somatic cells ml^{-1} (FDA, 2013). Somatic cells are typically composed of leukocytes from blood that enters into milk. Increased somatic cell counts (SCC) are found in milk when cows are affected by mastitis. Hydrolytic enzymes including plasmin and lipoprotein lipase can enter into the milk in conjunction with somatic cells (Barbano and Santos, 2006). These enzymes can lead to bitterness and rancidity in pasteurized milk (Ma et al., 2000).

Effect on Pasteurized Milk

The characteristics and quality of raw milk will affect the subsequent characteristics and quality of pasteurized products. Ravanis and Lewis (1995) examined the effect of raw milk quality and the subsequent shelf-life of pasteurized milk. The keeping quality of raw milk pasteurized up to 14 days after storage was at least 22 days (as determined by a microbial limit of 10^6-10^7 cfu/ml standard plate count in the pasteurized milk), indicating that the control of post pasteurization contamination (**PPC**) was the main contributing factor (Ravanis and Lewis, 1995). Gillis et al. (1985) found that raw milk samples with < 10^4cfu/ml standard plate count had significantly less proteolysis than samples with >10^5 cfu/ml for UHT milk (Gillis et al., 1985). Recent studies have also identified and characterized psychrotolerant sporeformers as a main issue limiting the shelf life of fluid milk. Psychrotrophic *Bacillus* spp. can produce proteinases and lipases that are heat stable, reducing the shelf life of products. The bacilli can also produce spores that can survive heat treatment and produce enzymes after germination to degrade pasteurized products (Meer et al., 1991).These microorganisms have been found not only in raw milk, but throughout the entire milk process from the farm to the packaged, pasteurized product (Huck et al., 2008).

These results indicate that contamination by spoilage bacteria must be further controlled if the shelf life of fluid milk is to be extended.

Interestingly, raw milk microbiological tests (standard plate count, somatic cell count, psychrotrophic bacteria count, coliform count, preliminary incubation count, laboratory pasteurization count, and spore pasteurization count) do not appear to accurately predict pasteurized milk quality as measured by microbial, sensory, or shelf-life analyses (Fromm and Boor, 2004; Martin et al., 2011). The tests can give an indication of the quality of the raw milk, but there lacks a correlation between the raw milk tests and pasteurized milk quality. PPC and plant factors such as pasteurization temperature are hypothesized explanations for the poor correlation between raw milk tests and subsequent pasteurized milk quality (Martin et al., 2011). Ranieri et al. (2009) reported on the effect of pasteurization temperature and found that an increase in pasteurization temperature from 72.9°C to 85.2°C resulted in higher aerobic plate counts over the shelf-life for the higher pasteurization temperatures. Identification by DNA sequencing revealed that >85% of the microorganisms at day 0, 1, and 7 post-processing were *Bacillus* spp. which were followed by >92% *Paenibacillus* spp. at day 14 and 21 post-procesisng (Ranieri et al., 2009). Both genera are associated with spoilage during refrigerated storage (De Jonghe et al., 2010). PPC has also been identified as an important limiting factor currently for pasteurized milk shelf life and quality (Ralyea et al., 1998; Martin et al., 2011). A recent study reported that PPC by coliforms can affect sensory and shelf life quality due to lipolytic and proteolytic activity of the microorganisms (Masiello et al., 2016). From the on-farm practices to transportation to cooling and storage to processing, stringent controls are necessary at each point to ensure safety and quality of the raw milk for pasteurized fluid milk processing.

Pasteurized Fluid Milk

Pasteurization of fluid milk allows milk to be consumed safely and increases the shelf life. The PMO defines pasteurization as "the process of heating every particle of milk or milk product, in properly designed and operated equipment, to one (1) of the temperatures given in Table 1.2 and held continuously at or above that temperature for at least the corresponding specified time" (FDA, 2013). Thermal processing has been applied since the late 1800s to the early 1900s to destroy pathogens and reduce spoilage microorganisms. The first *Standard Milk Ordinance* was developed in 1924 to serve as a model for milk pasteurization, then the *Code* in 1927 provided more thorough guidance for compliance (FDA, 2013).

Processing is needed because milk is an excellent growing environment for microorganisms. Milk processing usually follows the following procedure: clarification to remove dirt and body cells, separation by centrifugation to separate cream from skim milk, fat content standardization, then pasteurization, homogenization, and lastly packaging (Goff and Griffiths, 2006). A variety of heat treatments exist including low temperature long time (LTLT), high temperature-short time (HTST), ultra-pasteurization (UP), and ultra-high temperature (UHT) processing. Non-thermal treatments have also been recently proposed as a method of further reducing microbial loads in milk.

The parameters for the LTLT method were established in 1924 with the first pasteurized milk ordinance (Westhoff, 1978). The minimum requirement for this treatment was 61.1°C (142°F) for 30 minutes to destroy *Mycobacterium tuberculosis*. Work by North and Park (1927) summarized the numerous reports on the thermal death of *M. tuberculosis* to validate the LTLT method (North and Park, 1927). Concurrent with the research for long temperature-long time processing, plate heat exchangers were also considered as an option to

more quickly process milk. *M. tuberculosis* was again the microorganism of concern when establishing parameters for what would be known as HTST pasteurization. The work by North and Park (1927) did not review temperatures higher than 65.6°C for destroying *M. tuberculosis*, making it difficult to set minimum requirements. The first HTST standards were publisheds in 1933 in the U.S. Public Health Service Milk Ordinance and Code (Westhoff, 1978). Workman (1941) studied the effects of time and temperature on various pathogenic bacteria, including those that cause tuberculosis and brucellosis, and found that all were destroyed by heating to 71.1°C (160°F) for 15 seconds (Workman, 1941). Based on these results, a standard of 71.7°C (161°F) for 15 seconds was established as the minimum for HTST pasteurization. In 1957, the parameters for both LTLT and HTST pasteurization were updated to account for the thermal tolerance of *Coxiella burneti*, the microorganism which causes Q fever. The new parameters increased the minimum temperature and time of LTLT pastuerization to 62.8°C (145°F) for 30 minutes and 71.7°C (161°F) for 15 seconds for HTST pasteurization; products with higher solids were recommended to be pasteurized at higher temperatures to account for varying suspensions of the pathogenic microorganism (Enright et al., 1957). Current standards for grade "A" pasteurized milk requires milk to be cooled to 7°C (45°F) or less, contain less than 20,000 cfu/ml for overall bacteria (less than 10 cfu/ml for coliforms), contain less than 350 milliunits per liter active alkaline phosphatase, and show no positive results for drug residues (FDA, 2013).

Concurrent with the development of LTLT and HTST pasteurization, ultra-high temperature (**UHT**) was also examined as a method of pasteurization or sterilization. UHT pasteurization indicates that all pathogens have been destroyed and it differs from UHT sterilization which destroys both pathogens and most bacterial spores (Westhoff, 1978). UHT

pasteurization includes all temperatures greater than 88.3°C (191°F) held for at least one second whereas UHT sterilization utilizes temperatures between 130 to 150°C and held for 2-10 seconds (Mehta, 1980; Holsinger et al., 1997). UHT sterilization is similar to ultra-pasteurization (**UP**) in terms of thermal treatment, but UHT sterilized milks are usually packaged aseptically for shelf-stable storage. "Ultra-pasteurization" is defined by the PMO as thermal processing "at or above 138°C (280°F) for at least two (2) seconds, either before or after packaging, so as to produce a milk and/or milk product, which has an extended shelf-life under refrigerated conditions" (FDA, 2013). UP milks can be packaged aseptically or under near aseptic conditions to limit post processing contamination. The standards for grade "A" UP milk are the same as for conventional grade "A" fluid milk. Both indirect and direct forms of heating can be used for UHT and UP processing. Indirect treatments can utilize plate or tubular heat exchangers; direct treatments include direct injection of superheated steam into the product line and steam infusion, where milk is heated by falling through a chamber filled with superheated steam (Datta et al., 2002).

Physicochemical changes

The heat treatment and processing of milk lead to physicochemical changes that can affect the composition, functionality, and sensory characteristics of the milk. Enzymes are denatured by heat, which can inactivate them. This effect can prevent enzymatic spoilage of the milk by proteinases or lipases, inactivate antimicrobial enzymes like lactoperoxidase, and can also be used as an indicator for adequate pasteurization as is the case with alkaline phosphatase (Walstra et al., 2006). Heat treatment in excess of about 70°C can result in denaturation and aggregation of alpha-lactalbumin and beta-lactoglobulin; caseins are much more heat-stable than whey proteins and show fewer changes, even at UHT sterilization

conditions (Fox et al., 2015). The degree of whey protein denaturation can affect the functionality of UHT milk with a greater percentage denaturation resulting in delayed gelation during shelf life (Datta and Deeth, 2001). Exposure to heat also induces Maillard reactions, the products of which can be used as indicators of heat treatment.

Heat indicators

Heat indicators are described by Pellegrino et al. (1995) as falling into two categories – Type 1 which includes products formed by denaturation, degradation, or inactivation such as with whey proteins, enzymes, and vitamins; and, Type 2 which includes formation of new or originally minimal substances (Pellegrino et al., 1995). Useful thermal indicators should be robust and easily quantified for a wide range of time/temperatures. Indicators that fit this criteria include denaturation of whey proteins – alpha-lactalbumin, beta-lactoglobuin, bovine serum albumin, and immunoglobulins – and reaction products furosine, hydroxymethylfurfural, and lactulose (Morales et al., 2000).

As previously mentioned, alkaline phosphatase is a useful indicator of pasteurization as it is a native raw milk enzyme that denatures at temperatures slightly above the minimum for destroying pathogens. Lactoperoxidase can be used to determine higher temperature processing as a negative result indicates pasteurization temperatures above 85°C for 20 seconds (Walstra et al., 2006). Measurements of undenatured protein for both individual whey protein components and overall whey protein can be used as thermal treatment indicators (Mortier et al., 2000). Beta-lactoglobulin is the predominant whey protein fraction and is more heat labile than alpha-lactalbumin, making beta-lactoglobulin a more useful indicator for measuring thermal treatment of milk than alpha-lactalbumin. Beta-lactoglobulin has also been studied in more detail than alpha-lactalbumin (Walstra et al., 2006). Beta-

lactoglobulin is measured by reversed-phase HPLC after acidic pH adjustment, providing precise and accurate analysis; however, variations of the absolute and relative abundance of beta-lactoglobulin can limit comparisons between samples (Mortier et al., 2000; Datta et al., 2002).

The Maillard reaction causes browning, flavor changes, and compositional changes to milk. The degree of these changes is dependent on the heat treatment, making analysis of the byproducts a useful measure of the thermal load experienced by the milk. Lactulosyllysine, furosine, and hydroxymethylfurfural (**HMF**) are compounds that are formed by Maillard reactions or by Maillard reaction products (van Boekel, 1998). Figures 1.1 and 1.2 illustrate the formation reactions for these compounds. Lactulosyllysine is created early in the Maillard reaction by lactose forming a Schiff base with the amino group of lysine. Amadori rearrangement of this sugar-protein compound forms the Amadori product lactulosyllysine (van Boekel, 1998). Acid hydrolysis of lactulosyllysine produces furosine, a more easily quantified compound than lactulosyllysine (van Boekel, 1998). Furosine is typically quantified using HPLC (Resmini et al., 1990). Quantification of furosine is usually given in mg furosine/100g protein as furosine concentration is highly dependent on the protein content (Montilla and Olano, 1997). Furosine content is also dependent on storage temperature has been shown to increase over time when stored at room temperature due to continued Maillard reaction forming lactulosylysine (van Boekel, 1998; Elliot et al., 2005). The presence of fat affects furosine analysis with a positive correlation being found by Claeys et al. (2003), although the difference was not significant in the context of heat treatment comparisons (Claeys et al., 2003). HMF can be quantified spectrophotometrically after reaction with thiobarbituric acid or by HPLC (Fox et al., 2015). Concentrations of HMF

were found to range from ~1.0 μmol/L in raw or minimally heat treated milk up to 22.0μmol/L for in-bottle sterilized milk (Morales et al., 2000). However, HMF can be formed by both isomeration/degradation and by the Maillard reaction, making it less useful as a heat indicator (van Boekel, 1998). Lactulose is another commonly used indicator of heat treatment. Lactulose is formed by the isomeration of lactose, the process of which is more dependent on temperature and pH than furosine (van Boekel, 1998). Lactulose is measured in mg/L and is only found after higher temperature treatments (Mortier et al., 2000). For each heat indicator, differences can be discerned between indirect and direct modes of UHT treatment with values generally being lower for direct modes of heating (van Boekel, 1998; Elliot et al., 2005).

Shelf life

The effect of pasteurization temperature on shelf life is another important processing consideration. HTST milks typically last two to three weeks under refrigerated storage, UP milks for one to two months refrigerated, and UHT sterilized milks can last one to two years under ambient conditions. The end of shelf life can be determined by microbial count or by sensory analysis upon the loss of product quality. For HTST pasteurized milks, shelf life is typically dictated by the growth of psychrotrophic microorganisms that survive pasteurization and by PPC. In a study by Fromm and Boor (2004) of three commercial plants, the percentage of shelf life samples with >20,000 cfu/ml total bacteria count increased from 8% at day 7, to 58% on day 14, then 92% by day 17. *Paenibacillus*, *Bacillus*, and *Microbacterium* comprised the majority of the microorganisms found after bacterial spoilage and quantitative descriptive analysis results showed that undesirable flavors, sulfur/putrid, were most apparent at day 17 (Fromm and Boor, 2004). For extended shelf life milks, Blake

et al. (1995) reported that milks pasteurized by direct steam injection at 100° or 110°C for 4 seconds had high total (10^7 and 10^6 cfu/ml, respectively) and psychrotrophic (10^6 cfu/ml) counts after 15 days stored at 7°C. Spore-forming *Bacillus* spp. were identified in milks pasteurized between 120° and 132°C for 4 seconds, but no organisms were found above 134°C (Blake et al., 1995). These results highlight the importance of these microorganisms as determinants of shelf life. Adequate processing and appropriate packaging are also very important with regard to the shelf life of milks; any process deviation or post pasteurization contamination can greatly reduce the shelf life of UHT sterilized milks (Mehta, 1980; Ravanis and Lewis, 1995).

Historically, higher pasteurization temperatures were thought to destroy more microorganisms, but recent evidence shows that this may not be the case for HTST milks. Stored at 7ºC, 2% fat milk pasteurized at 77, 79, 82, and 85ºC did not have significantly different shelf lives as determined by sensory analysis; all lasted 13-15 days (Gandy et al., 2008). In contrast, Ranieri et al. (2009) concluded that higher processing temperatures for 2% milk were inversely related to the length of shelf life as determined by bacterial counts (i.e. higher processing temperature resulted in higher counts during shelf life). The higher bacterial counts were attributed to endospore-forming psychrotolerant bacteria which germinated under higher pasteurization temperatures. A case study by Martin et al. (2012) of a milk processing plant found that milk pasteurized at a lower temperature supports the conclusion by Ranieri et al. (2009); total bacteria counts for skim, 2%, and whole milk pasteurized at 76.1ºC for 18.25 seconds were lower than milk pasteurized at the higher 79.4ºC temperature with the same hold time for all time points up to 21 days. The initial

quality of the milk tested in this study was high with 75.7% of samples with <10,000 cfu/ml total bacteria count and <50 cfu/ml coliform count (Martin et al., 2012).

Flavor and Sensory Properties of Milk

Typical US market milks (HTST) are characterized by a light and clean, but slightly sweet flavor. A basic lexicon for flavor, aroma, mouthfeel, and appearance terms for milks is given in Table 1.3. The flavor of milks can be influenced by a number of factors including animal source and feed, fat content, processing treatment, storage and storage conditions, and spoilage by enzymes or bacteria. These characteristics will ultimately dictate consumer acceptance and purchase.

Cattle provide milk for consumers all around the world. Milk from one region may be obtained from different species of cattle or from cattle fed different diets. Urbach (1990) reviewed the effects of various feed types on volatile compounds found in milk. Low lipid cattle diets were reported to produce milks with high fatty acids which were qualitatively assessed to contain cheesy flavor and coconut-like lactones. In contrast, high lipid diets resulted in raspberry-like lactones (Urbach, 1990). Bendall (2001) investigated the effect of diet on aroma compounds from New Zealand milks. The Friesian cows were fed a total mixed rations (**TMR**) diet or were pasture-based. Using solvent-assisted flavor evaporation (**SAFE**), Bendall (2001) concluded that the majority of aroma compound types were similar between the two different milks; thus, the flavor differences could be attributed to differences in the common compounds, rather than compounds from specific feeds (Bendall, 2001). Croissant et al. (2007) reported greater intensities of cowy/barny and grassy flavors for milks from pasture-based cattle compared to those fed with TMR. The consumer study associated with this experiment showed that consumers could not consistently tell the difference

between the milk types and that milk type did not affect overall acceptance (Croissant et al., 2007).

Fat

The fat content of milk influences the appearance, texture, and flavor of milk. Phillips et al. (1994) utilized a trained sensory panel to characterize milks of different fat percentages. As fat percentage increased, scores for raw cream aroma, opacity, residual glass coating, mouth coating, and thickness increased; however, when red lighting was used to mask the appearance, the differences were diminished such that the scores for 2% milk under red lights were more similar to the scores for 1% milk under normal lighting. Untrained panelists were also less capable of detecting a difference when appearance attributes were eliminated, 46% correct compared to 75% (Phillips et al., 1995). Pangborn et al. (1985) used paired difference tests under red light and found that few subjects could discriminate between skim milk and 0.25, 0.75, 1.5, 3.0, and 5.0% fat levels. Phillips and Barbano (1997) were able to use fat substitutes and titanium dioxide in skim milk to emulate the appearance of higher fat milks (Phillips and Barbano, 1997). Frost et al. (2001) utilized a combination of thickener, whitener, cream aroma, and homogenization to also match the sensory properties of higher fat milk, approximately 1.3% fat (Frost et al., 2001). Chojnicka-Paszun et al. (2012) used descriptive analysis (**DA**) to show that milk with fat contents below 1% were indistinguishable from each other in creamy texture and taste values. Francis et al. (2005) concluded that milk composition, especially with regard to fat, most greatly influenced the characteristics of milk by sensory and volatile compound analysis. Fat-derived compounds were significantly correlated with sensory descriptors regarding fat (i.e. fat flavor, sweet flavor, sweet aromatic) and were also reported to decrease sour aromatic. McCarthy et al.

(2016) reported that milk consumers preferred different fat content milks depending on the fat content consumed most often. Skim milk drinkers preferred 1 and 2% milk over skim and whole milks. Low-fat milk drinkers preferred 1%, 2%, and whole milks. Liking decreased for higher fat contents due to flavors and textures associated with milk fat. Whole milk drinkers preferred samples with higher milkfat (McCarthy et al., 2016). These studies support the influence of appearance, texture, and flavor on the sensory perception of milk.

