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EFFECT OF THERMOSONICATION ON VISCOSITY OF MILK CONCENTRATES
AND MILK QUALITY AND SHELF LIFE

by

Vidita Deshpande

A dissertation submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Nutrition and Food Sciences

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ABSTRACT

Effect of thermosonication on viscosity of milk concentrates and
milk quality and shelf life.

by

Vidita Deshpande, Doctor of Philosophy

Utah State University, 2020

Major Professor: Dr. Marie K Walsh

Department: Nutrition, Dietetics, and Food Science

Thermosonication is the application of sound waves at high temperatures (≥ 50 °C). It can be used to improve functional properties, overall quality, and shelf life of dairy products. Milk concentration is limited to 30% total solids (TS) for milk protein concentrates (MPC) and 50% TS for skim milk powder (SMP) due to increased viscosity. The first part of this study investigated if thermosonication can be used along with modulation of temperature and TS to decrease the viscosity of these concentrates, so that a higher TS can be spray dried. Results showed that thermosonication significantly reduced the viscosity of reconstituted MPC (rMPC) and SMP (rSMP) at each TS and temperature tested. After 30 s of thermosonication at 60 °C, rMPC at 34% TS showed viscosity lower than that of 30% TS, and the viscosity of rSMP significantly decreased.

This study also investigated if thermosonication could improve the overall milk quality by reducing the thermophilic and total bacteria in milk. Thermosonication at 72°C significantly reduced the thermophilic bacterial cells and spores in a batch system by ≤ 1 log and thermosonication along with heat in a continuous system reduced *G. stearothersophilus* cells by ≤ 0.5 log. Reductions in thermophilic bacterial cells and

spores although statistically significant, may not have a major impact on the overall quality of milk in practical applications. Thermosonication at 72°C for 11 s along with pasteurization in a continuous system resulted in significantly higher log reductions of 4.1 seen as compared to 2.8 seen for control at week 0. Thermosonicated milk samples had lower bacterial counts than control during shelf life. Microbial quality, pH, free fatty acid (FFA) content were significantly improved due to thermosonication. Consumer acceptance studies showed that thermosonicated milk had an unknown flavor after processing, but the flavor declined after 4 days. Thermosonication in a continuous system coupled with pasteurization improved the overall quality of whole raw milk as compared to 11 s of heat with pasteurization alone which could potentially improve milk quality and increase the shelf of milk with minimal changes in consumer acceptance.

(211 pages)

PUBLIC ABSTRACT

Effect of thermosonication on viscosity of milk concentrates and
milk quality and shelf life.

by

Vidita Deshpande

Thermosonication is the application of soundwaves at temperatures $\geq 50^{\circ}\text{C}$. It can be used to improve functional properties, overall quality, and shelf life of dairy products. Milk is concentrated before being spray dried and currently milk concentration is limited by increased viscosity. Decreasing the viscosity of concentrates prior to spray drying could be economically beneficial to dairy processors. Results from this study showed that that thermosonication significantly reduced the viscosity of concentrates such as reconstituted milk protein concentrate (rMPC) and reconstituted skim milk powder (rSMP). This would allow for spray drying of these concentrates at a higher total solids with a lower viscosity.

Currently, the shelf of pasteurized fluid milk stored at refrigeration temperatures is 2-3 weeks and is limited due to bacterial growth during refrigerated storage. Increasing milk shelf life would potentially minimize product loss and consumers can enjoy their refrigerated milk for longer time. Some thermophilic bacterial cells and spores can survive high temperatures involved in dairy processes and decrease milk product quality. Minimizing losses due to product quality loss could potentially minimize losses for dairy producers and It would be beneficial to improve dairy product shelf life and quality by eliminating these microorganisms using thermosonication.

The effect of thermosonication at pasteurization temperatures on survival of total

and thermophilic bacteria such as *Geobacillus stearothermophilus*, *Anoxybacillus flavithermus*, and *Bacillus subtilis* in both batch and continuous systems was studied.

Results showed that thermosonication successfully reduced thermophilic bacterial cells but not spores. Moreover, the cell reductions observed may not have an impact on milk product quality. Thermosonication along with pasteurization successfully improved the milk microbial quality along with other quality indicators such as pH and free fatty acid (FFA) content. Milk shelf life was extended by 2 weeks and minimal changes in consumer acceptance of milk were observed. Thus, thermosonication along with pasteurization could be used to improve milk quality and shelf life and decrease the viscosity of milk concentrates.

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LIST OF IMPORTANT ABBREVIATIONS

ANOVA	Analysis of Variance
CN/TP	Casein as a percentage of total protein
DF	Diafiltration
FDA	Food and Drug Administration
FFA	Free fatty acid
FFDCA	Federal Food, Drug, and Cosmetic Act
GRAS	Generally Recognized as safe
HTST	High temperature short time
LTLT	Low temperature long time
MD	Malondialdehyde
MPC	Milk Protein Concentrate
NFDM	Nonfat dry milk
PHE	Plate heat exchanger
REGWQ	Ryan, Einot, Gabriel, Welsh Studentized Range Q test
rMPC	Reconstituted milk protein concentrate
rSMP	Reconstituted skim milk powder
SMP	Skim milk powder
TBA/TBARS	Thiobarbituric acid reactive substances (TBARS)
TS	Total solids
UF	Ultrafiltration
UHT	Ultra-high temperature
UP	Ultra-pasteurization

USDA	United States Department of Agriculture
WPC	Whey protein concentrate
WPI	Whey protein isolate

CHAPTER I

INTRODUCTION

Milk is a major part of the Western diet as it provides nutrients such as proteins, lipids, and minerals. Raw milk when refrigerated has a shelf life of about a week which can be extended to 2-3 weeks with pasteurization, the most commonly used for refrigerated milk being high temperature short time (HTST; 72 °C for 15 s) (Gandy et al., 2008; Pasteurized Milk Ordinance-FDA, 2017). The shelf life of milk can also be extended by different techniques such as concentration and drying. Skim milk powder (SMP) is a dried form of pasteurized skim milk and milk protein concentrates (MPC) are dried protein products with higher protein concentrations, both produced from concentrated skim milk. In comparison with SMP, MPCs are higher in protein (42-85%) and lower in lactose (4-46 % depending on protein content) and minerals (6-7%) because ultrafiltration (UF) followed by diafiltration (DF) is employed in MPC manufacturing (Agarwal, Beausire, Patel, & Patel, 2015; Patel & Patel, 2014).

Milk is available in the market as a ready to drink beverage (flavored or unflavored) and also in the form of a variety of products such as yogurts, cheese, ice-creams and frozen desserts, infant formulas, and powdered beverages. MPCs are used in protein-fortified foods and beverages and bars targeted for meal replacement and nutrition needs due to its high protein and lower lactose content (Patel & Patel, 2014). SMP is used in infant formulas, nutritional products for children, and fortification of dairy products along with serving as a functional ingredient in bakery products, snacks,

and chocolate confectionaries due to its varied functionality (Agarwal et al., 2015; Lagrange, Whitsett, & Burris, 2015; Patel & Patel, 2014).

The current shelf of HTST pasteurized milk is limited to 2-3 weeks (Gandy et al., 2008). This is mainly due to the growth of bacteria that can grow during refrigerated storage, some of which maybe thermophilic (Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz, 2013; Sørhaug & Stepaniak, 1997). Furthermore, thermophilic bacteria and their spores survive pasteurization and degrade quality of milk and milk products upon germination (Burgess, Lindsay, & Flint, 2010; Khanal, Anand, & Muthukumarappan, 2014a; Khanal, Anand, Muthukumarappan, & Huegli, 2014b). Thermophilic bacteria form heat-resistant biofilms on equipment such as plate heat exchangers (PHE) and evaporators which in turn can contaminate the processed product (Burgess et al., 2010; Scott, Brooks, Rakonjac, Walker, & Flint, 2007; Sharma & Anand, 2002).

Survival and growth of bacteria after pasteurization can deteriorate the quality of milk products due to the enzymes they secrete. Enzymes such as proteases, lipases, and phospholipases can be active even if the microbe involved in their production was eliminated. Lipases and proteases can release free fatty acids and bitter peptides in milk, respectively, causing rancid odors and flavors and bitterness in milk (Sørhaug & Stepaniak, 1997). It is beneficial to reduce the number of bacteria that grow in milk and milk products during storage.

Increasing the pasteurization temperatures does not help eliminate bacteria that limit the shelf life of fluid milk products stored at refrigeration temperature. Gandy et al. (2008) reported that higher pasteurization temperatures (up to 85 °C) did not increase the

shelf life of milk and decreased acceptance by some consumers. Moreover, some thermophilic bacteria associated with dairy products can survive high temperature heat treatment involved in ultra- pasteurization (UP; at ≥ 138 °C for 2 s), ultra-high pasteurization (UHT; at 135-150 °C for 4-15 s in aseptic environment), and even milk powder processing (Burgess et al., 2010; Sadiq et al., 2016).

It would be economical to obtain a solution of higher % total solids (TS) prior to spray drying in processing of SMP and MPC. It is difficult to do so because an increase in viscosity is observed with a high solids content. The viscosity of milk concentrates decreases at temperatures > 40 °C and at higher temperatures (> 70 °C) the viscosity of milk concentrates increases. Increased viscosity prior to spray drying can lead to negative effects such as reduced flow rates, high pressure drops, decreased turbulence, and fouling in heating operations (Fernández-Martín, 1972; O'Donnell & Butler, 2008). Alternative technologies such as high-pressure processing, pulsed electric field, and sonication could be explored to tackle the issue of heat resistant microbes and increased viscosity associated with dairy products. Sonication combined with heat (thermosonication) and pressure (manosonication) or both (manothermosonication) has been effective in reducing microorganisms in foods and reducing viscosity of milk concentrates (Villamiel, Schutyser, & De Jong, 2009; Zisu, Schleyer, & Chandrapala, 2013).

Sonication is the application of ultrasonic waves (≥ 20 kHz) that leads to a phenomenon called cavitation and when applied at high temperatures, is referred to as thermosonication. Sonication can be used in numerous applications in the food industry such as emulsification, filtration, viscosity modification, improvement of whey protein heat stability, improvement of meat tenderness, and inactivation of spoilage microbes

(Chandrapala, Oliver, Kentish, & Ashokkumar, 2012; Chemat, Zill-E-Huma, & Khan, 2011; Ganesan, Martini, Solorio, & Walsh, 2015; Knorr, Zenker, Heinz, & Lee, 2004). Sonication has been explored to increase the shelf life and maintain the quality of milk by destruction of indigenous microflora or added (Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009; Bermúdez-Aguirre, Mawson, Versteeg, & Barbosa-Cánovas, 2009). A reduction in viscosity of skim milk concentrate and reconstituted whey protein powders was seen upon application of high power, low frequency ultrasound (Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010; Zisu et al., 2013).

Milk inoculated with *Anoxybacillus flavithermus* cells showed a 1.1 log reduction after 1 min of batch sonication (with sonication vessel immersed in an ice bath) and after 10 min of batch sonication, 4 log reduction in *A. flavithermus* vegetative cells was seen aonly 1.27 in *A. flavithermus* vegetative cells after pasteurizing at 63 °C for 30 min. In contrast, Lim, Benner, & Clark (2019), showed that thermosonation and cold sonaication in a continuous heating and batch sonication system (for 10-60 s) did not significantly reduce the microbial numbers when raw milk was inoculated with *Paenibacillus amolyticus*. A 2.9 log reduction in overall milk microflora was seen after 1.7 min of sonication in a continuous system; however, the effect of thermosonation along with pasteurization was not evaluated (Mar Villamiel & De Jong, 2000).

Previous studies showed that batch sonication did significantly reduce *A. flavithermus* cells but thermosonation did not have an effect on microbial numbers of *P. amolyticus*. So further investigation is needed on the effect of thermosonation on survival of cells and spores of different thermophilic bacteria such as *Geobacillus stearothermophilus* and *Bacillus subtilis* which are associated with spoilage of dairy

products. Khanal et al. (2014b) showed that when cold sonication in a batch system for 1 min was used with batch pasteurization, the log reductions in vegetative cells of *A. flavithermus* were doubled; however, treatment time of 10 min did not significantly affect the reduction as compared to batch sonication alone. Thus, the effect of shorter and practical residence times (≤ 1 min) for thermosonication in a continuous system combined with pasteurization on thermophilic bacteria in milk and indigenous microflora of milk needs to be investigated. Previous studies also lacked evaluation of the effect of thermosonication in a continuous system on other quality parameters in milk such as pH, free fatty acid (FFA) content, and casein as a percentage of total protein (CN/TP) content (Fromm & Boor, 2004; Lim et al., 2019).

Based on the shortcomings of previous studies, the current study aimed at using thermosonication conditions that are more realistic in terms of time and temperature for both batch and continuous systems. This study investigated the effect of thermosonication on survival of thermophilic and indigenous microflora of milk and also focused on effect of thermosonication on the milk quality during its shelf life along with the effect on sensory attributes.

Previous studies on viscosity reduction have investigated the effects of sonication in dairy systems (Ashokkumar et al., 2009; Sun et al., 2014; Zisu et al., 2010, 2013). However, there are no published studies that investigated the effects of batch and continuous sonication on reconstituted MPC and SMP at different solids (30-44% TS MPC, and 46-64% TS SMP) and at different temperatures (40-60 °C). Based on this information, the current study aimed at using temperatures and TSs that mimic the conditions used during the processing of milk concentrates.

HYPOTHESES

Hypotheses of this study are as follows:

1. Thermosonication of concentrated milk products (SMP and MPC) will decrease the viscosity of these products.
2. Use of thermosonication as a processing treatment for milk will reduce the thermophilic spoilage population allowing for an extended shelf life and improved quality of milk
3. Use of thermosonication as a processing treatment for milk will not affect the physicochemical properties of milk (pH, sulfur, color, and lipid oxidation).

OBJECTIVES

Objectives of this study are as follows:

1. Measure the influence of temperature and solids on the viscosity of reconstituted milk products (rSMP and rMPC) before and after thermosonication (batch and continuous).
 - a. Evaluate the effect of temperature, TS, and batch sonication on the viscosity of rMPC samples (TS of 30-44 %) and rSMP samples (TS of 46-64 %) at 40, 50, and 60 °C.
 - b. Evaluate the effect of temperature, flow through sonication (versus no sonication), and TS on the viscosity of rMPC samples (TS of 30-34 %) and rSMP samples (TS of 50-54 %) at 60 °C.

2. Evaluate the effect of thermosonication in a batch and a continuous flow setting on the survival of thermophilic/thermoduric organisms.
 - a. Evaluate the survival of *G. stearothermophilus* (cells and spores), *A. flavithermus* (cells and spores), *Bacillus licheniformis* (spores), *B. subtilis* (spores) in a batch system (thermal versus thermosonication) in media and 2% milk.
 - b. Evaluate the survival of *G. stearothermophilus* cells in a continuous system (thermal versus thermosonication) along with the effect of different residence times (flow rates) and location of the PHE.
3. Evaluate the effect of thermosonication in a batch and a continuous flow setting on the microbial quality (microbial count, lipase and protease activity) over a period of the shelf life of milk.
 - a. Raw unhomogenized milk treated at optimum settings from Objective 2 and samples evaluated for microbial growth, FFA content, sulfur content, and CN/TP ratio.
4. Evaluate the effect of thermosonication in a continuous flow system (pilot scale) on the physicochemical (sulfur, pH, color, lipid oxidation, and viscosity), and sensory properties of milk.
 - a. Pasteurized homogenized milk treated in a pilot scale continuous system and samples evaluated for their sensory properties and consumer acceptance.

- b. Pasteurized homogenized milk treated in a pilot scale continuous system will be evaluated for its pH, color, viscosity, sulfur content, FFA content, and for any potential lipid oxidation product through its shelf life.

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CHAPTER II

LITERATURE REVIEW

INTRODUCTION TO MILK AND MILK POWDERS

Milk and Milk Powders

Milk is a source of proteins, lipids, and minerals in the Western diet. The typical composition of bovine milk is approximately, 87.7% water, 4.9% lactose, 3.4% fat, 3.3% protein, and 0.7% minerals (Smith, 2008). The composition of milk is affected by various factors such as species of animal, breed, animal feed, animal to animal variation, and stage of lactation. Out of all the animal species, bovine milk is more commonly produced and consumed in the United States. In 2017, about 97 million kilograms of bovine milk was produced annually (USDA, 2018). Although milk can be processed into several products such as concentrates, powders, cheese, yogurt, about 22 % of milk produced is consumed as a liquid (USDA Economic Research Service, 2019). Liquid milk undergoes a process called pasteurization to extend its refrigerated shelf life. Liquid milk can be sold as full fat or whole milk (3.25% fat), 1% fat milk, 2% fat milk, skim milk (0.1- 0.2 % fat), or flavored milk as examples.

Skim milk powder (SMP) is a dried form of pasteurized skim milk that contains \leq 5% moisture and \leq 1.5% milkfat (by weight) (Smith, 2008). SMP has a standardized milk protein content of 34% as opposed to nonfat dry milk (NFDM), which does not (Smith, 2008). NFDM is most commonly produced in the U.S. SMP is used in this research to have a standardized product and for ease of comparison of results obtained from this

study with related literature. Milk protein concentrates (MPCs) are high protein content products that have various roles in terms of functionality and nutrition. MPCs and SMPs contain both casein and whey proteins as opposed to whey protein concentrates (WPC) or isolates (WPI). In comparison with SMP, MPCs are higher in protein (42-85%) and lower in lactose (4-46 % depending on protein content) and minerals (6-7%) (Agarwal, Beausire, Patel, & Patel, 2015; Patel & Patel, 2014) . Table 2.1 displays a comparative composition of milk, SMP, and commonly used MPC's in the industry.

Milk Processing

Due to variation in milk obtained from the same farm or different farms, milk must be standardized, especially for its fat content. Milk is standardized using centrifugal separators which separate the skim component ($\approx <0.01$ % fat) from the cream portion ($\approx 40\%$ fat). Known amount of cream is added back to the skim component to obtain a fat content of $\approx 3.25\%$. As mentioned before, milk is pasteurized to extend its shelf life. Pasteurization is a heat treatment process that inactivates major pathogenic and some spoilage bacteria in milk. Milk can be pasteurized using different temperature and time treatments as well in batch and continuous settings. Table 2.2 shows the different treatments commonly utilized in milk pasteurization. These conditions are designed to produce milk that is safe to consume while having minimal cooked flavor. The majority of U.S. fluid milk is pasteurized using a high temperature short time (HTST) continuous process of at least 72°C for 15 seconds (IDFA, n.d.).

Production of SMP and MPC

SMP is standardized to 34 % protein by using either the milk retentate or permeate from ultrafiltration (UF) of milk. SMP is manufactured using pasteurized skim milk that is concentrated using evaporation followed by spray drying as shown in Figure 2.1 (Smith, 2008). The solubility of SMP is increased by agglomeration. MPC's are produced using skim milk, which is concentrated by UF. UF results in segregation of caseins, whey proteins, micellar salts, and residual fat in the retentate, whereas lactose, soluble salts, and non-protein nitrogen are removed with the permeate (Bastian, Collinge, & Ernstrom, 1991). Diafiltration (DF) is commonly applied to remove residual lactose and soluble minerals and to obtain a product with a high protein content (Patel & Patel, 2014). MPC is produced by further concentration of this UF retentate using evaporation followed by spray drying as shown in Figure 2.2.

Applications of SMP and MPC

MPCs provide a range of functionalities such as water binding, viscosity, gelling, foaming/whipping, emulsification, are relatively heat stable, and are used in many protein-fortified foods but primarily in meal replacements, nutritional beverages and bars (Agarwal et al., 2015; Patel & Patel, 2014; Sahay, Singh, Panjagari, & Arora, 2017). MPCs, due to their lower lactose content can reduce Maillard browning in final products. MPCs also have a clean and milky flavor with minimal aftertaste. Apart from serving as a substitute for milk, SMP can be used in infant formulas, nutritional products for children, and fortification of dairy products along with serving as a functional ingredient in bakery products, snacks, and chocolate confectionaries (Lagrange, Whitsett, & Burris, 2015).

Milk microflora, milk quality, and shelf life

As in any other foods, milk contains psychrophilic, psychrotrophic, mesophilic, and thermophilic bacteria. The psychrotrophic bacteria can be long or short rods, cocci, or vibrios; gram-positive or gram-negative bacteria; spore formers or non-sporeformers; and aerobic, facultative anaerobic or anaerobic microorganisms (Cousin, 1982; Vithanage, Yeager, Jadhav, Palombo, & Datta, 2014). Most of the fluid milk has a shelf life of up to 3 weeks when pasteurized using high temperature short time (Gandy et al., 2008; Pasteurized Milk Ordinance-FDA, 2017). Pasteurization results in destruction of pathogens along with reducing other vegetative bacteria. The shelf life of fluid milk is affected by raw milk quality (somatic cell count; SCC), processing conditions, microbial growth, packaging materials, temperature abuse, and exposure to light (Barbano, Ma, & Santos, 2006; Gandy et al., 2008).

Spoilage of fluid pasteurized milk stored at refrigeration temperatures is most commonly attributed to growth of bacterial cells and spores that survive pasteurization and are able to grow at refrigeration temperatures and microbial enzymatic degradation (Sørhaug & Stepaniak, 1997). Post-pasteurization contamination of milk with heat labile psychrotrophs such as *Pseudomonas* post-pasteurization can also limit the shelf life of milk. Improvement in filling and packaging technologies has limited post-pasteurization contamination to a minimum, with shelf life of fluid milk made from good quality raw milk being about 3 weeks (Barbano et al., 2006).

Some inherent thermotolerant psychrotrophic spore forming species survive pasteurization temperatures (HTST, LTLT) and can possibly grow during refrigerated storage. Most of the thermotolerant psychrotrophic species are Gram-positive, and

especially belong to the genus *Bacillus* and *Paenibacillus*, which have been associated with dairy farms and processing facilities (Ivy et al., 2012; Ledenbach and Marshall, 2010). Other than *Pseudomonas* (which accounts for 65-70% of the psychrotrophs in raw milk), psychrotrophic microbial genera of *Micrococcus*, *Aerococcus*, and *Lactococcus* also can be found in raw milk (Ledenbach & Marshall, 2009). If milk is kept at refrigeration temperatures and post-pasteurization contamination is prevented, the only microbes present would be bacteria that survive pasteurization some of which may be thermotolerant psychrotrophs.

Psychrotrophic population of 10^6 - 10^7 CFU mL⁻¹ produce an adequate quantity of enzymes which can lead to quality defects in milk (Ledenbach & Marshall, 2009).

Microbial enzymes such as proteases, lipases, phospholipases indirectly cause spoilage in dairy products and are mostly extracellular. Most of these enzymes are active even if the microbe involved in their production was eliminated. The lipases can release free fatty acids which may account for rancid flavors in milk. Unsaturated free fatty acids are susceptible to oxidation, resulting in off odors and flavors. Proteases, on the other hand can break down peptide chains and cause bitterness due to release of bitter peptides (Sørhaug & Stepaniak, 1997).

Fromm & Boor (2004) demonstrated that shelf life of fluid milk was limited due to bacteria that survive pasteurization and are capable of growing at refrigerated storage. In this study, the bacterial counts showed a dramatic increase after two weeks with an average of a 4-log increase after 17 days. Chemical analyses were performed to assess lipolysis (free fatty acid content; FFA) and proteolysis (casein as a percent of true protein; CN/TP) over the shelf life which showed both FFA content increased and CN/TP

levels decreased over time. A descriptive analysis of the milk by a trained panel identified rancid, sour, nutty, metallic, and hay or grainy as defects which can be attributed to off flavor caused by increased lipolytic and proteolytic activity (because of bacterial growth). These off flavors are unacceptable to consumers, hence, there is a need to eliminate these heat resistant microorganisms that are also psychrotrophic.

Higher temperature treatments such as ultra-pasteurization (UP; at ≥ 138 °C for 2 s) and ultra-high temperature pasteurization paired with aseptic packaging (UHT; at 135-250 °C for 4-15 s) will yield longer shelf life of 1-3 months (until package is opened) and ≥ 6 months, respectively. High temperature treatments result in cooked flavors which are not generally acceptable to consumers in the U.S (Schiano, Harwood, & Drake, 2017). High temperature processing can lead to losses such as water-soluble vitamin losses, denaturation of proteins, production of Maillard reaction products, off flavors, and other chemical reactions (Mehta, 1980; Oamen, Hansen, & Swartzel, 1989).

Thermophilic microorganisms can grow at temperatures from 40-65°C and thermophilic bacterial cells may survive pasteurization as seen in Appendix A, Table A.1 and produce acids, enzymes (lipases and proteases) which deteriorate milk quality (Burgess, Lindsay, & Flint, 2010; Khanal, Anand, Muthukumarappan, & Huegli, 2014b; Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz, 2013). Even if the vegetative cells are destroyed, spores of thermophilic bacteria can survive pasteurization and the spores can germinate and grow during storage to cause off flavors in milk products (Fromm & Boor, 2004; Ledenbach & Marshall, 2009; Ranieri et al., 2012). Sweet curdling, flat sour, bitterness, ropiness, and off flavor production are some types of spoilages associated with dairy products. Thermophilic bacterial cells and spores of

Anoxybacillus flavithermus, *Geobacillus stearothermophilus*, *Bacillus licheniformis*, *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus cereus*, *Bacillus pumilus*, and *Bacillus sporothermodurans* have also been associated with UHT pasteurized milk and other milk products (Burgess et al., 2010; Lücking et al., 2013; Sharma & Anand, 2002).

Rückert, Ronimus, & Morgan (2004) after analyzing milk powder samples from 28 countries, reported that 96.8% of spoilage organisms belonged to the genus *Bacillus* (*A. flavithermus*, *G. stearothermophilus*, *B. licheniformis*). Also, dairy powders could have about 10^4 - 10^6 CFU g⁻¹ thermophilic bacilli resulting either from the survival of vegetative cells after heat treatment or due to activation of spores as a result of heat treatment resulting in their germination (in optimal growth conditions) (Burgess et al., 2010). Thermophilic bacteria and their spores form heat-resistant biofilms on equipment such as plate heat exchangers, evaporators, and can contaminate the product stream resulting in products with spore counts 1000 times higher than the original milk (Burgess et al., 2010; Scott, Brooks, Rakonjac, Walker, & Flint 2007).

Thermophilic bacteria and spores are difficult to eliminate in high temperature processing and it would be beneficial to reduce their concentrations in incoming milk prior to any other harsh heat treatment. Thermophilic *bacilli* can grow fast and have a generation time of about 15-20 min (Scott et al., 2007). Although most of the thermophilic bacteria and spores found in dairy processing are not pathogenic, they can cause product spoilage during storage (Watterson, Kent, Boor, Wiedmann, & Martin, 2014).

Gandy et al. (2008) showed that increasing pasteurization temperatures (up to 85°C) did not increase the shelf life of milk and the use of pasteurization temperatures

over 79 °C resulted in decreased consumer acceptance. Increasing processing temperatures have shown limited impact on elimination of some thermophilic bacteria which are associated with spoilage of dairy products (Burgess et al., 2010). Therefore, alternative technologies need to be explored to tackle the issue of heat resistant microbes which may also be psychrotrophic.

Viscosity of milk concentrates

Rheologically, milk acts as a Newtonian fluid. Factors such as temperature, fat content, protein content, total solids, and solid to liquid fat ratio have an effect on viscosity of milk (Bienvenue et al. 2003; Fernández-Martín 1972). An increase in viscosity with an increase in solids content and the decrease in viscosity with an increase in temperature was observed in skim milk by Fernández-Martin (1972) and Morison, Phelan, & Bloore (2013) and in reconstituted MPC (rMPC) by O'Donnell & Butler (2008). Milk concentrates are produced using both evaporation and spray drying which involve high temperature processing and an increase in solids content of milk concentrates. Hence, to understand the viscosity behavior of concentrates, it is important to study the combined effect of temperature and solids content on the viscosity of milk concentrates.

A decrease in viscosity of milk concentrates is seen with an increase in temperature at temperatures up to 80 °C (Fernández-Martín, 1972). Fernández-Martín (1972) have attributed the decrease in viscosity to decrease in average intermolecular forces seen with an increase in temperature. No studies were found that studied the viscosity of milk concentrates at temperatures > 80 °C, possibly due to increase in viscosity seen at higher temperatures for these concentrates. Anema & Skelte (2007)

showed that heat treatment ($>70\text{ }^{\circ}\text{C}$) of reconstituted skim milk resulted an increase in casein micelle size as a result of denatured whey proteins associating with casein micelles. This association of whey protein with casein micelles increases with an increase in temperature resulting in increase in viscosity at higher temperatures.

Concentration by evaporation increases the total solids content to between 30-55 %, and the increase in viscosity is non-linear at high concentration levels (Bienvenue et al. 2003). Morison et al. (2013) studied the rheology of evaporated low-fat milk concentrates and showed that up to 20% total solids, the liquid was Newtonian, but above 30% concentration skim milk concentrates exhibited pseudoplastic (shear thinning) behavior. An increase in solids content is a result of water removal from milk which in turn causes an increase in volume fraction of dispersed particles and interactions between casein micelles; this in turn reduces the distance between the micelles (Bienvenue et al., 2003).

An increase in viscosity poses a problem in the dairy processing industry since it leads to reduced flow rates, high pressure drops, decreased turbulence (lower rate of heat transfer), and fouling in heating operations. The production of concentrated skim milk, which is used in the production of both SMP and MPC, is limited to approximately 50% TS for SMP and 32% TS for MPC, because large increases in viscosity are observed at $\text{TS} \geq 45\%$ (Enríquez-Fernández, Camarillo-Rojas, & Vélez-Ruiz, 2013). Fluid milk with $\geq 45\%$ concentration is difficult to atomize due to increase in viscosity that leads to large droplets being formed in the atomizer; thus, decreasing the thermal efficiency of the spray dryer (Enríquez-Fernández et al., 2013; Zisu, Schleyer, & Chandrapala, 2013). Additionally, the viscosity of concentrated skim milk increases with time in a process

called “age thickening” which is a result of structural build via noncovalent interactions between casein micelles (Bienvenue et al., 2003).

SONICATION

Sonication and its applications

Waves are disturbances that travel from one point to another while transferring energy between those points. Sound waves are mechanical longitudinal waves. Sound waves are mechanical because they need a medium to transfer energy from one point to another such as air, water, and solid materials like steel. Sound waves are longitudinal because the particles in the medium through which they travel, oscillate in a direction similar to that of the wave (Mohammadi, Ghasemi-Varnamkhasti, Ebrahimi, & Abbasvali, 2014).

Ultrasound is sound waves that have a frequency of greater than 20 kHz and produced using a sonication power source composed of a transducer and a probe or a sonotrode (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). A transducer changes the electrical input based on the input given by the operator of an ultrasound machine. The piezoelectric nature of the sonotrode attached to the transducer converts these electrical signals to sound waves. A piezoelectric material has the ability to produce a piezoelectric effect, which is the ability to convert mechanical energy (soundwaves) to electrical energy and vice versa. Many materials exhibit piezoelectric properties such as quartz, potassium sodium tartrate, barium titanate, polyvinyl chloride, and polyvinyl fluoride. The ultrasound assembly currently used in food industry applications consists of titanium alloys (Atchley & Crum, 1988).

Application of ultrasound in a fluid medium is referred to as sonication and the terms ultrasound and sonication are used interchangeably with each other. When sonication is applied with heat it is termed as thermosonication. Sonication can result in generation of thermal energy or cavitation depending on the frequency used. Passing of these sound waves through a liquid causes the formation of alternating high-pressure (compression) and low-pressure (rarefaction) cycles (Leighton, 1994). During rarefaction, small gas filled bubbles or voids form which increase in size and can collapse during compression and rarefaction cycles. Formation and collapse of these small bubbles or voids in a liquid due to sonication is referred to as acoustic cavitation (Ashokkumar, 2011). As a result of bubble collapse, very high temperatures ($\approx 5,000$ K) and pressures ($\approx 2,000$ atm) are reached locally (Zisu et al., 2013).

Different applications of sonication are being explored or implemented in food processing for emulsification, filtration, viscosity modification, improvement of whey protein heat stability, improvement of meat tenderness, and inactivation of spoilage microbes (Chandrapala et al., 2012; Chemat, Zill-E-Huma, & Khan, 2011; Knorr, Zenker, Heinz, & Lee, 2004). Sonication has caught the interest of the food industry due to its potential to alter the functionality of foods as well as improve the shelf life and quality (Chandrapala et al., 2012; Knorr et al., 2004). There have been other studies that investigated the effects of sonication on viscosity in dairy systems (Ashokkumar et al., 2009; Sun et al., 2014; Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010; Zisu et al., 2013). But none of the studies focused on evaluation effect of sonication (both batch and continuous setting) at different solids and temperatures.

Regulation of sonication in the food industry

One approach to understanding how sonication will be regulated by the Food and Drug Administration (FDA), would be to understand why irradiation is regulated as an additive. Earlier studies on food irradiation used sources of high energies to induce irradiation and the radioactivity could be detected in the final food product. As technology progressed sources with lower energies could be used for irradiation whose radioactivity could not be detected. Hence, the source of radiation needed to be defined and only approved sources can be used; any new sources for inducing irradiation need to go through an approval process. Toxicological safety of food additives is typically measured by feeding high dosage amounts to laboratory animals and checking if a toxic effect is produced. Initial studies conducted tried to induce an exaggerated amount of irradiated food in the diet of the lab animals, which is not a true representation. These studies showed adverse effects on the nutritional components in food and produced confounding variable in these studies (Ioannis & Tserkezou, 2007).

