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IMPACT OF NEW PROCESSING TECHNOLOGIES ON THE PHYSICAL AND
FUNCTIONAL PROPERTIES OF SKIM MILK AND SKIM MILK
CONCENTRATE

BY
HARSH DAHIYA

A thesis submitted in partial fulfillment of the requirement for the

Master of Science

Major in Biological Sciences

Specialization in Dairy Science

South Dakota State University

2016

IMPACT OF NEW PROCESSING TECHNOLOGIES ON THE PHYSICAL AND
FUNCTIONAL PROPERTIES OF SKIM MILK AND SKIM MILK
CONCENTRATE

This thesis is approved as a creditable and independent investigation by a candidate for the Master of Science degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidates are necessarily the conclusions of the major department.

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ABBREVIATIONS

HC	Hydrodynamic cavitation
HCT	Heat coagulation time
RCT	Rennet coagulation time
US	Ultra sound
UV	Ultra violet
β -LG	Beta lactoglobulin
α -LA	Alpha lactalbumin
TS	Total solids
NFDM	Non-fat dried milk
USDEC	US dairy export council
SMP	Skim milk powder
SMC	Skim milk concentrate
CFR	Code of federal regulation
MPC	Milk protein concentrate
MT	Metric ton
K	Consistency coefficient
ppm	Parts per million
UF	Ultrafiltration
nm	Nanometer
g	Gram

GDL	Glucono- δ -lactone
CCP	Colloidal calcium phosphate
G'	Shear elastic modulus
G''	Shear loss modulus
GRAS	Generally recognized as safe
HCl	Hydrochloric acid
SAOR	Small amplitude oscillatory rheology
SDS-	Sodium dodecyl sulfate polyacrylamide gel
PAGE	electrophoresis
HP	Horsepower
VFD	Variable frequency drive
L/h	Liters per hour
mL	Milliliter

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ABSTRACT

IMPACT OF NEW PROCESSING TECHNOLOGIES ON THE PHYSICAL AND
FUNCTIONAL PROPERTIES OF SKIM MILK AND SKIM MILK
CONCENTRATE

HARSH DAHIYA

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United States is one of the largest producer of cow's milk in the world and a substantial portion of this milk is converted into dried milk products, a category that is becoming increasingly important to the dairy industry. The processing treatments on skim milk and skim milk concentrate affect the functionality of resultant powders made from them, therefore processing treatments are crucial to modify physical and functional properties of skim milk, skim milk concentrates and the powders made from them.

The objective of this research was to explore impact of new processing technologies hydrodynamic cavitation (HC) and carbon dioxide (CO₂) injection on physical and functional properties of skim milk and skim milk concentrate (SMC) respectively. In one of the experiment it was found that HC had little effect on casein micelle size and heat stability, however it produced acid gels with final gel strength (G') almost similar to the gels from conventionally heated milk samples in the treatments that involved simultaneous heating along with cavitation. HC can therefore serve as an alternate technology to scale-free heat milk for yogurt

manufacturing. HC without involving heating at higher flow rates had a positive effect on rennet coagulation time. In another experiment effect of CO₂ injection technology to modify the pH of skim milk concentrate and decrease the apparent viscosity of high solids SMC was studied. This experiment was carried out first in lab and then scaled up at pilot plant to study the feasibility of this technique. In both the experiments significant difference ($p \leq 0.05$) in apparent viscosity was observed between CO₂ treated and control SMC samples. High solids (55-57% total solids) concentrate obtained from CO₂ treated SMC sample had significantly ($p \leq 0.05$) lower apparent viscosity compared to control. Also the powders produced from concentrate that was CO₂ treated had higher bulk density, improved solubility, better heat stability on reconstitution in comparison to powders from control concentrate. Overall the observation from these experiments suggest that both HC and CO₂ injection can serve as technologies that can help modify physical and functional properties of skim milk and concentrate for better.