Processing

Processing and packaging conditions are used to impart various shelf life characteristics to milks. However, these conditions can also affect the flavor and sensory attributes in milk, influencing consumer liking and acceptance. Calvo and de la Hoz (1992) reviewed the flavor of heat treated milks and classified compounds as those formed by denaturation of milk proteins which subsequently release sulphydryls and those formed by nonenzymatic browning reactions. Terms to describe the flavors of heat treated milks include cooked, sulfurous, caramelized, and scorched (Shipe et al., 1978). Cooked flavors result from sulfurous compounds released from whey proteins, specifically beta-lactoglobulin, and proteins in the milk fat globule membrane (Mehta, 1980; Calvo and de la Hoz, 1992). These flavors typically decrease over time (Deane et al., 1967; Shipe et al., 1978). For higher heat treatments of milk (i.e. ultra-pasteurized or UHT), the intensities of heat-related flavors are greater than for typical HTST pasteurized milks. Milks processed at 135° to 150°C for several seconds show strong sulfur or cooked aromatics (Shipe et al., 1978). The compounds identified as contributing to the sulfur and cooked notes include “methyl sulphide, hydrogen sulphide, carbonyl sulphide, methanethiol, carbon disulphide, dimethyl sulphide and dimethyl disulphide” (Calvo and de la Hoz, 1992). Numerous other compounds also

contribute to the sensory characteristics of milk. Over 100 peaks were obtained by gas chromatography-mass spectrometry on skim and whole milks (Francis et al., 2005). Maillard reactions, lactose breakdown components, and flavors from lipids contribute aldehydes, furanoids, lactones, aromatic carbonyls among many other compounds to impart "brown" flavors like caramelized flavor (Shipe et al., 1978; Calvo and de al Hoz, 1992). Chapman et al. (2001) used quantitative descriptive analysis to characterize commercial ultra-pasteurized milks of varying fat levels from two different dairy plants. Cooked flavor and aroma, dry mouthfeel, lingering aftertaste, sweet flavor, and bitter flavor explained 87.6% of the variability among samples tested (Chapman et al., 2001). Clare et al. (2005) reported that indirectly heated UHT milks exhibited higher caramelized flavor, astringency, fatty/stale flavor, and brown color than those processed by microwave processing (Clare et al., 2005). A study examining UHT milks from different countries suggested that the manufacturing process influenced the sensory characterics of fluid milks the greatest, more so than country of origin or fat content of UHT milk (Oupadissakoon et al., 2009).

Storage

After processing, storage and storage conditions can affect the flavor of milk. As previously mentioned, shelf life for conventional HTST milks are most often dictated by microbial spoilage. Bacteria with proteolytic or lipolytic activity can result in sulfur/putrid, bitter, and rancid flavors (Santos et al., 2003; Fromm and Boor, 2004). Milks were noted to decrease in sweet aromatic flavors and sweet taste, while fatty/stale flavor, astringency, and color intensity increased over time for both indirect UHT and microwave processed milks (Clare et al., 2005). Packaging material is also an important consideration for milk storage. In addition to securely containing milk, packaging should provide appropriate barriers to

microorganisms, light, and oxygen while preserving the flavor of the milk. ESL, UP, and UHT milks are packaged in hermetically sealed containers which are disinfected or sterilized by hydrogen peroxide, heat, and/or UV rays to prevent contamination by the container (Henyon, 1999). During retail display, milks can form oxidized flavors due to excessive light exposure and lack of light protection from packaging. This results in decreased consumer acceptence of oxidized milk, indicating a need for improved packaging and storage conditions for milk at retail (Walsh et al., 2015). Simon and Hansen (2001) examined several different packaging materials and found that the flavor of UP milks deteriorated with standard packaging (cardboard coated in polyethylene) compared to barrier (low density polyethylene, cardboard, and ethylene-vinyl alcohol) and foil packaging. Polyethylene packaging can adsorb and also allow milk volatiles to permeate through (Czerny and Schieberle, 2007). These studies show how storage and storage conditions affect milk flavor and why they must be monitored to ensure quality through shelf life.

Consumer Acceptance

A few studies have examined the effect of pasteurization temperature on consumer liking. Chapman and Boor (2001) reported that children 6 to 11 years old had a significant preference order for 2% milk of commercial 1) HTST, 2) UHT, and 3) UP. The HTST milk was tested within 36 hours of processing, the UP milk within 6 to7 days, and the aseptically packaged UP milk at 24 to 30 days after processing. The preference for HTST milk was largely attributed to familiarity with the taste from regular consumption. Gandy et al. (2008) examined consumer acceptability of 2% fat milk pasteurized at 77, 79, 82, and 85ºC for 15 seconds. Here, the majority of consumers found 79ºC milk to be highly acceptable at day 0 while both 79 and 82ºC milk were preferred on day 6. Higher temperature milks (82ºC and

85ºC) were characterized by greater cooked flavor and aroma on day 0. The lower liking scores for these milks were attributed to these cooked characteristics. These results reflect historical trends seen by Deane et al. (1966) where 78.9ºC milk was most preferred and higher pasteurization temperature milks (82.2ºC and 85.6ºC) was least preferred by adult consumers.

Objectives

As milk technology has progressed, processing methods have evolved to improve the quality of milk. These changes have resulted in better shelf life and safety, but they have also impacted the sensory perception of milk, affecting the acceptance and purchase intent. A better understand of the factors related with shelf life and consumer perception will allow the dairy industry to adapt and compete with the myriad of beverage competitors. To compete, fluid milk must have improved shelf life compared to current conventional milks while maintaining or improving the flavor of said milk. The objectives of this study are to understand the effects of processing parameters on the flavor and sensory quality of fluid milk. The influence of raw milk cooling rate will be investigated with regard to shelf life and consumer perception for skim milk. Also, the effect of heat treatment on 2% and skim milk processed by indirect and direct steam injection ultra-pasteurization and conventional HTST methods on sensory perception, consumer acceptance, and instrumental analyses will also be investigated.

REFERENCES

Barbano, D., and Santos, M. 2006. Influence of Raw Milk Quality on Fluid Milk Shelf Life. J. Dairy Sci. E15-E19.

Bendall, J. 2001. Aroma Compounds of Fresh Milk from New Zealand Cows Fed Different Diets. J. Ag. and Food Chem. 49:4825-4832.

Blake, M., Weimer, B., McMahon, D., and Savello, P. 1995. Sensory and Microbial Quality of Milk Processed for Extended Shelf Life by Direct Steam Injection. J. Food Protection. 1007-1013.

Boor, K. 2001. Fluid Dairy Product Quality and Safety: Looking to the Future. J. Dairy Sci. 84:1-11.

Boor, K., and Nakimbugwe, D. 1998. Quality and stability of 2% fat ultrapasteurized fluid milk products. Dairy, Food, and Environmental Sanitation. 18:78-82.

Brans, G., Schroen, C., van der Sman, R., and Boom, R. 2004. Membrane fractionation of milk: state of the art and challenges. J. Membrane Sci. 243:263-272.

Calvo, M., and de la Hoz, L. 1992. Flavour of Heated Milks. A Review. International Dairy J. 69-81.

Chapman, K., and Boor, K. 2001. Acceptance of 2% Ultra-Pasteurized Milk by Consumers, 6 to 11 Years Old. J. Dairy Sci. 84:951-954.

Chapman, K., Lawless, H., and Boor, K. 2001. Quantitative Descriptive Analysis and Principal Component Analysis for Sensory Characterization of Ultrapasteurized Milk. J. Dairy Sci. 84:12-20.

Chojnicka-Paszun, A., De Jongh, H., and De Kruif, C. 2012. Sensory perception and lubrication properties of milk: Influence of fat content. S. Int. Dairy J. 15-22.

Claeys, W., Cardoen, S., Daube, G., De Block, J., Dewettinck, K., Dierick, K., et al. 2013. Raw or heated cow milk consumption: Review of risks and benefits. Food Control. 251-262.

Claeys, W., van Loey, A., and Hendrickx, M. 2003. Kinetics of hydroxymethylfurfural, lactulose and furosine formation in milk with different fat content. J. Dairy Research. 70:85-90.

Clare, D., Bang, W., Cartwright, G., Drake, M., Coronel, P., and Simunovic, J. 2005. Comparison of Sensory, Microbiological, and Biochemical Parameters of Microwave Versus Indirect UHT Fluid Skim Milk During Storage. J. Dairy Sci. 88:4172-4182.

Croissant, A., Washburn, S., Dean, L., and Drake, M. 2007. Chemical Properties and Consumer Perception of Fluid Milk from Conventional and Pasture-Based Production Systems. J. Dairy Sci. 90:4942-4953.

Czerny, M., and Schieberle, P. 2007. Influence of the polyethylene packaging on the adsorption of odour-active compounds from UHT-milk. Eur Food Res Technol. 225:215-223.

Dairy, N. Y. 2003, 03. 1 NYCRR Part 2 Requirements for the Production, Processing, Manufacturing and Distribution of Milk and Milk Products. Accessed May 9, 2016. http://www.agriculture.ny.gov/DI/Laws%20in%20PDF/PART2_milk_control_law.pdf.

Darchuk, E., Waite-Cusic, J., and Meunier-Goddik, L. 2015. Effect of commercial hauling practices and tanker cleaning treatments on raw milk microbiological quality. J. Dairy Sci. 98:7384-7393.

Datta, N., and Deeth, H. 2001. Age gelation of UHT milk - A review. Food and Bioproducts Processing. 197-210.

Datta, N., Elliott, A., Perkins, M., and Deeth, H. 2002. Ultra-high-temperature UHT treatment of milk: Comparison of direct and indirect modes of heating. Australian J. Dairy Technology. 211-227.

De Jonghe, V., Coorevits, A., De Block, J., Van Coillie, E., Grijspeerdt, K., Herman, L., et al. 2010. Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. International J. Food Microbiology. 318-325.

Deane, D., Chelesvig, J., and Thomas, W. 1967. Pasteurization Treatment and Consumer Acceptance of Milk. J. Dairy Sci. 50:1216-1220.

Deeth, H. 2006. Lipoprotein lipase and lipolysis in milk. International Dairy J. 555-562.

Dickow, J., Larsen, L., Hammershoj, M., and Wiking, L. 2011. Cooling causes changes in the distribution of lipoprotein lipase and milk fat globule membrane proteins between skim milk and cream phase. J. Dairy Sci. 94:646-656.

Donlan, R. 2002. Biofilms: Microbial Life on Surfaces. Emerging Infectious Diseases. 8:881-890.

Elliot, A., Datta, N., Amenu, B., and Deeth, H. 2005. Heat-induced and other chemical changes in commercial UHT milks. J. Dairy Research. 72:442-446.

Elmoslemany, A., Keefea, G., Dohooa, I., Wichtela, J., Stryhn, H., and Dingwell, R. 2010. The association between bulk tank milk analysis for raw milk quality and on-farm management practices. Preventive Veterinary Medicine. 95:32-40.

Enright, J., Sader, W., and Thomas, R. 1957. Thermal inactivation of Coxiella burnetii and its relation to pasteurization of milk. United States Public Health Service. Public Health Monograph No. 47.

Euromonitor. 2016. Milk Market Size. Euromonitor Passport Database.

FAO. 2012. Dairy Production and products: Milk production. Acessed July 25, 2014. http://www.fao.org/agriculture/dairy-gateway/milk-production/en/#.U9KPLfldWNA

Farkye, N. Y. 2004. Cheese technology. International J. Dairy Technology.

FDA. 2013. Grade "A" Pasteurized Milk Ordinance. Washington, DC: US Department of Health and Human Services. Public Health Service.

FDA. 2015a. 21 CFR 1240.61. Accessed May 9, 2016. https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=1240.61

FDA. 2015b. 21 CFR 131.110. Accessed May 9, 2016. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=131.110

Fox, P., Uniacke-Lowe, T., McSweeney, P., and O'Mahony, J. 2015. Dairy Chemistry and Biochemistry, Second Edition. Springer Verlag.

Francis, L., Chambers, D., Kong, S., Milliken, G., Jeon, I., and Schmidt, K. 2005. Serving Temperature Effects on Milk Flavor, Milk Aftertaste, and Volatile Compound Quantification in Nonfat and Whole Milk. J. Food Sci. S413-S418.

Fromm, H., and Boor, K. 2004. Characterization of Pasteurized Fluid Milk Shelf-life Attributes. J. Food Sci. M207-M214.

Frost, M., Dijksterhuis, G., and Martens, M. 2001. Sensory perception of fat in milk. Food Quality and Preference. 327-336.

Gandy, A., Schilling, M., Coggins, P., White, C., Yoon, Y., and Kamadia, V. 2008. The Effect of Pasteurization Temperature on Consumer Acceptability, Sensory Characteristics, Volatile Compound Composition, and Shelf-Life of Fluid Milk. J. Dairy Sci. 91:1769-1777.

Gebre-Egziabher, A., Cheong, A., Blankenagel, G., and Humbert, E. 1985. Extended Storage of Raw Milk. I. Effect on Microbiological Quality. Canadian Institute of Food Sci. and Technology. 247-250.

Gillis, W., Cartledge, M., Rodriguez, I., and Suarez, E. 1985. Effect of Raw Milk Quality on Ultra-High Temperature Processed Milk. J. Dairy Sci. 68:2875-2879.

Goff, H. D., and Griffiths, M. W. 2006. Major Advances in Fresh Milk and Milk Products: Fluid Milk Products and Frozen Desserts. J. Dairy Sci. 1163-1173.

Griffiths, M. W., Phillips, J. D., and Muir, D. D. 1987. Effect of low-temperature storage on the bacteriological quality of raw milk. Food Microbiology. 285-291.

Guul-Simonsen, F., Christiansen, P. S., Edelsten, D., Kristiansen, J. R., Madsen, N. P., Nielsen, E. W., et al. 1996. Cooling, Storing and Quality of Raw Milk. Acta Agriculturae Scandinavica, Section A - Animal Sci. 105-110.

Hantsis-Zacharov, E., and Halpern, M. 2007. Culturable Psychrotrophic Bacterial Communities in Raw Milk and Their Proteolyic and Lipolytic Traits. Applied and Environmental Microbiology. 7162-7168.

Hayes, M. C., and Boor, K. 2001. Raw Milk and Fluid Milk Products. In Applied Dairy Microbiology pp. 59-76. New York: Marcel Dekker, Inc.

Henyon, D. 1999. Extended shelf-life milks in North America: a perspective. International J. Dairy Technology. 95-101.

Holsinger, V., Rajkowski, K., and Stabel, J. 1997. Milk pasteurisation and safety: a brief history and update. Scientific and Technical Review of the Office International des Epizooties. 441-451.

Huck, J., Sonnen, M., and Boor, K. 2008. Tracking Heat-Resistant, Cold-Thriving Fluid Milk Spoilage Bacteria from Farm to Packaged Product. J. Dairy Sci. 91:1218-1228.

Ma, Y., Ryan, C., Barbano, D., Galton, D., Rudan, M., and Boor, K. 2000. Effects of Somatic Cell Count on Quality and Shelf-Life of Pasteurized Fluid Milk. J. Dairy Sci. 83:264-274.

MacDonald, H. B. 2010. The role of milk in the diet. In H. B. MacDonald, Improving the safety and quality of milk. Volume 2: Improving quality in milk products pp. 3-27. Woodhead Publishing.

Marchand, S., De Block, J., De Jonghe, V., Coorevits, A., Heyndrickx, M., and Herman, L. 2012. Biofilm Formation in Milk Production and Processing Environments; Influence on Milk Quality and Safety. Comprehensive Reviews in Food Sci. and Food Safety. 133-147.

Martin, N., Ranieri, M., Murphy, S., Ralyea, R., Wiedmann, M., and Boor, K. 2011. Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk. J. Dairy Sci. 94:1211-1222.

Martin, N., Ranieri, M., Wiedmann, M., and Boor, K. 2012. Reduction of pasteurization temperature leads to lower bacterial outgrowth in pasteurized fluid milk during refrigerated storage: A case study. J. Dairy Sci. 95:471-475.

Masiello, S., Martin, N., Trmcic, A., Wiedmann, M., and Boor, K. 2016. Identification and characterization of psychrotolerant coliform bacteria isolated from pasteurized fluid milk. J. Dairy Sci. 99:130-140.

Masiello, S., Martin, N., Watters, R., Galton, D., Schukken, Y., Wiedmann, M., et al. 2014. Identification of dairy farm management practices associated with the presence of psychrotolerant sporeformers in bulk tank milk. J. Dairy Sci. 97:4083-4096.

McCarthy, K.S., Lopetcharat, K., Drake, M.A. 2016. Milkfat threshold determination and the effect of milkfat content on consumer preference of fluid milk. MS Thesis. North Carolina State Univ., Raleigh, NC.

Meer, R., Baker, J., Bodyfelt, F., and Griffiths, M. 1991. Psychrotrophic Bacillus spp. in Fluid Milk Products: A Review. J. Food Protection. 969-979.

Mehta, R. 1980. Milk Processed at Ultra-High-Temperatures - A Review. J. Food Protection. 212-225.

Montilla, A., and Olano, A. 1997. Effect of the protein content and dilution on the lactulose/furosine ratio in heat-treated milk. Milchwissenschaft, 52:506-507.

Morales, F., Romero, C., and Jimenez-Perez, S. 2000. Characterization of industrial processed milk by analysis of heat-induced changes. International J. Food Sci. and Technology. 35:193-200.

Mortier, L., Braekman, A., Cartyvels, D., van Renterghem, R., and De Block, J. 2000. Intrinsic indicators for monitoring heat damage of consumption milk. Biotechnology, Agronomy, Society, and Environment. 221-225.

Muir, D. D. 1996a. The shelf-life of dairy products: 1. Factors influencing raw milk and fresh products. J. the Society of Dairy Technology. 24-32.

Muir, D. D. 1996b. The shelf-life of dairy products: 2. Raw milk and fresh products. J. the Society of Dairy Technology. 44-48.

North, C., and Park, W. 1927. Standards for milk pasteurization. Am. J. Hyg. 147-154.

O'Connell, A., Ruegg, P., Jordan, K., O'Brien, B., and Gleeson, D. 2016. The effect of storage temperature and duration on the microbial quality of bulk tank milk. J. Dairy Sci. 99:3367-3374.

Oupadissakoon, G., Chambers, D., and Chambers IV, E. 2009. Comparison of the sensory properties of ultra-high-temperature UHT milk from different countries. J. Sensory Studies. 427-440.

Pangborn, R., Bos, K., and Stern, J. 1985. Dietary fat intake and taste responses to fat in milk by under, normal, and overweight women. Appetite. 6:25-40.