The FDA has referenced many studies to show that irradiation did not produce any toxicity in food at safe dosage levels. Due to the earlier studies which produced confounding variables, Congress formed a committee and published the U.S. Regulatory Requirements for Irradiating Foods (1986) that FDA follows to monitor irradiation as a food additive (Ioannis & Tserkezou, 2007). In terms of labeling, FDA requires a radura symbol or the words 'treated with irradiation' on whole fresh foods only, where a consumer needs to be informed that the product has been processed with irradiation. If any of these irradiated ingredients are added to foods that are not irradiated and to be sold in retail market, no labeling is required because the consumer knows that the food is

processed. If such a food is not to be sold in the retail market, it needs to be labelled as containing irradiated ingredients to ensure that this food product does not get irradiated multiple times (Irradiated Food & Packaging, 2009). To summarize, the FDA regulates irradiation as a food additive because of the earlier confounding studies considered by the Congress and the way it regulates is by ensuring approved sources and dosages are used.

Sound waves are not retained in the food medium after sonication and hence would not be considered as a food additive. FDA defines a food additive as any substance that is reasonably expected to become a component of food. Food additives need to be approved by the FDA, unless they are generally recognized as safe (GRAS) by the experts or meet one of the other exclusions from the food additive definition in section 201(s) of the Federal Food, Drug, and Cosmetic Act (FFDCA). However, the sonotrodes used to apply sonication in the foods do potentially deteriorate and leech into food when used at more than the recommended amplitudes, at which point food would be considered adulterated (Atchley & Crum, 1988). To prevent that from occurring, sonication will have to be regulated so that a manufacturer does not exceed the amplitude for that particular sonotrode and final product testing will have to include checking for presence of materials from the sonotrode.

EFFECT OF THERMOSONICATION ON MILK MICROFLORA AND MILK QUALITY

Effect on milk microflora

As stated before, thermosonication results in the inactivation of microbes. The mechanism of sonication-induced bacterial-cell death is still debated. There are three proposed mechanisms of how sonication induces cell death: i) damage to bacterial cell from high temperatures and pressures produced locally from acoustic cavitation ii) shear forces disrupt bacterial cells and iii) cavitation collapse cleaves water into highly reactive H and OH radicals, which may form hydrogen peroxide leading to microbial destruction (Chandrapala et al., 2012; Tiwari & Mason, 2012). The destruction of bacterial cells could be due to a combination of the three proposed mechanisms above.

Most of the studies performed have shown physical disruption of cell structure as a result of cavitation when samples were observed using scanning electron microscopy (SEM) (Gera & Doores 2011; Khanal et al. 2014b). Spores produced by bacteria on the other hand showed shrinking and water loss but no physical disruption as a result of sonication when observed under SEM (Khanal, Anand, & Muthukumarappan, 2014a). Microbial destruction increases when sonication is combined with other processing technologies such as high/ultra-high pressure, antimicrobials, UV light, and pulsed electric fields (Chemat et al., 2011) .

Bacterial cells have shown to be more susceptible to disruption by sonication when compared to spores due to inherent resistance of spores to stress (Chandrapala et al., 2012; Khanal et al., 2014a). The sensitivity of bacteria to sonication may also depend

on the cell's physiological state. Furthermore, inactivation efficacy of sonication is sensitive to properties of liquid medium such as pH, viscosity, and chemical composition (Chandrapala et al., 2012). Sonication has been used to increase the shelf life and maintain the quality of milk by destruction of indigenous microflora or added pathogens in milk in batch and continuous systems in previous studies (Bermúdez-Aguirre, Corradini, Mawson, & Barbosa-Cánovas, 2009a; Bermúdez-Aguirre, Mawson, Versteeg, & Barbosa-Cánovas, 2009b).

Bermúdez-Aguirre et al. (2009b) showed that when skim milk was pasteurized in a batch system (63 °C for 30 min) followed by sonication at 63 °C for 30 min, a 5 log reduction of mesophilic bacteria inherent to milk was seen. Previous studies in batch sonication (residence times between 1-10 min) have shown reduction of thermophilic bacteria (vegetative cells and spores) in milk when milk was immersed in an ice bath during sonication (Khanal et al. 2014 a, b). these researchers have not evaluated the effect of thermosonication (sonication with heat) on the survival of indigenous microflora in milk and thermophilic bacteria in milk products. Sonication when used with heat or pressure has shown to have more of an impact on bacteria reductions as compared to sonication alone (Villamiel, Schutyser, & De Jong, 2009). Moreover, these studies were performed in batch systems which may make scaling up of residence times to continuous system a little difficult (Villamiel et al., 2009). Residence times need to be designed keep practical residence times in mind (equivalent to 15-60 s in continuous systems).

Villamiel & De Jong (2000) performed sonication (46-79 °C) in a continuous system and showed 3.1-5.1 log reduction on total milk microflora. A batch sonication study performed by Lim, Benner, & Clark (2019) showed that thermosonication (at 72

°C) and cold sonication for 10-60 s did not help extend the shelf life of milk inoculated with *Paenibacillus amolyticus*. The effect of thermosonication has not been evaluated in other thermophilic bacteria such as *G.stearothermophilus*, *A.flavithermus*, and *B.subtilis* using practical residence times in both batch and continuous systems. Therefore, there was a need of a study that used thermosonication using practical residence times for processing milk and evaluated the effect on indigenous and added thermophilic bacteria in milk.

Effect on overall milk quality

Overall milk quality during shelf life can be monitored using indicators such as pH, FFA, CN/TP, lipid oxidation, and sensory analysis (Fromm & Boor, 2004). pH values are indicative of acid produced in milk by the bacteria capable of growing at refrigerated temperatures. pH of milk decreases during storage as bacterial population increases. FFA content is an indicator of the extent of lipolysis occurring in milk as a result of lipases produced by the bacteria which release free fatty acids in milk. Lipases cause hydrolytic rancidity in milk that can result in the formation of sour, rancid, and other off flavors (Ma, Barbano, & Santos, 2003). CN/TP content on the other hand is indicative of the proteolysis activity in milk, where proteases produced by microorganisms can break down proteins and cause bitter flavors in milk.

Previous studies performed using thermosonication using practical residence times (15-60 s) such as by Lim, Benner, & Clark (2019) and Villamiel & De Jong (2000) did not evaluate the effect of thermosonication on shelf life attributes that can be an indicator of overall milk quality. Bermúdez-Aguirre et al. (2009b) studied the effect of batch sonication on pH and color during shelf life. These researchers only evaluated the

effect for a total of 2 weeks and addition of thermal effect with sonication was not used. Therefore, there is a need of study that evaluates the effect of thermosonication on overall quality of milk during its shelf life.

Also, no significant effect on the pH, protein content, butterfat, and solids in non-fat milk have been observed as a result of thermosonication immediately after processing. Bermúdez-Aguirre et al. (2009b) did report that sonicated whole milk was whiter than the control due to decreased the fat globule size in whole milk. Alternatively, Khanan et al. (2014b) showed a decrease in whiteness after sonicating skim milk for 10 min due to a lower fat content.

Sonication has produced off flavors in food systems with a high lipid content such as oils made from sunflower, palm oil, and kiwi seed oil (Pingret, Fabiano-Tixier, & Chemat, 2013). Some off flavors and aromas have been associated with sonication of milk in a batch system as well (Chouliara, Georgogianni, Kanellopoulou, & Kontominas, 2010; Lim et al., 2019). These studies failed to accurately identify the cause of these off flavors or odors except for some volatile compounds not necessarily associated with lipid oxidation. Riener, Noci, Cronin, Morgan, & Lyng (2009) reported that production of volatiles such as benzene, toluene, and xylene was seen with sonication, but the researchers reported that the specifics of chemical reactions resulting in formation of these volatiles is difficult to identify in complex food systems such as milk. Also, application of sonication at 15 W for 15 min has not shown production of any off flavors in high protein beverages made from whey (Nam, Wagh, Martini, & Walsh, 2017). Also, Juliano et al. (2014) did not see production of any off flavors when milk was sonicated at energy densities $\leq 230 \text{ J mL}^{-1}$.

Chouliara, Georgogianni, Kanellopoulou, & Kontominas (2010) conducted a sensory panel with 16 participants to compare the odor and flavor of control versus batch sonicated samples (on a scale of 5, with 5 being more liked). A sensory panel was conducted from day 0 to 8 on every other day. No significant differences were found in odor ratings of control versus treatment samples. Flavor was rated significantly lower for treatment. Chouliara et al. (2010) used thermosonication residence times of ≥ 2 min in a batch system, but did not attribute off flavors in sonicated milk to formation of lipid oxidation compounds, confirmed by thiobarbituric acid or TBA test. Lim et al. (2019) evaluated the aroma of samples using a descriptive sensory panel to evaluate the effect of sonication on HTST pasteurized skim milk. They reported that the rubbery aroma was distinctly noticeable in treatment samples for longer sonication treatment of 1 min while shorter treatment times of about 10 s showed no significant differences in aroma for treatment and control samples.

Off flavors in milk can be due to many causes such as heating, oxidation, and micro-organisms and can be described using a variety of terms such as cooked, caramelized, scorched, rancid, bitter, cardboard, metallic, and foreign (Shipe et al., 1978). Terms such as cooked, caramelized, scorched are due to heating of milk, while rancid, metallic, and cardboard are due to either lipolysis or lipid oxidation. Shipe et al. (1978) described the foreign flavors in milk as flavors that are abnormal and whose cause or chemical nature is unknown. To describe the off flavor and aroma of sonicated samples, terms such as 'burnt', 'foreign like', and 'rubbery' have been used in past studies, but as discussed before, the cause of these flavors is unknown (Chouliara et al., 2010). Off flavors seen due to

sonication in high lipid systems have been identified as metallic or rancid in oil systems and could be due to production of compounds such as hexanal (Chemat et al., 2004).

Sonication has been associated with production of lipid oxidation products in high lipid systems such as sunflower oil when sonication was applied for 2 min at 20 kHz and 150 W (Chemat et al., 2004). High frequency high power sonication (20-2000 kHz) did not have an effect on production of lipid oxidation compounds in cheese whey samples at residence time of up to 30 min (Torkamani, Juliano, Ajlouni, & Singh, 2014). Riener et al. (2009) observed formation of volatile components in milk samples when sonication was applied at 45 °C for 20 min at a frequency of 24 kHz and a high power of 400 W. Lipid oxidation induced due to sonication is highly dependent on treatment time, and reducing the time treatment can significantly reduce the volatile compounds generated (Juliano et al., 2014). Marchesini et al. (2015) performed batch sonication at 24 kHz on HTST pasteurized milk for 50-300 s and reported that a treatment time of >100 s led to the formation of volatile compounds associated with lipid oxidation that led to decrease in sensory acceptability of samples.

Similarly, Juliano et al. (2014) reported that sonication had no significant on volatile compounds detected for batch sonication even at high frequencies and treatment times. Flow-through sonication did have an effect on volatiles, but after treatment time of 5 min. Shanmugam & Ashokkumar (2014) have shown that application of sonication (20 kHz, 176 W) for 1- 8 min in 7% flaxseed oil/milk emulsion (obtained by sonication) did not significantly affect lipid oxidation products produced when conjugated dienes were measured.

Juliano et al. (2014) studied the effect of batch and flow through sonication on production of lipid oxidation products at low (20 kHz) and high frequencies (400- 2000 kHz) in raw non- homogenized milk and pasteurized skim milk for 5 and 20 min at temperatures between 4-63 °C. Volatiles were analyzed using solid phase micro extraction gas chromatography (SPME-GC). In the batch system, volatiles associated with lipid oxidation detected for sonicated samples were not significantly different than control samples at treatment times of 5 min for all temperatures and frequencies. At treatment time of 20 min in the batch system, volatile components were above the threshold limits at 4 °C (at frequencies ≥ 1000 kHz) and at 45 °C (at frequencies ≥ 400 kHz). Flow through sonication was performed only at 20 kHz at 20 °C, and the volatile compounds exceeded their threshold detection values after 5 min of treatment time for raw milk and after 16 min for skim milk. The volatile compound production in the continuous system was attributed to high shear forces generated in the sonicator vessel when milk was recirculated multiple times to achieve a residence time of 5 or 16 min.

To summarize, sonication had a significant effect on production of lipid oxidation products in batch systems that used low frequency (20-24 kHz) and high power at residence times > 100 s. The production of lipid oxidation products in milk was significantly affected in flow through systems (with multiple passes) at residence times of 5 min and no lipid oxidation volatiles were detected in milk at sonication energy densities $\leq 230 \text{ J mL}^{-1}$. This study aimed at identifying sonication conditions that will minimize the physicochemical changes in milk as a result of sonication which may potentially affect the acceptability of milk by consumer while decreasing the numbers of spoilage organisms. The current study applies thermosonication at a low frequency (20 kHz) with a

residence time of 11.1 s in a continuous system (only one pass through the system) which results in an energy density of 18.6 J mL^{-1} .

To evaluate the effect of thermosonication of milk on overall milk quality, this study aimed at evaluating the pH, FFA content, and CN/TP content of milk during its shelf life along with evaluating the total microbial count. Previous studies mostly focused on identifying and scoring the intensity of off aromas and flavors associated with sonication of milk, but an attempt to study the effect on consumer liking due to these off odors and flavors has not been made (Chouliara et al., 2010; Lim et al., 2019). Therefore, this study also aimed to identify the effect of thermosonication on consumer liking of milk.

EFFECT OF THERMOSONICATION ON VISCOSITY OF MILK CONCENTRATES

A decrease in viscosity upon treatment with sonication could be a result of breaking down of noncovalent protein interactions. These interactions are broken down by the shear forces created as a result of cavitation (Zisu et al., 2013). A decrease in viscosity was observed by Zisu et al. (2013), where sonication reduced the viscosity of skim milk concentrate in both batch and continuous processing. In their study, sonication could not prevent age thickening, however, sonication reduced the viscosity of the aged concentrate similar to that of the starting material. A reduction in viscosity of skim milk concentrate and reconstituted whey protein powders was seen sonication (Ashokkumar et al., 2009; Sun et al., 2014; Zisu et al., 2010, 2013).

This study will investigate the effects of total solids (TS) and temperature on the viscosity of rMPC and reconstituted skim milk powder (rSMP). Additionally, the

influence of sonication on the viscosity of rMPC and rSMP at different TS and temperatures will be investigated using both batch and flow-through sonication systems in a laboratory setting. Although there have been other studies that investigated the effects of sonication in dairy systems (Ashokkumar et al., 2009; Sun et al., 2014; Zisu et al., 2010, 2013), there are no published studies that investigated the effects of batch and continuous sonication on reconstituted MPC and SMP at different solids (30-44% TS MPC, and 46-64% TS SMP) and at different temperatures (40 to 60 °C).

The temperatures and TSs used for this study were an attempt to mimic the conditions used during the processing of milk concentrates. Since concentrated skim milk is evaporated at temperatures between 50-70 °C, rMPC and rSMP will be treated at 40, 50, and 60 °C to investigate the influence of temperature on the viscosity (Singh, 2007). Also, MPC and SMP are evaporated to obtain solids contents of about 30 and 50 % solids respectively, prior to spray drying (Agarwal et al., 2015). Using this rationale, the TS used for this research were ≥ 30 % TS for rMPC and ≥ 46 % TS for rSMP.

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Tables

Table 2.1. Composition of milk, Skim Milk Powder (SMP), and Milk Protein Concentrate (MPC).

Components	Milk ¹	SMP ¹	MPC 70 ²	MPC 80 ²	MPC 85 ²
	%				
Protein	3.3	34	70	80	85
Lactose	4.9	53	16	6	4
Ash	0.7	4	7	7	7
Fat	3.4	1.5	1.5	1.5	1.5
Moisture	87.7	3.5	5	5	5

¹ Adapted from Smith, (2008); ² Adapted from Agarwal, Beausire, Patel, & Patel (2015).

Table 2.2. Different pasteurization conditions used for milk processing.

Pasteurization Type	Process setting	Typical Storage after processing	Processing Temperature, Holding Time ¹
Low Temperature Long Time (LTLT)	Batch/ vat	Refrigerated	63-69 °C, 30 min
Continuous, high temperature short time (HTST)	Continuous	Refrigerated	72-83 °C, 15-25 s
Ultra-pasteurization (UP)	Continuous	Refrigerated, extended storage	≥138 °C, 2 s
Ultra-high temperature (UHT), aseptic processing	Continuous	Room temperature	135-150 °C, 4-15 s

¹ Adapted from the Grade “A” Pasteurized Milk Ordinance (PMO), 2017 revision.

Figures

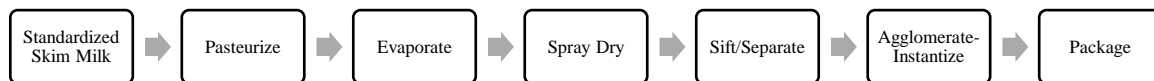


Figure 2.1. Different pasteurization conditions used for milk processing (Smith, 2008).

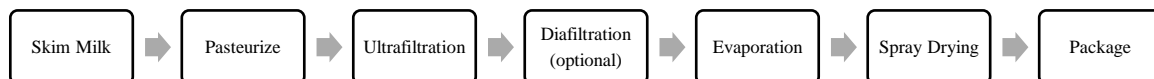


Figure 2.2. Milk Protein Concentrate Production (Patel & Patel, 2014).

CHAPTER III

EFFECT OF THERMOSONICATION ON THE VISCOSITY OF RECONSTITUTED SKIM MILK POWDER AND MILK PROTEIN CONCENTRATE AS INFLUENCED BY SOLIDS CONCENTRATION, TEMPERATURE, AND SONICATION¹

ABSTRACT

Skim milk powder (SMP) and milk protein concentrates (MPCs) are manufactured by evaporation followed by spray drying and are widely used as functional and nutritional ingredients. This study investigated the effects of temperature (40-60 °C) and total solids content (TS) on the viscosity of reconstituted MPC (rMPC) (≥ 30 % TS) and SMP (rSMP) (≥ 46 % TS) in laboratory conditions. Additionally, the influence of sonication in batch (70 % amplitude) and flow through systems (90% amplitude) was studied in a laboratory setting. The viscosity increased for all treatments with an increase in TS and decreased with an increase in temperature. Overall, sonication in both batch (30 s) and flow through systems (10.1, 20.2, and 30.2 s) resulted in significant decreases in viscosity for both rSMP and rMPC. An increase in viscosity was observed after post-sonication circulation; however, the viscosity did not return to the pre-sonication values.

¹ International Dairy Journal. Effect of sonication on the viscosity of reconstituted skim milk powder and milk protein concentrate as influenced by solids concentration, temperature and sonication. Volume 78, March 2018, Pages 122-129. Vidita K. Deshpande, Marie K. Walsh. (original copyright notice as given in the publication in which the material was originally published) “With permission of Elsevier”.

INTRODUCTION

Skim milk powder (SMP) is a dried form of pasteurized skim milk that contains ≤ 5 % moisture and ≤ 1.5 % milkfat (by weight) (Smith, 2008). SMP has a standardized milk protein content of 34 % as opposed to nonfat dry milk (NFDM), which does not (Smith, 2008). Milk protein concentrates (MPCs) are high-quality protein products that have various roles in terms of functionality and nutrition. MPCs and SMPs are complete proteins that contain both casein and whey proteins as opposed to whey protein concentrates (WPC) or isolates (WPI). In comparison with SMP, MPCs are higher in protein (42-85 %) and lower in lactose (4-46 % depending on protein content) and minerals (6-7 %) (Agarwal, Beausire, Patel, & Patel, 2015; Patel & Patel, 2014).

SMP is standardized to 34 % protein using either the milk retentate or permeate from ultrafiltration (UF) of milk. SMP is manufactured using pasteurized skim milk that is concentrated by evaporation followed by spray drying (Smith, 2008). MPCs are generally produced using skim milk, which is concentrated by UF. UF results in segregation of caseins, whey proteins, micellar salts, and residual fat in the retentate, whereas lactose, soluble salts, and non-protein nitrogen are removed with the permeate (Bastian, Collinge, & Ernstrom, 1991). Diafiltration (DF) is commonly applied to remove residual lactose and soluble minerals and to obtain a product with a high protein content (Patel & Patel, 2014). MPC is produced by further concentration of this UF retentate using evaporation followed by spray drying.

MPCs provide a range of functionalities such as water binding, viscosity, gelling, foaming/whipping, emulsification, and heat stability, and are used in many protein-fortified foods but primarily in meal replacements, nutritional beverages and bars

(Agarwal et al., 2015; Patel & Patel, 2014). MPCs, due to their lower lactose content, can impart a clean dairy flavor with reduced Maillard browning. Apart from serving as an excellent substitute for milk, SMP can be used in infant formulas, nutritional products for children, and fortification of dairy products along with serving as a functional ingredient in bakery products, snacks, and chocolate confectionaries (Lagrange, Whitsett, & Burris, 2015).

Processing of both SMP and MPC involves evaporation and spray drying, which are both high heat treatments. It would be economical to obtain a liquid feed of higher % total solids (TS) prior to spray drying. However, it is difficult to do so because an increase in viscosity is seen with a high solids content (Fernández-Martín, 1972; O'Donnell & Butler, 2008). An increase in viscosity poses a problem in the dairy processing industry since it leads to reduced flow rates, high pressure drops, decreased turbulence (lower rate of heat transfer), and severe fouling in heating operations. The production of concentrated skim milk, which is used in the production of both SMP and MPC, is limited to approximately 50 % TS since large increases in viscosity are observed at $TS \geq 45 \%$ (Enríquez-Fernández, Camarillo-Rojas, & Vélez-Ruiz, 2013). Fluid milk with $\geq 45 \%$ concentration is difficult to atomize due to increase in viscosity that leads to large droplets being formed in the atomizer; thus, decreasing the thermal efficiency of the spray dryer (Enríquez-Fernández et al., 2013; Zisu, Schleyer, & Chandrapala, 2013). Additionally, the viscosity of concentrated skim milk increases with time in a process called “age thickening” that is a result of structural build via noncovalent interactions between casein micelles (Bienvenue, Jiménez-Flores, & Singh, 2003).

A reduction in viscosity of skim milk concentrate and reconstituted whey protein powders was seen upon application of high power, low frequency ultrasound (Ashokkumar et al., 2009; Sun et al., 2014; Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010; Zisu et al., 2013). Ultrasound is sound waves that have a frequency of greater than 20 kHz and produced using a sonication power source (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). Acoustic cavitation is a phenomenon where passing of these sound waves through a liquid causes the formation of alternating high pressure (compression) and low-pressure (rarefaction) cycles. During rarefaction, small vacuum bubbles or voids form that increase in size during every compression and rarefaction cycle. These bubbles reach a volume at which no additional energy can be absorbed during the compression cycle, leading to bubble collapse. As a result of cavitation and bubble collapse, very high temperatures (≈ 5000 K) and pressures (≈ 2000 atm) are reached locally (Zisu et al., 2013).

Different applications of ultrasound are being explored or implemented in food processing for emulsification, filtration, viscosity modification, improvement of whey protein heat stability, improvement of meat tenderness, and inactivation of spoilage microbes (Chandrapala et al., 2012; Chemat, Zill-E-Huma, & Khan, 2011; Knorr, Zenker, Heinz, & Lee, 2004). Sonication has caught the interest of the food industry due to its potential to alter the functionality of foods as well as improve the shelf life and quality (Chandrapala et al., 2012; Knorr et al., 2004). Few studies have investigated the influence of sonication on the viscosity of concentrated milk (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011; Zisu et al., 2013).

This study investigated the effects of total solids (TS) and temperature on the viscosity of reconstituted milk protein concentrates (rMPC) and reconstituted skim milk

powder (rSMP). Additionally, the influence of sonication on the viscosity of rMPC and rSMP at different TS and temperatures was investigated using both batch and flow-through sonication systems in a laboratory setting. Although there have been other studies that investigated the effects of sonication in dairy systems (Ashokkumar et al., 2009; Sun et al., 2014; Zisu et al., 2010, 2013), there are no published studies that investigated the effects of batch and continuous sonication on reconstituted MPC and SMP at different solids (30-44 % TS MPC, and 46-64 % TS SMP) and at different temperatures (40-60 °C).

The temperatures and TSs used for this study were an attempt to mimic the conditions used during the processing of milk concentrates. Since concentrated skim milk is evaporated at temperatures between 50 and 70 °C, rMPC and rSMP were treated at 40, 50, and 60 °C to investigate the influence of temperature on the viscosity (Singh, 2007). Samples treated at 70 °C stuck to the containers used for heating and an even sample could not be obtained. Also, MPC and SMP are evaporated to obtain solids contents of about 30 % and 50 % solids respectively, prior to spray drying (Agarwal et al., 2015). Using this rationale, the TS used for this research was ≥ 30 % TS for rMPC and ≥ 46 % TS for rSMP.

MATERIALS AND METHODS

Experimental design

For the first part of the study, the effect of temperature and TS on the viscosity of rMPC samples with TS of 30-44 % and rSMP samples with TS of 46-64 % was evaluated at 40, 50, and 60 °C. For the second part of the study, the effect of temperature, batch sonication (versus no sonication), and TS on the viscosity of rMPC samples with TS of 30-44% and rSMP samples with TS of 46-64 % were evaluated at 40, 50, and 60 °C. For the

third part of the study, the effect of temperature, flow through sonication (versus no sonication), and TS on the viscosity of rMPC samples with TS of 30-34% and rSMP samples with TS of 50-54 % were evaluated at 60 °C. All experiments were replicated 3 times and analyzed in triplicate.

Sample preparation

MPC 70 (low heat; Darigold, Seattle, Washington, USA) evaporated to 32 % TS and low heat SMP (Darigold; High Dessert Milk, Burley, ID, USA) evaporated to 45 % TS before spray drying were used. Powders were stored at temperatures between 20 and 25 °C. The moisture percent of the powders stated as 5.25 ± 0.10 for MPC and 4.37 ± 0.28 for SMP and this was confirmed using a moisture analyzer (Sartorius AG MA 150, Göttingen, Germany). The moisture content of the powders was monitored over the time frame of the experiments and no changes were observed. Thus, any changes in solubility as a result of prior storage history are assumed to be consistent in all samples and did not contribute to significant changes in viscosity.

Both rMPC and rSMP of known TS were made by weighing the solute (MPC or SMP) with the addition of distilled water to make 400 mL solutions. Distilled water was heated to the required temperature (40, 50, or 60 °C) before being added to the solute. This mixture was blended with a high shear blender (Ultra-Turrax with S25N-18G 10 dispersion tool) for 15 min and kept in a water bath for 30 min at temperatures between 45 and 65 °C depending on the experiment to be performed to maximize solubility. The TS content of samples was determined using a moisture analyzer (Sartorius).

Overnight rehydration time was not possible in a laboratory setting at the % TS (30-44 % for MPC and 46-64 % for SMP) we worked with because the samples would show

age thickening and would render viscosity measurements invalid. However, we do believe the steps taken (mixing with a high-speed rotor blender and high-water temperature) during sample preparation were sufficient to achieve almost complete rehydration such that the powders were in solution.

Sonication treatment

For batch sonication, a 30 mL sample of reconstituted concentrated milk was placed in a double walled glass vessel (50 mL) at a constant temperature and sonicated at 70 % amplitude for 30 s using a 12.7 mm microtip and a Sonicator power source (QSonica Q500, Newtown, CT, USA). A circulating water bath was used to maintain the appropriate sample temperature (40, 50 or 60 °C). The viscosity of the samples was measured before and after sonication as described below.

To simulate continuous operation, samples were reconstituted as described above and pumped using a Masterflex 7529 pump (Cole-Palmer, Vernon Hills, IL, USA) at a flowrate of 1.8 L min⁻¹ for a total of 60 min and 15 min for rMPC and rSMP, respectively before being sonicated and a sample was collected at this time point. The sonication flow cell had a 261 mL volume resulting in an 8.4 s residence time (time sample was exposed to sonication) for the 1.8 L min⁻¹ flow rate. For flow through sonication, the total volume of rMPC or rSMP used was 3 L and the samples were recirculated through the system. Samples were collected for viscosity measurements at 2, 4, and 6 min, which corresponded to total sonication residence times of 10.1, 20.2, and 30.2 s, respectively. For the continuous operation, rSMP and rMPC were sonicated (Heischler UIP500hd, Ringwood, NJ, USA) at 90 % amplitude. Samples were recirculated through the flow through system post sonication and samples were collected for viscosity measurements at

45 min for rMPC and 30 min for rSMP. Schematics of the sonication systems are shown in Appendix B, Figure B.1. Two water baths were used: one water bath was to maintain the sample temperature at 60 °C; the stainless-steel flow cell had a water jacket and was connected to the other water bath to maintain the temperature of the sample during sonication at 60 °C.

The energy density (J mL^{-1}) for the samples sonicated in the batch and flow through system was calculated according to Chandrapala, Martin, Kentish, and Ashokkumar (2014). The power readings ranged from 190 to 192 W in the flow through system. An average of 191 W was used and the calculated energy density at 10.0, 20.2, and 30.2 s of residence time was 0.64 J mL^{-1} , 1.28 J mL^{-1} , and 1.92 J mL^{-1} , respectively. The power readings for the batch sonication were an average of 63 W so the energy density for batch sonication was 63 J mL^{-1} with a 30 mL sample volume and 30 s sonication time.

Viscosity measurement

The viscosity was measured for all samples using a viscometer (Fungilab-Expert series, Hauppauge, New York, NY, USA) and a rheometer (AR-G2, TA Instruments, New Castle, DE, USA) equipped with a concentric cylinder geometry. Viscometer spindles TL 5, 6, and 7 were used at the highest rpm (ranged between 10-200 rpm) attainable for that sample with type of spindle used to obtain a % torque between 20 and 100 %. Measurements were taken at the three highest rpm attainable and a mean of the viscosity values was calculated to be used for further analysis. For rheometer viscosity measurements, a steady state flow procedure was used to measure the viscosity as a function of shear rate (1×10^{-4} - 300 s^{-1}) for both rMPC and rSMP and the mean of the viscosity at a steady state (highest shear rates) was recorded. Data from the viscometer

were compared with that of the rheometer (for the solids and temperature experiments only). The viscosity measured was reported in Pa s.

Statistical analysis

Analysis of variance (ANOVA) and t-tests were performed to test for statistical significance ($\alpha = 0.05$) using SAS 9.4 and Excel statistics. Statistical significance of differences between viscosity measurements were tested using t-tests. ANOVA was used to determine if solids and temperature have a combined effect on the viscosity of rSMP and rMPC at the given temperature and TS parameters. For ANOVA, the data obtained for both rMPC and rSMP was transformed to get a greater normal distribution. rMPC was transformed using the logarithmic function and rSMP was transformed using the square root function. ANOVA was performed using a complete block design for both rSMP (46, 50, and 54% TS) and rMPC (30, 32, 34, and 36% TS) treated at 40, 50, and 60 °C.

RESULTS AND DISCUSSION

Effect of solids and temperature

Effect of solids and temperature on the viscosity of rMPC and rSMP can be seen in Figure 3.1. The viscosity measurements with a viscometer when compared with that of rheometer were not significantly different (data shown in Appendix B, Figure B.2), therefore viscometer measurements are given. Since rMPC and rSMP are commonly evaporated at temperatures between 50 and 70 °C to a TS of 30 and 50%, respectively (Agarwal et al., 2015; Singh, 2007), rMPC and rSMP were reconstituted at $\geq 30\%$ and $\geq 46\%$ TS, respectively, and treated at 40, 50, and 60 °C. Overall, there was an increase in

viscosity with an increase in solids content at each temperature tested, for both rMPC and rSMP. For both rMPC and rSMP, the increase in viscosity at 60 °C was initially linear, but was subsequently exponential at $\geq 42\%$ and $\geq 60\%$ TS, respectively. However, the overall increase in viscosity was exponential in all other rSMP treatments while the viscosity increase was linear at 40 °C and exponential at 50 °C in rMPC (with linear or exponential regression $R^2 > 0.94$)². For all TS, the 60 °C samples showed the lowest viscosity followed by 50 °C then 40 °C.

From ANOVA of rMPC and rSMP (Table 3.1), the effects of TS, temperature and their interactions were statistically significant, indicating that both TS and temperature have a combined effect on the viscosity of rMPC and rSMP. ANOVA determined the significant variables with the largest effect for rMPC as temperature, followed by TS, and the interaction, while for rSMP, the largest effect was TS followed by temperature, and the interaction.

When comparing Figure 3.1 A and 3.1 B, it should be noted that the ordinate of rSMP (Figure 3.1B) is ten times greater than that of rMPC (Figure 3.1A). However, it should also be noted that rMPCs are treated at relatively lower TS as compared with rSMP in this study. At 50 °C, the viscosity of 44% TS rMPC was 0.6 Pa s, and that of a 46% TS rSMP was 0.09 Pa s. Also, at 60 °C, the viscosity of 44% TS rMPC was 0.3 Pa s, and that of a 46% TS rSMP was 0.07 Pa s. Hence, it can be said that at same temperatures and approximately the same TS, rMPC has a higher viscosity as compared with rSMP. This

² Viscosity behavior of rMPC and rSMP at different temperatures is shown using different trend lines in Appendix B. Figure B.3

may be attributed to the higher protein content of rMPC. Moreover, rSMP thickened with aging faster than rMPC.