Chapter I

Review of literature

Non-fat dried milk industry overview

The United States is the second largest producer of cow's milk in the world behind European Union. Dried milk products which are mainly used as ingredients in various dairy and food products represent an increasingly important category of US dairy industry. Non-fat dried milk (NFDM) or Skim milk powder (SMP) is the largest dry dairy ingredient manufactured in the United States. Of the total production around 52% of US skim milk powder is exported as per USDEC data in 2014. SMP exports from United States constituted 30% of the total global supply of during 2013, making it the global leader in export of SMP. These figures emphasize the importance that this product holds in the total dairy export basket from United States.

Continued economic growth in Asia and Africa has fueled the demand for dairy and dairy ingredients. Economic growth combined with population growth in this part of the world presents a huge supply opportunity in the global market for manufacturers of dry dairy ingredients which are able to meet the specification required by recombined milk products and infant formula manufacturers. The largest use of SMP is as an ingredient in dairy products (Drake et al., 2003) which involve reconstitution or recombination with anhydrous milk fat depending upon the intended end use.

The opportunity becomes even bigger due to a projected supply shortage owing to slower growth in exports from major global suppliers. As per current trends, USDEC estimates a potential latent demand gap for SMP of up to 280,000 MT by 2020. Most of the volume will be needed in Asia to be used in recombined products, infant and other nutritional formulations. Historically, US has faced challenges in securing exports to these areas and is not perceived as a preferred supplier. United States, however is well positioned to improve upon its status and increase its share in the global NFDM market. In order to strengthen its position as a preferred supplier of NFDM globally US manufacturers must identify opportunities and implement best manufacturing practices to improve upon their abilities to consistently meet both rigorous quality and quantities their customers are looking for. Functional properties important for recombining and infant formula applications which are important to the end user should be main area of focus for US manufacturers to seize this opportunity. Another aspect apart from consistent quality and functionality is the price of NFDM for them to become preferred supplier in global market and for US manufacturers to become competitive on pricing they should focus on improving efficiency of manufacturing processing involved in NFDM production.

Evaporation and concentration of skim milk

Water can be removed from milk, skim milk, whey and other milk streams by evaporation for the concentration process. Concentration is usually done to reduce product volume and to improve keeping quality. Concentration of milk causes lot of

changes in the milk system. The milk salt equilibria is affected and Ca^{2+} ion activity is slightly increased, viscosity increases as the concentration increase, liquid behave as non-Newtonian fluid exhibiting viscoelastic character and shear thinning (Walstra, 2013). The changes in pH and salt equilibria also affect milk proteins, as the amount of solvent (water) decreases with concentration the tendency of protein particles to associate increases. Casein micelles tend to associate with each other thus increasing their size, however this phenomenon can be controlled to great extent if the milk is preheated causing whey proteins to associate with casein. Evaporation is an important process applied to milk, skim, whey to manufacture products such as sweetened condensed milk, evaporated milk, yogurt, lactose, cheese in some cases and it also serves as a processing step to manufacture dry milk products. It is far more economical to remove same quantity of water through evaporation than by drying which makes evaporation an important process step in manufacturing of dry ingredients. In order to facilitate boiling at a much lower temperature and avoid heat damage to heat sensitive milk constituents evaporation is always carried out under vacuum. Another benefit of carrying out evaporation under vacuum is that it is more energy efficient.

Viscosity

Concentrated milk is an intermediate dairy product which can be further processed to produce either evaporated milk, sweetened condensed milk or can be dried into milk powder. As water is removed from milk during evaporation the flow behavior of milk changes from Newtonian to non-Newtonian. This transition in flow

behavior with increase in concentration is attributed to the decrease in spacing among milk constituents like casein, whey proteins, lactose etc. (Prentice, 1992, Vélez-Ruiz et al., 1997). Temperature, total solids, storage time, milk composition and preheat treatment are some of the factors that affect rheological properties of concentrated milk (Bloore and Boag, 1981, Vélez-Ruiz and Barbosa-Cánovas, 1995). Snoeren et al. (1981) found that it is the viscosity of volume fraction of milk macromolecules and viscosity of milk serum phase that together govern viscosity of SMC. Concentration, protein content, heat treatment and composition of milk in turn determine the volume fraction of protein (Snoeren et al., 1981).