Pellegrino, L., Resmini, P., and Luf, W. 1995. Assessment indices of heat treatment of milk. In P. Fox, Heat-induced changes in milk pp. 409-453. Brussels, Belgium: International Dairy Federation.

Phillips, L., and Barbano, D. 1997. The Influence of Fat Substitutes Based on Protein and Titanium Dioxide on the Sensory Properties of Lowfat Milks. J. Dairy Sci. 80:2726-2731.

Phillips, L., McGiff, M., Barbano, D., and Lawless, H. 1995. The influence of fat on the sensory properties, viscosity and color of low-fat milks. J. Dairy Sci. 78:1258-1266.

Popkin, B. 2010. Patterns of beverage use across the lifecycle. Physiology and Behavior. 26:4-9.

Ralyea, R., Wiedmann, M., and Boor, K. 1998. Bacterial tracking in a Dairy Production System Using Phenotypic and Ribotyping Methods. J. Food Protection. 1336-1340.

Ranieri, M., Huck, J., Sonnen, M., Barbano, D., and Boor, K. 2009. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. J. Dairy Sci. 92:4823–4832.

Ravanis, S., and Lewis, M. 1995. Observations on the effect of raw milk quality on the keeping quality of pasteurized milk. Letters in Applied Microbiology. 20:164-167.

Resmini, P., Pellegrino, L., and Batteli, G. 1990. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. Italian J. Food Sci. 173-183.

Santos, M., Ma, Y., Caplan, Z., and Barbano, D. 2003. Sensory Threshold of Off-Flavors Caused by Proteolysis and Lipolysis in Milk. J. Dairy Sci. 86:1601-1607.

Shipe, W., Bassette, R., Deane, D., Dunkley, W., Hammond, E., Harper, W., et al. 1978. Off Flavors of Milk: Nomenclature, Standards, and Bibliography. J. Dairy Sci. 61:855-869.

Simon, M., and Hansen, A. 2001. Effect of Various Dairy Packaging Materials on the Shelf Life and Flavor of Ultrapasteurized Milk. J. Dairy Sci. 84:784-791.

Song, W., Chun, O., Kerver, J., Cho, S., Chung, C., and Chung, S. 2006. Ready-to-Eat Breakfast Cereal Consumption Enhances Milk and Calcium Intake in the US Population. Journal of the American Dietetic Association. 11:1783-1789.

Steele, J. 2000. History, trends, and extent of pasteurization. Journal of the American Veterinary Medical Association. 175-178.

Teh, K., Flint, S., Palmer, J., Andrewes, P., Bremer, P., and Lindsay, D. 2014. Biofilm - An unrecognized source of spoilage enzymes in dairy products? International Dairy J. 32-40.

Urbach, G. 1990. Effect of Feed on Flavor in Dairy Foods. J. Dairy Sci. 73:3639-3650.

USDA. 2015. Estimated Fluid Milk Products Sales Report. USDA.

USDA ERS. 2015. Food Availability Per Capita Data System. USDA, Economic Research Service.

van Boekel, M. 1998. Effect of heating on Maillard reactions in milk. Food Chemistry. 403-414.

Varnam, A. H., and Sutherland, J. P. 2001. Milk and Milk Products. Gaithersburg, Maryland: Aspen Publishers.

Vissers, M., Te Giffel, M., Driehuis, F., De Jong, P., and Lankveld, J. 2007. Predictive Modeling of Bacillus cereus Spores in Farm Tank Milk During Grazing and Housing Periods. J. Dairy Sci. 90:281-292.

Walsh, A., Duncan, S., Potts, H., and Gallagher, D. 2015. Comparing quality and emotional responses as related to acceptability of light-induced oxidation flavor in milk. Food Research International. 293-300.

Walstra, P., Woulters, J. T., and Geurts, T. J. 2006. Dairy Sci. and Technology. Boca Raton, FL: CRC Press.

Weimer, P. J. 2001. Microbiology of the Dairy Animal. In Applied Dairy Microbiology pp. 1-58. New York: Marcel Dekker, Inc.

Westhoff, D. 1978. Heating Milk for Microbial Destruction: A Historical Outline and Update. J. Food Protection. 122-130.

Wiking, L., Bertram, H., Bjork, L., and Nielsen, J. 2005. Evaluation of cooling startegies for pumping of milk - Impact of fatty acid composition of free fatty acid levels. J. Diary Research, 476-481.

Workman, T. 1941. Short time high temperature pasteurization. Bulletin of the International Association of Milk Dealers. 585-588.

Zall, R., and Chen, J. 1981. Heating and Storing Milk on Dairy Farms Before Pasteurization in Milk Plants. J. Dairy Sci. 1540-1544.

TABLES

Table 1.1 Standards for Grade "A" raw, pasteurized, and ultra-pasteurized milk (Taken from the 2013 Pasteurized Milk Ordinance, FDA, 2013)

GRADE "A" RAW MILK AND MILK PRODUCTS FOR PASTEURIZATION, ULTRA-PASTEURIZATION, ASEPTIC PROCESSING AND PACKAGING, OR RETORT PROCESSED AFTER PACKAGING	Temperature	Cooled to 10ºC (50ºF) or less within four (4) hours or less, of the commencement of the first milking, and to 7°C (45ºF) or less within two (2) hours after the completion of milking. Provided, that the blend temperature after the first milking and subsequent milkings does not exceed 10ºC (50ºF).
	Bacterial Limits	Individual producer milk not to exceed 100,000 per mL prior to commingling with other producer milk. Not to exceed 300,000 per mL as commingled milk prior to pasteurization.
	Drugs	No positive results on drug residue.
	Somatic Cell Count	Individual producer milk not to exceed 750,000 per mL.
GRADE "A" PASTEURIZED MILK AND/OR MILK PRODUCTS	Temperature	Cooled to 7ºC (45ºF) or less and maintained thereat.
	Bacterial Limits	Not to exceed 20,000 per mL, or gm **NOTE:** Tested in conjunction with the drug residue/inhibitory substance test.
	Coliform	Not to exceed 10 per mL. Provided, that in the case of bulk milk transport tank shipments, shall not exceed 100 per mL. **NOTE:** Tested in conjunction with the drug residue/inhibitory substance test.
	Phosphatase	Less than 350 milliunits/L for fluid products and other milk products by approved electronic phosphatase procedures.

Table 1.1 (continued)

	Drugs	No positive results on drug residue detection.
GRADE "A" ULTRA- PASTEURIZED (UP) MILK AND/OR MILK PRODUCTS	Temperature	Cooled to 7ºC (45ºF) or less and maintained thereat.
	Bacterial Limits	Not to exceed 20,000 per mL, or gm **NOTE:** Tested in conjunction with the drug residue/inhibitory substance test.
	Coliform	Not to exceed 10 per mL. Provided, that in the case of bulk milk transport tank shipments, shall not exceed 100 per mL.
	Drugs	No positive results on drug residue detection.

Table 1.2 Minimum time and temperature requirements for fluid milk pasteurization (Taken from the 2013 Pasteurized Milk Ordinance, FDA, 2013)

Batch (Vat) Pasteurization	
Temperature	**Time**
63°C (145°F)*	30 minutes
Continuous Flow (HTST and HHST) Pasteurization	
Temperature	**Time**
72ºC (161ºF)*	15 seconds
89ºC (191ºF)	1.0 second
90ºC (194ºF)	0.5 seconds
94ºC (201ºF)	0.1 seconds
96ºC (204ºF)	0.05 seconds
100ºC (212ºF)	0.01 seconds

*If the fat content of the milk product is ten percent (10%) or greater, or a total solids of 18% or greater, or if it contains added sweeteners, the specified temperature shall be increased by 3ºC (5ºF).

Table 1.3 Descriptive lexicon for fluid milks

Attribute	**Description/Reference**
Flavor/Aroma	
Sweet aromatic	Sweet aromatics similar to cake mix or those found in fresh dairy products
Fat/milkfat/lactone	Flavors and aromas associated with dairy fat
Cooked/caramelized	"Brown" flavors and aromas associated with heated milk
Sulfur/eggy	Sulfurous or egg-like flavors and aromas; Referenced by hard-boiled egg white
Cowy/barny/phenolic	Barnyard or animal-like flavors and aromas
Mothball	Aromas associated with protein decomposition Referenced by indole, skatole
Grassy	Sweet flavor and aroma like that of cut grass
Feed/malty/silage	Flavors and aromas like mixed grains, fermented hay, and cattle feed
Bitter	Basic taste referenced by caffeine in water
Sour	Basic taste referenced by citric acid in water
Sweet	Basic taste reference by sucrose in water
Salty	Basic taste references by sodium chloride in water
Texture	
Chalky	Dry, powdery mouthfeel
Fat feel	Oily mouthfeel between tongue and palate
Viscosity	Fluid flow across tongue
Astringent	Drying sensation of the mouth; Referenced by alum in water
Appearance	
Opacity	Degree of opacity; Referenced by water and whole fat milk
Color intensity	Degree of color compared to standard references (e.g. paint chips)

Adapted from Clare et al. (2005), Croissant et al. (2007), Oupadissakoon et al. (2009), and McCarthy et al. (2016)

FIGURES

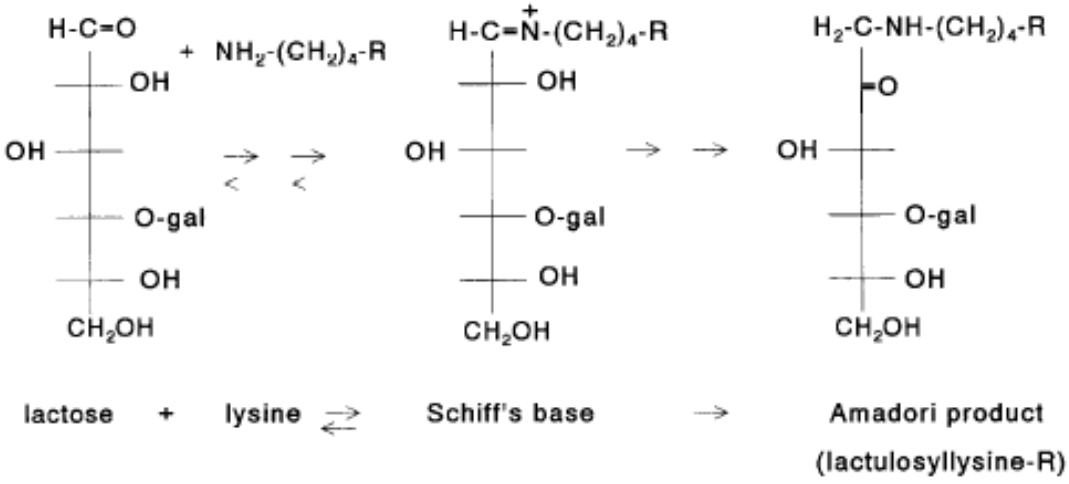


Figure 1.1 Schematic overview of the early Maillard reaction in milk, leading to the Amadori product. (gal = galactose, R = protein chain)

Taken from van Boekel (1998)

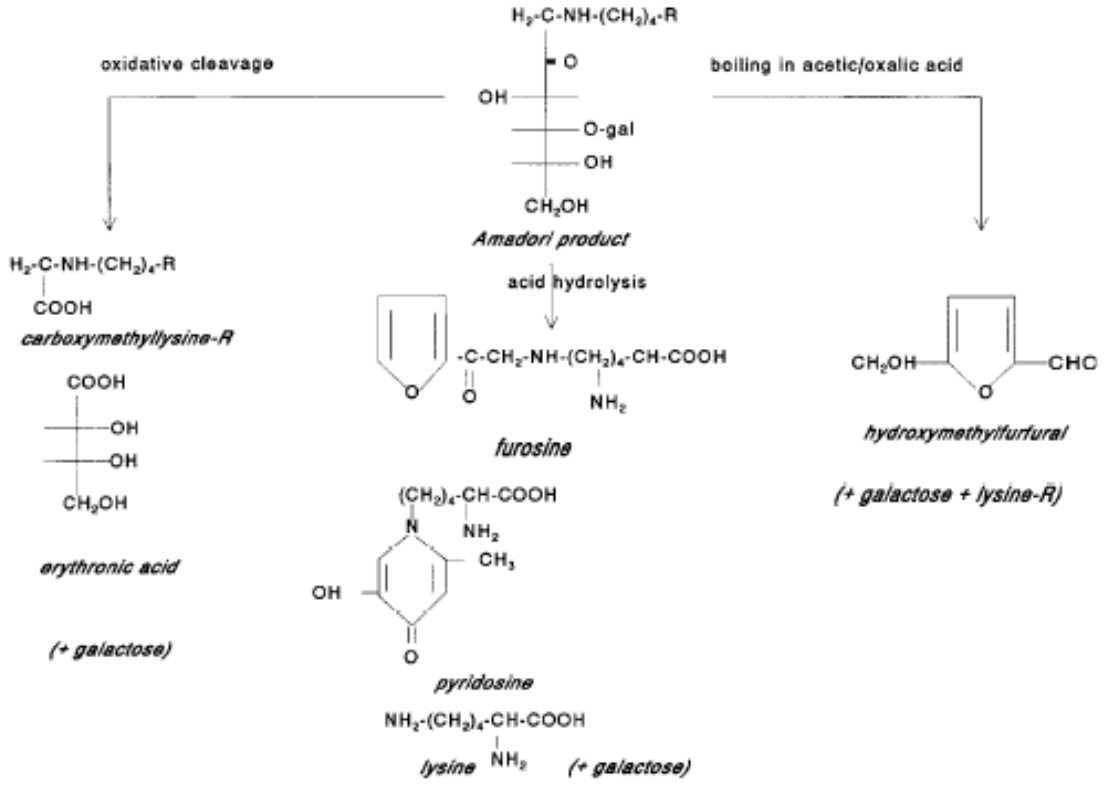


Figure 1.2 Degradation of the Amadori product via oxidative cleavage, acid hydrolysis, or boiling in acetic/oxaclic acid. (gal – galactose, R = protein chain).

Taken from van Boekel (1998)

CHAPTER 2:

Short communication: The impact of raw milk cooling on sensory perception and shelf life of HTST skim milk

Short communication: The impact of raw milk cooling on sensory perception and shelf life of HTST skim milk

A.P. Lee[1], D.M. Barbano[2], M.A. Drake[1]

[1]Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

[2]Northeast Dairy Research Center, Cornell University, Ithaca, NY 14850

The contents of this chapter have been submitted to:

Journal of Dairy Science

Interpretive Summary

The handling and processing of raw milk influences the quality of pasteurized milk, affecting consumer acceptability and purchase. Raw milk cooling rate and pasteurization temperature are two possible factors influencing sensory properties and shelf life of pasteurized milk. This study found that neither cooling rate nor pasteurization temperature significantly affected sensory quality or shelf life when initial raw milk quality was excellent, indicating that other factors during handling and processing are more important and impactful on the sensory properties and shelf life of pasteurized milk.

Abstract

The cooling rate of raw milk may influence sensory properties and pasteurized shelf life. Under the Pasteurized Milk Ordinance (PMO) for Grade A milk, raw milk may be cooled instantaneously by on-farm heat exchangers but is also acceptable if "cooled to 10°C or less within four (4) hours of the commencement of the first milking". The objective of this study was to determine the effect of raw milk cooling on consumer perception and shelf life. Raw milk (18 to 21°C) was obtained and transported within 1 h of milking to North Carolina State University. The batch of raw milk was split in two portions and a plate heat exchanger was used to quickly cool one portion to <6°C within 1 min. The second portion was stored in a jacketed bulk tank and slowly cooled over 4 h to <10°C. Milk from 3 consecutive milkings were collected every 12 h with subsequent milkings added to the previous collections. The bulk milk was kept below 10°C while adding milk for the slow cool milk treatment. After 72 h, each whole milk was separated; the skim milk was pasteurized at 73 or 78°C for 20 s, homogenized, and held at 4°C. Difference tests (n=75) and consumer acceptance tests (n=100) were conducted to determine if consumers could detect differences among milks. Descriptive analysis (DA) and microbial testing for aerobic, psychrotrophic, and psychrotolerant spore counts were conducted through shelf life. The entire experiment was repeated in triplicate. Raw milks averaged 3.3 log CFU/mL by aerobic plate count, <25 CFU/mL coliforms, 300,000 SCC and 3.15 ± 0.07% protein. Psychrotolerant spores were not found in the raw milk. Consumers could not detect differences (P>0.05) between cooling treatments of the same pasteurization temperature nor between different temperatures of the same cooling treatment. Milks reached sensory failure 49 ± 4 d on average after processing, and aerobic counts were between 5 to 7 log cfu/mL. Cooling treatment had no effect on shelf

life. These results suggest that pasteurized milk quality is due to other factors. Raw milk cooling rate is not the largest impact on milk quality, when raw milk quality is excellent and cooling is conducted within the limits of the PMO.

Keywords: milk, raw milk, cooling, pasteurization, shelf life, consumer

Short Communication

Per capita milk consumption has declined steadily from 112.0 kg of fluid milk consumed in 1975 to 72.1 kg in 2014, a 35% decrease (USDA ERS, 2015). To compete against other, more shelf stable beverage offerings, fluid milk must improve product quality and shelf life (Boor, 2001; Caplan and Barbano, 2013). Along with post-pasteurization contamination, the major obstacles for milk shelf life are bacterially related, typically from psychrotrophic bacteria and their heat resistant enzymes, milk somatic cell enzymes and native milk proteases (Ma et al., 2000; Santos et al., 2003a,b), and heat-resistant spore formers (Fromm and Boor, 2004; Huck et al., 2007). Although standards exist for raw milk quality and production of fluid milk in the US, different farms and processing facilities have different management practices, leading to a wide variation in the overall quality and microbial community of the raw milk (Huck et al., 2007; Masiello et al., 2014). Gram-positive *Bacillus* spp. and *Paenibacillus* spp. are among the main microorganisms of concern in raw milk that may limit the shelf life of milk (Meer et al., 1991; Fromm and Boor, 2004; Huck et al., 2007; Ranieri et al., 2009; Masiello et al., 2014). These organisms exist as heat-resistant spores in raw milk that can germinate after pasteurization, which can result in early spoilage of fluid milk by proteolysis or lipolysis (Collins, 1981; Meer et al., 1991; Deeth et al., 2002; Santos et al., 2003a,b; Barbano and Santos, 2006; Huck et al., 2007). The entire milk processing chain is subject to contamination by these microorganisms, from raw milk procurement to final packaging.