With rMPC, a significant % increase in viscosity was observed at each TS (30, 32, 34, and 36 %) at 40 °C and 50 °C as compared with 60 °C (Table 3.2). For rMPC, the greatest % increase in viscosity (784.3 %) was observed at 36 % TS at 40 °C. For rMPC, the % increase at 40 °C as compared with 60 °C was 304.2, 489.4, 513.9, and 784.3 % at 30, 32, 34, and 36 % TS, respectively. rMPC at 50 °C showed a % increase of 228.9, 194.3, 197.2, and 215.9 %, respectively, at 30, 32, 34, and 36 % TS, as compared with 60 °C. The % increase of 36 % TS for rMPC at 40 °C, was approximately 3, 2, and 1.5 times higher when compared with 30, 32, and 34 % TS. At 50 °C, the increase in viscosity as compared with 60 °C was relatively proportional in terms of TS. This implies that temperature had a greater effect than TS for the viscosity increases observed in rMPC within the ranges tested.

With rSMP, a significant % increase in viscosity was observed at each TS (46, 50, and 54 %) at 40 °C and 50 °C as compared with 60 °C (Table 3.2). For rSMP, the % increase in viscosity at 54 % TS at 40 °C and 50 °C was extreme (2446.2 and 1147.2, respectively) as compared with 60 °C. The % increase in viscosity at 46 % and 50 % TS at 40 °C and 50 °C compared with 60 °C was significant, but not as extreme, with values being 40.5 % and 24.5 %, respectively, for 46 % TS, and 64.5 % and 37.8 %, respectively, for 50 % TS. At 40 °C, the % increase for 54 % TS rSMP was 61 and 38 times higher than at 46 % and 50 % TS, respectively. Also, at 50 °C the % increase for 54 % TS rSMP was 47 and 30 times higher than at 46 % and 50 % TS, respectively. This implies that TS had a greater effect than temperature on the viscosity of rSMP within the ranges tested.

The increase in viscosity with increase in solids content and the decrease in viscosity with an increase in temperature seen with rMPC and rSMP was similar to the effect of temperature and solids content observed in skim milk by Fernández-Martín (1972) and in rMPC by O'Donnell & Butler (2008). However, for rSMP, temperatures ≤ 40 °C had a more dramatic effect on the viscosity as compared with temperatures greater than 40 °C, at ≤ 30 % TS (Fernández-Martín, 1972). A similar trend was seen in this study with rSMP, where the increase in viscosity was exponential for all rSMP treatments while the viscosity increase was linear at 40 °C in rMPC.

For rMPC, the greatest % increase in viscosity (784.3 %) was observed at 36 % TS at 40 °C and that for rSMP was observed at 54 % TS at 40 °C. The viscosity of rSMP (0.14 Pa s) measured in this experiment was lower than the viscosity of a skim milk concentrate from an evaporator (0.40 Pa s) measured by Zisu et al. (2013), when both had a 50 % TS concentration and treated at 50 °C.

In milk, at solids content of ≥ 40 %, the viscosity increases in a nonlinear manner with an increase in total solids content, which is similar to the exponential increase in viscosity at high solids seen in this study. In skim milk, an increase in solids content is accompanied by reduction in the volume fraction of water which in turn causes an increase in volume fraction of dispersed particles and the micelle-micelle interactions as the distance between the micelles becomes smaller (Bienvenue et al., 2003). Thus, the increase in viscosity seen with increase in solids content is due to increased intermolecular interactions between proteins. The decrease in viscosity with an increase in temperature has been attributed to a possible decrease in protein-protein interactions and an increase in protein-water interactions (Fernández-Martín, 1972; Herceg & Lelas, 2005).

During spray drying of milk powders, the temperature of the milk droplet does not exceed 70 °C and the powders are heated only for a few seconds, thus very minimal changes are observed in the behavior of milk components post spray drying when compared with the pre-drying concentrate (Singh, 2007). However, both evaporation and spray drying alter the soluble salt equilibrium of milk where a decrease in the solubility of calcium and phosphate is seen.

Previous research has shown that rehydration of milk powders is a function of dissolution (solubility) and mineral equilibration and is influenced by spray drying heat treatment, powder storage time and temperature (Anema, Pinder, Hunter, & Hemar, 2006), and rehydration temperature, times and shear (Chandrapala, Martin, Kentish, & Ashokkumar, 2014; Martin, Williams, Choong, Lee, & Dunstan, 2008; Martin, Williams, & Dunstan, 2010; Mimouni, Deeth, Whittaker, Gidley, & Bhandari, 2009). Low heat SMP is rapidly dissolved with just vigorous shaking at room temperature for 20 s (Martin et al., 2008). This is not to state that a mineral equilibrium was reached, but the sample is in solution. In contrast, MPC is known for having a low solubility. The complete rehydration of milk powders is a result of two processes that occur simultaneously. Dissolution of powder particles in the solvent and the transfer of water to the core of the powder particles. Sikand, Tong, Roy, Rodriguez-Saona, & Murray (2011) found that the reason for low solubility of high protein MPCs is due to decreased rate of water transfer to the core of the protein particles. Mimouni et al. (2009) concluded that the rate limiting step in the complete rehydration process of MPC 85 was the dissolution rate. They showed that there was a large acceleration in rehydration of MPC 85 with an increase in temperature from 24 to 35 °C. In addition, Martin et al. (2010) showed that MPC 80 could be rapidly solubilized with

vigorous shaking followed by heating at 60 °C for 5 min. Chandrapala et al. (2014) showed that a 10 % (w/w) solution of MPC 80 achieved dissolution at 90-95 % using high shear for less than 10 min. We used 15 min of high shear at temperatures greater than 40 °C on the reconstitution of our samples, therefore the rSMP and rMPC samples may not have been 100 % soluble prior to sonication so the decrease in viscosity may also be due to an increase in solubility as a result of sonication as well as the disruption of protein aggregates.

Effect of batch sonication

Effect of sonication on the viscosity of rMPC and rSMP at 40 °C, 50 °C, and 60 °C in a batch sonication system are displayed in Figures 3.2 and 3.3, respectively. Overall, there was a decrease in viscosity after sonication for both rMPC and rSMP. An overall greater % decrease in viscosity due to batch sonication was seen with an increase in % TS for rMPC. For rMPC, the % decrease in viscosity as a result of batch sonication was greater at 50 °C, followed by 40 then 60 °C. We were unable to determine the effects of sonication at % TS > 36 % at 40 °C because the sample was too viscous.

In the case of rSMP, the highest values for % decrease in viscosity were seen at 54, 60 and 64 % TS at 60 °C for batch sonication. We were unable to determine the effects of sonication at TS > 52 % at 40 and 50 °C as the samples were too viscous. Zisu et al. (2013) reported a 10 % reduction in viscosity when skim milk concentrate was sonicated for a total of 1 min at 55 °C and at 50 % TS which is similar to the 22.1 % reduction seen in this study.

At 50 °C, the % decrease in viscosity of 44 % TS rMPC was 54.6 % and that for a 46 % TS rSMP was 18.9 %. Also, at 60 °C, the % decrease in viscosity of 44 % TS rMPC was 44.3 % and that for a 46 % TS rSMP was 19.2 %. Hence, it can be said that at same

temperatures and approximately the same % TS, rMPC showed a higher reduction in viscosity as compared with rSMP in a batch sonication system. Samples were in solution prior to sonication, however, we do acknowledge that in a laboratory setting given our experimental parameters, 100 % solubility may not have been achieved. We believe the reduction in viscosity is majorly a result of breaking of protein aggregates due to sonication; however, an increase in solubility of reconstituted samples from sonication may have influenced the decrease in viscosity as well.

Effect of flow-through sonication

The effect of sonication on rMPC and rSMP at 60 °C in a flow-through recirculating sonication system is shown in Figure 3.4. Temperature and TS conditions were chosen to mimic the manufacturing conditions of SMP and MPC. For rSMP, % TS of ≥ 54 % in a continuous system required long heating times to form a continuous solution, which resulted in age gelation of samples, therefore the highest TS used was 54. To achieve a steady state viscosity, rMPC was run through the continuous system for 60 min. A steady state was determined by no change in viscosity. rSMP was run for a shorter time because an age thickening effect was observed when run for more than 15 min.

For rMPC, the decrease in viscosity with sonication is shown in Figure 3.4 A. When rMPC was run through the flow-through sonication system for 45 min after sonication, the decreases in viscosity were 33.2, 17.2, and 10.3 % for 30, 32, and 34 % TS, respectively, as compared with pre-sonication. For rSMP, the decrease in viscosity with sonication is shown in Figure 3.4 B. When rSMP was run through the flow-through system for 30 min after sonication, the decreases in viscosity were 24.15, 4.0, and 11.5 % for 50, 52, and 54 % TS, respectively, as compared with pre-sonication.

Overall, there was an increase in viscosity with an increase in solids content and a decrease in viscosity with sonication for both rSMP and rMPC in the flow system, similar to the batch system. Sonication in a continuous flow-through system significantly decreased the viscosity of samples collected after sonication times of 10.1, 20.2, and 30.2 s as compared with the baseline prior to sonication (60 min for rMPC and 15 min for rSMP). For rMPC, the mean viscosity of the 34 % TS sample after 30.2 s residence time of sonication was lower than the mean viscosity of 30 % TS sample prior to sonication. Also, the mean viscosity at 34 % TS after 10.1 s of residence time of sonication was equivalent to that at 30 % TS prior to sonication. Therefore, if MPC is concentrated to 34 % TS via evaporation, only 10 s of sonication may be needed to obtain an equivalent viscosity as seen at 30 % TS. Furthermore, sonication of the 34 % TS rMPC for 30 s would yield a viscosity which was lower than that at 30 % TS pre-sonication values.

A similar effect was not seen for rSMP when looking at the viscosity changes between 50 and 54 % TS with sonication. It would take at least 30.2 s of sonication for the viscosity of 54 % TS rSMP to be equivalent to the pre-sonication viscosity of the 52 % TS rSMP. The differences in viscosity decrease for rSMP compared with rMPC may have been due to an immediate aging effect seen in the samples prior to the viscosity measurements. Depending on the flow-through sonication system, an increase in total sonication time to achieve a desired level of viscosity may be obtained by addition of multiple sonication flow cells in sequence in a processing facility. The sonication times used in flow (10.1 s) that resulted in a significant decrease in viscosity for rMPC are within a practical range.

With rMPC at 60 °C, after 30 s of sonication, the % decrease in viscosity was greater for 30 and 32 % TS and lower for 34 % TS as compared with that seen in 30 s of batch sonication. Similarly, in the case of rSMP, the % decrease in viscosity after 30 s residence time in continuous sonication was greater for 46 and 50 % TS and lower for 54 % TS as compared with batch sonication.

In the flow-through system, a decrease in viscosity was seen after 10.1, 20.2, and 30.2 s of sonication respectively for both rMPC and rSMP as compared with pre-sonication observations for rSMP and rMPC, respectively (Figure 3.4). However, after 30 min (rSMP) and 45 min (rMPC) of post-sonication circulation through the continuous system, the viscosity increased but did not revert to the pre-sonication values.

Previous studies by Ashokkumar et al. (2009), Chandrapala et al. (2014), and Sun et al. (2014) have shown, via particle size analysis of sonicated dairy systems, that sonication breaks apart large aggregates leading to a decrease in particle size and a lower viscosity. Additionally, others (Martini, Potter, & Walsh, 2010) showed no change in whey protein sizes via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after sonication of a whey protein solution for 15 min at 60 °C. Sun et al. (2014) did not observe protein degradation in MPC sonicated for up to 5 min via SDS-PAGE and Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar (2011) observed no changes in reverse-phase high performance liquid chromatography of whey samples sonicated for up to 60 min. These authors concluded that the physiochemical properties of casein micelles is unaffected by sonication and the viscosity reduction in dairy systems is primarily caused by the shear forces generated during acoustic cavitation, which disrupt noncovalent interactions (casein-casein and/or casein-whey protein interactions) forming aggregates

(Zisu et al., 2010). After 30-45 min of recirculation post sonication, the increase in viscosity may be due to the ability of these non-covalent interactions to reform.

A similar effect of decrease in viscosity was observed by Zisu et al. (2013), where high power low frequency ultrasound reduced the viscosity of skim milk concentrate in both batch and continuous processing. In their study, sonication could not prevent age thickening, however, sonication reduced the viscosity of the aged concentrate similar to that of the starting material. Aging of milk concentrates may be a result of either weakening of casein micelle interactions (Karlsson, Ipsen, Schrader, & Ardö, 2005) or flocculation of these micelles that may be due to loss of electrostatic repulsion during storage (Bienvenue et al., 2003).

CONCLUSION

From this study, it can be said that both TS and temperature significantly influence the viscosity of concentrated milk and can be used to modulate the viscosity of SMP and MPC concentrates. Overall, there was an increase in viscosity with an increase in solids content at each temperature tested, for both rSMP and rMPC. At the same temperatures and approximately the same % TS, rMPC had a higher viscosity as compared with rSMP. This may be attributed to the higher protein content of rMPC. Moreover, temperature had a relatively greater effect on the viscosity for rMPC, while, for rSMP, TS had a greater effect on the viscosity.

An overall greater % decrease in viscosity as a result of batch sonication was seen with an increase in TS for rMPC and rSMP. The % decrease in viscosity as a result of batch sonication ranged from 27.3 to 54.6 % for rMPC and 18.7- 44.3 % for rSMP. Sonication

in a flow through continuous operation significantly decreased the viscosity of samples collected after sonication times of 10.1, 20.2, and 30.2 s as compared with pre-sonication. An increase in viscosity was observed after post-sonication circulation; however, the viscosity did not return to the pre-sonication values.

We do acknowledge that the decrease in viscosity seen may be a result of increased solubility along with the disruption of protein aggregates due to sonication. Increased solubility of rMPC along with aging of rSMP may have led to the differences in decrease in viscosity of these two reconstituted concentrates. If MPC is concentrated to 34 % TS via evaporation, only 10 s of sonication may be needed to obtain an equivalent viscosity as seen at 30 % TS. Furthermore, sonication of the 34 % TS rMPC for 30 s yielded a viscosity, which was lower than that at 30 % TS pre-sonication values. For practical application of this research, this work needs to be repeated with fresh concentrates to determine whether the effect of sonication on the decrease in viscosity seen in this research is due to break down of aggregates or in solubility in the reconstituted samples or a combination of both. Moreover, the effect of sonication on transient aggregates formed during the process of concentration can also be studied.

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Tables

Table 3.1. ANOVA for rMPC and rSMP samples when reconstituted at 30-36 % and 46-54 % TS, respectively and treated at 40, 50, and 60 °C.

Parameter	rMPC		rSMP	
	F statistic	P-value	F statistic	P-value
Total Solids	244.08	< 0.0001	1003.36	< 0.0001
Temperature	4679.22	< 0.0001	330.83	< 0.0001
Total Solids x Temperature	52.32	< 0.0001	315.13	< 0.0001

Table 3.2. Percent Increase in Viscosity of rMPC and rSMP at 40 and 50 °C as compared to 60 °C.

%Total Solids	% Increase at 40°C	p-value	% Increase at 50°C	p-value
rMPC				
30	304.2	0.0003	228.9	< 0.0001
32	489.4	0.0020	194.3	0.0001
34	513.9	0.0005	197.2	< 0.0001
36	784.3	< 0.0001	215.8	< 0.0001
rSMP (%TS)				
46	40.1	0.0015	24.5	0.0012
50	64.5	0.0068	37.8	0.0006
54	2446.2	0.0023	1147.2	< 0.0001

Figures

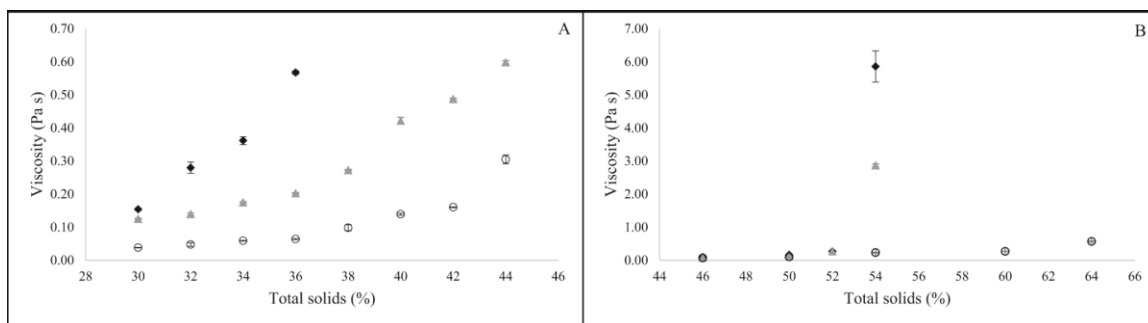


Figure 3.1. Viscosity of rMPC (A) and rSMP (B) at various solids content treated at 40°C, 50°C, and 60°C.

◆, 40 °C; ▲, 50 °C; ◻, 60 °C.

Error bars indicate standard deviation.

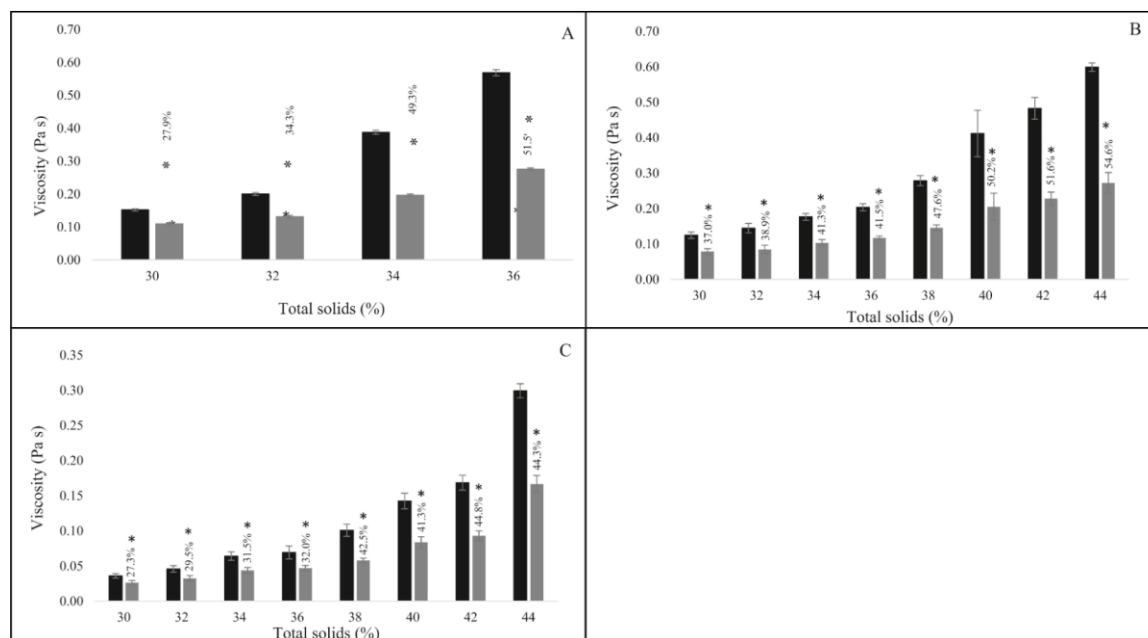


Figure 3.2. Effect of batch sonication on the viscosity of rMPC at various solids content at 40°C (A), 50°C (B), and 60°C (C) in a batch system.

■, mean viscosity (Pa s) before sonication; ■, mean viscosity (Pa s) after sonication. Error bars indicate standard deviation. Values above bars are % reduction in viscosity as a result of batch sonication; an asterisk indicates values are significantly different as compared with before sonication at $\alpha = 0.05$.

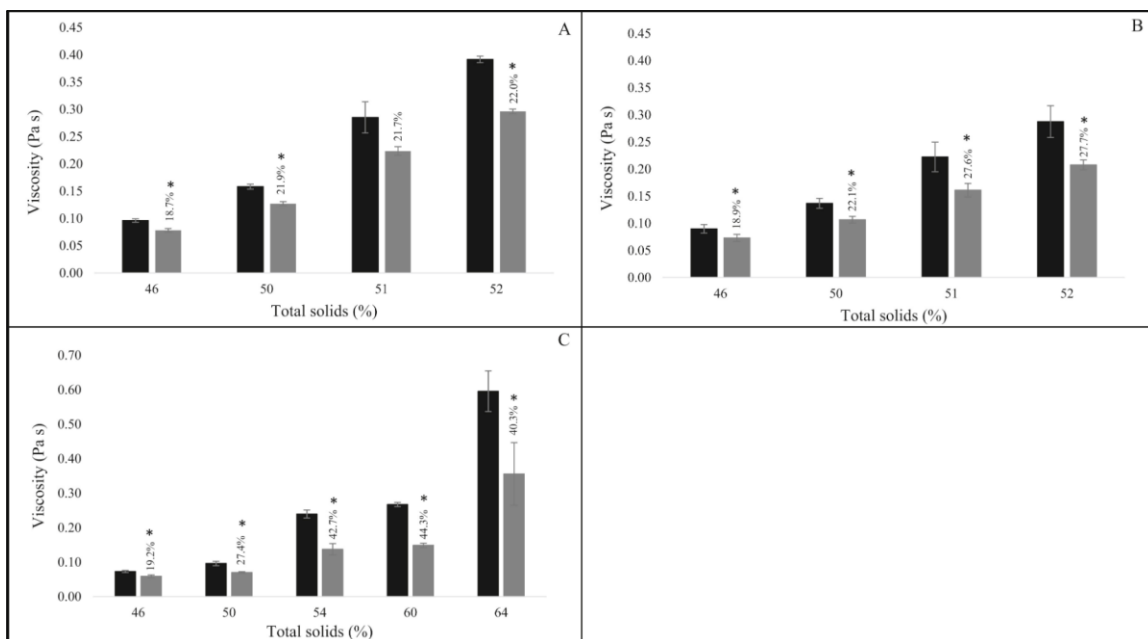


Figure 3.3. Effect of batch sonication on the viscosity of rSMP at various solids content at 40°C (A), 50°C (B), and 60°C (C) in a batch system.

■, mean viscosity (Pa s) before sonication; ■, mean viscosity (Pa s) after sonication. Error bars indicate standard deviation. Values above bars are % reduction in viscosity as a result of batch sonication.

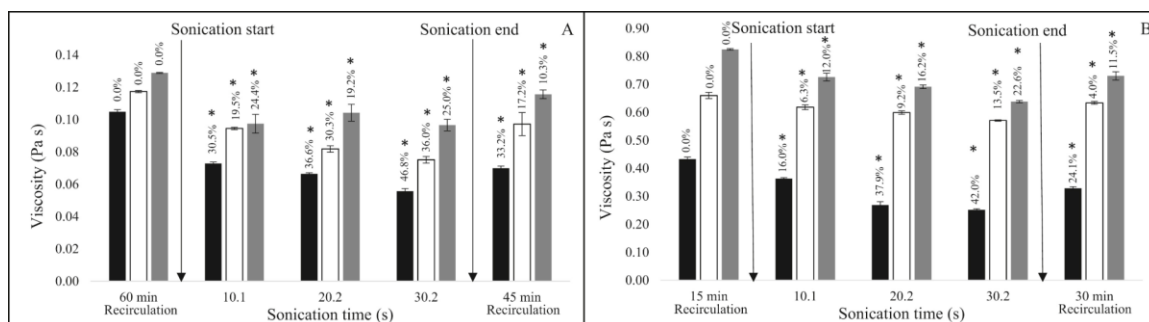


Figure 3.4. Effect of flow through sonication on the viscosity of rMPC and rSMP at various solids content at 60 °C in a continuous system as compared with pre-sonication. rMPC in panel A: ■, 30 % TS; □, 32 % TS; ■, 34 % TS and rSMP in panel B: ■, 50 % TS; □, 52 % TS; ■, 54 % TS

Error bars indicate standard deviation. Values above bars are % reduction in viscosity as a result of flow through sonication; an asterisk indicates values are significantly different as compared with before sonication at $\alpha = 0.05$. On the abscissa, numbers indicate residence time in seconds.

CHAPTER IV

EFFECT OF THERMOSONICATION IN A BATCH SYSTEM ON THE SURVIVAL OF THERMOPHILIC BACTERIA IN MILK³.

ABSTRACT

Thermosonication may help reduce thermophilic bacteria counts. Cells and spores of *Geobacillus stearothermophilus*, *Anoxybacillus flavithermus*, and *Bacillus subtilis* (spores only) were treated with either heat alone or thermosonication in a batch system from 0-120 s in tryptic soy broth and 2 % fat milk at 72 and 73 °C. D-values for cells were calculated and were reduced with thermosonication as compared to heat alone. Maximum reduction in cells after thermosonication was 1 log after 30-45 s and for spores was ≤ 0.5 log after 120 s, which may not influence milk product quality in scale up systems.

INTRODUCTION

Thermophilic bacterial cells and spores are capable of surviving pasteurization temperatures. Thermophilic bacteria grow in dairy products and produce acids and

³ This is the pre-peer reviewed version of the following article: Deshpande, V.K. and Walsh, M.K. (2020), Effect of thermosonication in a batch system on the survival of spore-forming bacteria. Int J Dairy Technol. doi:10.1111/1471-0307.12685, which has been published in final form at <https://doi.org/10.1111/1471-0307.12685>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions.

enzymes which deteriorate milk quality, and is a concern for the dairy industry (Burgess *et al.* 2010; Khanal *et al.* 2014b; Lücking *et al.* 2013). Thermophilic bacteria have been known to cause defects such as ropiness, flat sour spoilage, and production of lactic acid and off flavors in milk products (Burgess *et al.* 2010; Khanal *et al.* 2014b; Lücking *et al.* 2013). Thermophilic bacterial cells and spores are able to form heat-resistant biofilms on equipment surfaces and can contaminate the product stream resulting in products with spore counts 1000 times higher than raw incoming milk (Burgess *et al.* 2010).

Cells and spores of obligate thermophilic bacteria such as *Anoxybacillus flavithermus* and *Geobacillus stearothermophilus* and facultative thermophilic bacteria such as *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus sporothermodurans* have been associated with pasteurized milk and other milk products (Burgess *et al.* 2010; Gopal *et al.* 2015; Lücking *et al.* 2013; Sharma and Anand, 2002). Some strains of thermophilic bacteria such as *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis* have been shown to survive high processing temperatures employed in milk powder manufacturing (Burgess *et al.* 2010; Sadiq *et al.* 2016). Even if the thermophilic bacterial cells do not survive high temperature processing, their spores do and can germinate (Khanal *et al.* 2014a). Thermophilic bacteria and spores are difficult to eliminate in high temperature processing and it would be beneficial to reduce their concentrations in incoming milk.

Addition of heat alone may not reduce thermoresistant microbes and use of alternative technologies such as sonication could be explored. Application of ultrasound also referred to as sonication, can result in cavitation. As a result of cavitation, very high temperatures ($\approx 4726^\circ\text{C}$) and pressures ($\approx 2,000\text{ atm}$) are reached locally (Zisu *et al.*

2013). Sonication combined with heat (thermosonication) and pressure (manosonication) or both (manothermosonication) has been effective in reducing microorganisms in foods (Villamiel *et al.* 2009). Sonication has been explored to improve milk quality by reducing the indigenous microflora or added pathogens in batch and continuous systems (Bermúdez-Aguirre *et al.* 2009a, 2009b; Khanal *et al.* 2014a, 2014b).

Lim *et al.* (2019) studied the effect of batch thermosonication and cold sonication on milk inoculated with *Paenibacillus amolyticus* which is a thermotolerant psychrophilic organism. They showed that thermosonication did reduce the overall microbial count in milk, but the reductions were not significantly different than the pasteurized control samples. Previous studies showed that batch cold sonication of milk for 10 min reduced *A. flavithermus* cells by 4 logs and spores of *G. stearothersophilus* by < 1 log (Khanal *et al.* 2014a, 2014b).

Previous studies on sonication of thermophilic bacteria in milk did not explore the use of temperature with sonication (thermosonication) on *G. stearothersophilus*, *A. flavithermus*, and *B. subtilis* cells and spores. Moreover, treatment times of 5-10 min do not seem practical for potential scale up systems. Hence, there is a need of a study that uses shorter treatment times (< 2 min) to study the effect of thermosonication on thermophilic bacteria in milk. Also, calculation of D-values as a result of thermosonication has not been studied before for thermophilic bacterial cells in milk. The aim of this study was to investigate the effect of thermosonication at pasteurization temperatures on the inactivation of thermophilic bacterial cells and spores such as *G. stearothersophilus*, *A. flavithermus*, and *B. subtilis* in a batch system with relatively short treatment times (< 2 min).

MATERIALS AND METHODS

Experimental design

To calculate the effect of heat and batch thermosonication on reductions of thermophilic bacterial cells and spores, tryptic soy broth (TSB) and 2 % fat sterile milk were inoculated with bacteria and treated for 0-120 s at both 72 and 73 °C. Cells and spores of *G. stearothermophilus* and *A. flavithermus* along with spores of *B. subtilis* were used. D-values (the amount of time required to destroy 90 % of the initial microbial population) were determined for *G. stearothermophilus* and *A. flavithermus* cells with heat alone and with thermosonication in TSB and 2 % fat milk in a batch sonication system. Note should be taken, HTST pasteurization was not actually performed on any of the samples. Treatment times at HTST pasteurization temperatures of 72-73 °C were studied to make potential application to a continuous system easier since HTST pasteurization is most commonly used in the U.S (IDFA n.d.).

Growth of thermophilic cells and spores

G. stearothermophilus spores (NAMS A *G. stearothermophilus* 2.4 × 10⁶ in 0.1 mL, VWR, Atlanta GA, USA) were germinated and frozen stocks were prepared as described by Beatty and Walsh (2016). Cultures for experiments were grown by inoculating 25 mL TSB with 0.1 mL of frozen stock and incubated at 55 °C in a shaker at rpm for 15 h. Optical density (OD₆₀₀) was measured at 600 nm using a spectrophotometer (Bio Spec 1601, Shimadzu, USA) after every overnight growth and was between 0.70-0.98 which corresponded to approximately 10⁸ CFU/mL as determined by plating on tryptic soy agar (TSA) plates. For spore samples, *G. stearothermophilus*

spores were obtained directly from the commercial stock solution. Diluted stock solution was prepared by inoculating 10 mL of sterile water with 0.1 mL of commercial spore stock solution.

Cultures for *A. flavithermus* TNO-09.006 were kindly provided by Remco Kort (Vrije University, Amsterdam, the Netherlands) in the form of slant tubes. One loop from the slant tube was used to make a streak plate on TSA and was incubated at 55 °C for 48 h. A subculture was grown by inoculating 25 mL of TSB with one loop of the culture grown on the streak plate in a sterile 250 mL Erlenmeyer flask covered with sterile foil. Cells were grown aerobically at 55 °C in a shaker at 100 rpm for 15 h. The OD₆₀₀ was 0.81 after 15 h, which corresponded to 10⁸ CFU/mL as determined by plating on TSA plates. Frozen stocks were prepared by as described by Beatty and Walsh (2016). Cultures for experiments were grown by inoculating 25 mL TSB with 0.2 % (w/v) soluble potato starch (PS) with 0.5 mL of frozen stock in a sterile 250 mL Erlenmeyer flask covered with sterile foil and incubated at 55 °C in a shaker at 100 rpm for 12-13 h (Rueckert *et al.* 2005). OD₆₀₀ was checked after every overnight growth, and was between 0.70-0.81 and corresponded to 10⁸ CFU/mL as determined by plating on TSA plates.

Spores were prepared according to the method described by Rueckert *et al.* (2005) where 1 mL of frozen stock culture was inoculated in 1 L liquid Castenholz media and incubated at 55 °C for 48 h. Spores were harvested by centrifuging the media with the 48-h growth at 15,000 x g for 8 min at 4 °C, where pellets were obtained as residue at the bottom of the sterile centrifuge tubes and the filtrate was removed. The pellets were then washed with sterile deionized water. Washing with sterile water was repeated three times

while centrifuging between washings, and the residue (pellets) was saved and the filtrate was removed each time. After the last washing, the tubes were stored at 4 °C. Prior to use of the spore stock solutions, the pellets were resuspended in sterile water. *B. subtilis* spores were obtained directly from the commercial stock solution (*Bacillus subtilis* spore suspension, Merck KGaA, Darmstadt, Germany). Diluted stock solution was prepared by inoculating 10 mL of sterile water with 1 mL of commercial spore stock solution.

For vegetative cell experiments, a stock solution was prepared to be used for control or thermosonication treatments by inoculating 44.5 mL of either TSB or 2 % fat milk (Gossner Foods, Logan, UT, USA) with 5.5 mL of the cultures from the overnight growth. For spore experiments, 6 mL of the diluted stock solution was inoculated in 24 mL of TSB or 2 % fat milk in a sterile 250 mL Erlenmeyer flask covered with sterile foil. Spores, after being germinated at 85 °C for 15 min, were enumerated to be about 10^6 - 10^7 CFU/mL on plated on TSA and incubated at 55 °C (at 37 °C for *B. subtilis*) for 24 h.

Heating and thermosonication conditions

Treatments for heat and thermosonication were performed in batch using a 10 mL double-walled glass cylinder containing 5 mL of pre-inoculated sample. A circulating water bath was used to control the temperature and it took approximately 3-4 min to heat the solutions. Experiments for thermosonication were performed using a 3 mm microtip submerged about 4 cm in the glass cylinder (approximately halfway through the sample height) and a sonicator power source (500 W power; QSonica Q500, Newtown, CT) at 20 kHz and 30 % amplitude as shown in Appendix C, Figure C.1. The energy densities and temperature difference due to sonication are shown in Appendix C, Table C.1. The values for energy densities ranged between 60-263 J/mL and the temperature increases were

between 0.7-3.3 °C for times between 30-120 s for treatment samples. Temperature increases for control samples for the same time were between 0.5 to 1 °C. Samples with starting temperatures between 72-72.5 °C and 73-73.5 °C were used for 72 and 73 °C experiments, respectively. All materials were rinsed with 10 % (w/v) bleach solution, followed by sterile water before each treatment.