Problems associated with high viscosity

A large volume of milk that is concentrated is ultimately dried to produce dried milk powder; knowing the factors that affect functionality of powders will help produce powders with consistent properties and better quality. This makes it even more important to understand the process of evaporation and various factors that affect functionality of SMC. During powder manufacturing the focus should be to remove as much as possible amount of water in evaporator. This is where high viscosity of concentrate becomes a limiting factor. High viscosity of concentrate decreases flow rate along the heating surface in the calendria tubes thus decreasing the heat transfer, the heating surface fouls more readily (Walstra, 2013). It is difficult to produce milk concentrate with high total solids due to the large increase in apparent viscosity when total solids reaches 45% and beyond (Vélez-Ruiz and Barbosa-Cánovas, 1998, VÉLEZ-RUIZ and BARBOSA-CÁNOVAS, 2000). Milk

concentrate with such high total solids is difficult to atomize, and the large droplets resulting from it decrease the thermal efficiency of the spray dryer (Hayashi and Kudo, 1990). Weberschinke and Filková (1982) developed a relationship for the milk drop diameter as a function of apparent viscosity. Similarly many others have stated that viscosity of milk concentrate is important parameter to determine the size of milk droplets formed in the spray dryer, which eventually affect the drying process.

Strategies to reduce viscosity of skim milk concentrate

Temperature, total solids, holding time, preheat treatment and protein content are five main factors that influence viscosity of skim milk concentrate (Bloore and Boag, 1981). So when working on viscosity reduction researchers have worked on these factors or the parameters that influence these factors to reduce viscosity of concentrates. Walstra and Jenness (1984) found that in skim milk systems, main contributors to viscosity are the casein micelles. It is the volume fraction of casein micelles along with whey protein that is the most important variable affecting viscosity of skim milk concentrate (Snoeren et al., 1981). Therefore any parameter or factor that affect their voluminosity or aggregation such as pH, preheat treatment, salt balance, casein genetic variants will in turn affect the viscosity of skim milk concentrate (Walstra, 1979, Snoeren et al., 1981, Anema and Creamer, 1993, Bienvenue et al., 2003, Anema et al., 2004).

Numerous researchers have studied different strategies to reduce viscosity of skim milk concentrates (Bienvenue et al., 2003, Zisu et al., 2013, Anema et al., 2014, Lee, 2014). Treatment with high intensity low frequency ultrasound was found to

reduce viscosity of skim milk concentrate (50-60% TS) by 10% (Zisu et al., 2013). Decrease in viscosity of concentrate during storage was observed with gradual decrease in mineral content of milk (Bienvenue et al., 2003). Milk samples heated with pH adjusted to 7.1 produced concentrates with lower viscosity than concentrate from milk samples heated with pH adjusted to pH 6.5 (Anema et al., 2014). Lee (2014) observed decrease in viscosity of concentrate obtained from ultra filtration and reconstitution when such concentrated were treated with CO₂ first and then decarbonated.