The Pasteurized Milk Ordinance (**PMO**) regulates the production, packaging, and sale of Grade A milk and milk products (FDA, 2013). Included in the PMO are standards for raw milk quality and handling. Raw milk must be cooled within four hours to 10°C or less

from the start of milking, then to 7°C or less within two hours after the completion of milking. Also, subsequent milk collections added to previously collected milk must not raise the blended bulk milk above 10°C (FDA, 2013). To meet these requirements, dairy producers may utilize plate heat exchangers to rapidly cool the milk before storage or they may use bulk tanks to more slowly cool the raw milk. During the slower cooling process, microbial growth may continue, which can affect the subsequent quality of milk pasteurized by high temperature-short time (**HTST**) processing due to heat-resistant bacteria or enzymes (Muir, 1996; Barbano and Santos, 2006).

Previous studies suggest that the cooling and storage temperature of raw milk may or may not influence the shelf life of raw milk. Guul-Simonsen et al. (1996) reported that instantaneous cooling by heat exchanger to 4°C before bulk tank storage reduced bacterial growth compared to a batch cooling process which cooled milk from 35°C to 4°C over 2.5 hours (Guul-Simonsen et al., 1996). At 2°C, the time for the psychrotrophic bacteria count to exceed 10^6 cfu/mL was increased from 2.9 days at 6°C to 5 days when the initial count was below 10^4 cfu/mL (Griffiths et al., 1987). A more recent study by O'Connell et al. (2016) reported that storage at 2° or 4°C did not significantly affect microbial quality of bulk tank raw milk over 96 hours as long as raw milk entering the bulk tank had very low microbial count. The effects of cooling and storage on raw milk may also affect subsequent pasteurized milk quality and shelf life. Milks pasteurized at 85.2°C had higher microbial counts than milks pasteurized at 72.9°C with the same hold time. The predominant microorganisms found in the pasteurized milks up to 7 days after processing were from genus *Bacillus*, then genus *Paenibacillus* after 14 days (Ranieri et al., 2009). A case study of a single milk processing plant supported the findings by Ranieri et al. (2009); total bacteria counts for

skim, 2%, and whole milk pasteurized at 76.1ºC for 18.25 s were lower than milk pasteurized at the higher 79.4ºC temperature with the same hold time for all time points up to 21 days (Martin et al., 2012).

Pasteurization temperature can not only affect microbial quality, but also sensory quality. Thermal treatment results in the generation of volatile compounds from denaturation of milk proteins which subsequently release sulfydryls and those formed by nonenzymatic browning reactions (Calvo and de la Hoz, 1992). Sulfydryl compounds are responsible for cooked/sulfur/ eggy characteristics of heated milks, which consumers may or may not accept (Shipe et al., 1978). Deane et al. (1967) previously reported that adult consumers preferred whole milk pasteurized at 78.9°C for 17s compared to those at lower (72.2° and 75.6°C) and higher (82.2° and 85.6°C) temperatures; children younger than 13 y were least discriminating of the pasteurization temperatures. Gandy et al. (2008) examined adult consumer preference for HTST 2% fat milks pasteurized at 77, 79, 82, and 85°C and reported that 79°C milk was most acceptable by all consumers. Of these consumers, some consumer clusters were distinguished by acceptability of cooked flavor in the 82° and 85°C treatments while others discriminated against these treatments (Gandy et al., 2008).

The objective of this study was to understand the effect of raw milk cooling rates on shelf life and sensory perception of HTST skim milk. Skim milk was selected for this study as it is the most flavor sensitive milk matrix compared to higher fat milks which may mask sensory characteristics. Two different HTST pasteurization temperatures were also compared for each cooling treatment to see if consumers could distinguish between either cooling or heat treatments.

For this study, 200L of raw milk was obtained from the North Carolina State University (NCSU) dairy farm per milking every 12 h across three milkings. The milk was received at 18-21°C and transported to the NCSU pilot plant facilities in 38L (10 gallon) sanitized milk cans for cooling. Transportation from the farm to the pilot plant facilities took on average 20 min. Chlorine (200ppm, Multi-Chlor II, Diversey, Sturtevant, WI) was used to sanitize all equipment. The three milkings were collected, cooled, and stored before processing. The total mixed rations feed was held constant throughout the weeks of milk collection to minimize influence of feed. Continuously recording thermocouple readers (model HH306A, OMEGA Engineering Inc., Stamford, CT) were used to track all temperatures.

At the NCSU pilot plant, the raw milk collected from a milking was blended together before being partitioned for quick-cooling (**QC**) by a plate heat exchanger (model T4 RGS-16/2, SPX Flow Technology, Greensboro, NC) or slow cooling (**SC**) by a jacketed bulk tank (Precision Stainless, Inc., Springfield, MO) with agitator (model XD33VM with 6.0" prop, Lightnin, Rochester, NY). Ninety ± 3kg of each treatment was stored per milking for a total of 270 kg per treatment after the third milk collection. For all milkings, the QC milk was cooled to 6°C within 1 min by the plate heat exchanger then stored in a jacketed bulk tank at 3°C with any QC milk collected previously. The first milking of the SC milk was cooled linearly to 6°C across a 4 hour period and stored at 6°C. For the second and third milk collections, warm raw milk (17-20°C) was slowly added to the SC bulk tank over the course of 4 h to prevent the bulk milk from exceeding 10°C as stated in the PMO. After the third milk collection, the milks assigned to SC or QC were stored for an additional 48 h such that the storage time from the first milk collection was no longer than 72 h.

Following storage, the cooled milks were warmed to 50°C for separation to skim and cream by a hot bowl centrifugal separator (model SI600E, Agri-Lac, Miami, FL). The skim milk portion was then pasteurized at either 73°C or 78°C for 20s with a Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a two-stage homogenizer at 13.8 MPa total pressure with 3.4 MPa on the second stage (GEA Niro Soavi, Parma, Italy). The milks were cooled to 15°C by an in-line cooler before packaging in half-gallon light shielding containers (Upstate Niagara Cooperative, Inc., Buffalo, NY). The milks were stored at 4°C over shelf life. The order of processing for the cooling treatments was randomized between replications. The entire experiment was repeated in triplicate across a one month time frame.

Raw milk microbial quality was determined by aerobic plate count (**APC**) (AOAC method 990.12) and coliform count (**CC**) (AOAC method 991.14) using Petrifilm plates (Aerobic Count Plates and Coliform Count Plates, 3M, St. Paul, MN) incubated at 32°C for 24 or 48 h, respectively. Psychrotrophic bacteria counts (**PBC**) were determined Wehr and Frank, (2004) by plating on plate count agar (BD Difco, Franklin Lakes, NJ) and incubating at 7° ± 1°C for 10 days. Raw milk somatic cell count (**SCC**) was measured by SomaScope fluorescence flow cytometry (Delta Instruments, Drachten, the Netherlands), drug residue testing was determined by SNAP beta-lactam test kit (Idexx Laboratories Inc., Westbrook, ME), and proximate analyses for fat, protein, and solids was measured by a Fourier-transform mid-infrared milk analyzer (LactoScope FTIR; Delta Instruments BV, Drachten, the Netherlands). Psychrotrophic spore count (**PSC**) was evaluated using the methods described by Huck et al. (2007) with modifications. Briefly, raw milks were heated to 80°C for 12 min then plated on plate count agar weekly and incubated at 7 ° ± 1°C for 10 d.

Pasteurized milk quality was determined by APC and CC using the same methods as for raw milk. Samples were plated weekly through shelf life. Pasteurization was confirmed by the Scharer Rapid Visual alkaline phosphatase method (Wehr and Frank, 2004) using a Phos-Kit (Weber Scientific, Hamilton, NJ).

Sensory testing was conducted in accordance with the NCSU Institutional Review Board for the Protection of Human Subjects in Research regulations. A trained panel was used to characterize the flavor profiles of the milks across shelf life and to confirm the end of shelf life by sensory failure. Each panelist (4 females, 2 males, ages 21-55 y) had a minimum of 50 h of experience evaluating flavor and mouthfeel/texture using the Spectrum^{TM} method (Meilgaard et al., 2007), and at least 20 h of previous experience with evaluating sensory properties of fluid milk using an established sensory language (Croissant et al., 2007; McCarthy et al., 2016). Due to the general shelf life objective of this study, four attributes were selected for evaluation (sweet aromatic and cooked flavor, sweet taste, and astringency). Milks were evaluated twice a week until the end of shelf life. Samples (30 mL) were dispensed into lidded 59 mL soufflé cups (Dart Container Corp., Mason, MI) with random three digit blinding codes. Samples were prepared with overhead lights off to prevent light oxidation. Milks were tempered to 10°C and evaluated using paper ballots. Each panelist evaluated each milk in duplicate. The trained panel was also used to determine shelf life failure, which was determined sensorially by spoilage aromas and flavors.

Difference testing with milk consumers was conducted with four pairwise comparisons (Pair 1: QC-73°C vs QC-78°C; Pair 2: QC-73°C vs SC-73°C, Pair 3: SC-73°C vs SC-78°C, Pair 4: QC-78°C vs SC-78°C) to determine if consumers could distinguish between cooling treatments or between pasteurization treatments. Testing was conducted by

balanced reference duo-trio tests (ASTM E2610) on the milks 72 h after processing. A minimum of 50 self-reported milk consumers were recruited from the university community using email listservs for each experimental replicate. Milks (88mL) were served in 177mL Styrofoam cups (Dart Container Corp.) with random three digit blinding codes. Each consumer evaluated the four pairwise comparisons in one seating that lasted approximately 20 m. Samples were served at 10°C and the pairwise duo-trio tests were served in a randomized balanced order. A two minute enforced rest was implemented between difference tests and consumers were provided with unsalted crackers and deionized water to cleanse their palates. Panelists were compensated with a food treat after each test and a $5 gift card after completing two separate test sessions. Data was collected by Compusense Cloud software (ver 6.9, Compusense Inc., Guelph, Canada).

Consumer acceptance testing was also conducted at four days after processing to determine if differences in liking existed. Based on descriptive sensory analysis and consumer difference testing results, two samples were selected for consumer acceptance testing, SC-73°C and SC-78°C. Milk consumers (n=104) were recruited from the NCSU campus. Milks (88mL) were dispensed into the same containers and temperature as for difference testing. Panelists were presented samples monadically using a Williams design serving order. Consumers were asked to evaluate appearance, aroma, overall liking, flavor, freshness, cooked flavor, mouthfeel/thickness/viscosity, and aftertaste on a 9-point hedonic scale where 1 is “Dislike Extremely” and 9 is “Like Extremely”. Just About Right (JAR) questions were asked for sample flavor, mouthfeel/thickness/viscosity, and aftertaste on a 5-point scale where 1 was “Not nearly enough flavor”, “Not nearly thick enough”, or “Much too mild” and 5 was “Much too much flavor”, “Much too thick”, or “Much too strong”, for

each question category, respectively. There was a two minute enforced rest between samples to allow panelists to cleanse their palate using unsalted crackers and deionized water. Lastly, consumers answered demographic questions which included questions on frequency of milk consumption, frequency of purchase, fat contents consumed, and fat content most often consumed. Panelists were compensated with a $5 gift card for participating. Compusense Cloud software was used for data collection.

The study was conducted using a split plot design where the whole plot factor was cooling treatment and the sub plots were one of the two different pasteurization treatments. Least squares means for cooling and pasteurization treatment effects at each time point were determined using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC). Response variables measured were log-transformed APC and PBC and descriptive panel attributes. Proximate analyses and consumer acceptance data were analyzed by ANOVA with means separation (Fischer's least significant difference) using XLSTAT (Version 2015.5.01, Addinsoft, New York, NY). Just about right (JAR) scores were evaluated using chi-squared tests. Critical values for duo-trio results were taken from Bi (2006). All statistical analyses were performed at the 0.05 significance level.

This study examined the effect of raw milk cooling rate on shelf life and sensory perception of pasteurized skim milk. Our hypothesis was that raw milks quickly cooled by a plate heat exchanger before storage would have improved pasteurized shelf life and sensory quality, whereas milks slowly cooled by bulk tank cooling were thought to encourage microbial growth which would limit both shelf life and sensory quality

Fat (4.15 ± 0.06%), protein (3.12 ± 0.03%), and solids (13.04 ± 0.13%) content of the raw milks remained consistent within replications and there were no significant differences

between replications (P>0.05). The initial microbial quality of the raw milk was also assessed before processing. The upper limit in the PMO for grade "A" raw milk total bacterial count is 5 log cfu/mL (100,000 cfu/mL) for a single producer, 5.48 log cfu/mL (300,000 cfu/mL) when the milk is commingled (FDA, 2013). Milk generally considered as high quality contains less than 4 log cfu/mL (10,000 cfu/mL) standard plate count and less than 2 log cfu/mL (100 cfu/mL) coliform count (Martin et al., 2012). With these standards, the raw milk used for this study can be considered high quality, indicating that on-farm practices were well-controlled and appropriate measures were taken against contamination between milk collection at the farm and treatment at the pilot plant facilities. The raw milks quickly cooled to 6°C then stored at 3°C did not differ in microbial counts compared to raw milks slowly cooled to 6°C then stored at 6°C for 72 h - 3.32 vs 3.25 log APC; 3.48 vs 3.62 log PBC; 1.70 vs 1.72 log CC, respectively (P>0.05). No psychrotolerant spores were detected (<10 spores/mL) from any samples. Our results agree with the study by Miller et al. (2015) which examined 33 dairy farms and found that <1% of samples had ≥10 psychrotolerant spores/mL.

Previous studies have addressed the impact of raw milk storage temperature. Grebre-Egziabher et al. (1985) reported that high quality milks stored at 4°C did not have significant increases in standard plate count and PBC after 3 d bulk storage. This is consistent with the study by Guul-Simonsen et al. (1996) who compared milks cooled by batch cooling over 1.5 h and instant cooling/continuous process; both milks maintained microbial quality up to 3 d. O'Connell et al. (2016) reported that raw milks cooled to 14.5°C before bulk storage and stored at 6°C had significantly greater total bacterial growth after 48h (3.43 log cfu/mL at 0h to 3.54 log cfu/mL at 72h) with counts reaching >100,000 cfu/mL after 96 h compared to

milks stored at 2° or 4°C. The milks stored at 6°C in our study did not have significantly different growth however, likely due to the shorter incubation time compared to some previous studies and by differing bacterial populations which have different growth rates (Muir, 1996). Although raw milk bacterial populations were low in our study, recent evidence also suggests that raw milk microbial tests (i.e. standard plate count, PBC, CC, laboratory pasteurization count, direct microscopic count, and preliminary incubation count) do not accurately predict the shelf-life performance of pasteurized milks. These tests were all examined for correlation with pasteurized fluid milk shelf life, but the majority showed R^2 values <0.25 with none greater than 0.45 (Martin et al., 2011).

Aerobic plate counts and psychrotrophic bacteria counts for the pasteurized milk samples stored at 4°C during shelf life are shown in Figure 2.1 and Figure 2.2, respectively. No significant differences were observed for APC or PBC among milks within any time point (P>0.05). The shelf life of HTST milks are typically dictated by the growth of psychrotrophic microorganisms. Fromm and Boor (2004) reported that off flavors in milk were associated with the growth of *Paenibacillus, Bacillus*, and *Microbacterium* spp. At approximately 6 log cfu/mL PBC, spoilage of pasteurized milk can occur due to extracellular enzymatic activity (Muir, 1996). PBC for the milks increased from <2 log cfu/mL at d 28 to between 4 and 6 log cfu/mL at d 35. By d 49, all milks displayed between 6 to 8 log cfu/mL PBC (Figure 2.2). APC counts followed a similar trend (Figure 2.1) as the PBC with increasing counts beginning at about d 35. Day 49 correlated with the average shelf life of the milks as determined by sensory failure. The extended shelf life of the milks in this study far exceed the typical shelf life of HTST milks, between 2 to - 3 weeks; however, Bang et al. (2005) reported that HTST milks from the NCSU dairy spoiled at 42 days after pasteurization

and storage at 4°C, reflecting the historic cleanliness of the NCSU dairy farm. Varying spoilage patterns in the milk towards the end of shelf life resulted from variation of both APC and PBC. Once all milks from a replicate were deemed spoiled by sensory analysis, microbial data was no longer collected, resulting in fewer data points for the final weeks of shelf life.

The results from our study showed that neither raw milk cooling rate, raw milk storage temperature, nor pasteurization temperature had significant effects on the microbiological quality of the milks, if the microbial quality of the raw milk was excellent (i.e. <4 log cfu/mL APC and <2 log cfu/mL CC) and the spore counts of the raw milk were very low (i.e. <10 spores/mL). However, other studies have found differences due to these factors. Ranieri et al. (2009) had previously reported that higher pasteurization temperatures resulted in *Paenibacillus* spore germination, resulting in increased bacterial numbers during storage for pasteurized 2% fat milks. The milk used in the current study had no detectable psychrotolerant spore counts. This may account for the differences between the reported literature and this study. Longer shelf life observed for low spore count raw milk in the present study is consistent with the effect of spore removal from milk on fluid milk shelf life. Elwell and Barbano (2006) reported that commercially pasteurized raw skim milk had a SPC that exceeded 20,000 cfu/mL in 29 days at 4.1°C, while the same milk that was microfiltered (MF) to remove spores prior to pasteurization had 90% of containers with < 20,000 cfu/mL after 92 d of shelf life at 4.1°C storage. A 3.79 log reduction in total bacteria was achieved by MF and a further 1.84 log reduction was achieved by following MF with minimum pasteurization, resulting in a 5.63 log reduction for the combined process. Similar results for removal of spores from raw milk by MF from for minimally pasteurized milk with 2% fat

were reported by Caplan and Barbano (2013). Gravity separation has also been demonstrated as an approach to remove spores from raw milk (Caplan et al., 2013; Geer and Barbano, 2014). Future studies should examine the impact of alternative spore removal technologies (e.g., spore removal by centrifugation or gravity separation) on fluid milk shelf life, sensory properties and the cost of processing.

Trained panelists could not detect differences in sensory attributes intensities between quick cooled and slow cooled milk at any time point ($P>0.05$) (results not shown). Small but significant differences ($P<0.05$) were noted for 78°C pasteurized milks compared to 73°C pasteurized milks in cooked and sweet aromatic flavor intensities at days 3 and 7 (3.4 vs 3.1 and 2.0 vs 1.7), but not at subsequent time points. Differences were not detected between 78°C and 73°C pasteurized milks for other attributes ($P>0.05$) (results not shown). Overall aroma intensity and sweet aromatic and cooked flavors decreased with storage time for all milks ($P<0.05$) (results not shown). Sensory failure for milks occurred at 49 ± 4 days after processing and was not statistically different among treatments ($P<0.05$). For all replicates, the four paired comparisons no significant differences were detected (Table 2.1), indicating that consumers were unable to differentiate between cooling treatments or between pasteurization treatments at four days post-processing ($P>0.05$). Although difference tests revealed that consumers could not detect differences between milks, trained panelists detected small differences in cooked and sweet aromatic flavors between 73 and 78°C pasteurized milks through d 7. As such, two milks (SC-73°C and SC-78°C) were subjected to consumer acceptance testing. Consumer acceptance testing (n=104) at four days post-processing detected no differences in any attribute ($P>0.05$). Liking for all attributes (overall,

appearance, aroma, flavor, freshness, and mouthfeel) was at parity and was between "Liked slightly" (6) and "Liked moderately" (7) on the 9-point hedonic scale.