The pre-inoculated sample after reaching the appropriate temperature was termed as time zero samples and was placed on ice before plating. The remaining sample was then treated with thermosonication or heat only. After treatment, samples were collected in sterile 2 mL microcentrifuge tubes and kept on ice until ready to plate. Dilutions of samples were prepared in sterile phosphate buffered saline (PBS) and plated on TSA and incubated for 24-48 h in a humidified incubator at 55 °C to determine log reductions. Spores were thermosonicated or heated without sonication as described above for vegetative cells. After treatment, samples with spores were diluted in sterile water and germinated at 85 °C for 15 min (Burgess *et al.* 2010). Germinated samples were plated on TSA and incubated for 24-48 h in a humidified incubator at 55 °C (at 37 °C for *B. subtilis* spores) to determine log reductions.

Microbial counts reported as CFU/mL were used to calculate log reductions at each time by taking a \log_{10} of N_0 (initial population) and N_t (population after control and treatment at a specific time). D-values were determined for *G. stearothermophilus* and *A. flavithermus* cells at 72 and 73 °C with and without sonication in TSB and 2 % fat milk using at least 3 replicates. D-values were determined from the negative reciprocal of the slope of the regression line (treatment time versus microbial count; representative plot shown in Appendix C, Figure C.2) and calculated using the equation:

$$D = t / (\log N_0 - \log N_t)$$

where D : decimal reduction time, t : duration of treatment, N_0 : initial bacterial population, and N_t : surviving bacterial population after treatment (Beatty and Walsh, 2016).

Statistical analysis

All experiments were conducted in triplicate. ANOVA was used to test for statistical significance ($\alpha=0.05$), followed by the Ryan, Einot, Gabriel, Welsh Studentized Range Q (REGWQ) test and Tukey HSD for post-hoc analysis in SAS 9.4. ANOVA followed by REGWQ test was used to determine if time of treatment and thermosonication had a significant effect on the cell log reductions of organisms tested and Tukey HSD test was used for spores of the microorganisms tested in both TSB and 2 % fat milk. Statistical significance between log reductions after 30 s of treatment time was calculated using t-tests.

RESULTS AND DISCUSSION

Effect of thermosonication on vegetative cells

The effect of temperature, time, and thermosonication on microbial count of *G. stearothermophilus* (Figure 4.1) and *A. flavithermus* cells (Figure 4.2) was evaluated in TSB (Figure 4.1 A and 4.2 A) and 2 % fat milk (Figure 4.1 B and 4.2 B). The initial concentration was approximately 10^7 CFU/mL for both organisms. For both organisms, as the time progressed, the microbial count decreased for both control and treatment samples. Treatment samples at both temperatures showed lower microbial count as

compared to the control samples when compared at each time. At each time tested, samples treated at 73 °C for both control and treatment had lower microbial count as compared to the control and treatment at 72 °C. After 30 s, thermosonication significantly increased the log reductions obtained for *G. stearothermophilus* and *A. flavithermus* cells at both 72 and 73 °C in both media as seen in Appendix C, Table C.2 and C.3. For *G. stearothermophilus*, log reductions in TSB increased from 0.03 to 1.22 at 72 °C and from 0.68 to 1.76 at 73 °C after 30 s of thermosonication. Whereas, in 2 % fat milk, thermosonication increased the log reductions from 0.17 to 1.50 at 72 °C and 0.26 to 1.94 at 73 °C. For *A. flavithermus*, log reductions in TSB increased from 0.62 to 0.87 at 72 °C and from 0.67 to 0.91 at 73 °C after 30 s of thermosonication. Whereas, in 2 % fat milk, thermosonication increased the log reductions from 0.35 to 0.86 at 72 °C and 0.40 to 0.89 at 73 °C.

D-values were calculated for the vegetative cells of the organisms tested to evaluate their susceptibility to heat versus thermosonication and are shown in Table 4.1. D-values in both media were comparable for *G. stearothermophilus* and *A. flavithermus* cells. For *G. stearothermophilus*, after heating at 72 °C for 120, a complete one log reduction was not observed for control samples therefore, the D-values were extrapolated to be 129.35 s and 119.98 s, in TSB and 2 % fat milk respectively. D-values were reduced significantly for control samples at 73 °C compared to control at 72 °C and were 60.03 and 77.65 s for TSB and 2 % fat milk, respectively. The D-values after treatment at 72 °C were reduced significantly to 25.16 and 19.98 s in TSB and 2 % fat milk, respectively. The D-values of all treatments at both 72 and 73 °C for both media for *G. stearothermophilus* were significantly different from the controls but not from each other.

For *A. flavithermus*, the D-values for control samples at 72 °C were 70.81 and 88.22 s in TSB and 2 % fat milk, respectively. A decrease in D-values was seen for control samples at 73 °C as compared to control at 72 °C in TSB (48.41 s) and 2 % fat milk (56.11 s), however the decrease was not statistically significant. Treatment at 72 °C did significantly decrease the D-values to 44.39 and 38.54 s, in TSB and 2 % fat milk, respectively. Although treatment at 73 °C decreased the D-values in both TSB (36.91 s) and 2 % fat milk (36.89 s) as compared to control at 73 °C, the decrease was not statistically significant. Additionally, the D-values of all treatments at both 72 and 73 °C for both media for *A. flavithermus* were not significantly different from each other.

Temperature and treatment had an interactive significant effect on the D-values of *G. stearothermophilus* (Table 4.2). For *G. stearothermophilus*, thermosonication significantly reduced the time required to achieve a one log reduction at both 72 and 73 °C as compared to the controls at each temperature in both TSB and 2 % fat milk. For *A. flavithermus*, thermosonication successfully reduced the time required to achieve a one log reduction in both media with the effect being significant at 72 °C. Temperature did not have a significant effect on the D-values for *A. flavithermus* (Table 4.2). Overall, application of thermosonication may potentially reduce the D-values obtained for *G. stearothermophilus* and *A. flavithermus* cells in both TSB and 2 % fat milk. However, increasing the temperature of thermosonication may not significantly affect the D-values in a batch thermosonication system.

Similar to the current study, Beatty and Walsh (2016) showed that log reductions of *G. stearothermophilus* cells were almost doubled (0.77-0.50 log reductions after treatment) after application of batch thermosonication (45-75 °C) for 5-30 s in

reconstituted skim milk powder. The overall log reductions were lower in the current study as compared to Beatty and Walsh (2016), which may be due to differences in sonication conditions and in material composition. Palanisamy *et al.* (2019) showed a one log reduction in *G. stearotheophilus* cell suspensions in media after 1 min of batch cold sonication which is comparable to the log reductions obtained after 30 s in TSB and 2 % fat milk in the current study. Moreover, Palanisamy *et al.* (2019) showed that after 20 min of sonication, a 5.3 log reduction was seen, however 20 min of treatment time is not suitable for practical applications. Use of thermosonication could reduce the time required to achieve a one log reduction from 1 min as reported by Palanisamy *et al.* 2019 to 30 s as reported in the current study.

Khanal *et al.* 2014b reported that cold sonication alone showed a 1.1 and 4.26 log reduction in *A. flavithermus* cells inoculated in to skim milk after 1 and 10 min of treatment time. Similar to Khanal *et al.* 2014b, the log reductions increased in the current study as the treatment time progressed. In contrast to the log reductions due to batch sonication reported by Khanal *et al.* 2014b, a one log reduction in *A. flavithermus* cells was observed after thermosonication after 36-45 s (at 72-73 °C) in this study. Moreover, a treatment time of 30 min in a batch cold sonication system was required to achieve a 1.26 log reduction in *A. flavithermus* cells inoculated in media (Palanisamy *et al.* 2019). This disparity between time required for obtaining a one log reduction between current and previous studies may be due to application of thermosonication at 72-73 °C employed in this study as opposed to sonication at lower temperatures used in previous studies. The use of batch sonication for shorter treatment times and higher temperatures did not significantly change the log reductions for *A. flavithermus* cells.

To summarize, overall, thermosonication significantly reduced the D-values and log reductions after 30 s for *G. stearothermophilus* and *A. flavithermus* cells in both media. A greater impact of thermosonication was seen after 30 s on log reductions of *G. stearothermophilus* as compared to *A. flavithermus*. After 30-40 s of thermosonication, only a one log reduction could be observed for vegetative cells of both organisms, which may not have a major impact on overall milk quality.

Effect of thermosonication on spores

The log reductions achieved at different times for spores of *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis* at 72 and 73 °C in TSB are shown in Table 4.3 and in 2 % fat milk are shown in Table 4.4. For spores of all organisms, an increase in log reduction was seen with an increase in time, increase in temperature, and application of thermosonication. For *G. stearothermophilus* (Table 4.3) in TSB, there was a significant reduction in spores at each time for the treatments at each temperature compared to the controls. But for *A. flavithermus*, the only significant reduction in spores in seen at 120 s at 73 °C, although there was a significant difference at 90 s but not at 120 s at 72 °C. For *B. subtilis*, there was no significant difference between controls and treatments at any time or temperature tested. The highest log reduction for each organism (Table 4.3) was observed at 120 s with the log reductions for of *G. stearothermophilus* and *A. flavithermus* being 0.46 and 0.41 for *B. subtilis*.

For *G. stearothermophilus* (Table 4.4) in 2% fat milk, there was a significant reduction in spores at each time for the treatments at each temperature compared to the controls. But for *A. flavithermus* there was significant reduction in spores compared to the controls at 90 and 120 s at 72°C and at 120 s at 73 °C. For *B. subtilis*, the only

significant difference between treatment and controls was seen at 120 s at both temperatures. The highest log reductions for each organism were observed at 120 s. For *A. flavithermus*, the highest spore reductions were 0.53 at 120 s at both 72 and 73 °C. For *G. stearothermophilus* and *B. subtilis*, the highest spore reductions were 0.49 and 0.42 at 120 s and 73 °C. The highest log reductions observed were 0.49, 0.53 and 0.42 for *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis* respectively

Even though thermosonication in a batch system had a significant effect on the log reductions overall, the maximum log reductions were ≤ 0.5 , which may not have a significant impact on spores of thermophilic bacteria in practical applications. Overall, spore reductions seen were significantly lower than those seen for thermophilic bacterial cells at comparative times similar to observations made by Palanisamy *et al.* (2019) which is due to the resistance of spores to adverse conditions (Palacios *et al.* 1991).

Khanal *et al.* (2014a) reported a log reduction of 0.05 after 1 min and 0.16 after 10 min for *G. stearothermophilus* spores in non-fat milk when batch cold sonication was applied. Use of batch thermosonication in this study did showed higher log reductions in *G. stearothermophilus* spores than reported by Khanal *et al.* (2014a), which may be due to the use of thermosonication. Beatty and Walsh (2016), reported a maximum log reduction of 0.35 in *G. stearothermophilus* spores when skim milk powder was reconstituted at 31.5 %, after being thermosonicated for 5 s at a comparative temperature of 75 °C in a batch setting, which is comparative to the log reductions seen in this study. Palanisamy *et al.* (2019) showed <0.4 and 1.5 log reduction in *G. stearothermophilus* spores inoculated in media after 1 min and 30 min of batch sonication, but increasing

sonication times to 30 min may not be suitable for practical application in the dairy industry.

Palanisamy *et al.* (2019) observed no log reductions in spores of *A. flavithermus* inoculated in media even after batch sonication for 30 min in contrast to the 0.4-0.5 log reduction seen for spores of *A. flavithermus* in both media in this study.

Thermosonication at 70 °C combined with pressure reduced the spores of *B. subtilis* in media by 0.5 and 1 log after 4 and 12 min, respectively (Raso *et al.* 1998). Similarly, in the current study, 2 min of treatment time did help achieve log reductions up to 0.42 in both media for *B. subtilis* spores with use of thermosonication. Overall, the reductions obtained for spores of these thermophilic bacteria observed in previous and this study with sonication may not have a notable impact on milk quality.

Although thermosonication showed reductions for thermophilic bacterial cells, a one log reduction (after use of practical treatment times) may not be sufficient to significantly alter the overall quality of milk and milk products. However, use of thermosonication along with pasteurization has shown promising results (Khanal *et al.* 2014b). Future studies can explore the effect of thermosonication along with pasteurization in a continuous system at HTST pasteurization conditions. Spores showed even lower log reductions as compared to thermophilic bacteria cells after thermosonication for 120 s. Future studies can explore application of thermosonication before pasteurization in a continuous flow system for evaluating results in spores.

CONCLUSION

Thermosonication showed greater microbial reduction in thermophilic bacterial cells and spores of organisms such as *G. stearothermophilus* and *A. flavithermus* as compared to the application of heat alone. D-values for vegetative cells were reduced for both organisms after thermosonication as compared to controls. The D-values after treatment were significantly different than control for *G. stearothermophilus* cells in both media and at both temperatures and that of *A. flavithermus* only at 72 °C in both media. A greater impact of thermosonication was seen on log reductions of *G. stearothermophilus* as compared to *A. flavithermus* after 30 s of treatment time.

Thermosonication had a significant effect on the log reductions of spores of *G. stearothermophilus*. However, thermosonication of spores of *A. flavithermus* and *B. subtilis* showed a significant impact only at 120 s. Maximum log reductions after 120 s of thermosonication were only ≤ 0.5 for spores. Although thermosonication showed higher log reductions for thermophilic bacterial cells than heat alone, a one log reduction may not be sufficient to significantly alter the overall quality of milk and milk products.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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Tables

Table 4.1. D-values obtained for *G. stearothermophilus* and *A. flavithermus* cells in tryptic soy broth (TSB) and 2 % sterile milk after control and thermosonication at 72 and 73 °C.

Organism	Medium	Temperature (°C)	D-value (s)	
			Control	Treatment
<i>G. stearothermophilus</i>	TSB	72	129.35 ^a	25.16 ^c
		73	60.03 ^b	21.99 ^c
	Milk	72	119.98 ^A	19.98 ^C
		73	77.65 ^B	18.68 ^C
<i>A. flavithermus</i>	TSB	72	70.81 ^a	44.39 ^b
		73	48.41 ^{ab}	36.91 ^b
	Milk	72	88.22 ^A	38.54 ^B
		73	56.11 ^{AB}	36.89 ^B

Values with different letters are significantly different within each organism.

Lowercase letters are used for TSB, Uppercase letters are used for milk.

Table 4.2. ANOVA for D-values obtained for *G. stearothermophilus* and *A. flavithermus* cells in tryptic soy broth (TSB) and 2% sterile milk at 72 and 73 °C.

Parameter	<i>G. stearothermophilus</i>		<i>A. flavithermus</i>	
	F statistic	P-value	F statistic	P-value
Temperature	29.48	0.0056	5.82	0.0734
Treatment	199.99	0.0001	17.52	0.0139
Temperature x Treatment	25.09	0.0074	2.76	0.1719

Table 4.3. Log reductions for spores of *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis* after being treated with control and treatment in tryptic soy broth (TSB) at various residence times.

Temp ¹ (°C)	C or T ²	Log reductions			
		30 s	60 s	90 s	120 s
<i>G. stearothermophilus</i>					
72	C	0.05 ± 0.01 ^a	0.10 ± 0.03 ^{ab}	0.16 ± 0.03 ^{abc}	0.24 ± 0.01 ^{cd}
	T	0.18 ± 0.01 ^{bc}	0.24 ± 0.05 ^{cd}	0.33 ± 0.04 ^{de}	0.43 ± 0.03 ^e
73	C	0.09 ± 0.02 ^A	0.15 ± 0.02 ^{AB}	0.21 ± 0.02 ^{BC}	0.31 ± 0.01 ^{DE}
	T	0.19 ± 0.03 ^B	0.27 ± 0.08 ^{CD}	0.35 ± 0.06 ^E	0.46 ± 0.04 ^F
<i>A. flavithermus</i>					
72	C	0.02 ± 0.01 ^a	0.06 ± 0.03 ^{ab}	0.14 ± 0.02 ^{bcd}	0.22 ± 0.01 ^{de}
	T	0.05 ± 0.01 ^{ab}	0.10 ± 0.02 ^{abc}	0.19 ± 0.01 ^{cd}	0.31 ± 0.04 ^e
73	C	0.05 ± 0.02 ^A	0.08 ± 0.01 ^A	0.15 ± 0.02 ^{ABC}	0.23 ± 0.02 ^C
	T	0.06 ± 0.02 ^A	0.11 ± 0.02 ^{AB}	0.21 ± 0.04 ^{BC}	0.46 ± 0.03 ^D
<i>B.subtilis</i>					
72	C	0.02 ± 0.01 ^a	0.06 ± 0.02 ^a	0.14 ± 0.03 ^b	0.28 ± 0.01 ^c
	T	0.03 ± 0.00 ^a	0.06 ± 0.02 ^a	0.18 ± 0.02 ^b	0.31 ± 0.01 ^c
73	C	0.03 ± 0.01 ^A	0.06 ± 0.01 ^A	0.19 ± 0.01 ^B	0.33 ± 0.01 ^C
	T	0.05 ± 0.01 ^A	0.07 ± 0.02 ^A	0.21 ± 0.02 ^B	0.41 ± 0.01 ^D

¹Temp: temperature. ²C: control and T: treatment.

Values with different letters are significantly different within each temperature and within each organism. Lowercase letters are used for 72 °C and uppercase letters are used for 73 °C. ANOVA tables shown in Appendix C, Table C.4-C.6. Values displayed are Mean ± S.D.

Table 4.4. Log reductions for spores of *G. stearothermophilus*, *A. flavithermus*, and *B. subtilis* after being treated with control and treatment in 2 % fat milk at various residence times.

Temp ¹ (°C)	C or T ²	Log reductions			
		30 s	60 s	90 s	120 s
<i>G. stearothermophilus</i>					
72	C	0.06 ± 0.03 ^a	0.08 ± 0.02 ^{ab}	0.18 ± 0.03 ^{bcd}	0.26 ± 0.02 ^d
	T	0.16 ± 0.06 ^{bc}	0.25 ± 0.06 ^{cd}	0.37 ± 0.07 ^e	0.47 ± 0.01 ^f
73	C	0.06 ± 0.02 ^A	0.10 ± 0.01 ^{AB}	0.18 ± 0.02 ^C	0.29 ± 0.04 ^D
	T	0.16 ± 0.02 ^{BC}	0.27 ± 0.06 ^D	0.39 ± 0.07 ^E	0.49 ± 0.01 ^F
<i>A. flavithermus</i>					
72	C	0.06 ± 0.02 ^a	0.10 ± 0.02 ^{abc}	0.18 ± 0.01 ^c	0.29 ± 0.03 ^d
	T	0.08 ± 0.01 ^{ab}	0.15 ± 0.03 ^{bc}	0.31 ± 0.04 ^d	0.53 ± 0.02 ^e
73	C	0.07 ± 0.03 ^A	0.13 ± 0.04 ^{AB}	0.23 ± 0.01 ^{CD}	0.34 ± 0.02 ^E
	T	0.08 ± 0.01 ^A	0.20 ± 0.04 ^{BC}	0.31 ± 0.07 ^{DE}	0.53 ± 0.05 ^F
<i>B.subtilis</i>					
72	C	0.02 ± 0.01 ^a	0.05 ± 0.02 ^a	0.12 ± 0.02 ^b	0.19 ± 0.01 ^c
	T	0.04 ± 0.01 ^a	0.07 ± 0.02 ^{ab}	0.13 ± 0.01 ^{bc}	0.27 ± 0.01 ^d
73	C	0.04 ± 0.01 ^A	0.06 ± 0.02 ^{AB}	0.13 ± 0.02 ^B	0.25 ± 0.01 ^C
	T	0.05 ± 0.01 ^A	0.08 ± 0.02 ^{AB}	0.13 ± 0.02 ^B	0.42 ± 0.01 ^D

¹Temp: temperature. ² C: control and T: treatment.

Values with different letters are significantly different within each temperature and within each organism. Lowercase letters are used for 72 °C and uppercase letters are used for 73 °C. ANOVA tables shown in Appendix C, Table C.4-C.6. Values displayed are Mean ± S.D.

Figures

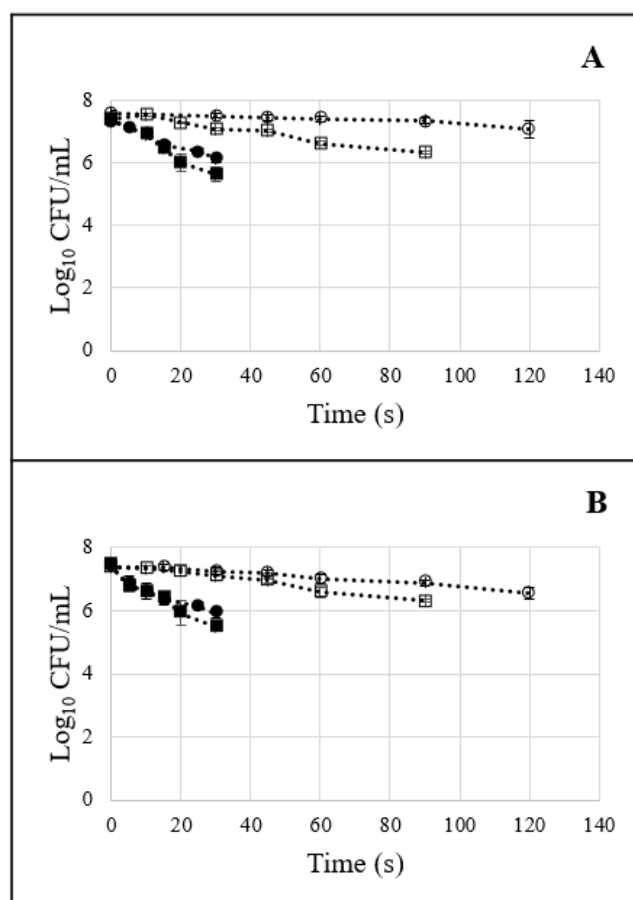


Figure 4.1. Microbial count (Log₁₀CFU/mL; Mean \pm S.D) of *Geobacillus stearothermophilus* cells in TSB (A) and 2% sterile milk (B) as a result of heat (control) and thermosonication (treatment) at 72 and 73 °C.

○ 72°C Control ● 72°C Treatment □ 73°C Control ■ 73°C Treatment

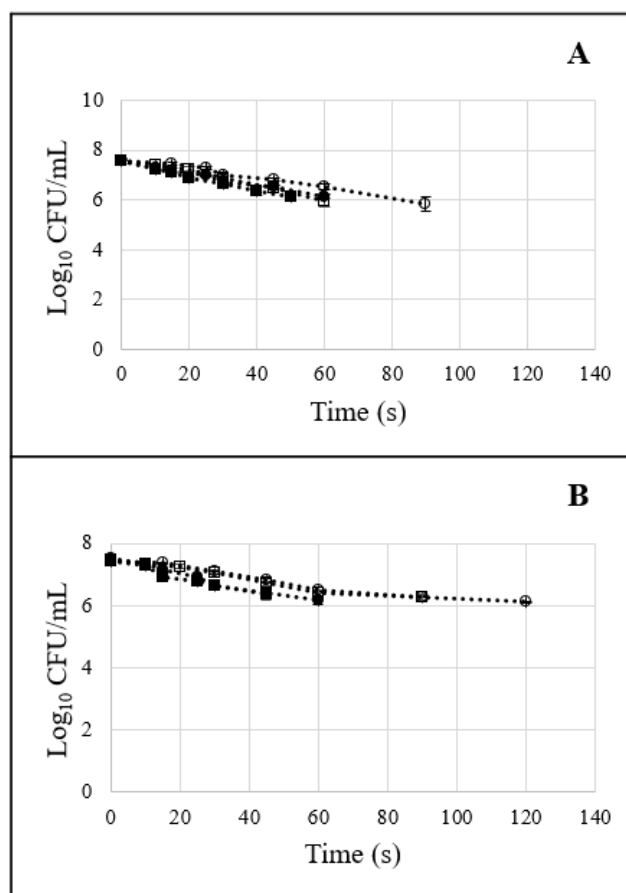


Figure 4.2. Microbial count (Log₁₀CFU/mL; Mean \pm S.D) of *Anoxybacillus flavithermus* cells in TSB (A) and 2% sterile milk (B) as a result of heat (control) and thermosonication (treatment) at 72 and 73 °C.
 ○72°C Control ● 72°C Treatment □ 73°C Control ■ 73°C Treatment

CHAPTER V

EFFECT OF THERMOSONICATION IN A LABORATORY-SCALE CONTINUOUS SYSTEM ON THE SURVIVAL OF THERMOPHILIC BACTERIA AND INDIGENOUS MICROFLORA IN MILK.

ABSTRACT

Thermophilic bacterial cells and spores can survive milk pasteurization and affect the quality of dairy products during shelf life. Coupling thermosonication with heat may reduce the microbial load in fluid foods and enhance the product quality during shelf life. This study evaluated the effect of thermosonication along with heating in a lab-scale continuous system on the survival of *Geobacillus stearothermophilus* in milk at two different settings (setting 1: 27.7 s total heating time with or without 11.9 s of sonication; setting 2: 20.3 s total heating time with or without 7.1 s of sonication) with all equipment set at 72°C. This study also investigated the effect of thermosonication along with heat on indigenous microflora in raw milk and milk quality assessed by pH, free fatty acid (FFA) content, and casein/total protein (CN/TP) content during storage at the two different settings. Overall, thermosonication with heat resulted in higher log reductions for *G. stearothermophilus*, but the reduction was not significant overall. The log reductions for control were between 0.25-0.37 and treatment were 0.45-0.54 at setting 1. Thermosonication with heat significantly decreased the indigenous microflora in milk as compared to heat alone at both settings. Longer residence times (setting 1) had significantly higher log reductions at week 0, and treatment samples had significantly higher reductions

than control during storage time at both the settings. Treatment samples at setting 1 had significantly higher pH, lower FFA content, and higher CN/TP content at week 4, as compared to control. Thermosonication using practical residence times along with heat may improve milk quality during its shelf life. Results from this study need to be verified in a scale up study employing pasteurization conditions.

INTRODUCTION

High temperature short time pasteurized (HTST; at 72°C for 15 s) milk has a shelf life of up to 3 weeks [1, 2]. Pathogens are destroyed and vegetative bacteria are reduced as a result of pasteurization, thus extending raw milk shelf life. About 19% of dairy products are lost at retail and consumer level due to its tendency to spoil [3]. Pasteurized milk quality and shelf life is limited mostly due to thermophilic organisms which may grow at both room and refrigeration temperatures and reduce the overall dairy product quality [4-7]. Extracellular hydrolytic enzymes produced by thermophilic bacteria have a major impact on the quality of dairy products during shelf life [4, 8].

Thermophilic bacterial cells and spores of organisms such as *Geobacillus stearothermophilus*, *Bacillus coagulans*, *Bacillus licheniformis*, *Bacillus subtilis*, and *Anoxybacillus flavithermus* may survive pasteurization and deteriorate milk product quality [9-11]. Thermophilic bacteria have been associated with spoilage of raw, HTST pasteurized, and ultra-high temperature (UHT) pasteurized milk along with canned milk products [9, 12]. Thermophilic bacteria such as *G. stearothermophilus* are not pathogenic and are used to assess milk product quality in the dairy industry and thus has been used as a representative organism for experiments conducted in this study [9, 13, 14]. *G.*

stearothermophilus is a thermophilic, endospore forming, Gram-positive organism found in dairy processing plants [9].

Elimination or reduction of some of these thermally resistant organisms that are responsible for milk product spoilage cannot be achieved by increasing pasteurization temperatures up to 85°C [2, 4, 6]. Moreover, use of high temperature processing such as ultra-pasteurization (UP) and UHT produce a cooked flavor which is not favored by some consumers, especially in the U.S [15]. Gandy, Schilling, and Coggins et al. [2] showed that increasing pasteurization temperatures up to 85°C did not lead to an increase in shelf life of milk. The consumer acceptance of milk declined when pasteurization temperatures were over 79°C. Use of alternative technologies such as sonication may help achieve a higher quality product and potentially extend the shelf life of pasteurized milk.

Sonication is the application of sound waves at frequencies ≥ 20 kHz in a fluid medium. When sonication is applied with heat, it is referred to as thermosonication. Sonication can result in cavitation. Cavitation and bubble collapse can generate very high temperatures ($\approx 4726^\circ\text{C}$) and pressures ($\approx 2,000$ atm) locally in the fluid medium [16]. Applications of sonication are being explored in different areas in food processing such as emulsification, filtration, viscosity modification, improvement of whey protein heat stability, improvement of meat tenderness, and inactivation of spoilage microbes [13, 17-21]. Sonication has been researched to improve milk quality and shelf life by destruction of indigenous microflora or added pathogens, with majority of studies being in batch systems [22-24].

Many studies showing reduction in thermophilic bacteria using batch sonication have been reported. *B. subtilis* cells were inactivated after up to 15 min of sonication [25].

Milk inoculated with *A. flavithermus* (5-6 log CFU/ml), showed a 1.1 log reduction after 1 min of cold batch sonication, and after 10 min, a 4-log reduction was seen [26]. Comparatively, Khanal, Anand, Muthukumarappan, and Huegli [26], reported a 1.27 log reduction of *A. flavithermus* after pasteurizing at 63°C for 30 min. Studies discussed above showed that batch sonication did significantly reduce the microbial numbers of *B. subtilis* and *A. flavithermus*. But the residence times of 10-15 min used in previous studies do not seem practical. Further investigation needs to be performed to see whether a combination of thermosonication at shorter residence times along with heat can reduce thermophilic bacteria such as *G. stearothermophilus*, which are associated with spoilage of dairy products. Lim, Benner, and Clark [27], showed that thermosonication and cold sonication along with pasteurization in batch sonication system (for 10-60 s) did not significantly reduce the milk microbial numbers when inoculated with *Paenibacillus amolyticus*. However, reductions were not evaluated in a continuous system.

A 2.9 log reduction in overall milk microflora was seen after 1.7 min of sonication in a continuous system; however, the effect of thermosonication was not evaluated [28]. Khanal, Anand, Muthukumarappan, and Huegli [26] showed that when cold batch sonication for 1 min was used with batch pasteurization, the log reductions in *A. flavithermus* cells were doubled. However, a 10 min treatment time of batch sonication with batch pasteurization did not significantly affect the reductions as compared to batch sonication alone. Most of the previously reported studies did not use sonication at practical residence times or additional heat which could possibly yield comparable or higher reductions compared to sonication alone. Thus, the effect of shorter and practical residence times (≤ 1 min) for thermosonication in a continuous system combined with

heat on milk microflora should be investigated. Previous studies also lacked the evaluation of the effect of thermosonication in a continuous system on milk quality parameters such as pH, free fatty acid (FFA) content, and casein as a percentage of total protein (CN/TP) content [5, 27].

Based on previous reports, this study aimed at evaluating the effect of thermosonication with heat treatment using a plate heat exchanger (PHE) in a lab-scale continuous system on *G. stearothermophilus* and indigenous microflora reductions. The first objective of this study was to evaluate the effect of thermosonication with heat on the survival of *G. stearothermophilus* at two different residence times. In this part of the study, the effect of location of the sonicator (before or after the PHE) was also evaluated. The second objective of this study was to evaluate the effect of thermosonication with heat on the survival of indigenous microflora in raw milk. Milk quality was assessed using microbial counts, FFA content, CN/TP content, and pH during storage.

MATERIALS AND METHODS

Experimental design

This study was designed with two components, first was to evaluate the effect of thermosonication with heat compared to heat only in a lab-scale continuous system on *G. stearothermophilus* reductions. The second component was to evaluate the effect of thermosonication with heat compared to heat alone in a lab-scale continuous flow system on indigenous microflora in milk and milk quality during storage. Samples that received only heat are termed control and samples that received thermosonication with heat are termed treatment.

To determine the effect of thermosonication (PHE set at 72°C, sonicator set at 72°C at 90% amplitude) on reductions of *G. stearothersophilus* cells in milk in a lab-scale continuous flow system, 2% fat sterile milk (Gossner Foods, Logan, UT, USA) was processed under control or treatment conditions for different residence times by adjusting the flow rate to give 2 different settings (Table 5.1). The effect of the position of the sonicator on microbial reductions was evaluated as well, by placing the sonicator before or after the PHE for the *G. stearothersophilus* experiments. All experiments at each residence time were performed in triplicate. To evaluate the effect of thermosonication (PHE set at 72°C, sonicator set at 72°C at 90% amplitude) on reductions of indigenous microflora in a lab-scale continuous system, raw whole milk (obtained from Aggie Creamery, Utah State University, Logan, UT, USA) was processed under control or treatment conditions for different residence times as described previously to achieve 2 settings.

At setting 1, samples received 27.7 s total heating time with or without 11.9 s of sonication. At setting 2, samples received 20.3 s total heating time with or without 7.1 s of sonication. All experiments at each residence time were performed in at least triplicate. An example of the lab-scale sonication assembly can be seen in Appendix D, Figure D.1-D.2. The inlet and outlet temperatures of milk were monitored. For *G. stearothersophilus* experiments, the inlet temperature of milk was 60°C while outlet temperature measured was 71 and 67°C, at setting 1 and 2, respectively. Whereas, the inlet temperature for raw milk was between 20-25°C and the outlet temperature for indigenous microflora experiments was 63 and 57°C, at setting 1 and 2, respectively.