Effect of pH on viscosity of skim milk concentrate

It is the volume fraction of milk particles along with serum phase viscosity that together determine the viscosity of a milk system. In case of skim milk systems, concentration of lactose determine viscosity of continuous phase and volume fraction of suspended particles depends on casein micelles, dissociated casein, native whey proteins and denatured and associated whey proteins (Snoeren et al., 1981, Jeurink and De Kruif, 1993, Anema et al., 2004). Concentrate viscosity will increase with factors that increase voluminosity of casein and whey proteins. Effect of pH on viscosity of reconstituted skim milk was studied by Anema et al. (2004) and they found out that pH of milk at heating is responsible for change in viscosity. They correlated change in viscosity with the change in casein particle size which in turn determines the casein micelle volume. The casein micelles size change and their interaction with other casein micelles depended on the amount of denatured whey proteins associated with casein particles, which is highly dependent on pH of the

milk at heating. Anema et al. (2014) studies effect of pH of skim milk at heating on milk concentrate viscosity for up to 40% total solids (TS). They found that milk concentrate viscosities at different pH of heating followed power law equation and up to 40% TS consistency coefficient (K) was different for different pH, however beyond 40% TS K did not depend on pH of milk at heating and was same for different pH.

Carbon dioxide injection as a technique to reduce pH of milk

Carbon dioxide injection is one of the techniques which has been explored in last decade to improve quality and functionality of milk and milk concentrates, modified milk protein concentrates etc. (Kosasih et al. 2016; Marella et al. 2015; Fuente 1998; Hotchkiss et al. 2006). CO₂ injection as a technique to lower milk pH have been used in cheese manufacturing prior to rennet coagulation (Nelson et al., 2004). Kosasih et al. (2016) studied the effect of partially acidifying concentrate on physical and functional properties of spray dried whole milk powder. Acidification using 2000 ppm of CO₂ was used and found effective in the modification of both function properties i.e. solubility and dispersibility and physical properties i.e. free fat and porosity of whole milk powders. The best results were achieved when warm concentrate at 30° C was treated with CO₂. Studies conducted by Marella et al. (2015) also used CO₂ injection as a tool to alter mineral environment of concentrate to improve functional properties of milk protein concentrate powders. They used 2,200 ppm of CO₂ injection during ultrafiltration of skim milk to produce milk protein concentrate which was further dried to produce milk protein concentrate

powder. The study resulted in improved solubility, decrease in z-poteintal and increase in micellar casein size. CO₂ addition modifies micellar structure and decreases inter-micellar interaction leading to improved solubility of resultant powders (Law and Leaver, 1998, Schokker et al., 2011, Marella 2015). Advantage of using CO₂ as acidulant in milk is that it can be easily and completely removed by applying heat or vacuum (Kosasih et al., 2016). Lee (2014) reported decrease in viscosity of Ultra filtered (UF) whey protein concentrate and UF and reconstituted skim milk concentrate, which were carbonated first and then decarbonated. They used CO₂ treatment resulting in pH induced modifications in salt distribution and improve milk properties. The use of CO₂ has been studied for its effectiveness in improving functional properties of whole milk concentrate, milk protein concentrate, reconstituted skim milk concentrate, UF whey protein concentrate and UF skim milk concentrate. However, the application of CO₂ injection and its impact on functional properties of evaporated skim milk concentrate is yet to be explored. In all these concentrate samples the physico-chemical properties return back to their original values when the CO₂ is removed, however change in viscosity still remains low even after decarbonation of concentrate. The injected CO₂ added to the concentrate was found to be completely removed after powder manufacturing using spray drying process (Lee, 2014).

Casein in milk

Caseins are a class of milk specific acid insoluble phosphoproteins that form the largest protein component (~80% of the total protein) in cattle milk and other