The trained panel results suggest that pasteurization temperature had a greater impact on sensory perception than raw milk cooling rate, but the majority of consumers were unable to differentiate between skim milks pasteurized at either 73° or 78°C by either difference or acceptance testing. These results differ from the results reported by Gandy et al. (2008) who reported that 2% fat milks pasteurized at 79°C were significantly more liked than milks pasteurized at 77°, 82°, or 85°C (6.7 on a 9-point hedonic scale compared to 6.1, 6.1, and 5.9, respectively) after 6 days of storage. Consumer clusters were distinguished by liking or disliking of cooked flavor (Gandy et al., 2008). Chapman and Boor (2001) also reported that children 6 to 11 years old preferred HTST milk over ultra-pasteurized (UP) and ultra-high temperature (UHT) milks. UP and UHT milks have prominent cooked characteristics (Chapman et al., 2001; Clare et al., 2005), which can influence consumer acceptance.

As previously mentioned, degradation by extracellular enzymatic activity from psychrotrophic bacteria and native milk enzymes associated with high milk SCC can result in the end of shelf life for fluid milk. If the cooling rate of raw milk influenced microbial numbers and subsequent enzyme production, then milks would be expected to exhibit greater proteolytic and lipolytic-related flavors earlier in the shelf life (Barbano and Santos, 2006). However, neither of these types of flavors was found in the milks prior to gross spoilage of all treatments, indicating that either the microbial growth was not sufficient to generate significant amounts of enzymes in the slow-cooled milks before the quick-cooled milks or that the microbial population in the milks did not produce degradative enzymes. No differences in raw milk microbial counts were detected between the slow or quick-cooled

milks prior to pasteurization; therefore, it is likely that bacterial growth and population of the slow-cooled milk was not sufficient for producing differences in pasteurized shelf life. Because the microorganisms in the raw milk were not identified however, it is unknown if the bacterial populations in the raw milks were capable of producing proteolytic and/or lipolytic enzymes. Previous research has utilized molecular subtyping (Huck et al., 2007; Masiello et al., 2014) and sequence analysis (De Jonghe et al., 2010) to identify spore-forming bacteria, mainly *Bacillus* and *Paenibacillus* spp, in raw milk and fluid milk processing systems. These bacteria have been shown to be capable of producing degradative enzymes (Fromm and Boor, 2004; De Jonghe et al., 2010). On-farm practices can influence the levels of spores found in raw milk, which can also contribute to post-processing contamination (Huck et al., 2007; Masiello et al., 2014; Miller et al., 2015). The data from this study show that the cooling temperatures and rates used in our study of high microbial quality raw milk does not impact microbial growth and subsequent shelf life. Instead, other on-farm practices that control microbial contamination of raw milk should be considered for their impact on pasteurized milk shelf life and quality.

The temperatures and rate of raw milk cooling used in our study did not impact the sensory characteristics or the shelf life of pasteurized skim milk. Instead, this study found that the largest factor on sensory perception was the pasteurization temperature, but only slight differences were noted by a trained panel; consumers were unable to distinguish between pasteurization temperatures. These results validate the processing standards in the PMO for the cooling of raw milk and reiterate the importance of quality management from farm practices through processing and packaging. Future studies should examine alternative methods of microbial and spore removal from raw milk combined with minimum time and

temperature for pasteurization. The effect of raw milk cooling rate should be examined with raw milks nearing the upper quality limits in the PMO.

Acknowledgements

This project was supported in part by the National Dairy Council (Rosemont, IL.)

REFERENCES

AOAC International. 2012. Official Methods of Analysis. 19th ed. AOAC International Gaithersburg, MD.

Bang, W., D. Clare, G. Cartwright, and M.A. Drake. 2005. Impact of a piggyback heat exchanger system on the shelf-life of pasteurized fluid milk. 66th Annual Meeting of the Institute of Food Technologists, July 16-20, New Orleans, U.S.A.

Barbano, D.M. and M.V. Santos. 2006. Influence of raw milk quality on fluid milk shelf life. J. Dairy Sci. E15-E19.

Bi, J. 2006. Standard discrimination tests. Pages10-11 in Sensory discrimination tests and measurements. 1st ed. Blackwell Pub., Ames, IA.

Boor, K. J. 2001. Fluid dairy product quality and safety: Looking to the future. J. Dairy Sci. 84:1-11.

Calvo, M., and L. de la Hoz. 1992. Flavour of heated milks. A Review. Int. Dairy J. 69-81.

Caplan, Z., and D.M. Barbano. 2013. Shelf life of pasteurized microfiltered milk containing 2% fat. J. Dairy Sci. 96:8035-8046.

Caplan, Z., C. Melilli, and D. M. Barbano. 2013. Gravity Separation of Fat, Somatic Cells, and Bacteria in Raw and Pasteurized Milks. J. Dairy Sci. 96:2011-2019

Chapman, K.W., and K. J. Boor. 2001. Acceptance of 2% ultra-pasteurized milk by consumers, 6 to 11 years old. J. Dairy Sci. 84:951-954.

Clare, D.A., W.S. Bang, G. Cartwright, M.A. Drake, P. Coronel, and J. Simunovic. 2005. Comparison of sensory, microbiological, and biochemical parameters of microwave versus indirect UHT fluid milk skim milk during storage. J. Dairy Sci. 88:4172-4182.

Collins, E. 1981. Heat resistant psychrotrophic microorganisms. J. Dairy Sci. 64:157-160.

Croissant, A., S. Washburn, L. Dean, and M.A. Drake. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. J. Dairy Sci. 90:4942-4953.

De Jonghe, V., A. Coorevits, J. De Block, E. Van Coillie, K.Grijspeerdt, L. Herman, P. De Vos, M. Heyndrickx. 2010. Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. Int. J. Food Micro. 136:318-325.

Deane, D., J. Chelesvig, and W. Thomas. 1967. Pasteurization treatment and consumer acceptance of milk. J. Dairy Sci. 50:1216-1220.

Deeth, H., T. Khusniati, N. Datta, and R. Wallace. 2002. Spoilage patterns of skim and whole milks. J. Dairy Res. 69:227-241.

Elwell, M. W., and D. M. Barbano. 2006. Microfiltration in the processing of extended shelf life skim milk. J. Dairy Sci. 89: E20-30E.

FDA. 2013. Standards for grade “A” milk and milk products. Pages 31-34 in Grade "A" Pasteurized Milk Ordinance. US Department of Health and Human Services, Public Health Service. Washington, DC.

Fromm, H., and K. J. Boor. 2004. Characterization of pasteurized fluid milk shelf-life attributes. J. Food Sci. M207-M214.

Gandy, A., M. Schilling, P. Coggins, C. White, Y. Yoon, and V, Kamadia. 2008. The effect of pasteurization temperature on consumer acceptability, sensory characteristics, volatile compound composition, and shelf-life of fluid milk. J. Dairy Sci. 91:1769-1777.

Gebre-Egziabher, A., A. Cheong, G. Blankenagel, and E. Humbert. 1985. Extended storage of raw milk. I. Effect on microbiological quality. Can. Inst. of Food Sci. Tech. 18:247-250.

Geer, S.R., and D. M. Barbano. 2014. The effect of Ig and somatic cells on the gravity separation of fat, bacteria, and spores in pasteurized whole milk. J. Dairy Sci. 97: 2027–2038.

Griffiths, M.W., J.D. Phillips, and D.D. Muir. 1987. Effect of low-temperature storage on the bacteriological quality of raw milk. Food Micro. 4:285-291.

Guul-Simonsen, F., P.S. Christiansen, D. Edelsten, J.R. Kristiansen, N.P. Madsen, E.W. Nielsen, and L. Petersen. 1996. Cooling, storing and quality of raw milk. Acta Agriculturae Scandinavica, Section A - Animal Science. 46:105-110.

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. 90:4872-4883.

Ma, Y., C. Ryan, D. M. Barbano, D. M. Galton, M. A. Rudan, and K. J. Boor. 2000. Effects of somatic cell count on quality and shelf-life of pasteurized fluid milk. J. Dairy Sci. 83:264-274.

Martin, N. H., M. L. Ranieri, S. C. Murphy, R. D. Ralyea, M. Wiedmann, and K. J. Boor. 2011. Results from raw milk microbiological tests do not predict the shelf-life performance of commercially pasteurized fluid milk. J. Dairy Sci. 94:1211-1222.

Martin, N. H., M. L. Ranieri, M. Wiedmann, and K.J. Boor. 2012. Reduction of pasteurization temperature leads to lower bacterial outgrowth in pasteurized fluid milk during refrigerated storage: A case study. J. Dairy Sci. 95:471-475.

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. 97:4083-4096.

McCarthy, K.S., K. Lopetcharat, and M.A. Drake. 2016. Milkfat threshold determination and the effect of milkfat content on consumer preference of fluid milk. J.Dairy Sci. Submitted.

Meer, R., J. Baker, F. Bodyfelt, and M. Griffiths. 1991. Psychrotrophic bacillus spp. in fluid milk products: A review. J. of Food Prot. 54:969-979.

Meilgaard, M., G.V. Civille, and B.T. Carr. 2007. Descriptive analysis techniques, Chapter 10. In: Sensory Evaluation Techniques, 4th ed. CRC Press, Boca Raton, FL/

Miller, R. A., D. J. Kent, K. J. Boor, N. H. Martin, and M. Wiedmann. 2015. Different management practices are associated with mesophilic and thermophilic spore levels in bulk tank milk. J. Dairy Sci. 98:4338-4351.

Muir, D. 1996. The shelf-life of dairy products: 1. Factors influencing raw milk and fresh products. J. of the Society of Dairy Tech. 49:24-32.

O'Connell, A., P. L. Ruegg, K. Jordan, B. O'Brien, and D. Gleeson. 2016. The effect of storage temperature and duration on the microbial quality of bulk tank milk. J. Dairy Sci. 99:3367-3374.

Ranieri, M. L., J. R. Huck, M. Sonnen, D.M. Barbano, and K. J. and Boor. 2009. High temperature, short time pasteurization temperatures inversely affect bacterial numbers during refrigerated storage of pasteurized fluid milk. J. Dairy Sci. 92:4823–4832.

Santos, M. V., Y. Ma, Z. Caplan, and D. M. Barbano. 2003a. Sensory threshold of off-flavors caused by proteolysis and lipolysis in milk. J. Dairy Sci. 86:1601-1607.

Santos, M. V., Y. Ma, and D. M. Barbano. 2003b. Effect of somatic cell count on proteolysis and lipolysis in pasteurized fluid milk during shelf-life storage. J. Dairy Sci. 86:2491-2503.

Shipe, W.F., R. Bassette, D.D. Deane, W.L. Dunkley, E.G. Hammond, W.J. Harper, D.H. Kleyn, M.E. Morgan, J.H. Nelson, and R.A. Scanlan. 1978. Off flavors of milk: Nomenclature, standards, and bibliography. J. Dairy Sci. 61:855-869.

USDA ERS. 2015. Food Availability Per Capita Data System. USDA, Economic Research Service. Accessed August 20, 2015. http://www.ers.usda.gov/data-products/dairy-data.aspx.

Wehr, H.M., and J.F. Frank. 2004. Chapter 6 Microbiological count methods, Chapter 7 Colfirm and other indicator bacteria, and Chapter 14 Alkaline phosphatase methods. Pages 153-226, 341-362 in Standard Methods for the Examination of Dairy Products. 17th ed. Am. Pub. Health Assoc. Washington, DC.

TABLES

Table 2.1 Consumer difference test results for pair-wise comparisons between cooling treatment (slow-cooled "SC" or quick-cooled "QC) or pasteurization temperature (73° or 78°C) at four days post-processing

	Replicate 1 (n=75)		Replicate 2 (n=77)		Replicate 3 (n=51)	
Pair-wise comparison	# Correct	Difference	# Correct	Difference	# Correct	Difference
QC-73°C vs QC-78°C	33	No	40	No	28	No
QC-73°C vs SC-73°C	36	No	37	No	28	No
SC-73°C vs SC-78°C	39	No	31	No	29	No
QC-78°C vs SC-78°C	40	No	42	No	20	No
Critical value ($p<0.05$)	***46***		***47***		***32***	

FIGURES

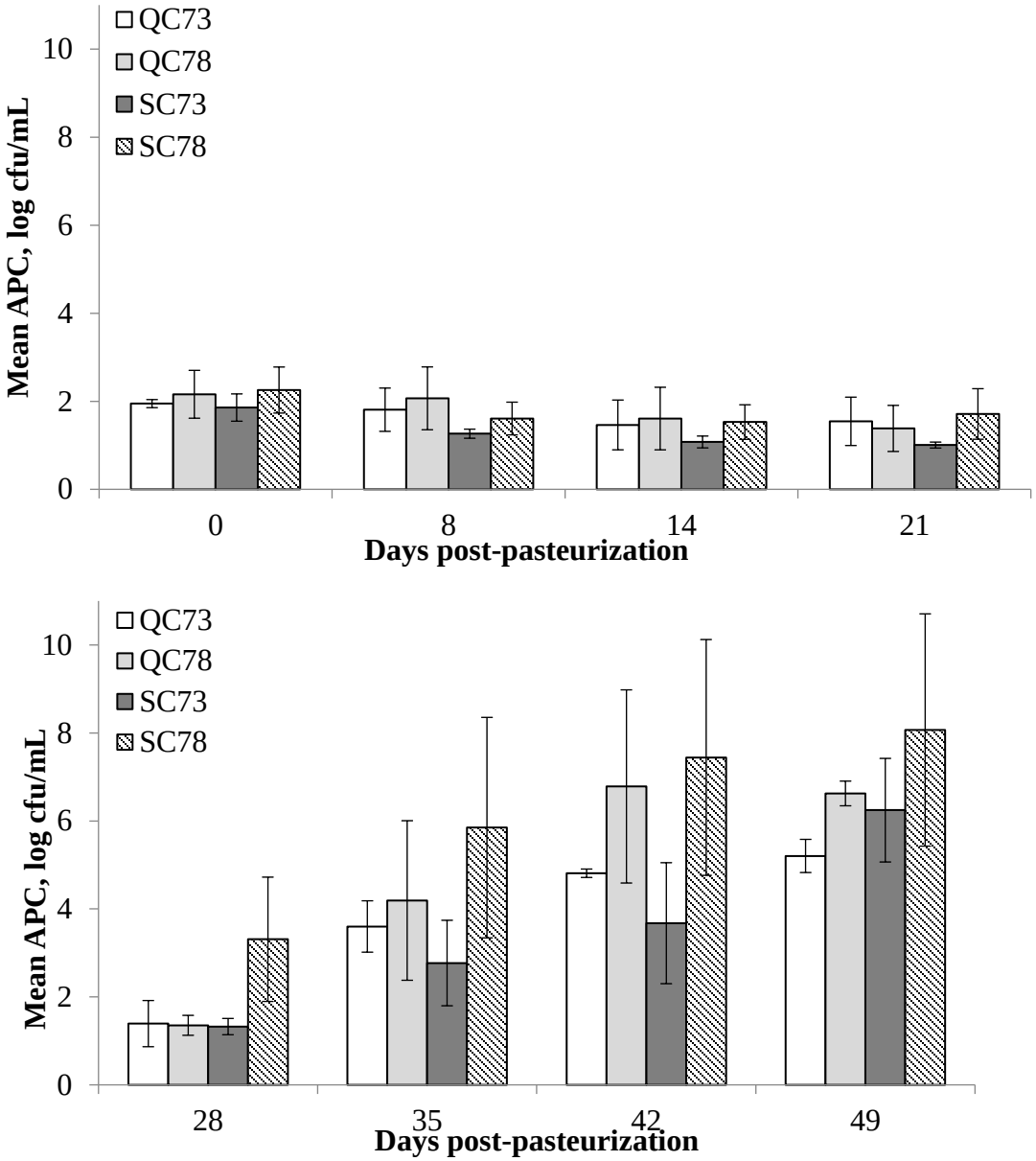


Figure 2.1 Aerobic plate count (APC) of quick-cooled (QC) and slow-cooled (SC) milk pasteurized at 73° or 78°C and stored at 4°C through shelf life. The data represent mean of 3 independent replicates. Bars represent the mean ± 1 SD for each treatment.

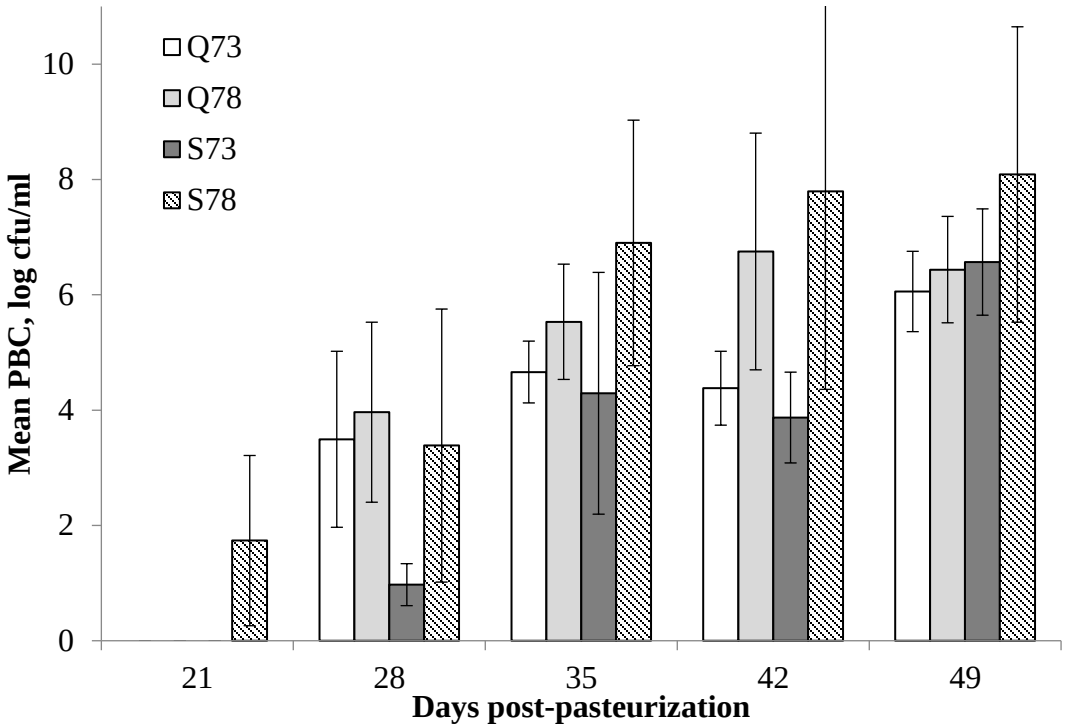


Figure 2.2 Psychrotrophic bacteria count (PBC) of quick-cooled (QC) and slow-cooled (SC) milk pasteurized at 73° or 78°C and stored at 4°C through shelf life. The data represent mean of 3 independent replicates. Bars represent the mean ± 1 SD for each treatment.