Growth of G. stearothermophilus cells

G. stearothermophilus spores were germinated using 0.1 ml of stock solution (NAMS *G. stearothermophilus* 2.4×10^6 in 0.1 ml, VWR, Atlanta GA, USA) inoculated into 10 ml of sterile water. The diluted stock was incubated for 10 min in a 80°C water bath to germinate the spores. For growing vegetative cells, 25 ml of TSB was inoculated with 1 ml of germinated bacteria in a sterile 250 ml Erlenmeyer flask covered with sterile foil and incubated at 55°C aerobically for 24 h in a shaker at 100 rpm. The optical density (OD) was measured at 600 nm using a spectrophotometer (Bio Spec-1601, Shimadzu, USA) and was found to be approximately 0.57 after 24 h, which corresponded to 7 log CFU/ml as determined by plating on tryptic soy agar (TSA: VWR, Atlanta GA, USA) [13]. A subculture was grown by inoculating 25 ml of TSB with 0.1 ml of culture grown from germinated cells in a sterile 250 ml Erlenmeyer flask covered with sterile foil. Cells were grown aerobically at 55°C in a shaker at 100 rpm for 15 h. Freezer stocks were made by inoculating 20 ml of TSB containing 30% (w/v) glycerol with 2 ml of subculture and aliquoted and stored in 2 ml cryo-vials at -6°C [13].

Cultures for experiments were grown by inoculating 600 ml TSB with 2.4 ml of freezer stock in a 1 L Erlenmeyer flasks and incubating at 55°C in a shaker at 100 rpm for 15 h. OD at 600 nm was checked after every overnight growth, and the culture was used when the OD was greater than 0.70. Overnight growth from the flasks was transferred to sterile 500 ml centrifuge bottles, which were then centrifuged at 500 rpm for 20 min at 10°C. Pellets containing cells were obtained and the filtrate was discarded. Pellets were resuspended in 400 ml of sterile 2% fat milk at room temperature. This milk was then

transferred to the pot with 3600 ml of pre-heated 2% fat milk (at 60°C) , and an initial concentration of 6 log CFU/ml was obtained.

Heating and thermosonication conditions for

G. stearothermophilus experiments

Milk was prepared by heating 3600 ml of 2% fat sterile milk to 60°C in a pot (cleaned and wiped with ethanol before use) and inoculated with *G. stearothermophilus* (described in section 2.2) before being treated either with control or treatment conditions. To simulate continuous operation at a lab-scale, milk was pumped using a Masterflex 7529 pump (Cole-Palmer, Vernon Hills, IL, USA) at different flow rates as described previously. A PHE was used for heating milk and the temperature of the PHE was maintained using a circulating water bath. PHE was placed either before or after the sonicator to evaluate the effect of location of the sonicator on *G. stearothermophilus* reductions. The sonication flow cell had a 160 ml volume resulting in different residence times (time sample was exposed to sonication or heating) at different flow rates. The flow rate also affected the heating residence time through the plate heat exchanger. The stainless-steel flow cell of the sonicator has a water jacket and was connected to a water bath to maintain the temperature of the sample during thermosonication (Appendix D, Figure D.1).

Samples were thermosonicated (Heischler UIP500hd, Ringwood, NJ, USA) at 90% amplitude and temperature of the flow cell was maintained at 72°C using a circulating water bath. All equipment were rinsed with detergent (Conquest, EcoLab, Saint Paul, MN, USA) followed by washing with hot water and sanitizer solution (Exelerate CIP Solution, EcoLab, Saint Paul, MN, USA) after each experiment and with

hot water between different treatments to avoid cross-contamination. Pre-treatment samples were placed on ice until ready to plate for time zero. The remaining sample was then subjected to control or treatment conditions. After being processed with control or treatment conditions, samples were collected in sterile 50 ml tubes and placed on ice before microbial plating. This entire procedure was performed each time for each experiment and its replicate.

*Heating and thermosonication conditions for
indigenous microflora experiments*

Raw milk, received at 4°C, was heated to 20- 25°C by placing sealed hot water bottles (between 85-90°C) in the milk can and occasional stirring over 1-2 h. The raw milk received had microbial counts of 2 log CFU/ml, and the warming step was performed to increase the initial microbial counts to 4 log CFU/ml, so that an effect of heat and thermosonication could be evaluated.

Experiments were performed using the same assembly and procedures used for *G. stearothermophilus* experiments, described in section 2.3, unless stated otherwise (Appendix D, Figure D.2). PHE was placed before the sonicator for indigenous microflora experiments. Pre-treatment samples were placed on ice until ready to plate for time zero and. The remaining sample was then subjected to control or treatment conditions. For each replicate, 8 samples of 200 ml of milk were collected post processing in sterile containers and transferred to a cold room at 4°C for various analyses over time.

The energy density (J/ml) for the samples sonicated in the lab-scale continuous flow system was calculated [29]. The power readings ranged from 168 to 180 W with an average

of 174.7 W. The residence times in the sonicator at setting 1 and 2 were 11.9 and 7.1 s, respectively. The energy density calculated at setting 1 (11.9 s residence time) was 12.99 J/ml and setting 2 (7.1 s residence time) was 7.75 J/ml.

Microbial and pH evaluation

For *G. stearothermophilus* experiments, dilutions of samples were made in sterile phosphate buffered saline (PBS) and plated on TSA and incubated for 24-48 h in a humidified incubator at 55°C to determine microbial reductions. Duplicate measurements were done for each sample.

For indigenous microflora experiments, dilutions of samples were made in sterile PBS and plated on standard plate count agar (SPC; for total aerobic bacteria) and TSA (for indigenous thermophilic bacteria). Plates were incubated for 24-48 h in a humidified incubator at 32°C (for SPC) and 55°C (for TSA). Plating was done each week, followed by pH measurements and the milk sample containers were transferred to the freezer at -29.9°C for remaining evaluations. pH of samples was measured at 4°C after standardization of the pH meter (Orion 3-star pH meter, Thermo Fisher Scientific, Waltham, MA, USA) with buffers of pH 4 and pH 7. Duplicate measurements were done for each sample.

The storage containers were then transferred to freezer at -29.9°C and stored for quality parameters testing as described below for FFA and CN/TP. Analyses during storage were stopped when sample appeared to be spoiled with CFU/ml > 6 log or when visible curdling of milk was observed [5, 27].

Free fatty acid (FFA) content

Lipolysis in milk samples was evaluated by measuring the increase in free fatty acid content from week 0 through week 4. The FFA content was measured using the copper soap method described in Shipe, Senyk, and Fountain [30], as modified by Ma, Barbano, and Santos [31]. Milk was thawed using a combination of a water bath at 20°C and microwave all the while keeping the temperature below 10°C. Reagents used included copper soap reagent, color reagent, solvent (chloroform-heptane-methanol; 49:49:2 vol:vol:vol), and solubilizing reagent and were prepared as described by methods described by Shipe, Senyk, and Fountain [30].

For the actual analysis, 0.2 ml aliquot of 0.7 N HCl was added to 1 ml milk samples in a test tube. The mixture was vortexed and 4 ml of the copper reagent and 12 ml of solvent were added. The sample was shaken for 30 min in shaker at 400 rpm and centrifuged for 10 min at 2500 x g in a centrifuge. Then 3.5 ml of the solvent layer was transferred to test tube containing 0.1 ml of the color reagent. Color was measured in cuvettes after mixing at 440 nm within 1 h using a spectrophotometer (BioSpec-1601, Shimadzu, Kyoto, Japan). Blanks were prepared by using deionized (DI) water instead of milk.

A standard curve was obtained by preparing six known concentrations of palmitic acid (0, 50, 100, 150, and 200 µg/ml) and mixed with 0.1 ml of 0.7 N HCl and 1 ml of DI water and a standard curve was plotted with absorbance measured at 440 nm. FFA content in milk was calculated in µg/ml from the standard curve. The final value is reported in meq FFA/kg of milk abbreviated as FFA/kg and was calculated as:

$$meq\ FFA = \frac{\frac{\mu g\ of\ FFA \times 0.001\ mg/\mu g}{256.43\ mg/meq}}{g\ of\ milk \times 0.001\ kg/g}$$

Duplicate reactions were conducted for each sample.

Casein/ Total Protein (CN/ TP) content

Casein (CN) as a percentage of total protein (TP) was measured at week 0, 2, and 4 to evaluate the proteolysis activity during storage of milk [31]. Milk was thawed using a combination of a water bath at 20°C and a microwave as described above. Whole milk samples were used for total protein (TP) measurements. Non- casein nitrogen (NCN) content was analyzed using whey portions from the whole milk.

Whey portions of milk were obtained by mixing 20 ml milk sample with 20 ml DI water in a 50 ml test tube which was then kept at 37°C for 30 min in a water bath. Two ml of acetic acid solution (10% v/v) was added and the mixture was vortexed for 30 s, followed by letting the mixture stand for 10 min. Two ml of 1 M sodium acetate was then added to the test tube and the mixture is cooled to 20°C in an ice bath. DI water was added to the 50 ml calibration mark and the test tubes were centrifuged at 3500 rpm for 15 min. The filtrate (whey) was collected for further analyses and casein was obtained as a pellet.

Whole milk and whey samples were then refrigerated and sent to Utah State University Analytical Laboratories (USUAL; Logan, UT, USA) for nitrogen content analyses performed using combustion. TP was calculate using nitrogen content from whole milk and CN was calculated from (TP- NCN). CN/TP is reported by taking a mean and standard deviation of three replicates.

Statistical analysis

Three replicates were used *G. stearothersophilus* experiments. A total of eight replicates were used for indigenous microflora experiments and outliers were identified using semi-studentized residual plot of microbial evaluation. Two replicates were identified as outliers and were eliminated from all analyses performed. Mixed model ANOVA was used to test for statistical significance ($\alpha=0.05$) for a repeated measures design in SAS 9.4. Ryan, Einot, Gabriel, Welsh Studentized Range Q (REGWQ) test and Tukey HSD for post-hoc analysis in SAS 9.4 and post hoc-analysis was performed within each setting.

ANOVA followed by Tukey HSD was used to evaluate the effect of thermosonication and sonicator position on reduction of *G. stearothersophilus* cells. Also, the effect of thermosonication, treatment time, and storage duration on microbial quality of milk was evaluated using ANOVA followed by Tukey HSD. One-way ANOVA followed by Tukey HSD were used to analyze the log reductions at week 0. ANOVA followed by REGWQ test was used to determine if thermosonication and storage time had a significant effect on the pH, FFA, CN/ TP, and reactive thiol content of the milk during its storage at each of the settings used.

RESULTS AND DISCUSSION

Effect of thermosonication in a lab-scale continuous system on thermophilic bacteria

The log reductions in *G. stearothersophilus* cells (initial counts of 5-6 log CFU/ml) for control and treatment conditions are shown in Table 5.1 when the sonicator

was placed before and after the PHE. Setting 1, having longer residence times, had the highest log reductions at both sonicator positions. The highest log reduction seen was 0.54 for treatment samples at setting 1. Thermosonication increased the log reductions of *G. stearothermophilus* cells at both the settings as compared to the control conditions. For example, at PHE- sonicator position, control had log reductions of 0.37 ± 0.05 (setting 1) and 0.05 ± 0.01 (setting 2), whereas treatment had higher log reductions of 0.45 ± 0.05 (setting 1) and 0.26 ± 0.04 (setting 2) as compared to control at each setting. The only significant increase in log reductions due to thermosonication was seen for sonicator-PHE samples at setting 1. Overall, the location of sonicator did not have a significant effect on control and treatment log reductions of *G. stearothermophilus* cells (Appendix D, Table D.1). The outlet temperature was not affected by the location of the sonicator.

The findings for *G. stearothermophilus* reductions are comparable to the ones reported in previous studies for thermophilic or facultative thermophilic bacteria in a continuous system. Villamiel and De Jong [28] studied the effect of thermosonication in a continuous system on *Streptococcus stearothermophilus* inoculated in growth media. They reported, that after 22.5 and 56.3 s of thermosonication, log reductions of 0.1 and 0.2 was observed for *S. stearothermophilus*. The results in this study are similar to those by Villamiel and De Jong [28] where higher log reductions were observed at longer residence times as compared to shorter residence times. When comparing to a batch sonication study of *A. flavithermus* in milk [26], lower log reductions for *G. stearothermophilus* were seen in this continuous system study. Khanal, Anand, Muthukumarappan, and Huegli [26] reported a 1.27 log reduction in *A. flavithermus* cells when heat alone was applied for 30 min at 63 °C. With cold sonication alone, a 1.1 log reduction was seen after 1 min of

treatment time and after application of sonication for 10 min, a 4 log reduction in *A. flavithermus* cells was observed [26].

The current research performed showed that application of 11.9 s of thermosonication (in addition to 15.9 s of heating) did not significantly reduce *G. stearothermophilus* cells as compared to heat alone. The highest log reduction was 0.54 for treatment and 0.37 for control samples. *G. stearothermophilus* was used as a representative microorganism for thermophilic bacteria in this study, and thermosonication may not decrease thermophilic bacterial cells associated with dairy foods under the conditions used in this study. Possible reductions in thermophilic bacteria could be seen if pasteurization temperatures are employed during processing.

Effect of thermosonication in a lab-scale continuous system on indigenous microflora in milk during storage

Log reductions for week 0 after raw whole milk (initial microbial count of 4 log CFU/ml) was processed either under control or treatment conditions at setting 1 and 2 are depicted in Table 5.2. Setting 1 had the highest log reductions in both control and treatment conditions. At setting 1, the microbial count was reduced to 3 log CFU/ml for control and 2 log CFU/ml for treatment at week 0. Whereas, for setting 2, both control and treatment conditions reduced the microbial population to 3 log CFU/ml. Thermosonication samples had significantly greater log reductions of 1.91 ± 0.06 (1.38 ± 0.04 for control) at setting 1 and 1.21 ± 0.07 (0.74 ± 0.13 for control) at setting 2.

The log reductions for both control and treatment conditions during milk storage at setting 1 and 2 are shown in Figure 5.1. The log reductions decreased during storage as the microbial count increased. End of analyses during storage was determined when samples

appeared spoiled with visible milk curdling [5, 27]. During storage, setting 1 could be analyzed for 4 weeks as compared to 3 weeks for setting 2. Significantly higher log reductions were seen for treatment samples at week 1 (1.29 logs), 2 (0.85 logs), and 3 (0.37 logs) at setting 1, and at setting 2 the same was observed till week 1 (0.64 logs). Setting 1 control and treatment samples had an average microbial count of 4 log CFU/ml after 4 weeks whereas for setting 2 it was 4 log CFU/ml after 3 weeks. Flow rate, thermosonication, and storage period had a significant three-way interactive effect on log reductions (Appendix D, Table D.3).

No thermophilic bacteria were observed at setting 1 after control or treatment processes from week 0 to 4. At setting 2, 1 log CFU/ml of thermophilic bacteria was recorded at week 0 for control samples and 1 CFU/ml for treatment samples. No growth of thermophilic bacteria was observed after week 1. Due to such lower microbial numbers seen for thermophilic bacteria in milk, any interpretation of thermosonication effect on indigenous thermophilic bacteria in milk would be speculative.

Villamiel and De Jong [28] applied thermosonication to raw milk at 76°C for 102.3 s in a continuous system and reported a 3.1 log reduction in overall microbial count. Cameron, McMaster, and Britz [33] observed a 2 log reduction for *Listeria monocytogenes* after application of sonication for 10 min in pasteurized milk. Gera and Doores [34] showed that sonication effectively inactivated the non-thermophilic bacteria *E. coli* and *Listeria* at 30-35°C, after more than 2.5 and 7-8 min of treatment time, respectively. Similarly, in the current continuous system study, thermosonication along with heat significantly reduced the total microbial count as compared to control alone. The log reductions observed immediately after treatment are lower than reported values,

which could be due to shorter residence times or application of low heat conditions in this study.

Thus, thermosonication of 11.9 s (after 15.8 s of heating; equipment held at 72 °C) along with heat employed in the current study did improve the microbial quality of milk by significantly reducing the microbial numbers after treatment when compared to control. Although treatment samples maintained the lower microbial count during storage, no significant difference was observed at week 4 (setting 1) and week 3 (setting 2) between control and treatment samples. Thus, future studies should consider applying thermosonication with pasteurization for possible extension of milk shelf life.

Effect of thermosonication in a lab-scale continuous system on overall milk quality during storage

The pH of whole milk for both control and treatment at settings 1 and 2 are given in Table 5.3. A decrease in pH could be a result of acids produced by microorganisms or due to lipolysis [24]. The pH of milk decreased during storage for both control and treatment at both the settings. At setting 1, pH of both control and treatment were not significantly different at week 0, with pH of 6.82 ± 0.02 for control and 6.85 ± 0.02 for treatment. After 4 weeks, setting 1 control samples (6.29 ± 0.03 at) had significantly lower pH than treatment samples (6.43 ± 0.04). Meanwhile, at setting 2, pH of both control and treatment samples were not significantly different, with week 0 pH values (6.73 ± 0.01 for control and 6.80 ± 0.03 for treatment) decreasing gradually to week 3 (6.35 ± 0.02 for control and 6.39 ± 0.03 for treatment).

Similar to this study, Bermúdez-Aguirre, Mawson, Versteeg, and Barbosa-Cánovas [24] observed no significant differences in pH between batch pasteurized and

thermosonicated (for 30 min) in milk during 2-week storage and a decrease in pH was observed for both the samples during storage. The pH value of setting 1 treatment samples after 4 weeks (6.4) was comparable to the value of 6.4 reported by Bermúdez-Aguirre, Mawson, Versteeg, and Barbosa-Cánovas [24] after 2 weeks. Therefore, thermosonication with heat resulted in improving the pH of milk during storage as compared to heat alone at setting 1.

The extent of lipolysis was evaluated using the FFA content of milk for both control and treatment samples. FFA content over time is shown in Figure 5.2, with A showing results for setting 1 samples and B showing results for setting 2. It is known that microbes in milk release lipases, therefore the higher FFA content may be due to microbial lipases. The FFA content of treatment were lower at setting 1 as compared to setting 2 from week 1 to 3. There was no significant difference between the FFA content of both control and treatment samples at week 0 at each setting. Also, the FFA content significantly increased for both the control and treatment as the weeks progressed at each setting. At setting 1, after 4 weeks, thermosonication samples had significantly lower FFA content of 0.24 ± 0.02 meq FFA/kg as compared to 0.34 ± 0.01 for control. At setting 2, after 3 weeks, treatment samples had a FFA content of 0.24 meq FFA/kg similar to 0.26 for control.

To compare if milk used in this study was still acceptable for FFA content, values from this study were compared to previously published data. In previous research, the FFA content of commercially pasteurized (HTST) milk after 2.5-week storage was 0.25 meq FFA/ kg[5]. In the current study, treatment samples had a FFA content of 0.24 meq FFA/ kg after 4 weeks at setting 1 and 3 weeks at setting 2, which is comparable to the value reported by Fromm and Boor [5] after 2.5 weeks. Thus, thermosonication along with heat

did significantly improve the FFA content of milk samples as compared to heat alone but only at setting 1.

During milk storage, the extent of proteolysis occurring in milk samples was tested using the CN/TP content and are shown in Figure 5.3. A decrease in the CN/TP represents an increase in the proteolysis of milk proteins as a result of proteases released from microorganisms. Overall, the CN/TP ratio decreased for both control and thermosonication at each setting. At each setting, treatment samples had a higher CN/TP ratio as compared to control for both the settings. Only at setting 1, treatment samples had a significantly higher CN/TP content of 0.85 ± 0.03 as compared to 0.74 ± 0.07 of control after 4 weeks. Fromm and Boor [5], when analyzing HTST pasteurized milk during storage for proteolysis, reported that the CN/TP content decreased from 0.87 to 0.85 in 2.5 weeks. After 4 weeks in this study, at setting 1, samples treated with thermosonication had CN/TP content of 0.85 which is comparative to the value reported in literature [5]. Therefore, thermosonication along with heat did significantly improve the CN/TP content of milk at setting 1 as compared to heat alone. Reactive thiol content of both control treatment samples was analyzed and no significant difference was found (Appendix D, Table D.8-D.9).

To summarize, thermosonication for 11.9 s with additional heating time of 15.9 along with heat in a lab-scale continuous system significantly improved the pH, FFA content, and CN/TP content of milk during storage as compared to heat alone. Thermosonication along with heat also significantly reduced the microbial count after processing as compared to heat alone, but did not affect the microbial counts after 4 weeks at setting 1 and 3 weeks at setting 2. This research helps establish the potential application

of thermosonication for improving the overall milk quality during its shelf life. Previous research has shown an increase in overall milk quality by application of sonication [24, 28]. These studies employed residence times ranging from 2- 30 min, making it difficult for practical applications. Whereas, based on the results obtained from this research, implementation of thermosonication along with heat at a scale up level could help improve milk quality during storage as compared to heat alone while keeping the residence times for thermosonication reasonable (10-15 s) for practical applications. Scale up systems should involve use of HTST pasteurization conditions, to evaluate the effect of thermosonication in achieving higher milk quality and possibly increase the shelf life. It would also be important to evaluate the effect of thermosonication on sensory properties of milk in a pilot scale which can be investigated in future scale up studies.

CONCLUSION

Overall, the effect of thermosonication along with heat in a lab-scale continuous system on *G. stearothermophilus* cell reductions was not significant as compared to heat alone. Longer residence times showed higher *G. stearothermophilus* cell reductions, with log reductions ranging between 0.45-0.54 for treatments as compared to 0.25-0.37 that of control. The log reductions observed were however not impactful enough to suggest scale up applications. Thermosonication along with heat in a lab-scale continuous system significantly decreased the indigenous microflora in milk as compared to heat alone at week 0. No significant differences were seen between log reduction for control and treatment samples after 4 weeks at setting 1 and 3 weeks at setting 2.

For all the samples, the pH and CN/TP content decreased while FFA content increased during storage. Thermosonication samples had significantly higher pH and CN/TP and lower FFA values as compared to control at setting 1. Application of thermosonication for 11.9 s along with heat helped improve the overall milk quality when applied in a lab-scale system as compared to heat alone. Scale up studies should use pasteurization along with heat to help achieve greater overall milk quality and shelf life. Future studies implemented in a scale up system should focus on keeping the residence times for thermosonication suitable for industrial application and should include a sensory evaluation component.

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Tables

Table 5.1. Mean Log₁₀ reduction of *G. stearothersophilus* cells after control or treatment at each setting.

Setting	Residence Time in PHE ¹ (s)	Residence Time in Sonicator ¹ (s)	Total Residence Time ¹ (s)	Exit Temperature ² (°C)	Control Reductions ³	Treatment Reductions ⁴
Plate Heat Exchanger-Sonicator						
1	15.8	11.9	27.7	71	0.37 ± 0.05 ^{abc}	0.45 ± 0.06 ^{ad}
2	13.2	7.1	20.3	67	0.05 ± 0.01 ^c	0.26 ± 0.04 ^{bc}
Sonicator-Plate Heat Exchanger						
1	15.8	11.9	27.7	71	0.25 ± 0.02 ^{bc}	0.54 ± 0.04 ^d
2	13.2	7.1	20.3	67	0.13 ± 0.01 ^{bc}	0.35 ± 0.02 ^{abd}

¹Equipment at 72 °C; ²Temperature before collection of samples;

³Control: treated with heat alone with sample flowing through the PHE and sonicator with sonicator off;

⁴Treatment: with sample flowing through the PHE and sonicator with sonicator on.

Values with different letters are significantly different. Values displayed are (Mean ± S.D). Setting 3 was performed and is shown in Appendix D, Table D.2.

Table 5.2. Mean Log₁₀ reductions of indigenous microflora on Day 0 at two different settings (described in Table 5.1).

Setting	Exit Temperature ¹ (°C)	Control Reductions ²	Treatment Reductions ³
1	63	1.38±0.04 ^a	1.91±0.06 ^b
2	57	0.74±0.13 ^c	1.21±0.07 ^d

¹Temperature before collection of samples; ²Control: treated with heat alone with sample flowing through the PHE and sonicator with sonicator off; ³Treatment: with sample flowing through the PHE and sonicator with sonicator on.

Values with different letters are significantly different. Values displayed are (Mean ± S.E). Statistical analysis shown in Table D.4.

Table 5.3. pH of whole milk during its shelf life after control or treatment at two different settings.

Week	Control ¹	Treatment ²	Control ¹	Treatment ²
	Setting 1		Setting 2	
0	6.82 ± 0.02 ^a	6.85 ± 0.02 ^a	6.73 ± 0.01 ^{AB}	6.80 ± 0.03 ^A
1	6.67 ± 0.03 ^b	6.81 ± 0.02 ^a	6.62 ± 0.03 ^{BC}	6.72 ± 0.03 ^{AB}
2	6.52 ± 0.03 ^{cd}	6.67 ± 0.04 ^{cb}	6.49 ± 0.04 ^{CD}	6.55 ± 0.05 ^{CD}
3	6.41 ± 0.03 ^{de}	6.55 ± 0.04 ^{cbd}	6.35 ± 0.02 ^E	6.39 ± 0.03 ^{DE}
4	6.29 ± 0.03 ^e	6.43 ± 0.04 ^d	-	-

¹Control: treated with heat alone with sample flowing through the PHE and sonicator with sonicator off; ²Treatment: with sample flowing through the PHE and sonicator with sonicator on.

Values with different letters are significantly different within each setting (Lowercase: Setting 1, Uppercase: Setting 2). Values displayed are (Mean ± S.D). - Measurements not taken because milk was curdled. Statistical analysis shown in Table D.5.

Figures

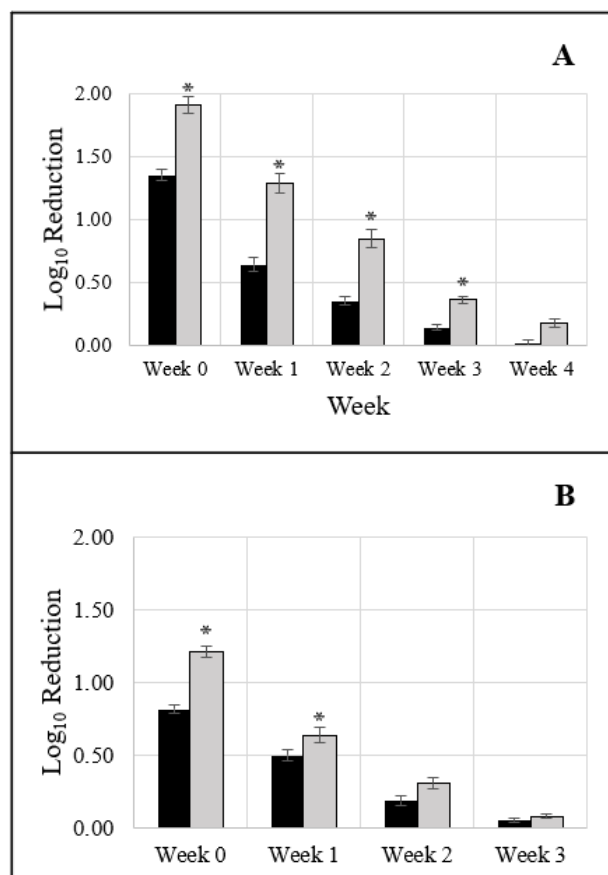


Figure 5.1. Log₁₀ reduction (Mean \pm S.E) of indigenous microorganisms in whole milk during its shelf life after being processed with control or treatment at setting 1 (A) and setting 2 (B).

* Signifies significant difference compared to control.

■ Control ■ Treatment

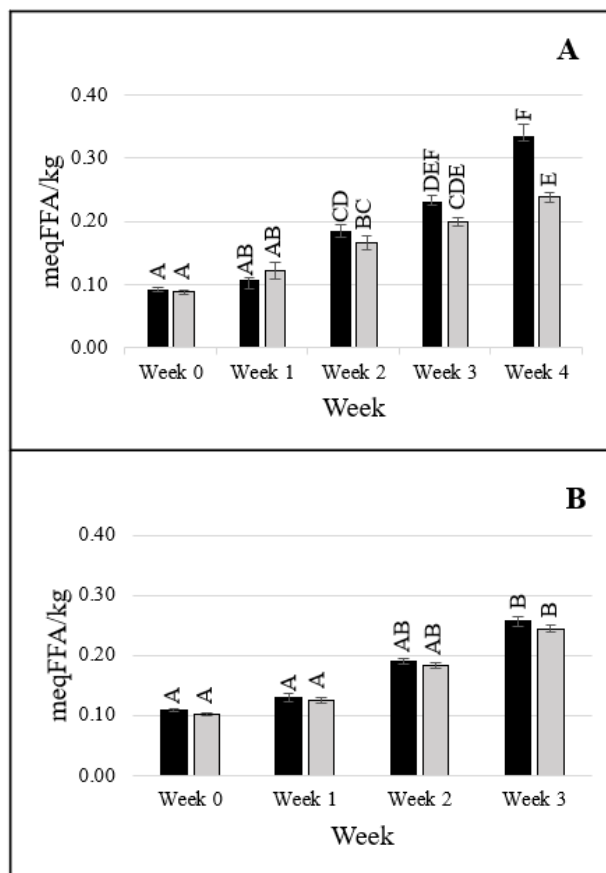


Figure 5.2. Free fatty acid content expressed as meq FFA/ kg in whole milk during its shelf life after being treated with control or treatment at setting 1 (A) and setting 2 (B).

Statistical analysis shown in Table D.6.

■ Control □ Treatment

Values with different letters are significantly different within each figure.

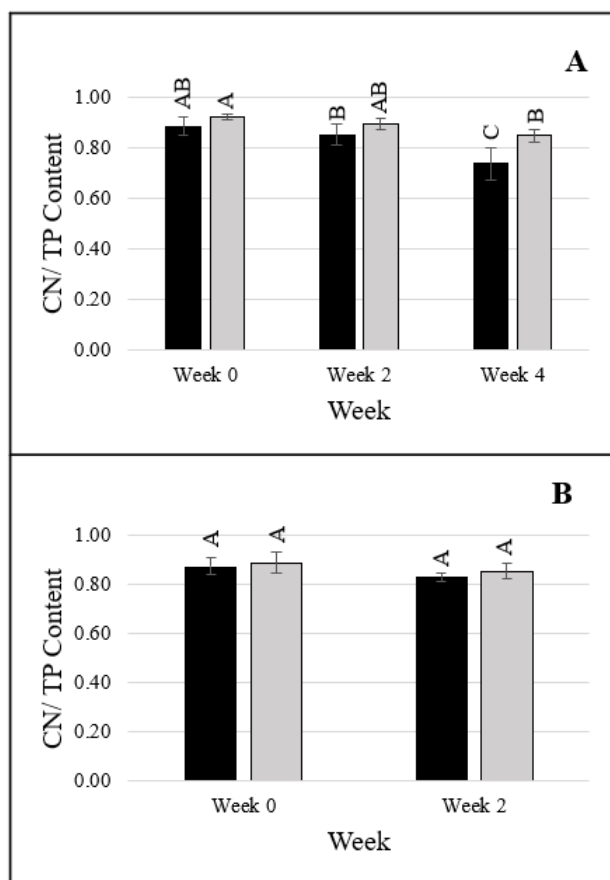


Figure 5.3. Casein/Total Protein content of whole milk during its shelf life after being treated with control or treatment at setting 1 (A) and setting 2 (B).

■ Control □ Treatment

Values with different letters are significantly different within each figure.

CHAPTER VI

EFFECT OF THERMOSONICATION IN A CONTINUOUS SYSTEM ON INDIGENOUS MICROFLORA AND MILK QUALITY DURING SHELF LIFE ALONG WITH THE EFFECT ON CONSUMER ACCEPTANCE.

ABSTRACT

Increasing pasteurized milk shelf life has been a concern for the dairy industry. This study evaluated the effect of pasteurization (control) coupled with thermosonication (treatment) (11.1 s) in a continuous system on shelf life parameters during storage along with consumer liking. Treatment samples had significantly lower microbial count than control during shelf life. Lower free fatty acid and higher pH and casein/total protein values were observed for treatment samples during shelf life. In a consumer panel, panelists commented on treatment samples having an unknown off flavor. This unknown flavor declined after 4 days and the consumer liking of treatment samples after 4 days in a second consumer panel was not significantly different than control. Thermosonication in a continuous system coupled with pasteurization successfully improved the overall quality of whole milk as compared to heat only. Future studies could investigate thermosonication conditions to minimize changes in sensory attributes in milk.

PRACTICAL APPLICATIONS

Thermosonication when applied with pasteurization could improve the microbial quality of milk during shelf life and extended the shelf life by 2 weeks as established by

this study. Increased shelf life would not account for milk loss due to temperature abuse. But it could possibly lead to reduced milk loss on the consumer level by extending the window milk can be consumed. This study also established that use of practical residence times (10-15 s) could minimize changes in sensory quality of milk as a result of thermosonication. Minimal effect of sensory quality may increase consumer acceptance of thermosonicated milk. Scale up systems will need to evaluate the effect of thermosonication at specific residence times and power levels on milk quality during shelf life and effect on consumer acceptance.

INTRODUCTION

Fluid milk in the U.S is most commonly pasteurized with high temperature short time pasteurization (HTST; at 72°C for 15 s), which extends the raw milk shelf life to about 3 weeks (Gandy et al., 2008; Pasteurized Milk Ordinance-FDA, 2017). Increasing pasteurized milk shelf life has been a concern for the dairy industry, which is limited mainly due to bacterial growth during refrigerated storage (Deeth, 2017).

Microorganisms that grow during refrigerated storage produce extracellular hydrolytic enzymes such as lipases and proteases that affect the milk shelf life (Rawat, 2015; Sørhaug & Stepaniak, 1997). Treating milk with higher processing temperatures could help reduce the total bacteria count and possibly improve milk quality and shelf life (Ivy et al., 2012).

High temperature processing (such as ultra-pasteurization and ultra-high temperature) results in milk having a cooked flavor, which is not desirable to some consumers, especially in the U.S (Deeth, 2017; Schiano, Harwood, & Drake, 2017).