commercially significant milks (Horne, 2006, Fox and Brodtkorb, 2008, O'mahony and Fox, 2013). Usual method of casein preparation involves isoelectric precipitation at pH 4.6 (Fox and Brodtkorb, 2008). Generally in milk caseins exist as aggregates or micelles of casein fractions and colloidal calcium phosphate with colloidal particle size ranging between 50-600nm in diameter with average around 150nm (Fox and Brodtkorb, 2008). Caseins in bovine milk consists of four major proteins namely α_{s1} -casein, α_{s2} -casein, β -casein and κ -casein (McMahon and Oommen, 2013), they have different charge distribution and exhibit different tendencies to aggregate when Ca^{2+} ions are present or absent (Walstra and Jenness, 1984). Since caseins do not possess a fixed three dimensional tertiary conformation they can react quickly to changes in environment and take various conformations therefore being rheomorphic in nature (Holt and Sawyer, 1993). Casein micelles are highly hydrated containing ~4g water per gram of protein, however only 15% of this water is bound to protein the rest is just occluded within the micelle (De Kruif and Holt, 2003, Farrell Jr et al., 2003). Variety of forces maintain casein micelle structural stability including hydrophobic interactions, hydrogen bonds, disulfide bonds, ionic and electrostatic interactions and steric stabilization (McMahon and Oommen, 2013). Caseins exhibit aggregation behavior based on various kinds of interactions namely calcium mediated interaction because of phosphoserine groups (Dalglish and Parker, 1979), hydrophobic regions are responsible for hydrophobic interactions, hydrophilic regions interact with water, hydrogen bonding and electrostatic interactions. A casein micelle is comprised of protein ~92%, inorganic matter mainly calcium phosphate ~8%, magnesium and

citrate in small amounts (O'mahony and Fox, 2013). β - and κ -casein are attached to the micelle only through hydrophobic interactions this is evident from the fact that at low temperatures when hydrophobic attraction are weakest β -casein and κ -casein tend to diffuse out from the micelle as compared to α_{s1} -casein and α_{s2} -casein (McMahon and Oommen, 2013). Highly phosphorylated α_{s1} -casein, α_{s2} -casein and also β -casein held together by calcium bridges with calcium phosphate nanoclusters. The glycomacro-peptide portion of κ -casein which is highly hydrophilic and extends into the serum phase is responsible for providing steric stability against aggregation (McMahon and Brown, 1984).

Casein Structure

Casein micelle structure have been explained through various models proposed over last 60 years and then there have been additions, improvements and revisions in these models from time to time. One of the early attempts to model casein structure was made by Slattery and Evard (1973), Slattery (1976) who proposed submicelle model and further additions to this model were made by Schmidt (1980). Submicelle model stated that caseins first aggregate through hydrophobic interactions to form subunits, these subunits differ in their κ -casein content. Subunits rich in κ -casein form the micelle surface whereas subunits poor in κ -casein content form the interior of micelle and are linked by colloidal calcium phosphate. Holt (1992) produced a model in which casein fragments in micelle were held together by interactions between caseins and calcium phosphate. Horne (1998) proposed dual binding model in which caseins are inter-linked by hydrophobic

interactions and also calcium bridges between caseins and calcium phosphate nanoclusters with κ -casein acting as size limiter at the micelle surface. With all the proposed models one thing that required a satisfactory explanation was the location of κ -casein in the micelle structure. A location that allow for κ -casein whey protein complexes to form on heating, allow chymosin action and allow steric stabilization of calcium sensitive casein fractions (Fox, 2003, Horne, 2006).

Whey Proteins

Whey protein or serum proteins are a class of bovine milk protein that remain soluble at pH 4.6 and make about 20% of total bovine milk protein (O'mahony and Fox, 2013). Whey proteins are compact, globular proteins in their native form and are highly soluble due to presence of surface hydrophilic residues (LEE et al., 1992). Also whey proteins possess excellent foaming and emulsifying properties. Two most abundant whey proteins are; β -LG constituting ~50% and α -LA constituting ~20% of total whey proteins. Other smaller components of whey proteins include bovine serum albumins, lactoferrin, immunoglobulins and proteose peptones. β -LG contains two disulfide bonds linking Cys66 with Cys160 near the C-terminal and Cys106 with Cys119 which leaves Cys121 as a free thiol group, although it is unexposed (O'mahony and Fox, 2013). Heat which is one of the denaturing agent causes the β -LG dimer to dissociate leading to unfolding and revealing the free thiol of Cys121 and other hydrophobic residues thereby making it possible for covalent, hydrophobic interactions and disulfide interchange reactions to take place (LEE et al., 1992, Qi et al., 1995, Iametti et al., 1996, Creamer et al., 2004, O'mahony and Fox, 2013). α -LA