CHAPTER 3:

The influence of ultrapasteurization by indirect and direct steam injection processing on sensory perception of skim and 2% fat milks

The influence of ultrapasteurization by indirect and direct steam injection processing on sensory perception of skim and 2% fat milks

A.P. Lee[1], D.M. Barbano[2], M.A. Drake[1]

[1]Southeast Dairy Foods Research Center, North Carolina State University, Raleigh, NC 27695

[2]Northeast Dairy Research Center, Cornell University, Ithaca, NY 14850

The contents of this chapter will be published in:

Journal of Dairy Science

Interpretive Summary

Thermal processing of milk can affect the sensory characteristics and consumer acceptability of milk. To improve the shelf life of milk, ultra-pasteurization utilizes more severe heat treatment to destroy additional spoilage organisms. Two different methods of ultra-pasteurization – direct steam injection and indirect heating – were investigated for their effects on the sensory properties and consumer acceptance of milk and compared to traditional HTST milk. Instrumental analysis and trained panelists documented differences between the milks, but adult and child consumers did not have differences in liking between the ultra-pasteurized (**UP**) milks 10 days post-processing, preferring typical HTST milk over either UP milk for both skim and 2% fat milk.

ABSTRACT

Fluid milk is traditionally pasteurized by high temperature short time (**HTST**) pasteurization which requires heating to at least 72C for 15s. Ultrapasteurization (**UP**) extends milk shelf life and is defined as heating to at least 138°C for 2s. The UP process can be done by indirect heating (**IND**) or by direct steam injection (**DSI**). The influence of these two UP methods on milk flavor has not been widely investigated. The objective of this study was to compare the effect of HTST, IND-UP, and DSI-UP on sensory perception of fluid milk. Raw skim and standardized 2% milks were pasteurized at 140°C for 2.3s by IND or DSI or by HTST (78°C, 15s) and homogenized at 20.7 MPa. The processed milks were stored in light shielded opaque high-density polyethylene (**HDPE**) containers at 4°C and examined by descriptive analysis and microbial analysis on days 3, 7, and 14. Furosine and serum protein (SP) denaturation analyses were performed on day 0 and day 14 as an indicator of heat treatment. Lastly, consumer acceptance testing was conducted at day 10 with adults (n=250) and children (ages 8-13 y, n=100) who were self-reported consumers of skim or 2% milk; consumers only received samples for either skim or 2% milk. The entire experiment was repeated in triplicate. Milks treated by HTST had lower cooked flavor than either UP milk ($p<0.05$). Milks heated by DSI-UP were characterized by sulfur/eggy and cooked flavors while IND-UP milks had higher sweet aromatic and sweet taste compared to DSI-UP milk ($p<0.05$). Aromatic flavor intensities of all milks decreased across 14 d storage ($p<0.05$). Furosine concentrations and SP denaturation were highest for the IND treatments, followed by DSI, and lastly HTST ($p<0.05$). Adult and child consumers preferred HTST milk over either UP milk, regardless of fat content ($p<0.05$). Ultrapasteurization by IND or

DSI did not impact consumer acceptance 10 days post processing, but traditional HTST milks were preferred by consumers of all ages.

INTRODUCTION

Extended shelf life milks are a current industry trend to meet supply chain demands in the US. With per capita fluid milk consumption declining 35% over the past 40 years (USDA ERS, 2015), the implementation of non-conventional processing methods for milk are necessary to compete in the beverage industry. Typical US milks are processed by HTST pasteurization (minimum of 72°C for 15 sec), resulting in shelf lives of two to three weeks. This is compared to soft drinks and juices which can have 2-9 mo shelf life. Ultra-pasteurization (**UP**) is one method that can extend the shelf life of milk to periods comparable to soft drinks and juices. However, the extreme thermal treatment of this process can affect the sensory properties of milk, resulting in changes to consumer perception and acceptance of the milk compared to typical HTST milks. Ultra-pasteurization is defined by 21 CFR 131.3 as thermal processing of milk "at or above 138°C (280°F) for at least two (2) seconds" to produce a product for extended shelf life under refrigeration. The thermal treatment of the UP process destroys not only pathogenic bacteria, but also spoilage microorganisms which are not completely destroyed during conventional HTST pasteurization.

Direct heating systems for UP treatment utilize superheated steam that is applied directly to the product, either by injecting the steam in-line into the product (direct steam injection) or by allowing product to pass through a steam-filled chamber (steam infusion) (Bylund, 2003). The addition of steam to products necessitates the removal of water by vacuum, which also acts to instantaneously cool the product (Datta et al., 2002). Mehta (1980) also previously reported that the cooked flavor of direct heat treated milks was reduced due to removal of sulfhydryl groups by vacuum cooling. Direct methods also have

advantages over indirect heating by tubular or plate heat exchangers in that the direct contact with the heating medium followed by instant cooling by vacuum allows for more efficient heat transfer. This produces smaller areas under the curve in the time-temperature profile of the treated product, limiting product quality loss from excess heat exposure (Datta et al., 2002; Bylund, 2003). Direct heat transfer by steam also greatly limits burn-on and fouling as there are no heat transfer surfaces for the final heating step (Jelen, 1982).

Heat treatment, both time and temperature, impacts milk sensory properties. Previous studies have noted several sensory differences in milks treated by UP methods, including cooked flavor and aroma , caramelized flavor, sweet, bitter, astringency, and color differences. The sulfurous, eggy flavor in heated milk has been attributed to sulfhydryl compounds released from whey proteins, specifically beta-lactoglobulin, and proteins in the milk fat globule membrane due to thermal treatment (Mehta, 1980; Calvo and de la Hoz, 1992). Caramelized and other "brown" flavors are attributed to nonenzymatic browning reactions such as from protein or sugar breakdown or by Maillard reaction (Shipe et al., 1978; Calvo and de la Hoz, 1992). These flavors typically decrease over time (Deane et al., 1967; Shipe et al., 1978); however, flavor differences are still present in milks even after several weeks of storage (Chapman and Boor, 2004; Grabowski et al., 2013). These flavor differences contribute to consumer perception of the milks. Chapman and Boor (2004) reported that children ages 6 to 11 y preferred HTST milk 1 day post-processing over UHT milk at 24-30 days post-processing, which were both liked more than UP milk at 6-7 days post-processing. Gandy et al. (2008) reported consumer preference for HTST milks pasteurized at 79°C at 6 days post-processing compared to milks pasteurized at 77°, 82°, and 85°C. Consumer clusters were distinguished by liking or disliking of cooked flavor (Gandy et

al., 2008). In the previous studies, descriptive analysis was not conducted. Furthermore, no published research has directly addressed the sensory properties of UP milks processed by IND or DSI.

This study was designed to evaluate the sensory effects of these two UP techniques compared to traditional HTST milk. Two fat levels, skim and 2%, were also utilized. The objective of this study was to understand the role of heat treatment, ultra-pasteurization with either direct steam injection or indirect heating and HTST, and fat content on sensory perception and consumer acceptance.

MATERIALS AND METHODS

Sample Preparation

For this study, 200L of raw skim milk (3.1% protein, 0.07% fat) and raw cream (45.4% fat) were obtained from the North Carolina State University dairy facility. The cream was separated from raw whole milk by a cold bowl separator (Model 590, Separators Inc., Indianapolis, IN). 100L of the raw skim milk was standardized to 2% fat milk with the raw cream. A Microthermics EHVH pasteurization unit (Microthermics, Raleigh, NC) with a two-stage homogenizer (GEA Niro Soavi, Parma, Italy) was used to process the milks. For the HTST treatment, raw skim and raw 2% milk were preheated to 60°C, homogenized, and pasteurized at 78°C for 15 s before cooling to 10°C (Figure 1a). The IND-UP milk was processed by preheating the raw milks to 90°C then pasteurizing at 140°C for 2.3s by the indirect heater. The milk was cooled to 85°C before homogenization and then cooled by a second cooler to 10°C before packaging (Figure 1b). For the DSI-UP milk, raw milks were also preheated to 90°C, heated to 140°C for 2.3s by direct steam injection, then cooled to 85°C by vacuum cooling to remove both heat and added water. The DSI-UP milk was then

homogenized and cooled to 10°C (Figure 1b). All milks were homogenized at 20.7 MPa total pressure with 3.4 MPa on the second stage. Milks were packaged in half gallon, light shielded milk jugs (Upstate Niagara Cooperative, Inc., Buffalo, NY) and stored at 4°C. The order of processing was randomized between replicates to account for process order effects. The experiment was repeated in triplicate.

Microbiological and Proximate Analysis

All milks were tested for aerobic plate count (**APC**) (AOAC method 990.12) and coliform count (**CC**) (AOAC method 991.14) using Petrifilm plates (Aerobic Count Plates and Coliform Count Plates, 3M, St. Paul, MN). APC and CC plates were incubated at 32° ± 1°C for 24 and 48 h, respectively. Pasteurized milks were tested at 3, 7 and 14 days after processing. Samples were plated in triplicate. Proximate analyses for fat, protein, and solids were measured by a Fourier-transform mid-infrared milk analyzer (LactoScope FTIR; Delta Instruments BV, Drachten, the Netherlands). Total nitrogen (**TN**), non-casein nitrogen (**NCN**), non-protein nitrogen (**NPN**), and total protein content ((TN – NPN) x 6.38) was also evaluated by Kjeldahl analysis (AOAC method 991.22) (Barbano and Clark, 1990; Barbano and Lynch, 1991; Lynch and Barbano, 1998). Milk pasteurization was confirmed by alkaline phosphatase test (AOAC method 946.03) (Phos-Kit, Weber Scientific, Hamilton, NJ). Fat particle size was determined using a Mastersizer 3000 (Malvern Instrument Ltd., Worchestire, UK) for the 2% fat milks. Hunter L, a, and b values were measured at d 0 to determine whiteness, greenness to redness, and blueness to yellowness, respectively. Values were generated from the diffuse reflectance in the range of 360-750 nm at 10 nm intervals using illuminant A as described by Quinones et al. (1997, 1998) and Misawa et al. (2016).

Serum Protein Denaturation and Furosine Analysis

Pasteurized milks were examined for serum protein (**SP**) denaturation and furosine content as indicators of thermal treatment. The percentage of SP denaturation was calculated by dividing the difference between NCN and NPN content from Kjeldahl analysis by the SP content of the raw skim or 2% milks times 100%. Furosine analysis was conducted on the pasteurized milks at d 0 and 14. The analysis was conducted using reversed phase HPLC analysis according to the method by Resmini et al. (1990) with modifications. Briefly, milks were hydrolyzed by mixing 2 mL with 6 mL 10.6M HCl, followed by bubbling with nitrogen, then heating at 110°C for 23 h. A reversed-phase solid phase extraction microcolumn (Discovery DSC-18, Supelco, Bellefonte, PA) was conditioned with 3 mL methanol followed by 6 mL of HPLC grade water before extracting 0.5 mL of the milk hydrolysate with 3 mL of 3M HCl. Furosine was quantified by reversed phase HPLC (Breeze HPLC, Waters, Milford MA) with a 0.4% acetic acid solution mobile phase filtered through a 0.45 μm filter (Nylaflow, Pall Corporation, Port Washington, NY). 50 μL of the filtered hydrolysate was injected (Waters 2707 autosampler) onto the column (Luna 5μm C8 100A 250 x 4.6 mm, Phenomenex Inc., Torrance, CA) at a flow rate of 1.2 mL/min by a binary pump (Water 1525). A photodiode array detector (Waters 2998) was used to analyze the spectra at 280 nm. The run time for each sample was 10 min. Samples were evaluated in duplicate.

Descriptive Sensory Analysis

Sensory testing was conducted in accordance with the NCSU Institutional Review Board for the Protection of Human Subjects in Research regulations. A trained sensory panel documented sensory attributes of milk at d 3, 7, and 14. Each panelist (4 females, 2 males,

ages 21-55 y) had a minimum of 50 h of experience evaluating flavor and mouthfeel/texture attributes of foods using the Spectrum™ method (Meilgaard et al., 2007), and at least 40 h of previous experience with evaluation of fluid milk sensory properties using an established sensory language (Croissant et al., 2007; McCarthy et al., 2016). Milks (30 mL) were dispensed into lidded 59 mL soufflé cups (Dart Container Corp., Mason, MI) with random three digit blinding codes. Samples were prepared with overhead lights off to prevent light oxidation. Milks were tempered to 10°C and evaluated using paper ballots. Each panelist evaluated each milk in duplicate.

The trained panel also documented color, opacity, and viscosity attributes in separate sessions (McCarthy et al., 2016). Briefly, 80 mL of milk were dispensed into 100 mm x 10 mm clear, plastic petri dishes (Thermo Scientific, Waltham, MA) and placed onto a white paper background. Paint chips were used as references for yellow color (Behr "ultra pure white" PPU18-06 = 0 and "glass of milk" P260-1u = 3.5). For opacity, milks were dispensed into 118 mL black soufflé cups with random three digit blinding codes. Water was used as a reference of 0 and whole fat, HTST milk was assigned a 12 for opacity. Viscosity was determined by determining the amount of force needed to slurp 1 tsp of milk from a spoon. The references for viscosity were water = 0 and heavy cream = 3.2 (Meilgaard et al., 2007).

Consumer Acceptance Testing

Consumer acceptance testing was conducted in accordance with the NCSU Institutional Review Board for the Protection of Human Subjects in Research regulations. Testing was conducted ten days after processing. Self-reported milk consumers were recruited using a survey constructed in SSI Web (version 8.4.8, Orem, UT) launched into a database of >7500 consumers maintained by NCSU. Children were recruited by parental

consent. Consumers were recruited based on milk fat content most often consumed for a minimum of 100 adult consumers and 50 child consumers per each milkfat treatment. Skim milk drinkers were presented the three skim milk treatments and low-fat milk drinkers received the 2% fat milks. Milks (88 mL) were served at 10°C in 177 mL Styrofoam cups (Dart Container Corp.) with random three digit blinding codes. Consumers were presented samples monadically using a Williams design serving order. Adult consumers were asked to evaluate appearance, aroma, overall liking, flavor, freshness, cooked flavor, mouthfeel/thickness/viscosity, and aftertaste on a 9-point hedonic scale where 1 was "Dislike Extremely" and 9 is "Like Extremely". Just About Right (**JAR**) questions were asked for sample flavor, mouthfeel/thickness/viscosity, and aftertaste on a 5-point scale where 1 was "Not nearly enough flavor", "Not nearly thick enough", or "Much too mild" and 5 was "Much too much flavor", "Much too thick", or "Much too strong", for each question category, respectively. Children received a modified ballot which asked questions in the same categories as the adult ballot. A 7-point, "smiley face" hedonic scale was used for children where 1 was "Super Bad" and 7 was "Super Good". For mouthfeel/thickness/viscosity, children were asked a JAR question where 1 was "Too thin, needs to be thicker", 2 was JAR, and 3 was "Too thick, needs to be thinner" (Li et al., 2015). There was a two minute enforced rest between milks to allow panelists to cleanse their palate using unsalted crackers and deionized water. A forced choice preference was asked after all milks were tasted. Panelists were compensated with a $20 (adult) or $30 (children) gift card. Compusense Cloud software (ver 7.6, Compusense Inc., Guelph, Canada) was used for data collection.

Statistical Analyses

The study was conducted using a split plot design where the whole plot factor was fat content and the subplot factor was pasteurization treatment (HTST, IND-UP, or DSI-UP). Least squares means for pasteurization treatment effects within each fat content across time points were determined using the MIXED procedure in SAS 9.4 (SAS Institute, Cary, NC) for microbial, furosine, and trained panel profiles. Proximate analysis, instrumental color, serum protein denaturation, and consumer acceptance data were analyzed by ANOVA with means separation within each fat content (Fischer's least significant difference) using XLSTAT (Version 2015.5.01, Addinsoft, New York, NY). Just about right (JAR) scores were evaluated using chi-squared tests. All statistical analyses were performed at 0.05 significance.

RESULTS AND DISCUSSION

Raw milk aerobic plate counts averaged 2.63 ± 2.26 log cfu/mL and 2.09 ± 1.98 log cfu/mL coliforms. HTST skim and 2% milks at d 3 averaged <20 cfu/mL aerobic plate count, while the UP treatments did not have any microbial counts. At d 7, all samples had <20 cfu/mL growth. At d 14, the HTST samples averaged 1.54 log cfu/mL and the UP milks had <20 cfu/mL microbial count. No coliforms were detected at any time point. Skim milk composition averaged 0.068% fat, 4.72% lactose, 3.11% protein, and 8.95% solids. Two percent fat milks averaged 1.96% fat, 4.63% lactose, 3.06% protein, and 10.71% solids. The D90 (the maximum diameter at which 90% of the particles are below) for milk fat particle size was <1.5 μm for all milks, indicating appropriate homogenization. Milkfat particle sizes were not different ($p>0.05$) between milks (data not shown). Milk composition was not different between treatments in each fat level ($p>0.05$), indicating that added water from the

direct steam injection system was completely removed. All samples were negative for alkaline phosphatase, indicating complete pasteurization.

Hunter L, a, b values showed differences ($p<0.05$) among the milks initially after processing (Table 1). Hunter L-value (whiteness) for skim milks was different for each treatment, IND-UP > DSI-UP > HTST ($p<0.05$). The L-values were at parity for the UP 2% milks and were higher than for HTST 2% milk. The values for the 2% milks were higher than those for skim due to the presence of fat. Hunter a-value (greenness to redness) for IND-UP skim milk was significantly greater than DSI-UP and HTST milks ($p<0.05$), which were at parity; a-values for UP 2% milk were at parity ($p>0.05$) and were greater than HTST milk. Lastly, Hunter b-values (blueness to yellowness) were significantly different for each skim milk (IND-UP > DSI-UP > HTST), but only IND-UP was significantly greater among the 2% milks. Hunter a and b-values were greater for 2% milk than skim milk as skim milk is more noticeably blue, which is a combination of the green and yellow color measured by Hunter a and b-values. Misawa et al. (2016) reported that the casein percentage of the total protein was strongly correlated to the Hunter L, a, b values. After heat treatment, serum proteins are denatured, decreasing NCN, which in turn increases the casein percentage of the total protein. Serum protein (**SP**) denaturation was greatest in the IND-UP skim milk (Table 2); thus, it is likely that the L, a, and b values were affected by SP denaturation. SP denaturation was not different between the UP 2% milks, so it is likely that the lack of differences in the L and a-values can also be explained by this. Appearance of milk (i.e. the whiteness and opacity) can affect the perception and overall liking of milks by consumers (Quinones et al., 1997; Palacios et al., 2009; McCarthy et al., 2016). Thus, appearance

differences between fat contents and between heat treatments within a fat content can directly influence consumer acceptance.