Furthermore, increases to the pasteurization temperatures (up to 85°C) did not increase the milk shelf life and temperatures over 79°C resulted in decreased consumer acceptance (Gandy et al., 2008). A potential solution to extend the milk shelf life could be using alternative technologies such as sonication coupled with thermal treatment for extension of milk shelf life, which has been successful in microbial inactivation in foods (Villamiel, Schutyser, & De Jong, 2009).

Sonication is the passing of sound waves (≥ 20 kHz frequency) in a liquid medium that results in generation of thermal energy or cavitation (Chandrapala, Oliver, Kentish, & Ashokkumar, 2012). As a result of cavitation and bubble collapse, very high temperatures ($\approx 4726^\circ\text{C}$) and pressures ($\approx 2,000$ atm) are generated locally in the fluid medium (Zisu, Schleyer, & Chandrapala, 2013). Sonication with its potential to alter food functionality and enhance the shelf life and quality is being explored for food industry applications (Beatty & Walsh, 2016; Chandrapala et al., 2012; Chemat, Zill-E-Huma, & Khan, 2011; Deshpande & Walsh, 2018; Ganesan, Martini, Solorio, & Walsh, 2015; Knorr, Zenker, Heinz, & Lee, 2004). In general, thermosonication has shown minimal changes in the pH, protein content, butterfat, and solids of whole milk after processing (Bermúdez-Aguirre, Mawson, Versteeg, & Barbosa-Cánovas, 2009).

Several batch sonication studies have used processing times between 2-10 min to show significant reductions of added microorganisms in milk such *Listeria*, *Escherischia coli*, *Anoxybacillus flavithermus*, and *Bacillus coagulans* (Cameron, McMaster, & Britz, 2008; Gera & Doores, 2011; Khanal, Anand, Muthukumarappan, & Huegli, 2014). However, these studies were limited to batch systems, used long processing times (2-10 min), and did not use thermosonication at pasteurization temperatures. A batch sonication

study by Lim, Benner, and Clark (2019) showed that 10-60 s of residence time (cold and thermosonication) could not extend the shelf life of milk inoculated with a psychrotrophic thermophilic *Paenibacillus spp* (inoculated at 6.00 log CFU/ml). Chouliara, Georgogianni, Kanellopoulou, and Kontominas (2010) showed that batch sonication of milk for 2-16 min at 15-25°C could not extend milk shelf life. Previous batch sonication studies for milk lacked data on evaluation of shelf life attributes. Thus, the current study focused on evaluating the effect of thermosonication at 72°C along with pasteurization in a continuous system on overall milk microflora.

In a continuous system, Villamiel and De Jong (2000) showed a 3.10 log reduction of overall microbial count in milk after thermosonication for 102.3 s at 76°C. A previous study performed by Bermúdez-Aguirre et al. (2009) showed batch pasteurization and thermosonication reduced the mesophilic bacteria in raw milk and sonicated samples had significantly lower counts than pasteurized milk after 2.5 weeks. However, a study by Villamiel and De Jong (2000) did not include analysis of any shelf life attributes and Bermúdez-Aguirre et al. (2009) performed pH and color measurements for only 2.5 weeks and an estimated shelf life and milk quality over time for sonicated samples was not determined. Moreover, processing times of 2-30 min may not be suitable for practical applications.

Batch studies performed in the past have included sensory panels for aroma and taste of sonicated samples but consumer liking has not been evaluated. A descriptive panel on aroma of samples performed by Lim et al. (2019) found a rubbery aroma in thermosonication samples, which declined over storage period; however, taste was not evaluated. Moreover, Chouliara et al. (2010) showed a decrease in taste acceptability

with 17 panelists in sonicated milk. There is a need for a sensory analysis including aroma and taste with a focus on consumer acceptance to evaluate the effect of thermosonication in a continuous system. Evaluation of shelf life attributes such as pH, free fatty acid content (FFA), and casein as a percentage of total protein (CN/TP) content along with microbial analysis and sensory evaluation, may help establish the benefits of sonication on overall milk quality (Fromm & Boor, 2004).

To summarize, there is a lack in the use of practical treatment times (2-30 min) for sonication in a continuous system. Also, there is a need of a comprehensive study on effect of thermosonication on milk that includes shelf life attribute evaluation during storage. Using this rationale, this study investigated the effect of thermosonication along with pasteurization on milk quality during shelf life while using practical residence times in a continuous system. The first objective was to study the effect of thermosonication in a continuous system on survival of indigenous microflora using practical residence times. The second objective was to evaluate the effect of thermosonication on shelf life attributes which were assessed during storage using microbial analyses, FFA content, CN/TP content, and pH. The third objective was to evaluate the effect of thermosonication on consumer acceptance of milk. Findings from this study can be used to construct parameters for thermosonication of milk in an industrial scale system.

MATERIALS AND METHODS

Experimental design

The effect of continuous thermosonication coupled with pasteurization on indigenous microflora in milk throughout its shelf life was investigated. The effect of

heat (at 72°C) and thermosonication (at 72°C at 60% amplitude) on reductions of indigenous microflora in whole milk in a continuous system was evaluated by treating raw whole milk either by heat or heat with thermosonication. Raw milk, 0.76 L/min flow rate, was heated using two plate heat exchangers (PHE). Heated milk was passed through a sonication vessel set at 73°C with a 11.1 s residence time, followed by passage through another PHE which was used for cooling. Outlets of all the PHEs were fitted with thermocouples and temperatures were constantly monitored (Appendix E, Figure E.1). Pasteurization conditions were simulated closely and the temperature of milk after passing through the second PHE was between 72-73°C; milk was held at that temperature for 15 s using insulated tubing. Control is defined as being treated with heat alone and treatment is defined as being treated with heat and thermosonication. Samples received 26.1 s total hold time at 72-73°C with or without 11.1 s of thermosonication at 72-73°C. Milk was collected in sample cups and evaluated for different parameters through its shelf life. A total of six replicates were performed.

The effect of thermosonication on consumer acceptance was evaluated using the sonication system described above without the PHEs, with milk just flowing through the sonicator, since the milk was commercially pasteurized. Commercially HTST pasteurized 2% fat milk was preheated to 70°C in a water bath before being either heat treated or thermosonicated for 11.1 s at 72-73°C.

Preparation of milk

Raw milk (obtained from George B. Caine Dairy Research Teaching Center, Utah State University, Wellsville, UT, USA) received at 4°C was heated to 25-30°C by placing three bottles containing hot water (between 85-90°C) in the milk cans with occasional

stirring for 1-2 hr. The raw milk received had microbial counts of 2.00 log CFU/ml, and the warming step was performed to increase the initial microbial counts to 5.00 log CFU/ml, so that an effect of heat along with thermosonication could be evaluated.

Heating and thermosonication conditions

Milk was pumped using a Masterflex 7529 pump (Cole-Palmer, Vernon Hills, IL, USA) at a flow rate of 0.76 L/min. Raw milk (19 L) was warmed to 25-30°C for each replicate. Two PHEs (PHE I set at 80°C and PHE II set at 75°C) were used for heating milk and the temperature of the PHEs was maintained using circulating water baths as shown in Appendix E, Figure E.1. The sonication flow cell had a 140 ml volume resulting in a residence time of 11.1 s with or without sonication for both control and treatment samples. Samples were passed through the third PHE (set at 0-4°C) to cool the milk; milk was bottled and kept in an ice bath for further cooling. Samples were thermosonicated (Heischler UIP1000hd, Ringwood, NJ, USA) at 60% amplitude and the temperature of the flow cell was maintained at 72-73°C using a circulating water bath set at 73°C. All equipment was rinsed with a detergent solution followed by washing with hot water and sanitizer solution after each experiment and with hot water between different runs to keep processing equipment sanitary.

The raw milk samples were placed on ice until ready to plate for initial microbial count. After being treated with control or treatment conditions, samples were collected in sterile 750 ml bottles and then redistributed in 200 ml sterile containers for shelf life experiments. Samples were kept on ice until ready to plate for week 0 measurements. Samples collected for shelf life experiments were transferred in a cardboard box to block

light and kept in a cold room at 4°C for shelf life monitoring. This entire procedure was repeated for each replicate and a total of six replicates were used for evaluations.

For sensory evaluation, commercially purchased HTST pasteurized 2% fat milk was pre-heated to 70°C in 750 ml sanitized bottles kept in a stationary water bath and passed through the sonication vessel using Masterflex 7529 pump at a flow rate of 0.76 L/min. For treatment samples, thermosonication was applied for 11.1 s at 72°C with the sonicator set at 60% amplitude. Control samples received 11.1 s of heating time at 72°C in the sonication vessel. Milk was collected in sanitized 750 ml bottles and transferred to an ice bath to be cooled immediately.

The energy density (J/ml) for the sonicated samples was calculated using power, residence time, and volume (Chandrapala, Martin, Kentish, & Ashokkumar, 2014). The power readings were between 225 and 250 W with an average of 234.3 W. With a residence time of 11.1s in the sonication vessel, the energy density calculated was 18.6 J/ml.

Microbial and pH evaluation

For shelf life experiments, dilutions of samples were made in sterile PBS and plated on standard plate count agar (SPC; for total aerobic bacteria). Plates were incubated for 24-48 hr in a humidified incubator at 32°C. Plating was performed before processing, immediately after processing and then at each week. After microbial testing, pH of samples was measured at 4°C after standardization of the pH meter (Orion 3-star pH meter, Thermo Fisher Scientific, Waltham, MA, USA) with buffers of pH 4 and pH 7. Duplicate measurements were done for each sample. The storage containers were then transferred to freezer at -29.9°C and stored for later shelf life testing as described below

for FFA and CN/TP. Milk shelf life was determined when samples appeared to be spoiled which was defined by either CFU/ml > 6.00 log or when visible curdling of milk was observed (Fromm & Boor, 2004; Lim et al., 2019).

Free fatty acid (FFA) content

Lipolysis in milk samples was evaluated by measuring the increase in FFA content from week 0 through week 6. The FFA content is expressed in meq FFA/kg of milk and has been abbreviated to meq FFA/kg. The FFA content was measured using the copper soap method described in Shipe, Senyk, and Fountain, (1980), as modified by Ma, Barbano, and Santos (2003). Milk was thawed using a combination of a water bath at 20°C and a microwave while keeping the temperature below 10°C. Reagents such as copper soap reagent, color reagent, solvent (chloroform-heptane-methanol; 49:49:2 vol:vol:vol), and solubilizing reagent were prepared as described in the methods by Shipe et al. (1980). For analysis, 0.2 ml aliquot of 0.7 N HCl was added to 1 ml milk samples in a test tube. The mixture was shaken using a Vortex test tube mixer, and 4 ml of the copper reagent and 12 ml of solvent were added. The sample was shaken for 30 min in shaker at 400 rpm and centrifuged for 10 min at 2500 x g in a centrifuge. Then 3.5 ml of the upper solvent layer was transferred to test tube containing 0.1 ml of the color reagent. Color was measured in cuvettes after mixing at 440 nm within 1 hr using a spectrophotometer (BioSpec-1601, Shimadzu, Kyoto, Japan). Blanks were prepared by using deionized (DI) water instead of milk.

A standard curve was obtained by preparing six known concentrations of palmitic acid (0, 50, 100, 150, and 200 µg/ml) and mixing with 0.1 ml of 0.7 N HCl and 1 ml of DI water and a standard curve was plotted with absorbance measured at 440 nm. FFA

content in milk was calculated in $\mu\text{g/ml}$ from the standard curve. The final value is reported in meq FFA/kg of milk and was calculated as:

$$\text{meq FFA} = \frac{\frac{\mu\text{g of FFA} \times 0.001 \text{ mg}/\mu\text{g}}{256.43 \text{ mg/meq}}}{\text{g of milk} \times 0.001 \text{ kg/g}}$$

Duplicate reactions were conducted for each sample.

Casein/ Total Protein (CN/ TP) content

Casein (CN) as a percentage of total protein (TP) was measured at alternate weeks from week 0 to 6 to evaluate the proteolysis activity during milk shelf life (Ma, Barbano, & Santos, 2003). Milk was thawed using a combination of a water bath at 20°C and microwave while keeping the temperature below 10°C. Whole milk samples were used for total protein measurements. Non-casein nitrogen content was analyzed using whey portions from the whole milk.

Whey portions of milk were obtained by mixing 20 ml milk sample with 20 ml DI water in a 50 ml test tube which was then kept at 37°C for 30 min in a water bath. Two ml of acetic acid solution (10% v/v) was added and the mixture was vortexed for 30 s, followed by letting the mixture stand for 10 min. Two ml of 1 M sodium acetate was then added to the test tube and the mixture is cooled to 20°C in an ice bath. DI water was added to the 50 ml calibration mark and the test tubes were centrifuged at 3500 rpm for 15 min. The filtrate (whey) was collected for further analyses and casein was obtained as a pellet.

Whole milk and whey samples, kept at 4°C, were sent to Utah State University Analytical Laboratories (USUAL; Logan, UT, USA) for nitrogen content analyses performed using combustion. Whole milk was used to generate TP and the whey was

used to generate non-casein nitrogen (NCN). CN was calculated from TP - NCN. CN/TP is reported by taking a mean and standard deviation of at least three replicates.

Grocery store milk analyses

To compare the milk obtained from control and treatment samples to commercially pasteurized milk, three whole milk samples of HTST pasteurized milk were purchased with their best by dates being within 2-2.5 weeks from the day they were purchased. Milk samples were stored at 4°C. Microbial analyses (SPC plating), pH measurements, and FFA content analyses were performed each week on the samples until any curdling in milk which was seen after 4 weeks of purchase. CFU/ml observed were > 6.00 log after 1 week.

Sensory Evaluation

Two sensory panels for consumer acceptance were conducted and both were approved by the Institutional Review Board (IRB) at Utah State University. The first consumer acceptance panel was conducted with 120 panelists to test for consumer acceptance of milk treated under control and treatment conditions. Commercially pasteurized 2% fat milk (Smiths, Logan, UT, USA) was passed through the sonication system as described above. Pasteurized milk was used to ensure the safety of milk provided to the consumers for tasting since the pasteurization system used for shelf life experiments was not verified by the FDA for its efficacy. Samples were processed a day before the sensory panel was conducted. Control and treatment milk samples were transferred to 60 ml cups and covered with lids with each cup containing 20-25 ml of milk samples. The samples were kept refrigerated before being served to the panelists.

SIMS 2000 (SIMS Software Cloud, New Jersey, USA) was used to conduct the consumer panel, to administer the questionnaire, and analyze the results. Samples were presented in a randomized manner, and panelists were asked to observe, smell, and taste each sample, and rinse their mouth with water and take a bite of a saltine cracker between each sample. An electronic questionnaire was used to ask the panelists to rate their liking for each sample for color, overall appearance, smell, flavor, and overall liking. Panelists were asked to rate their liking using a 9- point hedonic scale where 1 meant dislike extremely and 9 meant like extremely. Panelists were also asked to comment on each of the attributes.

A focus group of 6 panelist was conducted to gain more insights on flavor changes over 6 days where panelists tasted the treatment and control samples of commercially pasteurized 2% fat milk samples on days 1, 2, 4, and 6. Samples were coded and served randomly each day to remove any bias. Panelist were asked to comment on the appearance, aroma, and flavor of the samples each day and the comments were recorded. Focus group panelists were asked to comment on coded samples. The comments were then related back to control versus treatment samples when recording comments electronically. Based on the results of the focus group, a second consumer panel using control and treatment samples of 2% fat pasteurized milk was conducted with 110 panelists similar to the first consumer panel. However, instead of the 2 samples, 4 samples were provided. Control and treatment samples from day 1 and day 4 of processing were used. Day 1 control and treatment samples were also used for titanium content and particle size analysis.

SIMS 2000 (SIMS Software Cloud, New Jersey, USA) was used to conduct the second consumer panel and was used to administer the questionnaire and analyze the results similar to the first consumer panel conducted. An electronic questionnaire was used to ask the panelists to rate and comment on their liking for each sample for flavor and overall liking on a 9-point hedonic scale. The number of attributes tested was reduced from the first consumer panel due to an increase in the number of samples tested in order to avoid sensory fatigue.

Statistical analysis

All experiments were conducted at least in triplicate. Analysis of variance (ANOVA) was used to test for statistical significance ($\alpha=0.05$) considering a repeated measures design, followed by the Ryan, Einot, Gabriel, Welsh Studentized Range Q (REGWQ) test and Tukey HSD for post-hoc analysis in SAS 9.4. ANOVA followed by Tukey HSD was used to evaluate the effect of thermosonication and shelf life on the microbial quality of milk with measurements during shelf life treated as a repeated time measurement. ANOVA followed by REGWQ test was used to determine if thermosonication and shelf life had a significant effect on the FFA, CN/TP, and pH of the milk during its shelf life. Excel statistics was used to calculate the correlation coefficients microbial counts with pH, FFA, and CN/TP values and correlation coefficients were obtained using pooled data from control and treatment samples. Significant differences during the first sensory panel and particle size data were analyzed using a t-test, while ANOVA followed by Tukey-HSD were used to analyze the data from the second consumer panel and for titanium content.

RESULTS AND DISCUSSION

Effect of thermosonication on indigenous microflora

The average microbial count for raw milk before processing was 5.00 log CFU/ml and the average microbial count at week 0 was 2.00 log CFU/ml for control and 1.00 log CFU/ml for treatment as seen in Figure 6.1. Treatment conditions significantly reduced the microbial numbers by 4.06 ± 0.04 log as compared to 2.79 ± 0.08 for control conditions at week 0. Control conditions had a hold time of 15 s at 72°C along with 11.1 s of heating time in the sonication vessel, whereas treatment conditions consisted of a hold time of 15 s at 72°C along with 11.1 s of thermosonication in the sonication vessel.

The microbial count increased for both control and treatment samples as the shelf life progressed. However, at all weeks, treatment samples had significantly lower counts as compared to control samples. During shelf life, the microbial count increased from 3.00 log CFU/ml at week 1 to 5.00 log CFU/ml after 4 weeks, for control conditions and milk samples showed curdling at week 4. Meanwhile, treatment with thermosonication increased the microbial count from 2.00 log CFU/ml at week 1 to 5.00 log CFU/ml after 6 weeks and samples showed signs of curdling at week 6. The microbial count at week 4 for control was not significantly different than the microbial count at week 6 for treatment. Milk treated with control conditions had a shelf life of 4 weeks as compared to thermosonicated milk which had a shelf life of 6 weeks based on the criteria used by previous studies (Fromm & Boor, 2004; Lim et al., 2019). Microbial analyses performed on pasteurized whole milk purchased from the grocery store showed that the average microbial counts were 4.41 log CFU/ml the week the milk was purchased (Appendix E,

Table E.1). The average microbial counts increased to 7.93 log CFU/ml after 3 weeks, which was around the best by date for the milk samples. This indicates that the milk in a consumer's refrigerator may have microbial counts > 6.00 -7.00 log CFU/ml at the end of its shelf life.

Similar reduction in microbial count of milk as a result of sonication in a continuous system has been observed in previous studies. When only thermosonication at 150 W was applied to raw milk at 76°C in a continuous system, Villamiel and De Jong (2000) reported a 2.90 log reduction of overall microbial count at a residence time of 102 s as compared to 2.80 log with control. Comparatively, higher reduction of 4.06 log was seen after 11.1 s of thermosonication (234 W) along with heat for the current study as compared to 2.79 log seen for control samples. This higher reduction could be due to the higher power level and hence higher energy density seen in the current study compared to previous reports. Moreover, the results obtained in this study show that higher log reductions can be obtained when thermosonication is applied for a shorter residence time when coupled with pasteurization, instead of replacing it.

Bermúdez-Aguirre et al. (2009) batch sonicated samples for 30 min at different amplitudes following batch pasteurization (63°C for 30 min) and showed that the microbial counts of pasteurized milk increased to 5.00 log CFU/ml and of sonicated samples increased to 2.00 log CFU/ml after a 2-week shelf life. Similar to Bermúdez-Aguirre et al. (2009), the treatment samples from this study had significantly lower microbial counts than control after 2 weeks and at the end of the shelf life of 6 weeks. The microbial counts of 3.00 log CFU/ml reported for treatment samples in this study after 2 weeks, are slightly higher than reported by Bermúdez-Aguirre et al. (2009),

which may be due to their higher treatment time of 30 min which is not suitable for practical applications.

Fromm and Boor (2004), collected commercially pasteurized (HTST) 2% fat milk from three different plants and analyzed the total bacterial count. The microbial load increased from 2.00 log to 5.00 log CFU/ml in about 2.5 weeks. In this research, the microbial counts reached up 5.00 log CFU/ml after 4 weeks for control and 6 weeks for treatment. Thus, the microbial quality during milk shelf life could be enhanced and with possible shelf life extension using 11.1 s of thermosonication along with pasteurization as seen in this study.

Effect of thermosonication on shelf life attributes

The pH of whole milk for both control and treatment conditions is shown in Table 6.1. A decrease in pH was seen as the shelf life progressed from week 0 to 4 for control samples and week 0 to 6 for treatment samples, which could be due to microbially produced acids (Ziyaina, Govindan, Rasco, Coffey, & Sablani, 2018). The pH of both control and treatment samples were not significantly different at week 0 (6.78 ± 0.01 for both control and treatment). However, as the shelf life progressed, the pH of control samples was significantly lower than treatment samples. The pH of control samples significantly decreased from 6.53 ± 0.01 at week 2 to 6.43 ± 0.01 at week 4. For treatment samples, the pH significantly decreased from 6.64 ± 0.01 at week 2 to 6.50 ± 0.01 at week 6. From Appendix E, Table E.1, the pH of the milk purchased from grocery stores had a pH starting at 6.93 which reduced to 6.78-6.45 at the end of its shelf life (2-3 weeks). The pH values reported at the end of shelf life for control and treatment samples were higher to

the values reported for commercially pasteurized milk as shown in Appendix E, Table E.1.

Bermúdez-Aguirre et al. (2009) observed no significant differences in pH between batch pasteurized and thermosonicated (for 30 min) milk during 2-week shelf life and values at the end of the shelf life were between 6.5-6.7. Similar to this study, Bermúdez-Aguirre et al. (2009) observed a decrease in pH during the shelf life of milk for both pasteurized and thermosonicated samples which was attributed to acid production by bacteria. The pH values observed for thermosonation samples after a 6-week shelf life in this study are comparable to the pH values reported by Bermúdez-Aguirre et al. (2009) after a 2-week shelf life.

FFA content is related to the extent of lipolysis occurring during milk shelf life from microbially produced lipases. This was evaluated by comparing the FFA content at week 0 to the FFA content as the shelf life progresses for both control and treatment conditions which is shown in Table 6.1. The FFA content significantly increased for control samples from week 0 (0.09 ± 0.01 meq FFA/kg) to week 4 (0.32 ± 0.02 meq FFA/kg). For treatment samples as well, the FFA content significantly increased from week 0 (0.08 ± 0.01 meq FFA/kg) to week 6 (0.32 ± 0.02 meq FFA/kg). Treatment samples had a significantly lower FFA content as compared to control only at week 4. The FFA content observed for milk purchased from grocery stores at week 0 was 0.13 meq FFA/kg and increased to 0.41-0.53 meq FFA/kg after 2-4 weeks as seen in Appendix E, Table E.1. Milk samples purchased from grocery stores had higher FFA content than the control samples at week 0 and also at the end of the shelf life, which could be due to the differences in processing conditions and raw milk quality.

Fromm and Boor (2004), observed that the FFA content of commercially pasteurized (HTST) milk increased from 0.1 to 0.25 meq FFA/kg in a 2.5-week shelf life. In the current study, treatment samples had a FFA content of 0.20 meq FFA/kg and 0.32 meq FFA/kg after 4 and 6 weeks of shelf life, respectively. Meanwhile the control samples had a FFA content of 0.32 meq FFA/kg after 4 weeks of shelf life. The FFA content values observed for treatment samples at week 4 are lower and at week 6 are comparable to the values reported by Fromm and Boor (2004). Santos, Ma, Caplan, and Barbano (2003) established that consumers could detect off flavors as a result of lipolysis in milk at values between 0.32-0.35 meq FFA/kg. The values obtained for FFA content in thermosonication samples at 4 and 6 weeks were lower or near the threshold values reported by Santos, Ma, Caplan, and Barbano (2003).

The extent of proteolysis in milk samples during their shelf life was measured using CN/TP content. Greater extent of proteolysis can be seen with a decrease in the CN/TP content as the shelf life progresses due to production of proteases by microorganisms (Table 6.1). Overall, the CN/TP ratio decreased for both control and treatment samples. Control samples had a lower CN/TP content of 0.74 ± 0.02 at week 4 when compared to that of treatment samples (0.81 ± 0.01) at week 6. No significant differences were observed between control and treatment samples at all weeks.

Fromm and Boor (2004) when analyzing HTST pasteurized milk during its shelf life for proteolysis, reported that the CN/TP content decreased from 0.87 to 0.85 in a 2.5-week shelf life. In the current study, the values observed after 4 weeks for control samples was significantly lower than those reported in the literature; but the CN/TP content values after 4 and 6 weeks for treatment samples were still comparable. Santos,

Ma, Caplan, and Barbano (2003) showed that a CN/TP content of 0.73 was near the threshold value for detection of off flavors due to proteolysis. The CN/TP content of thermosonication samples after 6 weeks was ≥ 0.81 , which is above the threshold stated by Santos, Ma, Caplan, and Barbano (2003).

pH and CN/TP values were negatively correlated with microbial counts for both control and treatment samples with a correlation coefficient of 0.78 and 0.95, respectively (Appendix E, Table E.2). The FFA content was positively correlated with microbial counts for both control and treatment samples with a correlation coefficient of 0.95 (Appendix E, Table E.2). Similarly, Ziyaina et al. (2018), saw high correlations between microbial count and pH, lipase, and protease activity. Sharp decrease in pH and increase in lipase, and protease activity were reported when the microbial count reach 4.00-6.00 log CFU/ml, similar to observations of this study. Based on the results of this study, thermosonication improved the pH and FFA content of milk samples which could be due to the reduced bacterial count in treatment samples. Milk shelf life and quality can be assessed using microbial counts along with parameters such as pH, FFA, and CN/TP content (Ziyaina et al., 2018). Therefore, thermosonication of 11.1 s coupled with pasteurization may help improve milk quality during shelf life.

Effect of thermosonication on sensory attributes

To assess if thermosonication in a continuous system had an effect on consumer acceptance of milk, the first consumer panel performed asked panelists to indicate their liking for color, overall appearance, smell, flavor, and overall liking of the control and treatment samples using a 9-point hedonic scale. Majority of panelists were between 22-29 years of age (53.3%), 59.2% were female, 69.2% preferred 2% fat milk as compared

to whole milk, and about 80% of the panelists consumed milk between 2 to more than 6 times per week while 63.3% purchased milk at least once a week (Appendix E, Table E.3). Control samples were rated significantly higher by the panelists than thermosonication samples for all attributes, except for color, as seen in Table 6.2. All the attributes were rated at scores ≥ 6.04 for both control and treatment samples, except for flavor attribute for thermosonication sample which was 5.99.

Panelists were not provided with any particular words for describing attributes. However, from the selected panelist comments shown in Table 6.3, creamy or watery consistency, milky smell and flavor along with sweetness were the common themes observed in both the samples. Panelist commented that the control looked more yellow, creamy, and dull while milk after thermosonication appeared whiter, less creamy, and brighter. Treatment samples had a stronger milky or cooked flavor as compared to control as per the panelists' comments; however, overall both the samples had smells that were difficult to discern. Overall panelists thought that the control samples, had a slightly cooked flavor and were not as sweet. Whereas, treatment samples tasted more cooked with notes of maltiness, smokiness, and a strange after taste. Generally, the panelists liked both the samples and thought they both tasted similar to store-bought milk, but treatment samples were possibly rated lower due to a more enhanced cooked flavor with an unknown aftertaste.

Lim et al. (2019) performed a descriptive sensory panel on HTST pasteurized skim milk and evaluated the effect of batch thermosonication on the aroma of control versus treatment samples. The terms used to describe the aroma of treatment samples were rubbery, chemical like, and burnt, which have been used in past studies (Chouliara

et al., 2010). The unidentified strange taste identified by the panelists of the current study could possibly be rubbery or chemical, and the consumer panel participants may not have been able to accurately describe it. This could possibly be because the rubbery flavor was very mild and not all panelists could notice it. Lim et al. (2019) reported that the rubbery aroma for shorter sonication treatment of 10 s at a higher amplitude of 200 μm (energy density of 19.8 J/ml, similar to the current study) was 1.3 on a scale of 15, which was not significantly different than control samples which scored 2.1. They also reported that the rubbery aroma declined over storage time of 21 days.

To investigate if the unknown aftertaste declined in this study as well, a focus group was conducted with 6 panelists (3 males, 3 females) over a period of 6 days. Panelists were asked to comment on appearance, smell, and flavor of control and treatment samples on days 1, 2, 4, and 6, results of which can be seen in Table 6.4. On day 1, panelists could not identify any differences in the color of the samples but commented on the control samples looking more creamier than treatment. Moreover, treatments samples seemed to have a sweeter and stronger milk flavor as compared to control samples on day 1. Panelists from the focus group commented on treatment samples having an enhanced cooked flavor along with a slight sulfur and weird aftertaste. Panelist could easily identify a cooked flavor between control and treatment samples until day 2. After 2 days of storage, both control and treatment samples had a slightly pale appearance and a milder milk smell but no differences could be noticed between them. Control tasted watery and a very subtle cooked flavor was noticed in treatment samples; no weird flavor could be identified. Panelists found very minimal differences between appearance, smell, and flavor between control and treatment samples on 4, but both the

samples still tasted similar to store bought milk. Similar to day 4 samples, no differences were detected on day 6; however, panelists could detect hints of stale and old notes in smell and flavor.

Based on the results of the focus group, a second consumer panel was designed where samples from day 1 and day 4 were used for both control and treatment. Panelists were asked to indicate their liking for all the four samples for flavor and overall liking. Out of the 110 panelists, 54.2% were aged between 22-29 years, 64.2% were females, 71.7% preferred 2% fat milk, and 87.5% of panelists had a milk consumption frequency of 2 to ≥ 6 times a week and purchase frequency of at least once a week (Appendix E, Table 6.4). Figure 6.2 displays the second consumer panel results on a 9-point hedonic scale, where A shows the scores obtained for flavor and scores for overall liking are shown in B. Even though treatment samples scored lower than control samples, only the flavor and overall liking of treatment sample on day 1 was significantly lower.

Selected panelist comments were sorted on their preference of samples and compiled in Table 6.5. Similar to the first consumer panel, panelists were not provided with any specific descriptive words and the comments showed similar descriptive words such as sweet, creamy, cooked, and milky for both control and treatment samples. Panelists who preferred control day 1, liked that the samples had an overall good and appealing flavor, tasted similar to store bought milk, sweet, and cooked flavor. Whereas, panelists who preferred control on day 4, found lack of aftertaste and mild milk flavor more appealing. Panelists who highly rated treatment samples on day 1, preferred its sweet and milky flavor but noticed some cooked flavor.

Meanwhile, panelists who preferred treatment samples on day 4, liked it because it was sweet and had milk flavor and compared it to store bought milk. Control samples were described to have creamier and smoother mouthfeel with day 1 samples tasting thicker than day 4. Whereas treatment samples had smoother and less creamy mouthfeel, with day 4 samples having milk like consistency. Some of the panelists commented that treatment samples on day 1 had an unknown aftertaste and hints of metallic flavor.

Similar observations of decreased consumer acceptance of sonication samples were reported by Chouliara et al. (2010) where a total of 16 panelists were used to rate the odor and taste (on a scale of 5, with 5 being more favored) of pasteurized milk and pasteurized milk processed treated with batch sonication (24 kHz, 15-25°C) for 2-16 min and results from 2 min are compared due to their similarity with the current study. A sensory panel was conducted for up to 8 days with no significant difference seen between control and treatment samples during storage. The flavor scores for treatment samples were consistently lower) by approximately ± 1 from day 0 to 8, similar to the findings observed in the first consumer panel of this study.

Lim et al. (2019) hypothesized that the rubbery and strange aroma noticed by the panelists in their study was due to the formation of volatile compounds associated with secondary lipid oxidation. However, Chouliara et al. (2010) actually measured the lipid oxidation compounds by measuring malondialdehyde content of milk samples and showed that thermosonication residence times of ≥ 2 min in a batch system did not attribute off flavors in sonicated milk to formation of lipid oxidation compounds. Moreover, sonication in continuous systems showed the production of lipid oxidation products in milk significantly different than control in recirculating conditions (with

multiple passes) at total residence times of ≥ 5 min (Juliano et al., 2014). Thus, lipid oxidation may not have been a source of off flavors in thermosonicated milk with the residence time in the current study being 11.1 s in a continuous system.

Although descriptive panels in previous studies may have identified specific flavors associated with thermosonation milk, the effect of those flavors on consumer acceptance needs to be considered. Thermosonation milk had significantly lower scores for all attributes as compared to control on day 1 and a storage period of 2-4 days decreased the unknown off flavors significantly to an extent that minimal differences were observed. No significant differences were observed between control and treatment samples for titanium content and particle size analysis (Appendix E, Table E.11-E.14 and Figure E.2.). In the future, the industrial scale applications of thermosonation would need to verify effects on sensory attributes with extensive consumer panels for specific residence times and acoustic powers. Moreover, analysis of sensory attributes and consumer acceptance during milk shelf life may be helpful to establish the benefits of thermosonation on overall milk quality. Future studies should focus on investigating possible sources for the unknown off flavors noticed in thermosonation milk.