consists of 123 amino acids, binds Ca^{2+} , exists in halo form (O'mahony and Fox, 2013) and its structure derives stability from four disulfide bonds between Cys6-Cys120, Cys28-Cys111, Cys60-Cys77 and Cys73-Cys90 (Brew, 2003). Generally α -LA thermally unfolds at a lower temperature compared to β -LG, however unlike β -LG it does not contain free thiol group hence ruling out possibility of disulfide interchange reaction (Rüegg et al., 1977).

Heat induced interaction of casein and whey proteins

Heat treatment is a popular technique that brings about changes in functional properties of milk and milk products made from such milks (Oldfield et al., 2000). When subjected to heat treatment above denaturation temperature of major whey proteins (β -LG and α -LA) denaturation and aggregation occurs followed by formation of complexes between whey protein and caseins (Corredig and Dalgleish, 1999). Of all the whey proteins immunoglobulins and serum albumin are most readily denatured followed by β -LG and α -LA (Rüegg et al., 1977), however β -LG and α -LA being most abundant of all whey proteins determine the overall behavior of whey proteins to heat and hence the resultant functional properties of heated milk products (Singh et al., 1992). Whey protein casein micelle complexes are considered the most important outcome of heat treatment of milk and it is the free thiol group of β -LG that plays an important role in thiol-disulfide interactions with other denatured whey proteins and κ -casein (Anema and Li, 2003, Fox, 2003). The free thiol group of β -LG is exposed on heat treatment and this initiated a series of thiol-disulfide reactions of denatured β -LG with other denatured β -LG, α -LA and κ -casein. α_{s2} -

casein can also possibly be involved in thiol-disulfide interchange reaction with β -LG suggesting that all protein fractions that contain cysteine can possibly participate in whey protein casein micelle interactions (Dalgleish, 1990). Factors that affect denaturation of whey protein include whey protein concentration, pH, total solids, method of heat treatment, intensity of heat treatment and mineral content (Elfagm and Wheelock, 1978, Hillier et al., 1979, Smits and Van Brouwershaven, 1980, Mulvihill and Donovan, 1987, Anema and Li, 2003). The sequence of reactions involved in interaction of whey proteins with κ -casein on the micelle surface is still not clear. On heat treatment β -LG, α -LA along with other whey proteins first form soluble complexes among themselves that serve as intermediate entities in the interaction between whey proteins and casein micelles, also it is necessary for β -LG to be present for these interactions to occur (Corredig and Dalgleish, 1999). Mulvihill and Donovan (1987) reported that early in interaction between denatured whey proteins and casein micelles ionic and/or hydrophobic interactions play a crucial role. The pH at which milk is heated greatly affects the denatured whey proteins and casein micelle interactions that occur upon heat treatment (Anema, 2009). The pH of milk at heating determines degree of casein dissociation from the micelle and the extent of whey protein associated to the micelle (Smits and Van Brouwershaven, 1980, Singh and Fox, 1985, SINGH and CREAMER, 1991). (Singh and Fox, 1987) found β -LG/ κ -casein complexes to be dissociated from the micelle when milk was heated at pH>6.9 and β -LG/ κ -casein complexes to be attached with casein micelle when milk was heated at pH between 6.5-6.7. The exact sequence of

interaction reaction between denatured whey proteins and κ -casein is still a matter of debate and there are conflicting reports on whether it is a preferential serum phase interaction or preferential casein micelle surface interaction with early reaction between κ -casein and denatured whey proteins taking place in serum or casein micelle surface depending upon the pH at which milk is heated (Anema and Klostermeyer, 1997, Anema and Li, 2000, Donato and Dalgleish, 2006, Anema, 2007).