Heat Indicators

Serum protein denaturation indicated that HTST pasteurized milks had less ($p<0.05$) denaturation than the UP milks within each fat content, as expected (Table 3.2). Denaturation for HTST milks ranged from 15.98% in skim milk to 23.95% in 2% fat milk. The IND-UP skim milk had higher ($p<0.05$) SP denaturation than the DSI-UP skim milk (71.26% vs 67.89%); however, percent denaturation was not different between the UP milks at 2% milkfat (73.87% vs 72.27%). Furosine content did not differ ($p>0.05$) between skim and 2% milks treated by a specific pasteurization method nor between 0 and 14 d ($p>0.05$). IND-UP milks had the highest content of furosine at 168.72 mg/100g protein on average followed by DSI-UP milks at 43.81 mg/100g protein ($p<0.05$); HTST milks averaged 6.95 mg/100g protein. These furosine results are consistent with those found by Elliot et al. (2005).

The results from the heat indicator analyses indicate that both UP treatments received significantly more thermal load than the HTST treatment with IND-UP being the more severe of the two UP treatments. Others have reported that these indicators are dependent on degree, severity, and type of heat treatment (Pellegrino et al., 1995; Cho et al., 2012; Sakkas et al., 2014). Datta et al. (2002) reported that heat indices (lactulose, furosine, β-lactoglobulin, 2-hydroxymethyl-2-furfural) for commercially produced, direct heated ultra-high temperature (UHT) milks typically showed less severe heat treatment compared to indirectly heated UHT milks. Elliot et al. (2005) also reported that commercially produced UHT milk samples by direct heating methods had less denatured serum proteins, specifically α-lactalbumin, β-lactoglobulin, and bovine serum albumin. Storage time did not affect

furosine content until after 4 weeks of storage at room temperature for UHT milks (Elliot et al., 2005). Others (Cho et al., 2012; Smith et al., 2016) have reported changes in furosine content over time, but low temperature storage retards the increase. The milks were stored at 4°C for the duration of this study, so changes in furosine concentration were not expected.

Descriptive Analysis

Descriptive analysis was conducted on the milks at 3, 7 and 14 days post processing. Milks were distinguished by heat treatment at all timepoints ($p<0.05$) (Figure 3.2a, 3.2b), and these differences were consistent across skim and 2% fat milks. UP milks of either fat content had higher aroma intensity, cooked flavor and astringency than HTST milk ($p<0.05$). The UP milks within a fat content were also distinct from each other ($p<0.05$). IND milks had higher sweet aromatic flavor and lower sulfur/eggy flavor than DSI milks at day 3 and 7 ($p<0.05$). At day 14, sulfur/eggy flavor was not detected in IND milks of either fat content but remained at low intensity (1.0 + 0.2) on a 0 to 15-point scale in DSI milks. Aroma intensity and cooked and sulfur/eggy flavors decreased in UP milks of either fat content across 14 days storage ($p<0.05$), while sweet aromatic and cooked flavors decreased across 14 days storage for HTST milks ($p<0.05$).

The sulfur compounds contributing to cooked and sulfur/eggy flavor and aroma in milk have been associated with the denaturation of whey and milk fat globule membrane proteins (Mehta, 1980; Calvo and de la Hoz, 1992). Hydrogen sulfide, dimethyl sulfide, and methanethiol have been suggested as major sulfur containing volatiles contributing to cooked flavor (Blankenagel and Humbert, 1964; Vazquez-Landaverde et al., 2005; Vazquez-Landaverde et al., 2006). The distinct difference in the sensory profile of IND and DSI milks across both fat contents and through 14 d post processing has not been previously

reported.The difference in sulfur/eggy flavor between IND and DSI-UP milks suggests that additional sulfur volatiles are being generated during the DSI-UP process, perhaps due to the temperature difference between the milk and steam. Future studies should characterize volatile compound differences between UP milks from these two heating processes and should explore the mechanisms of sulfur volatile compound formation in these processes. The decrease in sweet aromatic and sulfur/eggy flavor and aroma during storage is consistent with previous studies which examined milk from several days to several months (Shipe et al., 1978; Chapman et al., 2001; Clare et al., 2005) and may be due to oxidation of sulfhydryl groups and lipid oxidation (Thomas et al., 1975) or by diffusion of volatiles through the packaging (Simon and Hansen, 2001; Zabbia et al., 2012). Future studies should address sensory changes beyond 14 days to account for the 60 day shelf life of UP milks.

Astringency is another attribute of milks that is affected by heat treatment. Whey or serum proteins denatured by heat and aggregated are suggested as one possible mechanism for the astringent mouthfeel of dairy products (Lemieux and Simard, 1994). As previously stated, serum protein denaturation was greatest in ultra-pasteurized milks. These UP milks had the greatest astringency, suggesting that the heat treatment directly impacted this sensory property. Cooked flavors, astringency, and other flavors not commonly associated with typical HTST pasteurized milk may be seen as flavor defects for US consumers (Liem et al., 2016). Palacios et al. (2009) reported that consumers typically preferred light or “medium-impact” products when evaluating skim and 2% milks and milk-substitute beverages. Therefore, focus should be given to understanding what compounds are inducing the various sensory attributes of milk as well as how processing conditions can affect their formation.

Trained panelists did not document differences in color or opacity of the milks within a fat content ($p>0.05$) (results not shown), but differences ($p<0.05$) were noted in viscosity between the HTST skim milk and both the IND-UP and DSI-UP skim milks (1.63 vs 1.85 and 1.80, respectively on a 0 to 15-point scale) and between the HTST 2% and IND-UP 2% milks (1.98 vs 2.20, respectively on a 0 to 15-point scale) ($p<0.05$). Chapman et al. (2001) also reported that viscosity of UP skim milks were approximately equal to the viscosity of HTST 1% fat milks. The differences in viscosity may be attributed to gelation or denaturation of proteins after UP heat treatment. From a consumer standpoint, viscosity can influence perception of fat in the product. McCarthy et al. (2016) reported that consumers typically preferred higher fat content milks than the one they stated they consumed most often. This means that milk which can imitate higher fat content may be more accepted by consumers. Additionally, appearance has been suggested as a more important factor than physical properties like viscosity with regard to fat content perception. With visual cues removed, only 46% of panelists were able to correctly determine which sample was different between skim and 2% milks (Phillips et al., 1995). This is consistent with the 4.4% milkfat threshold with no visual cues reported by McCarthy et al. (2016). Although the trained panel did not note visual differences in milk, previous studies have reported visual differences in the appearance of milks depending on the process used (Datta et al., 2002; Clare et al., 2005). These color changes in fluid milks have been attributed to browning by Maillard reactions. It is possible that differences would be noted between UP and HTST milks if milks were evaluated further into shelf life. Darker colored milks have been reported to decrease consumer liking of the product (Palacios et al., 2009), thus color is another consideration for processors when deciding the type of thermal treatment and fat content of fluid milks.

Adult and Child Consumer Acceptance of UP Milks

Adult skim (n=110) and 2% milkfat (n=135) consumers evaluated the milks after 10 days of storage. HTST milks were more liked than either UP treatments for both the skim and 2% treatments ($p<0.05$) (Table 3). IND-UP and DSI-UP were at parity for all liking attributes tested ($p>0.05$); however, consumers indicated that the DSI-UP skim milk was less preferred than the IND-UP skim milk in forced choice preference (16.4% vs 28.2%) ($p<0.05$). Both UP milks at both fat levels had significant penalties to overall liking due to the milk having "Too much flavor" and UP skim milks also received penalties due to having "Too thick" mouthfeel ($p<0.05$). Results from the children's testing revealed similar results (Table 4). Child skim milk (n=51) and 2% milkfat (n=54) consumers also preferred the HTST treatments over either UP treatments for skim and 2% milks ($p<0.05$). Aroma liking for DSI-UP 2% fat milk was significantly lower than for IND-UP 2% milk ($p<0.05$), but there were no other differences in liking among the attributes tested between IND-UP and DSI-UP milks at either fat level (results not shown) ($p>0.05$). In contrast with the adult consumer testing results, UP 2% milks had significant penalties from children to overall liking due to "Too thin" mouthfeel (results not shown) ($p<0.05$).

As previously mentioned, US milk consumers typically prefer light or medium milk flavor intensity (Palacios et al., 2009). This study agrees with these findings; the UP milks in this study were consistently penalized by adult consumers for having "Too much flavor". Based on trained panel profiles, high cooked flavor and sulfur/eggy flavor are the flavors that are not liked by consumers. Gandy et al. (2008) had also reported that some consumer clusters could be distinguished by bias towards or against cooked flavor in milk. This bias can be dependent on the type of milk most commonly consumed. Liem et al. (2016) reported

that consumers from China, where 60% of the milk consumed is UHT milk, preferred the flavor of UHT milk while consumers from Australia, where only 10% of milk consumed is UHT, preferred HTST milk. Chapman and Boor (2001) reported that children 6 to 11 y preferred HTST milk over UP and UHT milks, likely due to the cooked flavor differences compared to traditional HTST milks. These results support the direct impact of heat treatment on consumer acceptance of milks.

CONCLUSIONS

Heat treatment affects sensory properties and consumer acceptance of milk. Trained panelists were able to distinguish between the HTST and UP milks based on cooked flavor. Presence of sulfur/eggy flavor and aroma distinguished DSI-UP from IND-UP milk. Flavor intensities decreased with storage time, but HTST, IND-UP, and DSI-UP milks remained distinct at d 14 for both fat contents. UP milks exhibited greater thermal treatment by furosine and SP denaturation than the HTST milks. Color differences were also present by instrumental analysis at d 3, but the trained panel did not detect visual differences at any time point through d 14. Adult and child milk consumers indicated significant preference for HTST milk over either UP milk for skim and 2% fat milks, with no differences in liking between the UP milks at either fat content; however, IND-UP skim was preferred over DSI-UP skim by adults. These results indicate that consumers have preferences for certain sensory characteristics of milk, characteristics that are influenced by both heat treatment and fat content. Thus, processors must take the effects of these parameters into account when producing fluid milk to meet consumer expectations and to maximize acceptance.

ACKNOWLEDGMENTS

This project was supported in part by the National Dairy Council (Rosemont, IL.)

REFERENCES

Barbano, D.M. and J.L. Clark. 1990. Kjeldahl method for determination of total nitrogen content of milk: Collaborative study. J. Assoc. Off. Anal. Chem. 73:849-859.

Barbano, D.M. and J.M. Lynch. 1991. Direct and indirect determination of true protein content of milk by Kjeldahl analysis: Collaborative study. J. Assoc. Off. Anal. Chem. 74:281-288.

Blankenagel, G., and E. S. Humbert. 1963. Sulfhydryl groups and cooked flavor in ultra-high temperature processed skim milk. J. Dairy Sci. 46:614.

Bylund, G. 2003. Dairy processing handbook. Lund, Sweden. Tetra Pak Processing Systems AB.

Calvo, M. and de la Hoz, L. 1992. Flavour of Heated Milks. A Review. Int. Dairy J. 69-81.

Chapman, K., and Boor, K. 2001. Acceptance of 2% ultra-pasteurized milk by consumers, 6 to 11 years old. J. Dairy Sci. 84:951-954.

Chapman, K., Lawless, H., and Boor, K. 2001. Quantitative descriptive analysis and principal component analysis for sensory characterization of ultrapasteurized milk. J. Dairy Sci. 84:12-20.

Cho, Y.H., S.M. Hong, and C.H. Kim. 2012. Determination of lactulose and furosine formation in heated milk as a milk quality indicator. Korean J. Food. Sci. An. 32:540-544.

Clare, D.A., W.S. Bang, G. Cartwright, M.A. Drake, P. Coronel, and J. Simunovic. 2005. Comparison of sensory, microbiological, and biochemical parameters of microwave versus indirect UHT fluid milk skim milk during storage. J. Dairy Sci. 88:4172-4182.

Croissant, A., Washburn, S., Dean, L., and Drake, M. 2007. Chemical properties and consumer perception of fluid milk from conventional and pasture-based production systems. J. Dairy Sci. 90:4942-4953.

Datta, N., A. Elliott, M. Perkins, and H. Deeth. 2002. Ultra-high-temperature UHT treatment of milk: Comparison of direct and indirect modes of heating. Aust. J. Dairy Technol. 211-227.

Deane, D., Chelesvig, J., and Thomas, W. 1967. Pasteurization treatment and consumer acceptance of milk. J. Dairy Sci. 50:1216-1220.

Drake, M.A. 2007. Invited review: Sensory analysis of dairy foods. J. Dairy Sci. 90:4925-4937.

Elliot, A.J., N. Datta, B. Amenu, and H.C. Deeth. 2005. Heat-induced and other chemical changes in commercial UHT milks. J. Dairy Res. 72:442-446.

FDA. 2015. 21 CFR 131.3. Accessed May 9, 2016. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.3

Gandy, A., Schilling, M., Coggins, P., White, C., Yoon, Y., and Kamadia, V. 2008. The effect of pasteurization temperature on consumer acceptability, sensory characteristics, volatile compound composition, and shelf-life of fluid milk. J. Dairy Sci. 91:1769-1777.

Jelen, P. 1982. Experience with direct and indirect UHT processing of milk - A Canadian Viewpoint. J. Food Prot. 45:878-883.

Lemieux, L., and R.E. Simard. 1994. Astringency, a textural defect in dairy products. Lait. 74:217-240.

Li, X.E., Lopetcharat, K., Qiu, Y., and Drake, M.A. 2015. Sugar reduction of skim chocolate milk and viability of alternative sweetening through lactose hydrolysis. J. Dairy Sci. 98:1455-1466.

Liem, D.G., D.P. Bolhuis, X. Hu, and R.S.J. Keast. 2016. Short communication: Influence of labeling on Australian and Chinese consumers' liking of milk with short (pasteurized) and long (UHT) shelf life. J. Dairy. Sci. 99:1747-1754.

Lynch, J.M. and D.M. Barbano. 1998. Indirect and direct determination of the casein content of milk by Kjeldahl nitrogen analysis: Collaborative study. J. AOAC Int. 81:763-774.

McCarthy, K.S., K. Lopetcharat, and M.A. Drake. 2016. Milkfat threshold determination and the effect of milkfat content on consumer preference of fluid milk. J. Dairy Sci. Submitted.

Mehta, R. 1980. Milk processed at ultra-high-temperatures - A Review. J. Food Prot. 212-225.

Meilgaard, M., G.V. Civille, B.T. Carr. 2007. Descriptive analysis techniques, Chapter 10. Pages 173-186 in: Sensory Evaluation Techniques, 4th ed. Boca Raton, Fla.: CRC Press.

Misawa, N., D.M. Barbano, and M.A. Drake. 2016. Influence of casein as a percentage of true protein and protein level on color and texture of milks containing 1 and 2% fat. J. Dairy Sci. 99:5284-5304.

Palacios, O.M., J. Bardran, M.A. Drake, M. Reisner, and H.R. Moskowitz. 2009. Consumer acceptance of cow's milk versus soy beverages: Impact of ethnicity, lactose tolerance and sensory preference segmentation. J. Sens. Stud. 24:731-748.

Pellegrino L., I. Noni, and P. Resmini. 1995. Coupling of lactulose and furosine indices for quality evaluation of sterilized milk. Int. Dairy J. 5:647–659.

Phillips, L.G., M.L. McGiff, D.M. Barbano, and H.T. Lawless. 1995. The influence of fat on the sensory properties, viscosity, and color of lowfat milk. J. Dairy Sci. 78:1258-1266.

Quinones, H.J., D.M. Barbano, and L.G. Phillips. 1997. Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of skim and 1% milk. J. Dairy Sci. 80:3142-3151.

Quinones, H.J., D.M. Barbano, and L.G. Phillips. 1998. Influence of protein standardization by ultrafiltration on the viscosity, color, and sensory properties of 2 and 3.3% milks. J. Dairy Sci. 81:884-894.

Rauh, V.M., L.B. Johansen, M. Bakman, R. Ipsen, M. Paulsson, L.B. Larsen, and M. Hammershoj. 2015. Protein lactosylation in UHT milk during storage measured by liquid chromatography-mass spectrometry and quanitification of furosine. Int. J. Dairy Technol. 68:486-494.

Resmini, P., Pellegrino, L., and Batteli, G. 1990. Accurate quantification of furosine in milk and dairy products by a direct HPLC method. Italian J. Food Sci. 173-183.

Sakkas, L., A. Moutafi, E. Moschopoulou, and G. Moatsou. 2014. Assessment of heat treatment of various types of milk. Food Chem. 159:293-301.

Shipe, W.F., Bassette, R., Deane, D.D., Dunkley, W.L., Hammond, E.G., Harper, W.J., Kleyn, D.H., Morgan, M.E., Nelson, J.H., Scanlan, R.A. 1978. Off Flavors of Milk: Nomenclature, Standards, and Bibliography. J. Dairy Sci. 61:855-869.

Simon, M. and A. Hansen. 2001. Effect of various dairy packaging materials on the shelf life and flavor of ultrapasteurized milk. J. Dairy Sci. 84:784-791.

Smith, T.J., R.E. Campbell, Y. Jo, and M.A. Drake. 2016. Flavor and stability of milk proteins. J. Dairy Sci. 99:4325-4346.

Thomas, E.L., H. Burton, J.E. Ford, and A.G. Perkin. 1975. The effect of oxygen content on flavor and chemical changes during asceptic storage of whole milk after ultra-high-temperature processing. J. Dairy Res. 42:285–295.

USDA ERS. 2015. Food Availability Per Capita Data System. USDA, Economic Research Service. Accessed August 20, 2015. http://www.ers.usda.gov/data-products/dairy-data.aspx.

Vazquez-Landaverde, P.A., Velazquez, G., Torres, J.A., and Qian, M.C. 2005. Quantitative determination of thermally derived off-flavor compounds in milk using solid-phase microextraction and gas chromatography. J. Dairy Sci. 88:3764–3772.

Vazquez-Landaverde, P.A., Torres, J.A., and Qian, M.C. (2006). Quantification of trace volatile sulfur compounds in milk by solid-phase microextraction and gas chromatography-pulsed flame photometric detection. J. Dairy Sci. 89: 2919–2927.

Zabbia, A., E.M. Buys, and H.L. de Kock. 2012. Undesirable sulfur and carbonyl flavor compounds in UHT milk: A review. Crit. Rev. Food Sci. Nut. 52:21-30.