The findings from this study helped establish the potential application of thermosonation for improving the overall milk quality with a possible extension of milk shelf life. Thermosonation treatment for 11.1 s coupled with pasteurization in a continuous system could improve the shelf life attributes during storage and potentially increase the milk shelf life by about 2 weeks in the system used in the current study. Previous research has shown an improvement in milk shelf life attributes during storage by application of sonication (Bermúdez-Aguirre et al. 2009; Villamiel & De Jong, 2000).

However, these studies employed residence times ranging from 2-30 min, making it difficult for practical applications. Whereas, based on the results obtained from this research, implementation of thermosonication coupled with pasteurization at an industrial scale could help achieve an improved milk quality during shelf life while keeping the residence times for thermosonication practical (10-15 s) for industrial applications. It would also be important to verify that thermosonication does not affect the sensory attributes of milk during shelf life before any industrial applications are implemented.

CONCLUSION

Thermosonication in a continuous system coupled with pasteurization conditions successfully improved the milk shelf life attributes during storage as compared to pasteurization alone and could potentially increase the shelf of milk by 2 weeks. The average microbial count was lower for thermosonicated samples as compared to control throughout its shelf life and significantly higher log reductions were seen for thermosonication at week 0. Shelf life of control and thermosonication samples was estimated to be 4 and 6 weeks, respectively. Thermosonication significantly improved the pH and FFA content at the end of their shelf life as compared to control.

The first consumer panel showed significantly lower scores for overall appearance, smell, flavor, and overall liking of treatment samples but not for color. Panelists commented on thermosonication samples having an unknown off flavor, which when further investigated in a focus group seemed to decline after 2-4 days. The second consumer panel performed on day 1 and day 4 samples showed no significant difference between treatment samples on day 4 as compared to control samples on day 0 and 4.

Therefore, application of thermosonication in a continuous system using practical residence times (10-15 s) coupled with pasteurization may improve the overall milk quality and potentially increase the milk shelf life. Future studies should focus on investigating thermosonication conditions for industrial applications and changes in sensory attributes in scale up systems.

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Tables

Table 6.1. Whole milk samples evaluated during shelf life for pH, free fatty acid content (FFA), and casein/total protein (CN/TP) content.

Week	pH		FFA content (meq FFA/kg)		CN/TP	
	Control	Treatment	Control	Treatment	Control	Treatment
0	6.78 ± 0.01 ^a	6.78 ± 0.01 ^a	0.09 ± 0.01 ^a	0.08 ± 0.01 ^a	0.89 ± 0.03 ^a	0.92 ± 0.03 ^a
2	6.53 ± 0.01 ^c	6.64 ± 0.01 ^b	0.17 ± 0.01 ^{bc}	0.15 ± 0.01 ^b	0.86 ± 0.05 ^{ab}	0.89 ± 0.03 ^a
4	6.43 ± 0.01 ^d	6.55 ± 0.02 ^c	0.32 ± 0.02 ^d	0.20 ± 0.04 ^c	0.74 ± 0.02 ^b	0.82 ± 0.01 ^{ab}
6	-	6.50 ± 0.01 ^c	-	0.32 ± 0.02 ^d	-	0.81 ± 0.01 ^{ab}

Values with different letters are significantly different within each parameter tested.

Mean ± S.D are reported. - Control milk was curdled after week 4 and hence values not shown. Statistical analysis shown in Appendix E, Tables E.6-E.8.

Table 6.2. Consumer acceptance from the first consumer panel of 120 participants on day 1[†].

Attribute	Control	Treatment	P-Value
Color	7.00 ± 0.12 ^a	6.82 ± 0.12 ^a	0.0897
Overall Appearance	6.93 ± 0.13 ^a	6.65 ± 0.14 ^b	0.0293
Smell	6.39 ± 0.14 ^a	6.04 ± 0.15 ^b	0.0347
Flavor	6.91 ± 0.15 ^a	5.99 ± 0.20 ^b	0.0001
Overall Liking	7.00 ± 0.13 ^a	6.17 ± 0.18 ^b	0.0001

Values with different letters are significantly different within each row.

Control signifies heat treatment alone while treatment signifies thermosonication.

[†] Samples were tasted 24 hr after processing. Mean ± S.E are reported.

Table 6.3. Selected panelist comments from the first consumer panel for 2% fat milk on day 1[†].

Attributes	Control	Treatment
Color and Overall Appearance	Normal milk color, creamy white, looks like milk, slightly gray, slight yellow tinge, typical milk color, looks normal and good, looks creamy and thick.	Bright white color, looks white, slightly transparent, looks like normal milk, more white than normal, perfectly milky, less creamy.
Smell	No discernable smell, smells like fresh milk, strong milky fragrance, smells like milk, good, clean, less milky.	No smell detected, no distinct smell, fresh, neutral smell, more milky, sweet smelling, slightly milky, no smell.
Flavor	Creamy, slightly watery, not as sweet, more milk like, stronger milk flavor, good, slightly cooked.	Bland flavor with slight after taste, malty flavor, slightly different than normal milk, strange aftertaste, sweet, slightly sharp milk flavor, slightly smoky flavor.
Overall Liking	Like store bought, good milk flavor, nice and clean overall.	Slight off and cooked flavor, less creamy, slightly creamy, tasted delicious, tastes like milk.

[†] Samples were tasted 24 hr after processing. Control signifies heat treatment alone while treatment signifies thermosonication.

Table 6.4. Focus group comments post the first consumer panel for 2% fat milk on control and treatment milk over time (from day 1 to 6).

Attributes	Appearance	Aroma	Flavor
Day 1	Control looks creamier; treatment sample looks waterier. Color looks the same for both the samples.	Control smells like milk; treatment sample tastes sweeter, milkier.	Control has very light cooked but overall good milky flavor. Treatment samples have a cooked, slight sulfur or weird aftertaste.
Day 2	No difference can be seen between control and treatment. Both look white and slightly pale.	No difference between smells, have a light milk smell, very bland.	Control tastes slightly watery but tastes like milk. Slightly cooked flavor detected for treatment samples, but difficult to pinpoint.
Day 4	No difference can be seen between control and treatment. Look like milk. Slight watery appearance for control samples.	Very light milk smell detected between both the samples. Cannot tell the difference.	Both the samples taste slightly cooked. But no difference detected.
Day 6	Both samples look very pale and watery.	Both the samples smell slightly old, very milk flavor.	Both the samples have a slightly old milk flavor, no sourness detected, but does not taste fresh.

Control signifies heat treatment alone while treatment signifies thermosonication.

Table 6.5. Selected panelist comments from the second consumer panel for 2% fat milk on day 1 and 4.

Attributes	Preferred Day 1 Control	Preferred Day 1 Treatment	Preferred Day 4 Control	Preferred Day 4 Treatment
	Overall good flavor, taste like store bought milk, just the right sweetness, lightly cooked flavor, tastes like 2% fat milk.	Tasted sweet, slight after taste, some cooked flavor at the end, stronger milk flavor, tastes like typical milk, milky flavor.	Good flavor, no aftertaste, slightly sweet, mild milky flavor	Sweet, no aftertaste, very mild cooked flavor, like normal milk, tasted like store bought milk, mild milky flavor.
Mouthfeel	Smooth mouthfeel, creamy and smooth mouthfeel, thicker consistency.	Smoother mouthfeel, watery consistency, less creamy.	Slightly creamy, lighter, good consistency.	Creamier mouthfeel but slightly watery, right consistency, less creamy mouthfeel.

Control signifies heat treatment alone while treatment signifies thermosonication.

Figures

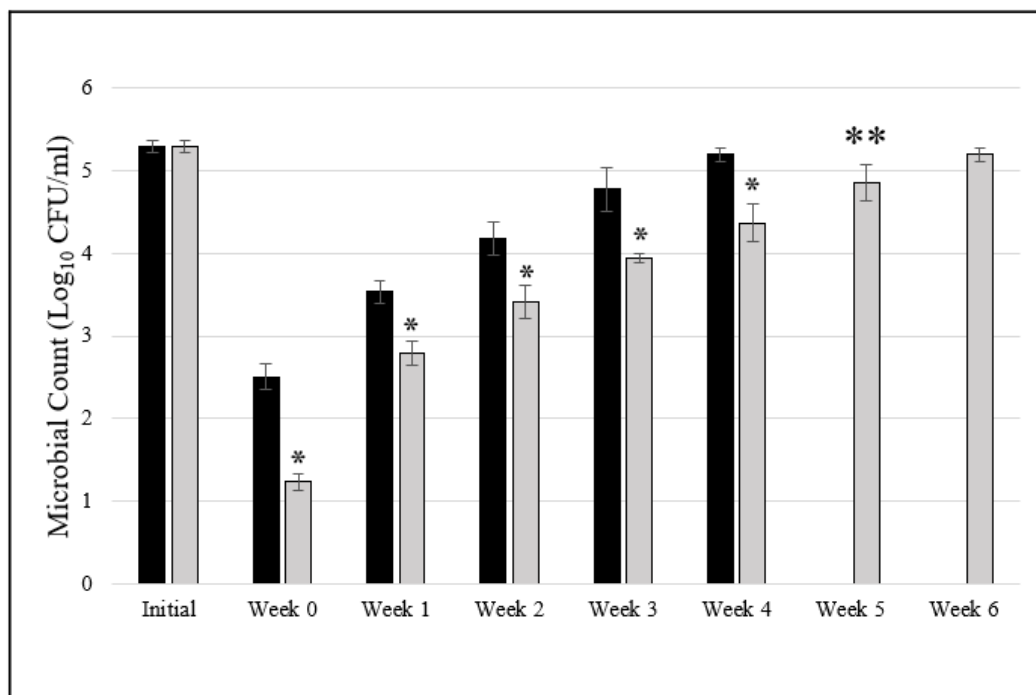


Figure 6.1. Microbial numbers (Mean \pm S.E) reported as Log₁₀CFU/ml in whole milk during its shelf life after raw milk was treated with heat alone (control) and heat with thermosonication (treatment).

Microbial counts represent total aerobic counts. Control milk was curdled after week 4 and hence values not shown.

Statistical analysis shown in Appendix E, Table E.5.

* Signifies significant difference between control and treatment samples within each week.

** Signifies significant difference between control and treatment when comparing week 5 and 6 treatment value with week 4 control.

■ Control □ Treatment

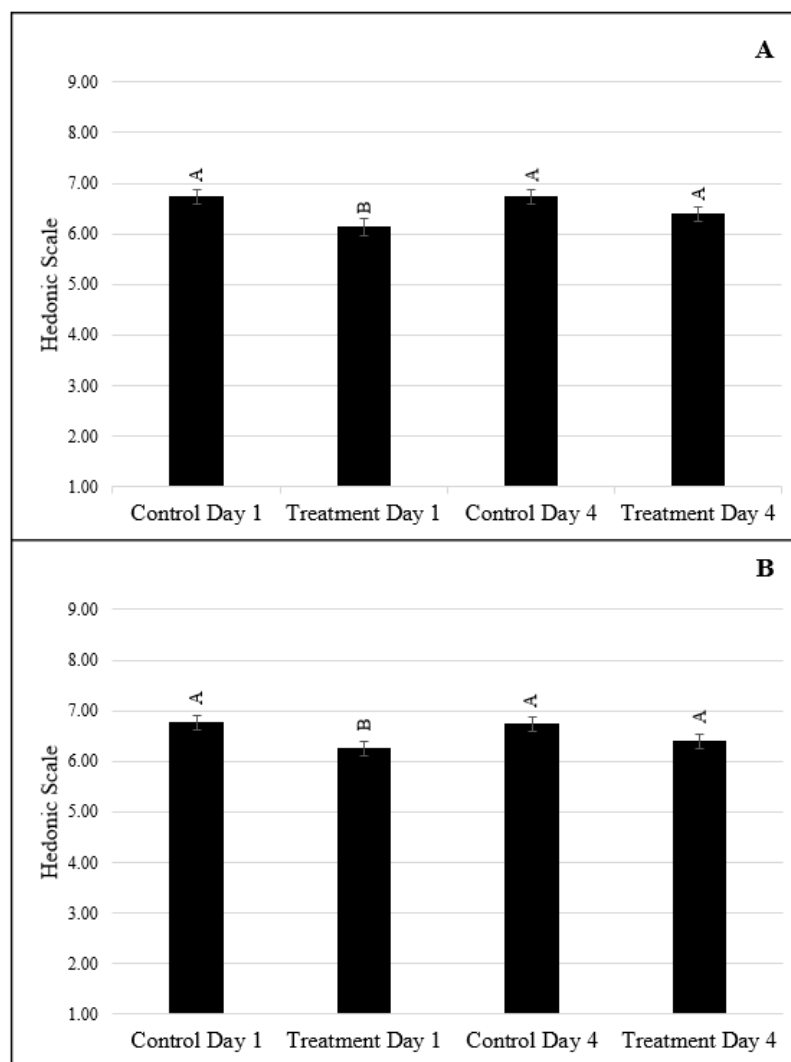


Figure 6.2. Consumer rating (Mean \pm S.E) for 2% fat milk for flavor (A) and overall liking (B) on a 9-point hedonic scale for control and treatment samples on Day 1 and Day 4 from the second consumer panel with 110 participants.

Control samples were heat treated and treatment samples received thermosonication.

Values with different letters are significantly different within each figure.

Statistical analysis shown in Appendix E, Table E.10.

CHAPTER VII

CONCLUSION AND FUTURE RESEARCH

EFFECT OF THERMOSONICATION ON VISCOSITY AS INFLUENCED BY SOLIDS AND TEMPERATURE

Viscosity of rMPC and rSMP can be significantly reduced by thermosonication, and together with temperature and TS it can be used to modulate the viscosity of these concentrates significantly prior to spray drying. When a flow through continuous operation system was used for thermosonication at 60 °C, the viscosity of samples decreased as the residence times increased from 10.1 to 30.2 s. Circulation post-sonication resulted in an increased viscosity, but the values were lower than the pre-sonication viscosity values for both rMPC and rSMP.

The decrease in viscosity could be attributed to possible disruption of large aggregates that increase the viscosity of milk concentrates. Previous studies by Fernández-Martín (1972) in skim milk and O'Donnell & Butler (2008) in MPC showed similar trends observed in this study of decrease in viscosity of rMPC and rSMP with an increase in temperature. Findings of our study agree about effect of thermosonication on viscosity of these concentrates are comparable to the ones observed by Zisu, Schleyer, & Chandrapala (2013) for viscosity of skim milk concentrate in both batch and continuous processing. With only 10 s of thermosonication, the viscosity of 34 % TS MPC could be equivalent to the viscosity seen at 30 % TS and after 30 s, the viscosity could be lower than that seen at

30 % TS. Moreover, 30 s of thermosonication could significantly reduce the viscosity of SMP concentrate prior to drying.

EFFECT ON THERMOPHILIC MICROBIOTA

Thermosonication (at 72-73 °C) in a batch system showed a statistically significant reduction in thermophilic bacteria used in this study (*Geobacillus stearothermophilus*, *Anoxybacillus flavithermus*, and *Bacillus subtilis*) as compared to application of heat alone. Thermosonication decreased the time required to achieve a one log reduction (D-value) for both the organisms at both the temperatures tested in media and 2 % fat milk. Thermophilic bacterial cells were more susceptible to inactivation by as compared to their spores which is similar to the observations seen in previous studies (Khanal, Anand, & Muthukumarappan, 2014a; Khanal, Anand, Muthukumarappan, & Huegli, 2014b). Spores showed significant reduction only after 120 s of treatment with a maximum log reduction of 0.5. Reductions seen in cells and spores are comparable to the ones seen in previous batch sonication studies performed by Beatty & Walsh (2016) and Khanal et al. (2014 a, b) in milk and milk products. A maximum of one log reduction was seen in thermophilic bacterial cells after 30-60 s of thermosonication which may not be impactful enough to improve quality of milk products. Based on previous studies, the effect of thermosonication along with pasteurization needed to be studied on thermophilic bacterial cells.

Based on observations of batch thermosonication study, a lab-scale continuous system was used to evaluate the effect of thermosonication (equipment set at 72 °C) along with heat (equipment set at 72 °C) on reduction in *G. stearothermophilus* cells. The effect of thermosonication along with heat in a lab-scale continuous system on *G.*

stearothermophilus cells was not significant when compared to heat alone. The log reductions obtained were not impactful enough for scale up operations. Use of pasteurization conditions along with thermosonication could potentially impact thermophilic bacterial reductions.

EFFECT ON TOTAL MICROBIOTA AND OVERALL MILK QUALITY

Thermosonication along with heat in a lab-scale continuous system significantly decreased the indigenous microflora in milk at week 0 as compared to heat alone (control). However, no significant differences in microbial numbers were seen between control and treatment samples at the end of storage. The pH, CN/TP, and FFA values were significantly improved as a result of thermosonication at the end of storage. Therefore, thermosonication did significantly improve the shelf life attributes during storage when practical residence times were used in a lab-scale continuous system. Pasteurization conditions could help achieve significant differences in microbial numbers at the end of storage. Therefore, these observations needed to be verified in a system where actual pasteurization conditions were implemented.

A continuous system with pasteurization conditions was implemented met to evaluate the effect of thermosonication on overall milk quality and shelf life. In this study, the average microbial count was lower for thermosonication samples as compared to control throughout its shelf life and a significantly higher log reductions were observed for thermosonication samples at week 0. Higher log reductions were observed in this system compared to lab-scale system possibly due to use of pasteurization temperatures and higher energy density. Shelf life of control and thermosonication samples was estimated to be 4

and 6 weeks, respectively. The pH, FFA, and CN/TP values of milk for both control and treatment were not significantly different immediately after processing. Thermosonication samples had improved pH, FFA, and CN/TP values at the end of shelf life. The first consumer panel showed significantly lower scores for overall appearance, smell, flavor, and overall liking of thermosonication samples but not for color. Panelists commented on thermosonication samples having an inexpressible off flavor. The inexpressible flavor when further investigated in a focus group seemed to decline after 2-4 days. This was confirmed by a second consumer panel, where flavor and overall liking of thermosonication samples at day 4 were not significantly different than day 1 of control samples.

The findings about improved microbial count, pH, FFA, and CN/TP values for thermosonication samples were in accordance to the findings made by Fromm & Boor (2004) in pasteurized milk and Bermúdez-Aguirre, Mawson, Versteeg, & Barbosa-Cánovas (2009) in pasteurized and sonicated milk. The inexpressible flavor of thermosonication samples is in accordance to observations made by Chouliara, Georgogianni, Kanellopoulou, & Kontominas (2010) in milk. However, no such flavor was reported by Nam, Wagh, Martini, & Walsh (2017) in high protein dairy beverages. The enhanced shelf life attributes including microbial quality could be correlated to significantly lower microbial numbers observed for thermosonication samples. Thermosonication in this continuous system coupled with pasteurization conditions successfully improved the overall milk quality of whole milk as compared to pasteurization alone which could potentially increase the shelf of milk by 2 weeks.

FUTURE WORK

Overall, thermosonication yielded significant reductions in viscosity of milk concentrates but failed to reduce the thermophilic bacteria in milk to have a major impact on quality of milk products. Future studies should focus on evaluating thermosonication effect on viscosity of milk concentrated using fresh concentrates, so that concentrates with a higher percent solids with relatively lower viscosity can be attained prior to spray drying. Application of thermosonication in a continuous system using practical residence times (~10-15 s) coupled with pasteurization may improve the overall milk quality during its shelf life and potentially increase the shelf life of milk. Repeating the shelf life experiments with homogenized milk could also be useful for more varied analyses. Future studies on thermosonication of milk with pasteurization to improve milk quality and shelf life should focus on investigating thermosonication conditions for industrial applications and changes in sensory attributes in scale up systems. Further investigation in the source of the unknown flavor or aftertaste is also needed before scaling up.

Future work in continuous thermosonication systems could incorporate use of pressure sensors in the system to adequately record pressure changes during processing. Although current work showed reductions in total bacterial count in milk due to thermosonication, a further analysis of surviving microbiota can be performed. Future work should include analyzing the effect of thermosonication on reductions in psychotropic, mesophilic, and facultative thermophilic bacteria instead of just total bacterial count. Thermosonication alone did not affect the added thermophilic bacteria in milk and future work could include application of manothermosonication (sonication with pressure and temperature) for reduction in thermophilic bacteria in milk.

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APPENDICES

APPENDIX A

Table A.1. Characteristics of thermophilic bacilli commonly found in dairy products.

Organism	Growth range (°C) ¹	Vegetative cells survival ¹	Spores survival ¹	Spoilage in dairy products ²
Obligate thermophile				
<i>Anoxybacillus flavithermus</i>	30-72	Yes	Not 121°C	Lactic acid production and off flavors
<i>Geobacillus stearothermophilus</i>	37-75	Yes/No	Retort	‘Flat sour’ in canned evaporated milk
<i>Geobacillus thermoleovorans</i>	35-70	NR*	NR	Lactic acid and lipase production
Facultative thermophiles				
<i>Bacillus licheniformis</i>	15-55	No	UHT	Production of slimy substance in cream, psychrotolerant
<i>Bacillus subtilis</i>	5-55	NR	UHT	Ropiness in pasteurized milk, UHT, and canned products
<i>Bacillus coagulans</i>	15-61	Yes	NR	Lactic acid production in UHT and canned milk products
<i>Bacillus cereus</i>		NR	UHT	Psychrotrophic Psychrotolerant; Off favors and
<i>Bacillus pumilus</i>	5-55	NR	UHT	spoilage from lipases and proteases
<i>Bacillus sporothermodurans</i>	20-55	No	Retort	Contaminant but no noticeable spoilage

*NR-Not Reported

^{1,2} Adapted from Burgess, Lindsay, & Flint, (2010).² Adapted from Khanal, Anand, Muthukumarappan, & Huegli (2014); Lücking, Stoeckel, Atamer, Hinrichs, & Ehling-Schulz (2013).

APPENDIX B

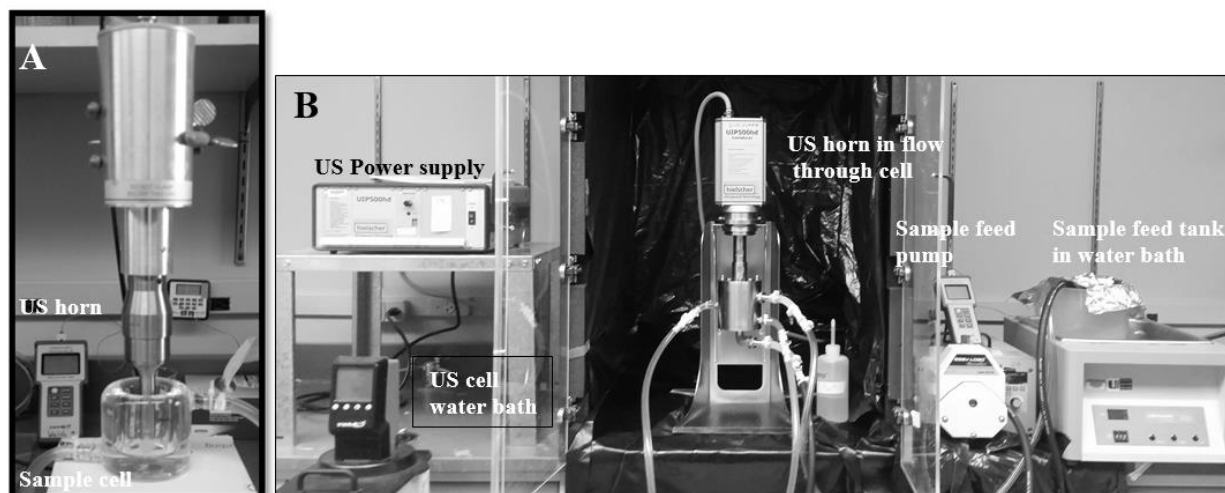


Figure B.1. Schematics of the application of ultrasound (US) in batch (A) and in the flow through system (B).

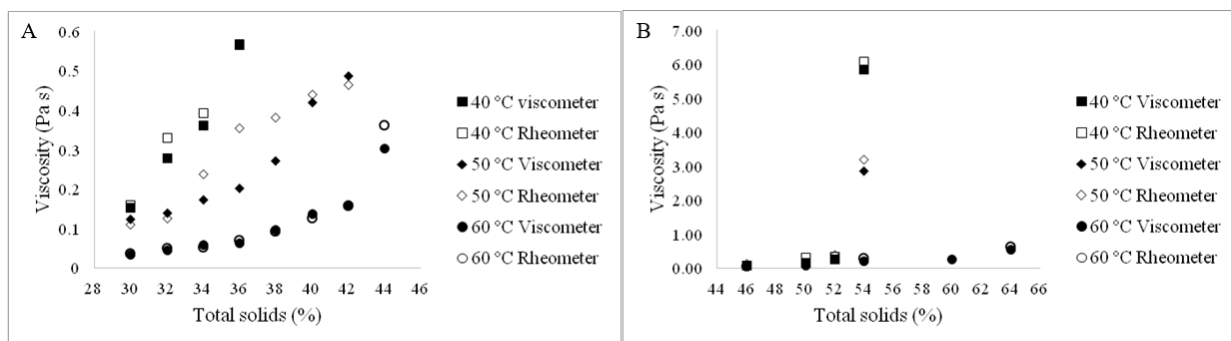


Figure B.2. Viscosity of rMPC (A) and rSMP (B) at various solids content treated at 40 °C, 50 °C, and 60 °C comparing viscometer readings with those of rheometer.

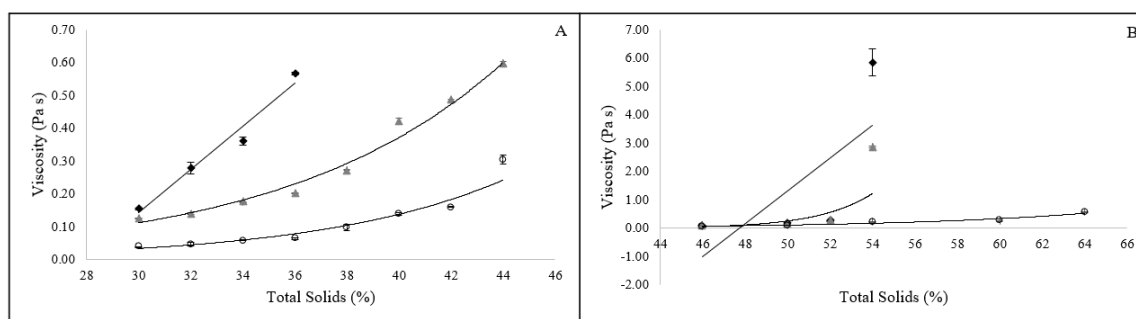


Figure B.3. Viscosity behavior of rMPC (A) and rSMP (B) at various solids content treated at 40°C, 50°C, and 60°C shown using trend lines.

◆, 40 °C; ▲, 50 °C; ◻, 60 °C

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Author: Vidita K. Deshpande, Marie K. Walsh

Publication: International Dairy Journal

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APPENDIX C

Table C.1. Energy density and temperature increase (denoted by Δ) observed in both 2 % fat milk and tryptic soy broth (TSB) samples at 72 and 73 °C.

Time (s)	Temperature (°C)	Energy density (J mL ⁻¹)	Δ Temperature (°C)
30	72	60.00 \pm 0.59	0.70 \pm 0.17
60	72	126.10 \pm 0.53	1.65 \pm 0.35
90	72	203.23 \pm 0.77	2.30 \pm 0.33
120	72	259.20 \pm 0.81	3.30 \pm 0.20
30	73	65.50 \pm 0.65	1.33 \pm 0.37
60	73	128.50 \pm 0.93	1.67 \pm 0.45
90	73	206.47 \pm 0.98	2.38 \pm 0.25
120	73	262.53 \pm 0.99	2.78 \pm 0.29

Values displayed are Mean \pm S.D.

Table C.2. Log reductions obtained in vegetative cells of *G. stearotheophilus* and *A. flavithermus* after 30 s in tryptic soy broth (TSB) after control and treatment conditions.

Organism	Time (s)	Temp ¹ (°C)	Log ₁₀ Reductions		p-value
			Control	Treatment	
<i>G. stearotheophilus</i>	30 s	72	0.03 \pm 0.04	1.22 \pm 0.11*	0.004
		73	0.68 \pm 0.08	1.76 \pm 0.28*	0.031
<i>A. flavithermus</i>	30 s	72	0.62 \pm 0.04	0.87 \pm 0.07*	0.008
		73	0.67 \pm 0.04	0.91 \pm 0.06*	0.025

¹Temp: temperature.

*Signifies statistical difference compared to control. Values displayed are Mean \pm S.D.

Table C.3. Log reductions obtained in vegetative cells of *G. stearotheophilus* and *A. flavithermus* after 30 s in 2 % fat milk after either control or treatment conditions.

Organism	Time (s)	Temp ¹ (°C)	Log ₁₀ Reductions		p-value
			Control	Treatment	
<i>G. stearotheophilus</i>	30 s	72	0.17 \pm 0.07	1.50 \pm 0.04*	0.001
		73	0.26 \pm 0.14	1.94 \pm 0.07*	0.001
<i>A. flavithermus</i>	30 s	72	0.35 \pm 0.03	0.86 \pm 0.02*	0.003
		73	0.40 \pm 0.04	0.89 \pm 0.10*	0.008

¹Temp: temperature.

*Signifies statistical difference compared to control. Values displayed are Mean \pm S.D.

Table C.4. ANOVA for spores of *G. stearothermophilus* in tryptic soy broth (TSB) and 2 % fat milk.

Parameter	In TSB		In 2 % fat milk	
	F-statistic	p-value	F-statistic	p-value
Temperature	12.84	0.0011	1.89	0.1786
Treatment	191.82	<0.0001	338.70	<0.0001
Time	90.71	<0.0001	163.81	<0.0001
Temperature x Treatment	1.71	0.1997	0.09	0.7630
Temperature x Time	0.25	0.8641	0.14	0.9381
Treatment x Time	1.41	0.2582	7.00	0.0009
Temperature x Treatment x Time	0.11	0.9565	0.17	0.9165

Table C.5. ANOVA for spores of *A. flavithermus* in tryptic soy broth (TSB) and 2 % fat milk.

Table C.5. Parameter	In TSB		In 2 % fat milk	
	F-statistic	p-value	F-statistic	p-value
Temperature	9.89	0.0036	8.09	0.0077
Treatment	41.67	<0.0001	118.04	<0.0001
Time	113.52	<0.0001	287.51	<0.0001
Temperature x Treatment	1.74	0.1966	1.55	0.2220
Temperature x Time	2.38	0.0879	0.53	0.6665
Treatment x Time	8.63	0.002	22.26	<0.0001
Temperature x Treatment x Time	2.59	0.0701	0.97	0.4198

Table C.6. ANOVA for spores of *B. subtilis* in tryptic soy broth (TSB) and 2 % fat milk.

Parameter	In TSB		In 2 % fat milk	
	F-statistic	p-value	F-statistic	p-value
Temperature	30.03	<0.0001	28.20	<0.0001
Treatment	11.83	0.0016	30.09	<0.0001
Time	443.06	<0.0001	268.30	<0.0001
Temperature x Treatment	0.02	0.9025	0.47	0.4969
Temperature x Time	5.63	0.0032	11.84	<0.0001
Treatment x Time	4.30	0.0117	18.34	<0.0001
Temperature x Treatment x Time	1.78	0.1703	4.50	0.0096

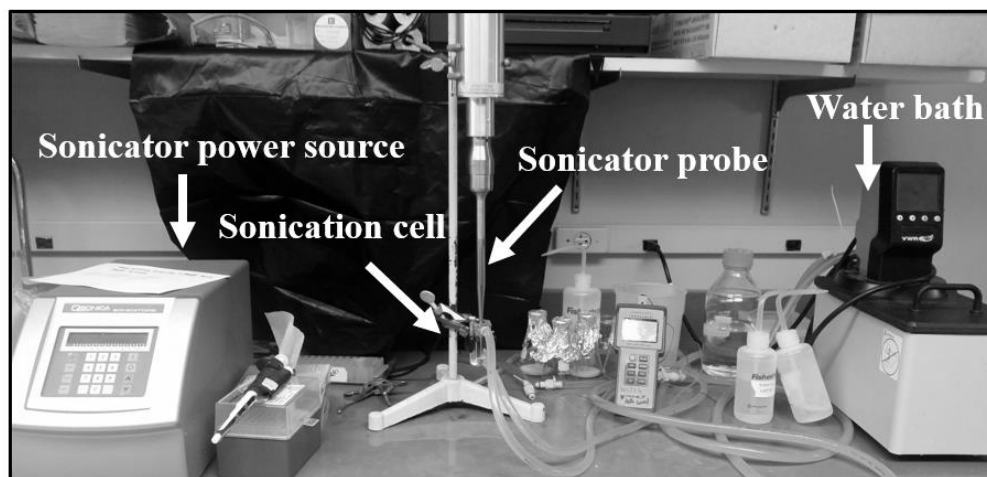


Figure C.1. Batch sonication assembly used for the experiment.