Casein voluminosity

Different researchers have determined voluminosity of casein micelles employing different methods such as microscopy, through measurement of hydrodynamic radius and sediment volume (Walstra, 1979). Reports from these studies indicate discrepancies in values of voluminosity reported for casein micelles. These discrepancies have been attributed to the fact that micelles are not perfect spheres and they have hairy κ -casein layer surrounding the micelles with degree of hairiness changing depending on surrounding environmental conditions like temperature, pH, free calcium ion content, rennet action etc. (Walstra, 1979, Snoeren et al., 1984). Snoeren et al. (1984) found that as the temperature and calcium content of milk decreased the micellar voluminosity increased because β -casein migrated into serum and micelles swelled. Amount of bound calcium to β -casein and α S1-casein alters hydration of these casein fraction and the casein micelle overall (Waugh et al., 1971). Sood et al. (1979) observed that as the calcium content of milk was

lowered micellar size increased and Sood et al. (1976) reported that voluminosity derived from hydrodynamic radii larger for micelles smaller in size and vice versa.

Acid gelation of milk

Products like yogurt which are classified as acidified milk products are produced and consumed worldwide. Acid gelation properties of milk are very important to improve manufacturing process and overall acceptability of such products. Acidification of milk can be brought about by use of bacterial cultures (lactic acid bacteria), addition of acids or addition of glucono- δ -lactone (GDL) (Lucey and Singh, 1997). Bacterial cultures convert lactose to lactic acid which lowers the milk pH, whereas GDL is an ester that is converted into gluconic acid upon hydrolysis thus reducing the pH of the milk (De Kruif, 1997). Most of the studies on acid gelation are based on gels prepared using GDL as an acidulant, however acid gels prepared using GDL differ in their rheological and physical properties from acid gels made using bacterial cultures particularly at higher temperature of gelation (Lucey and Singh, 1997). GDL exhibits a faster rate of acidification rapidly lowering the milk pH as it is quickly hydrolyzed into gluconic acid, whereas lactic acid bacteria do not change pH of the milk to a great extent immediately as they are added and slowly lower the milk pH. Changes are observed in several physicochemical properties of casein micelles as milk is acidified. These changes include loss of colloidal stability or casein fragments dissociation, voluminosity and viscosity changes, dissolution of colloidal calcium phosphate and migration of caseins into milk serum (Roefs et al., 1985, Dalgleish and Law, 1988,

Walstra, 1990). The removal of colloidal calcium phosphate from casein micelle and migration of casein fragments into serum phase on acidification of milk is temperature dependent; acidification to pH ~5.5 at 30° C causes almost no release of casein fragments into serum phase whereas if milk is acidified to pH 5.5 at 4° C close to 40% of casein fragments are released in the serum phase (Dalglish and Law, 1988). This observation along with study done by McGann and Fox (1974) where they used urea to disrupt micelle structure without solubilizing CCP establishes the fact that hydrophobic interactions and hydrogen bonding are equally essential along with CCP to maintain integrity of casein micellar structure. Even after all these changes with casein micelle when pH reduces to 5.5 the size of the micelle hardly changed because of the swelling that accompanied solubilization of CCP (Vreeman et al., 1989). As the pH is further lowered to 4.6, the isoelectric point, casein micelles begin to aggregate. Holt and Horne (1996) found that micelle-like particles that make up the acid gel network have structure and arrangement of casein fragments similar to that of original casein micelles. Milks used in yogurt manufacture are generally heat treated causing whey proteins to denature and associate with casein micelles through hydrophobic interactions and disulfide bonds (Anema and Li, 2000) hence affecting the properties of resultant acid gels through increasing the contact point and bridging casein micelles in the gel network (LUCHEY et al., 1997, LUCHEY et al., 1998, Bikker et al., 2000).