TABLES

Table 3.1 Hunter L, a, and b values for skim and 2% fat milks at day 3 after processing

	Skim milk		
Treatment	L	a	b
Raw	75.00[c]	-5.85[b]	2.40[c]
HTST	75.57[c]	-6.02[b]	2.66[c]
IND-UP	79.60[a]	-4.11[a]	6.28[a]
DSI-UP	77.53[b]	-5.82[b]	3.65[b]
	2% fat milk		
Treatment	L	a	b
Raw	78.29[c]	-3.76[b]	5.63[b]
HTST	81.91[b]	-3.12[a]	6.00[b]
IND-UP	82.98[a]	-2.93[a]	6.96[a]
DSI-UP	82.69[a]	-3.54[b]	6.13[b]

Different letters in columns following means signify significant differences ($p<0.05$)

Table 3.2 Serum protein denaturation and furosine concentration of skim and 2% fat milks processed by HTST pasteurization or UP

	Skim milk		
Treatment	Serum protein denaturation (%)	Furosine concentration (mg/100g protein)	pH
Raw	0	*	6.78[a]
HTST	15.98[c]	7.07[c]	6.78[a]
IND-UP	71.26[a]	169.04[a]	6.73[a]
DSI-UP	67.89[b]	42.49[b]	6.83[a]
	2% fat milk		
Treatment	Serum protein denaturation (%)	Furosine concentration (mg/100g protein)	pH
Raw	0	*	6.78[a]
HTST	23.95[b]	6.83[c]	6.78[a]
IND-UP	73.87[a]	168.41[a]	6.74[a]
DSI-UP	72.27[a]	45.12[b]	6.83[a]

*Furosine concentration not measured for raw milk samples
Different letters in columns following means signify significant differences ($p<0.05$)

Table 3.3 Adult consumer acceptance results for skim and 2% milks treated by HTST pasteurization or UP

	Skim milk (n=110)		
Treatment	Overall liking	Flavor liking	Preference[1]
HTST	7.0[a]	6.9[a]	55.5%[a]
IND-UP	6.2[b]	6.2[b]	28.2%[b]
DSI-UP	6.0[b]	5.8[b]	16.4%[c]
	2% fat milk (n=135)		
Treatment	Overall liking	Flavor liking	Preference[1]
HTST	7.1[a]	7.1[a]	51.1%[a]
IND-UP	6.1[b]	6.0[b]	21.5%[b]
DSI-UP	6.3[b]	6.2[b]	27.4%[b]

Liking attributes were scored on a 9-point hedonic scale where 1 = dislike extremely and 9 = like extremely. Different letters in columns following means signify significant differences ($p<0.05$)

Table 3.4 Child consumer acceptance results for skim and 2% milks treated by HTST pasteurization or UP

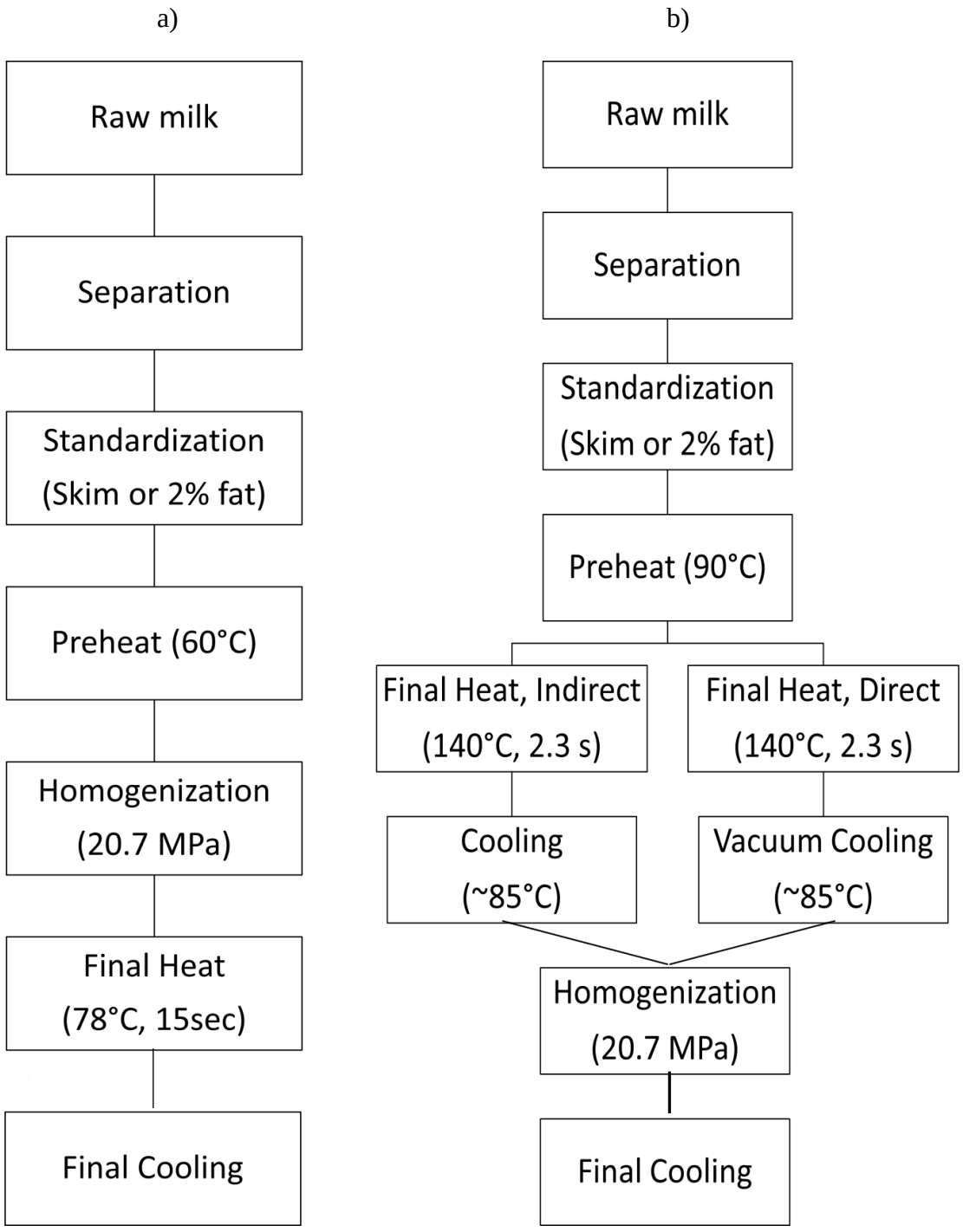
	Skim milk (n=51)		
Treatment	Overall liking	Flavor liking	Preference[1]
HTST	5.5[a]	5.4[a]	58.8%[a]
IND-UP	4.6[b]	4.5[b]	25.5%[b]
DSI-UP	4.4[b]	4.3[b]	15.7%[b]
	2% fat milk (n=54)		
Treatment	Overall liking	Flavor liking	Preference[1]
HTST	5.6[a]	5.6[a]	63.0%[a]
IND-UP	4.7[b]	4.6[b]	20.4%[b]
DSI-UP	4.5[b]	4.4[b]	16.7%[b]

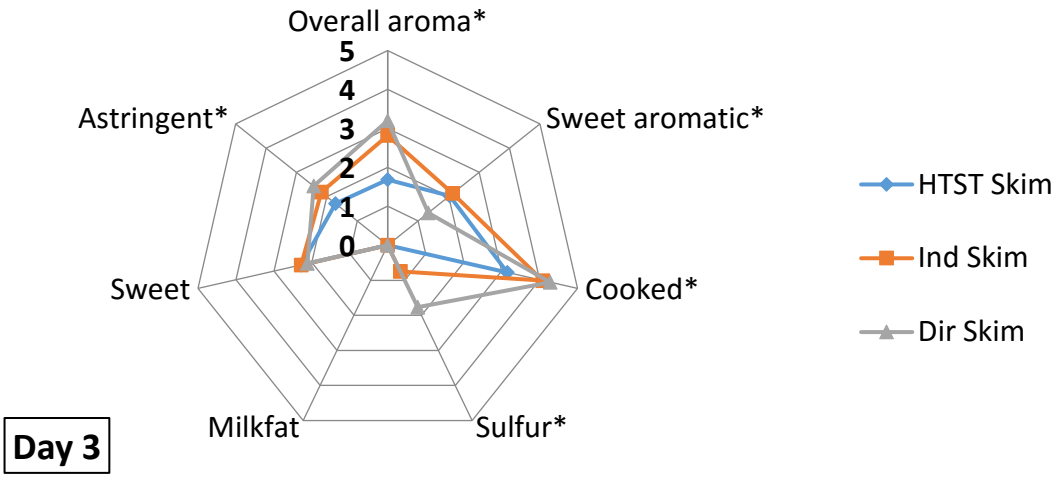
Liking attributes were scored on a 7-point hedonic scale where 1 = "Super bad" and 7 = "Super good". Different letters in rows following means signify significant differences ($p<0.05$)

[1]Preference is represented as the percentage of consumers who chose a particular sample

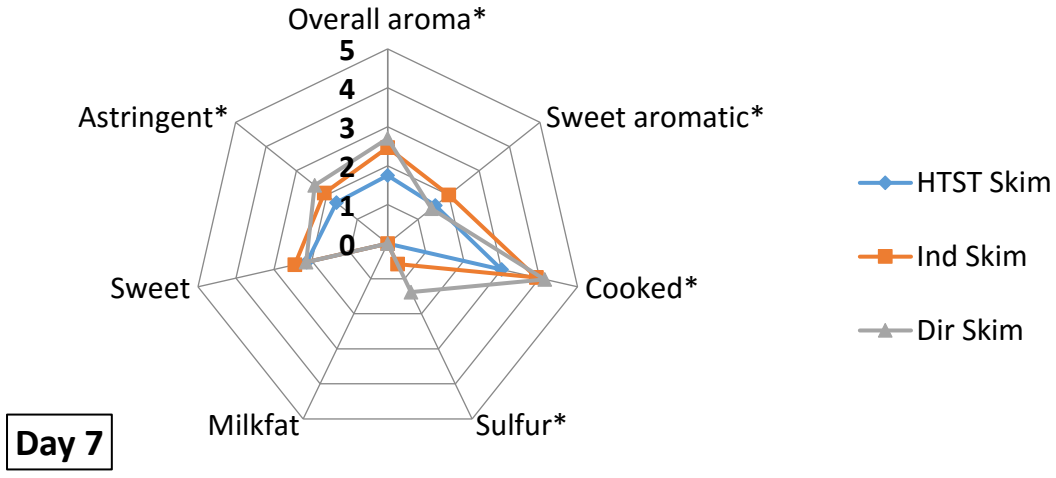
FIGURES

Figure 3.1 Process flow diagram for a) high temperature, short time and b) ultra-pasteurization

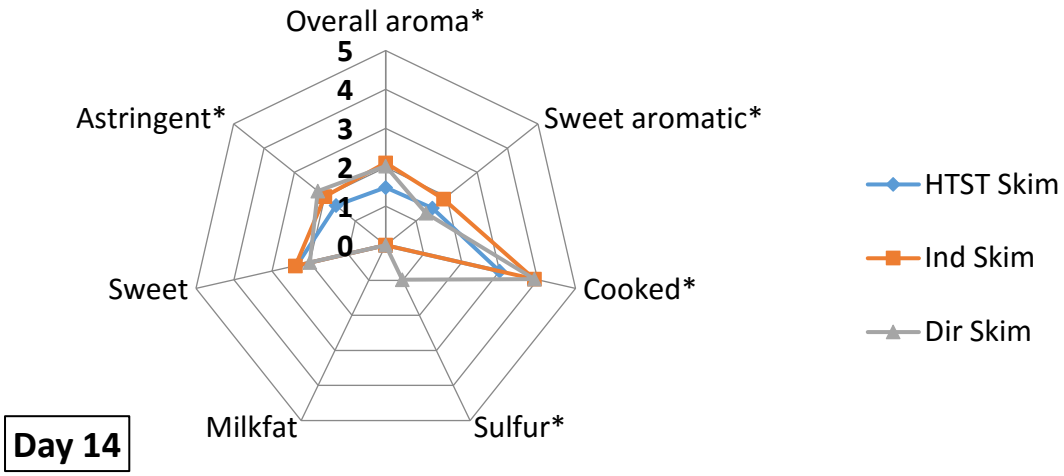



Overall aroma*
5
4
3
2
1
0
Sweet aromatic*
Cooked*
Sulfur*
Milkfat
Sweet
Astringent*
HTST Skim
Ind Skim
Dir Skim

Day 3

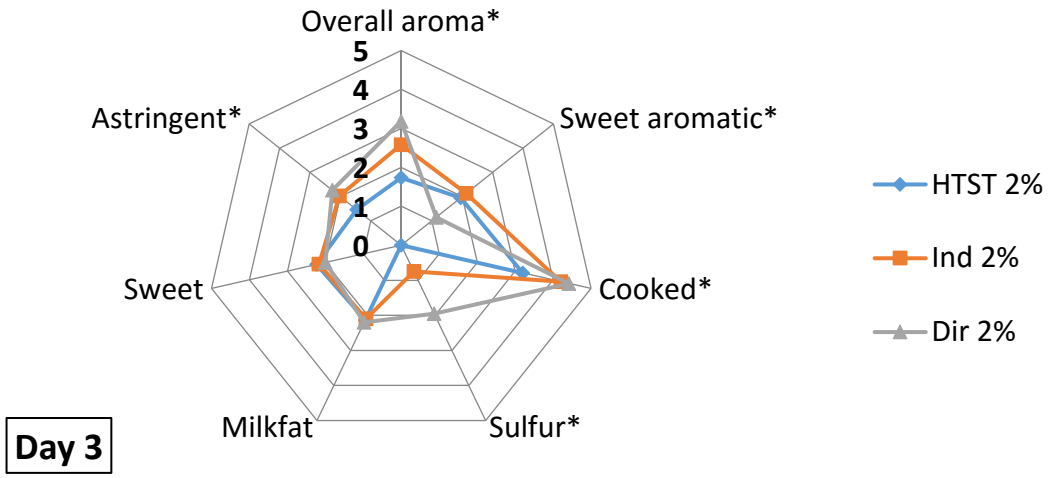

Overall aroma*
5
4
3
2
1
0
Sweet aromatic*
Cooked*
Sulfur*
Milkfat
Sweet
Astringent*
HTST Skim
Ind Skim
Dir Skim

Day 7

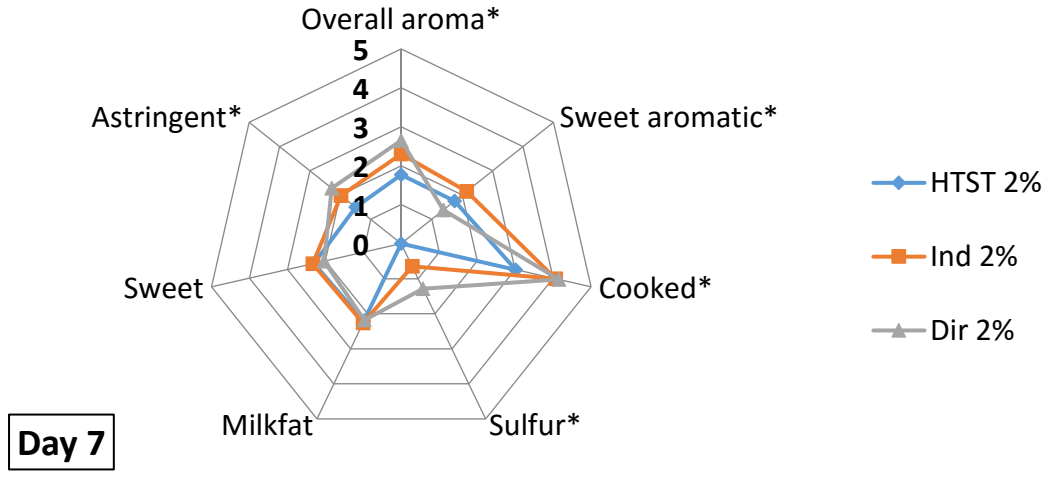


*indicates differences ($p<0.05$) for that attribute at that timepoint
Attribute intensities were scored on a 0 to 15 point universal scale. Fluid milk flavors fall between 0 and 4 on this scale (Drake, 2007; Croissant et al., 2007; McCarthy et al., 2016).

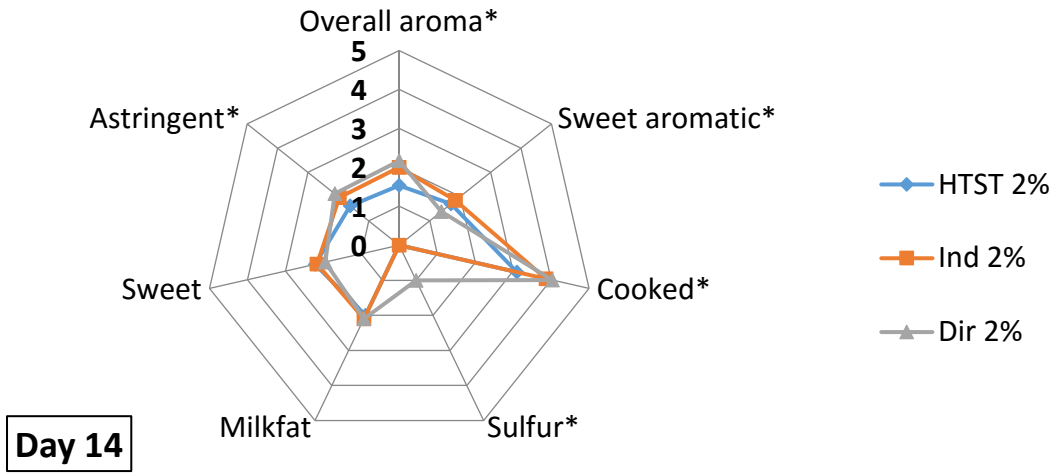
Figure 3.2a. Descriptive analysis results for skim HTST and UP milks at d 3, 7, and 14 post-processing


Overall aroma*
5
4
3
2
1
0
Sweet aromatic*
Cooked*
Sulfur*
Milkfat
Sweet
Astringent*
HTST 2%
Ind 2%
Dir 2%

Day 3


Overall aroma*
5
4
3
2
1
0
Sweet aromatic*
Cooked*
Sulfur*
Milkfat
Sweet
Astringent*
HTST 2%
Ind 2%
Dir 2%

Day 7



*indicates differences ($p<0.05$) for that attribute at that timepoint
Attribute intensities were scored on a 0 to 15 point universal scale Fluid milk flavors fall between 0 and 4 on this scale (Drake, 2007; Croissant et al., 2007; McCarthy et al., 2016)

Figure 3.2b. Descriptive analysis results 2% milkfat HTST and UP milks at d 3, 7, and 14 post-processing