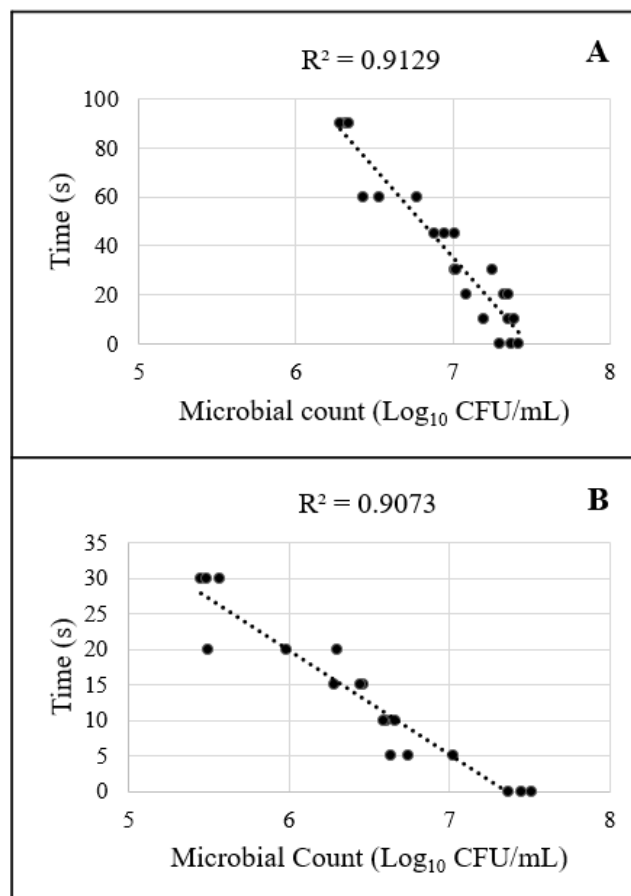


Figure C.2. Time (s) versus Microbial count (Log_{10} CFU/mL) plot used for calculation of D-value of *Geobacillus stearothermophilus* cells in 2 % fat milk at control (A) and thermosonication (B) setting at 73 °C. Figure is shown as a representative figure.

APPENDIX D

Table D.1. ANOVA on mean Log₁₀ reduction of *G. stearothersophilus* cells after treatment at each setting.

Parameter	F-statistic	P-value
Flow Rate	29.99	<0.0001
Treatment	47.24	<0.0001
Location	1.98	0.1788
Flow Rate x Treatment	0.43	0.5236
Flow Rate x Location	1.31	0.2701
Treatment x Location	0.13	0.7264
Flow Rate x Treatment x Location	0.09	0.7710

Table D.2. Mean Log₁₀ reduction of *G. stearothersophilus* cells after treatment at setting 3.

Setting	Residence Time in PHE *(s)	Residence time in Sonicator* (s)	Total residence time* (s)	Control Reductions ¹	Treatment Reductions ²
Plate Heat Exchanger-Sonicator					
3	12.1	5.1	17.2	0.02 ± 0.00	0.10 ± 0.01
Sonicator-Plate Heat Exchanger					
1	12.1	5.1	17.2	0.02 ± 0.00	0.08 ± 0.00

*Equipment at 72 °C; ¹Control: treated with heat alone with sample flowing through the PHE and sonicator with sonicator off; ²Treatment: with sample flowing through the PHE and sonicator with sonicator on. Values with different letters are significantly different. Values displayed are (Mean ± S.D).

Table D.3. ANOVA for microbial analyses for raw milk treated with control and treatment conditions at 2 settings.

Parameter	F-statistic	P-value
Flow Rate	243.71	<0.0001
Treatment	146.66	<0.0001
Storage time	454.32	<0.0001
Flow Rate x Treatment	7.99	0.0057
Flow Rate x Storage Time	13.69	<0.0001
Treatment x Storage Time	4.50	0.0023
Flow Rate x Treatment x Storage Time	13.13	<0.0001

Table D.4. One-way ANOVA for microbial analyses for raw milk treated with control and treatment conditions at 2 settings at week 0.

Parameter	F-statistic	P-value
Treatment	34.52	<0.0001

Table D.5. ANOVA for pH analyses for raw milk treated with control and treatment conditions at 2 settings.

Setting	Parameter	F-statistic	p-value
1	Treatment	55.31	<0.0001
	Storage time	130.99	<0.0001
	Treatment*Storage Time	1.48	0.2095
2	Treatment	9.74	0.0030
	Storage time	107.03	<0.0001
	Treatment*Storage Time	0.43	0.7885

Table D.6. ANOVA for FFA content analyses for raw milk treated with control and treatment conditions at 2 settings.

Setting	Parameter	F-statistic	p-value
1	Treatment	45.22	<0.0001
	Storage time	146.03	<0.0001
	Treatment*Storage Time	10.54	<0.0001
2	Treatment	4.07	0.0457
	Storage time	81.26	<0.0001
	Treatment*Storage Time	1.44	0.2236

Table D.7. ANOVA for CN/TP content analyses for raw milk treated with control and treatment conditions at 2 settings.

Setting	Parameter	F-statistic	p-value
1	Treatment	45.59	<0.0001
	Storage time	39.87	<0.0001
	Treatment*Storage Time	5.20	0.0236
2	Treatment	2.96	0.1108
	Storage time	9.91	0.0029
	Treatment*Storage Time	1.46	0.2698

Table D.8. Reactive thiol group content of whole milk during its shelf life after treatment at two different settings.

Week	Control ¹	Treatment ²	Control ¹	Treatment ²
	Setting 1		Setting 2	
0	1.08 ± 0.22 ^a	1.42 ± 0.33 ^a	0.37 ± 0.20 ^A	0.35 ± 0.19 ^A
1	0.66 ± 0.22 ^a	0.54 ± 0.17 ^a	0.09 ± 0.14 ^A	0.27 ± 0.16 ^A
2	0.27 ± 0.14 ^a	0.48 ± 0.14 ^a	0.04 ± 0.11 ^A	0.19 ± 0.11 ^A
3	0.22 ± 0.19 ^a	0.07 ± 0.17 ^a	0.01 ± 0.07 ^A	0.02 ± 0.03 ^A
4	0.47 ± 0.19 ^a	0.31 ± 0.31 ^a	-	-

¹Control: treated with heat alone with sample flowing through the PHE and sonicator with sonicator off; ²Treatment: with sample flowing through the PHE and sonicator with sonicator on.

Values with different letters are significantly different within each setting (Lowercase: Setting 1, Uppercase: Setting 2). Values displayed are (Mean ± S.E) and are reported as mM mL⁻¹.

- Measurements not taken because milk was curdled.

Table D.9. ANOVA for reactive thiol group analyses for raw milk treated with control and treatment conditions at 2 settings.

Setting	Parameter	F-statistic	p-value
1	Treatment	0.05	0.8325
	Storage time	3.32	0.0104
	Treatment*Storage Time	0.33	0.8945
2	Treatment	0.00	0.9834
	Storage time	1.33	0.2713
	Treatment*Storage Time	0.03	0.9977

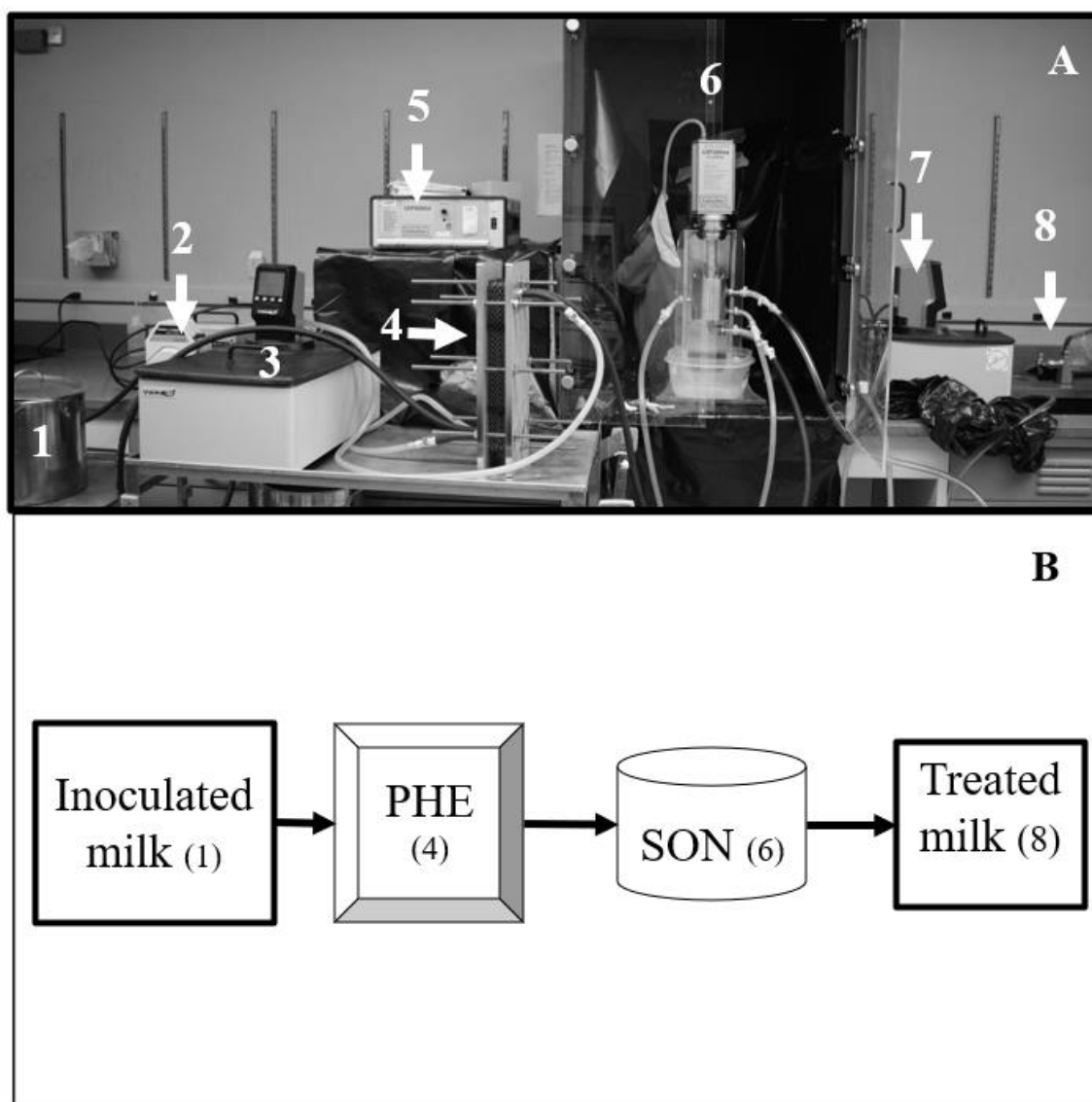


Figure D.1. Assembly used for *G. stearothermophilus* experiments shown in a photographic (A) and schematic format (B).

PHE : plate Heat Exchanger and SON: Sonicator vessel

In Figure A: 1- Milk inoculated with *G. stearothermophilus* at 60 °C, 2- Pump, 3- Circulating water bath for PHE, 4- PHE set at 72 °C , 5- Transducer, 6- Sonication vessel set at 72 °C, 7- Circulating water bath for sonication vessel, 8- Treated milk collected.

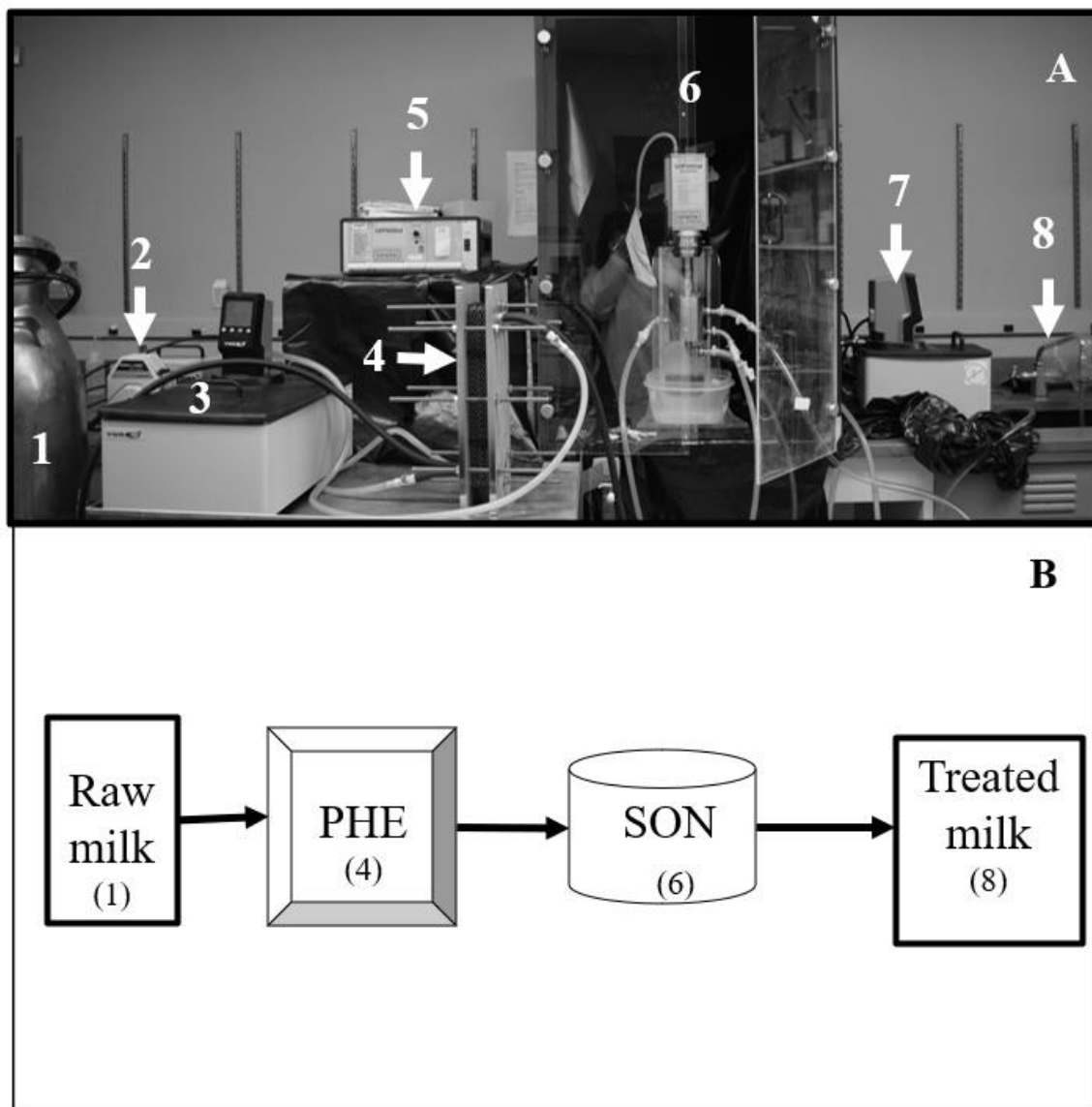


Figure D.2. Assembly used for raw milk experiments shown in a photographic (A) and schematic format (B).

PHE : plate Heat Exchanger and SON: Sonicator vessel

In Figure A: 1- Raw milk at 20-25 °C, 2- Pump, 3- Circulating water bath for PHE, 4- PHE set at 72 °C, 5- Transducer, 6- Sonication vessel set at 72 °C, 7- Circulating water bath for sonicator, 8- Treated milk collected.

APPENDIX E

Table E.1. Commercial HTST pasteurized whole milk samples evaluated during shelf life for microbial count, pH, and free fatty acid (FFA) content.

Week	Average Microbial Count (Log ₁₀ CFU/ml)	pH	FFA content (meq FFA/kg)
0	4.41 ± 0.06	6.93 ± 0.02 ^a	0.13 ± 0.07 ^A
1	6.83 ± 0.10	6.86 ± 0.05 ^a	0.25 ± 0.06 ^B
2	7.12 ± 0.05	6.78 ± 0.08 ^a	0.41 ± 0.03 ^C
3	7.45 ± 0.03	6.45 ± 0.14 ^b	0.48 ± 0.03 ^D
4	7.93 ± 0.11	6.24 ± 0.05 ^b	0.53 ± 0.02 ^E

Values with different letters are significantly different within each column (Lowercase: pH, Uppercase: FFA content). Mean ± S.D are reported. Statistical analysis shown in Appendix E, Table E.9.

Table E.2. Correlation coefficients between microbial counts and pH, free fatty acid (FFA), and casein/total protein (CN/TP) content of whole milk samples.

Parameter	Correlation coefficient
pH	-0.78
FFA	0.95
CN/TP	-0.89

Table E.3. Demographic information for 120 sensory panelists from the first consumer panel.

Category	Percent
<i>Age</i>	
18-21	29.2
22-29	53.3
30-39	13.3
40-49	1.7
Over 50	2.5
<i>Gender</i>	
Male	40.8
Female	59.2
<i>Product Preference</i>	
Milk	70.0
Dairy drinks and others (yogurt drinks, flavored milk, cream)	17.5
Non-dairy milk (soy, almond milk etc.,)	12.5
<i>Type of milk consumption</i>	
2% fat	69.2
Whole milk	30.8
<i>Milk consumption frequency</i>	
Less than once a month	5.0
0-1 times a week	10.0
2-5 times a week	42.5
6+ times a week	42.5
<i>Milk purchase frequency</i>	
More than 1/week	16.7
1/week	63.3
1/month	18.3
Never	1.7

Table E.4. Demographic information for 110 sensory panelists for the second consumer panel.

Category	Percent
<i>Age</i>	
18-21	17.5
22-29	54.2
30-39	14.2
40-49	5.0
Over 50	9.1
<i>Gender</i>	
Male	35.8
Female	64.2
<i>Product Preference</i>	
Milk	70.8
Dairy drinks and others (yogurt drinks, flavored milk, cream)	17.5
Non-dairy milk (soy, almond milk etc.,)	11.7
<i>Type of milk consumption</i>	
2% fat	71.7
Whole milk	28.3
<i>Milk consumption frequency</i>	
Less than once a month	4.2
0-1 times a week	8.3
2-5 times a week	38.3
6+ times a week	49.2
<i>Milk purchase frequency</i>	
More than 1/week	23.3
1/week	64.2
1/month	10.8
Never	1.7

Table E.5. ANOVA for microbial analysis obtained for whole milk throughout its shelf life.

Parameter	F statistic	P-value
Treatment	81.86	<0.0001
Shelf life	232.66	<0.0001
Treatment x Shelf life	2.75	0.0275

Table E.6. ANOVA for pH obtained for whole milk throughout its shelf life.

Parameter	F statistic	P-value
Treatment	57.18	<0.0001
Shelf life	221.68	<0.0001
Treatment x Shelf life	12.65	<0.0001

Table E.7. ANOVA for FFA content obtained for whole milk throughout its shelf life.

Parameter	F statistic	P-value
Treatment	59.76	<0.0001
Shelf life	313.30	<0.0001
Treatment x Shelf life	30.69	<0.0001

Table E.8. ANOVA for CN/TP content obtained for whole milk throughout its shelf life.

Parameter	F statistic	P-value
Treatment	11.05	0.0127
Shelf life	18.82	0.0010
Treatment x Shelf life	3.07	0.1101

Table E.9. One-way ANOVA for pH and FFA content of commercially bought HTST pasteurized whole milk samples during shelf life.

Attribute	F statistic	P-value
pH	40.73	<0.0001
FFA content	37.62	<0.0001

Table E.10. One-way ANOVA for the second consumer panel for 2 % fat milk on day 0 and day 4.

Parameter	F statistic	P-value
Flavor	3.81	0.01
Overall Liking	2.90	0.03

Table E.11. Titanium Content for pasteurized 2 % fat milk samples.

Sample	Titanium (ppm)
Treatment	0.034 ± 0.001
Control	0.032 ± 0.001
Grocery store samples*	0.029 ± 0.001

*Never treated in any of the experimental set up used in this study. Control signifies heat treat while treatment signifies thermosonication. Mean \pm S.D are reported.

Table E. 12. One- way ANOVA for titanium content of pasteurized 2 % fat milk.

Parameter	F statistic	P-value
Treatment	1.46	0.2930

Table E.13. Mean diameter size (arithmetic) obtained via particle size analysis of pasteurized whole milk samples.

Sample	Mean diameter size
Control	0.58 ± 0.02^A
Treatment	0.59 ± 0.02^A

Control signifies heat treatment, treatment signifies thermosonication. Values with different letters are significantly different. Mean \pm S.D are reported.

Table E. 14. t-test for performed on mean diameter size obtained via particle size analysis of pasteurized whole milk.

Parameter	T statistic	P-value
Treatment	4.30	0.0591

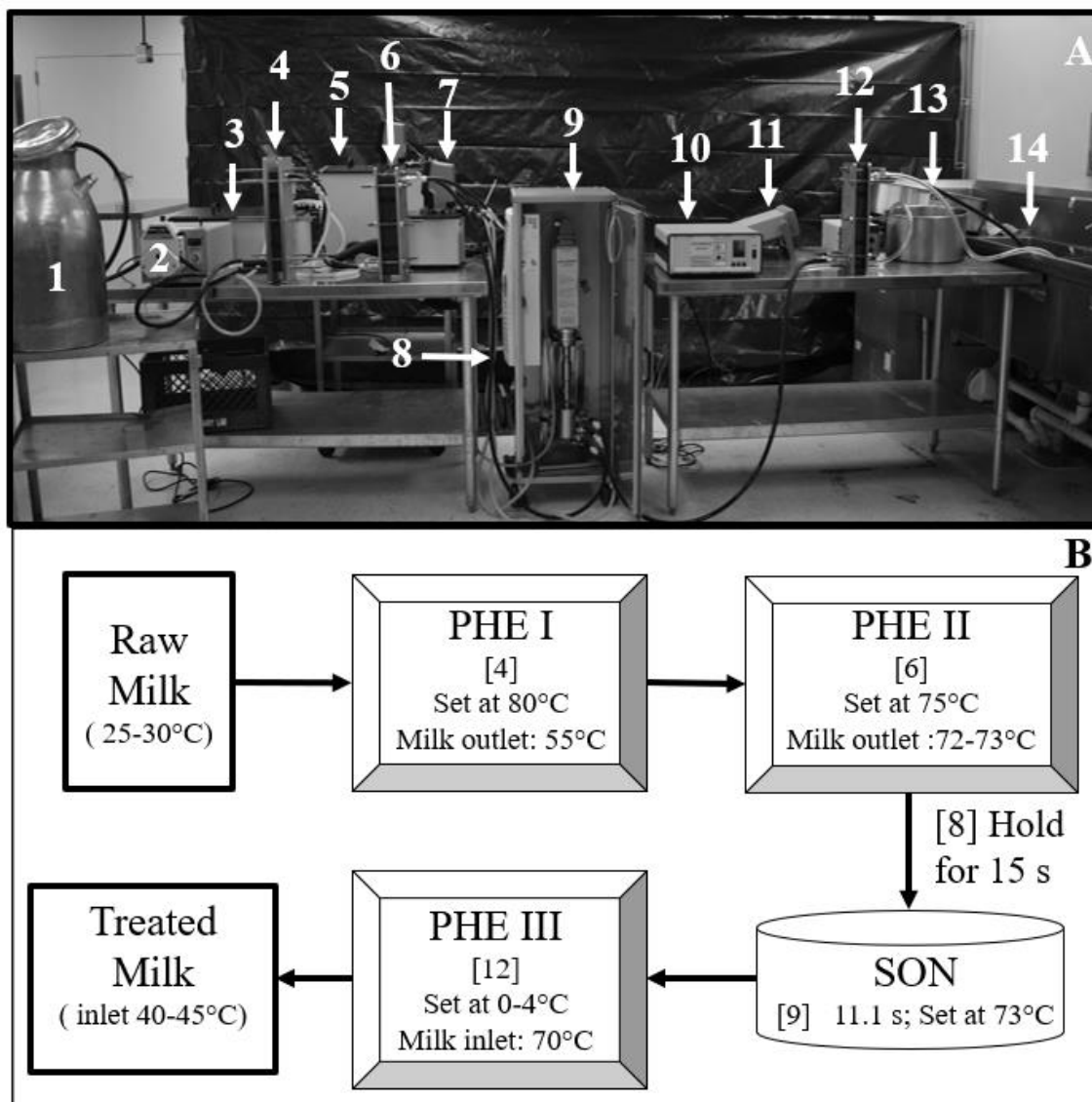


Figure E. 1. Assembly used for experiments shown in photographic (A) and schematic format (B).

PHE: Plate heat exchanger, SON: Sonicator.

PHE I and II: used for heating, PHE III: used for cooling

In Figure A, 1- Raw milk, 2- Pump, 3- Circulating water bath for PHE I, 4- PHE I, 5- Circulating water bath for PHE II, 6- PHE II, 7- Circulating water bath for sonication vessel, 8- Holding tube, 9- Sonication vessel in a sound proof cooling box, 10- Transducer, 11- Thermocouple reader, 12- PHE III, 13- Ice water bath with pump used for PHE III, 14- Treated milk

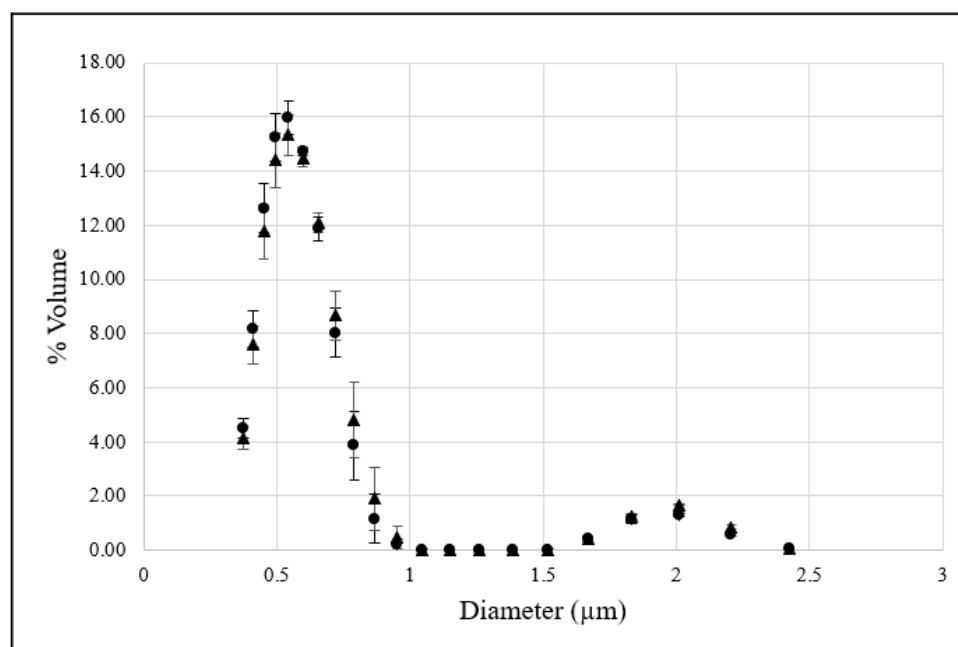


Figure E.2. Percent volume (Mean \pm S.E) for particle size diameters of commercially purchased HTST pasteurized whole milk after being treated with heat alone (control) and heat with thermosonication (treatment).

● Control ▲ Treatment

CURRICULUM VITAE

Vidita DeshpandeEmail: vidita.deshpande@gmail.comWebsite: <http://viditadeshpande.com/>**EDUCATION****Ph.D. in Food Science and Nutrition**

Utah State University, Logan, Utah, GPA: 3.95, 2015-2020

Bachelor of Science in Food ScienceCalifornia Polytechnic State University, San Luis Obispo, **GPA: 3.81**, 2012-2015**Bachelor of Science in Food Science and Quality Control**S.N.D.T Women's University College of Home Science, Pune, **GPA: 4.0**, 2010-2012**POSTER PRESENTATIONS AND PUBLICATIONS***Publications*

Deshpande, V.K., Walsh, M.K., (2018). Effect of sonication on the viscosity of reconstituted skim milk powder and milk protein concentrate as influenced by solids concentration and temperature. *International Dairy Journal.*, 78, 122-129.

Deshpande, V. K., Walsh, M. K. (2020). Effect of thermosonication in a batch system on the survival of spore-forming bacteria. *International Journal of Dairy Technology.*

Poster Presentations

Deshpande V.K., Walsh M.K. 2019. Effect of thermosonication in a batch system on the survival of thermophilic bacteria in milk. *ADSA Annual Meeting 2019.*

Deshpande V.K., Walsh M.K. 2019. Effect of sonication combined with heat to improve the microbial quality of milk. *ADSA Annual Meeting 2019.*

Deshpande V.K., Walsh M.K. 2017. Effect of batch sonication on the viscosity of reconstituted milk powders as influenced by total solids and temperature. *IFT & AACT's UTAH FOOD & CANDY EXPO 2017.*

Deshpande V.K., Walsh M.K. 2017. Effect of sonication on the viscosity of reconstituted milk powders as influenced by total solids and temperature. *ADSA Annual Meeting 2017.*

SKILLS

Computer: Statistical Analysis Software (SAS 9.4; Linear regression, ANOVA, design of experiments), Sensory Analysis (SIMS 2000), Genesis (R&D, product formulations, labels), Microsoft Office (Word, Excel, PowerPoint)

Analytical: Physicochemical and microbial testing for shelf life of milk and milk products, wine waste, and oatmeal samples.

EXPERIENCE

Utah State University

Graduate Researcher (2015- Present)

- Investigated and executed sonication procedures for dairy industry applications
- Designed experiments from lab to pilot scale successfully while trouble shooting any technical problems
- Researched and implemented protocols for spore germination and evaluating microbial reductions
- Developed methods and SOPs for sample preparation, sample analyses, and data analyses
- Implemented methods and SOP's for shelf life testing and physicochemical analyses such as free fatty acid content (FFA), sulfur content, color, and viscosity measurements

Cal Poly Food Science Department

Teaching Assistant - Food Chemistry, Food Analysis (2014-15)

- Planned, prepared, and assisted in creation and execution of food chemistry and analysis labs for 2 classes of 30 students each
- Maintained and trained students on use of analytical lab equipment: water activity meter, moisture analyzer, pH meter, spectrophotometer, conductivity meter

Student Production Assistant (2014-15)

- Trained new interns on tasks and documentations performed during production, cleaning, and sanitation
- Developed and reformulated Cal Poly products including jams/fruit spreads, bbq sauce, and dressings
- Performed quality and production tasks during production runs and resolved any technical issues with equipment set up

Research Assistant (2014-15)

- Planned and implemented DOE for wine waste filtration, performed chemical analysis and data analysis
- Executed experiments successfully and troubleshooting technical problems during trials or equipment setup

California Polytechnic State University Housing

Community Advisor (March 2014- June 2015)

- Interact with residents and communicate resident issues/concerns/requests to the Coordinator of Student Development
- Plan and execute various programs to meet University Housing Objectives

Gujarat Cooperative Milk Marketing Federation Ltd. Anand, Gujarat, India

QC Laboratory Assistant (April 2012-June 2012)

- Conducted quality control tests and evaluated samples using sensory evaluation and chemical analysis (fat content determination, particle size determination for powders, colorimeter measurements for butter)

PROJECTS AND CLASSES

Sensory panel for consumer liking of chocolates; 2019

- Executed a sensory panel to test for consumer liking for Aggie Chocolate Factory chocolate samples
- Coordinated and oversaw a team of 8 undergraduates
- Responsible for writing technical research proposal, establishing protocols for samples preparation and test administration using SIMS 2000

Undergraduate Senior Project: Extruded Oatmeal project; 2015

- Researched and studied the use of wet extrusion techniques to optimize oatmeal production
- Responsible for planning and executing DOE, data analysis, and data interpretation

Graduate level specialized classes: Proteins, Enzymes, Dairy Chemistry, Food Toxicology, Crystallization in Food Systems

Undergraduate level specialized classes: Food Packaging, Food Engineering (3 class series)

LEADERSHIP

IFTSA Student Officer Positions

Vice President of Volunteer Development (2017)

Fun Run Chair (2016-17)

Food Science Club- Utah State University

Vice President and Product Development Officer (2016-17)

IFT representative (2017-19)

Indian Student Association- Utah State University

Cultural Officer (2018-19)

International Student Friendship Club, Cal Poly, San Luis Obispo

Co-Event Planner (2013-15)

COMPETITIONS AND PROJECTS

Utah State University

IFTSA Smart Snacks for Kids Product Development Competition 2018, 2019

Second Prize at the Annual IFT 2019 Meeting with Cosmic Crackers

First Prize at the Annual IFT 2018 Meeting with Cauliflower Crust Pizza Bites

Idaho Milk Processors Association Product Development Competition 2016, 2018

First Prize at the Annual IMPA Meeting with SCOOPS (2018-frozen dessert using low value co-product of WPC processing)

First Prize at the Annual IMPA Meeting with PRO2GO (2016-frozen yogurt bar)

IFT College Bowl Competition 2016-19

Team Captain (2017) and Team Member (2016-19)

Disney IFTSA Product Development Competition 2016, 2017

Team lead for DEERTRAX (2016) and The Little Popping Sea (2017)

Cal Poly San Luis Obispo**Disney IFTSA Product Development Competition****2015**

Led team that won Grand Prize at Disney IFTSA Product Development Competition with Build A Snowman Kit

Undergraduate Senior Project: Extruded Oatmeal project**2015**

Researching wet extrusion techniques and settings to optimize oatmeal production

SCHOLARSHIPS AND AWARDSUtah State University

-Graduate Student Assistantship (USDA Grant Research Project) (2017-19)

-Dr. Niranjana R. Gandhi and Mrs. Josephine N. Gandhi Assistantship at Utah State University (2015-17)

-Tuition Award (Build Dairy Program and College of Agriculture and Applied Sciences) 2015-17

-Travel Awards from: School of Research and Graduate studies at Utah State University, Nutrition, Dietetics, and Food Science at Utah State University, Institute of Food Technologists Student Association, Build Dairy Program

Cal Poly-San Luis Obispo

-Foodsters Scholarship (2014-15)

-Travel Awards from: Food Science and Nutrition Department at Cal Poly, Institute of Food Technologists Student Association