Rennet coagulation of milk

Coagulation of milk or in simpler words converting milk from liquid to gel by destabilizing milk proteins is the basic first step in cheese manufacturing process (Guinee and Wilkinson, 1992). Rennet coagulation property of milk is of great economic importance to dairy industry as it directly impacts cheese making process and have been studied in details over the years. It is generally agreed that rennet coagulation of milk as a process occurs in two stages which although sufficiently separate from each other might overlap to some extent (Alais, 1956, Waugh and Von Hippel, 1956). First stage is enzymatic proteolysis and the second stage is aggregation of paracasein micelles (Guinee and Wilkinson, 1992), second stage overlaps first stage and milk clotting begins before κ -casein undergoes complete enzymatic proteolysis (Green et al., 1978, Dalgleish, 1979, Chaplin and Green, 1980). Casein micelles which are stabilized by κ -casein located on the surface of the micelles are eventually destabilized when chymosin acts on κ -casein and cleaves it leading to destruction of steric stabilization thus resulting in process of rennet coagulation (Sandra et al., 2007). Chymosin destroys the 'hairy' surface layer of κ -casein by specifically cleaving at Phe₁₀₅-Met₁₀₆ peptide bond of κ -casein causing the caseinomacropeptide (106-169) that provides stability to the micelle to diffuse in the serum phase (Hyslop, 2003). Removal of the hairy layer in the early stages of rennet coagulation leads to reduction in micelle radius, decrease in viscosity and turbidity (Walstra et al., 1981, Guthy et al., 1989, Horne and Davidson, 1993, Lomholt and Qvist, 1997, Fox et al., 2000, Hemar et al., 2004). As the cleavage of κ -casein

progresses and caseinomacropeptide diffuses into serum, charge on micellar surface is reduced, which is indicated by ζ -potential measurement (Fox et al., 2000). Dagleish (1979) and Green and Morant (1981) reported that it is only after 80-90% of glycol macro-peptide is released from casein micelle that the casein micelles begin to aggregate. (Van Hooydonk et al., 1986) and (Walstra, 1990) reported that at lower pH and higher temperature coagulation can occur even at a lower degree of κ -casein hydrolysis. Research suggests that Smoluchowski process is the mechanism behind the aggregation process where particles collide randomly to aggregate and there is no preference specific size of aggregates to be formed for the reaction to propagate (Sandra et al., 2007). Rennet coagulation of milk is affected by factors such as milk pH, coagulation temperature, enzyme concentration, Ca^{2+} ion concentration, casein content, heat treatment and temperature history of milk to list a few (Payne et al., 1993, Picon et al., 1995, Gunasekaran and Ay, 1996, Daviau et al., 2000). Milks subjected to various heat treatments and high pressure treatment have been studied thoroughly in recent years for rennet coagulation and gel formation properties especially with interest to improve cheese yield (Horne and Banks, 2004).

Measurement of rheological properties of Acid and Rennet Gels

Generally it is the number and strength of bonds between casein particles, their structure and dimensional distribution of strands that hold these particles together that determine the rheological characteristics of casein acid gels (Roefs et al., 1990). Useful information on the process of gel formation and gel structure can be obtained through dynamic rheology testing which involves application of

oscillatory strain or stress (Lucey, 2002). In a strain controlled experiment a fixed sinusoidally oscillating strain is applied to the sample and the corresponding oscillating stress response to this strain is measured along with amplitude of this stress and phase difference strain and stress (Ferry, 1980). Elastic or storage modulus (G'), viscous or loss modulus (G'') and loss tangent ($\tan \delta$) which represents the ratio of viscous to elastic component of gel are the important parameters that derived from above responses. Vélez-Ruiz et al. (1997) defined these parameters as below:

$$G' = (\tau_0/\gamma_0)\cos \delta$$

$$G'' = (\tau_0/\gamma_0)\sin \delta$$

$$\tan \delta = G''/G'$$

Where τ_0 represents amplitude of shear stress, γ_0 represents amplitude of strain and δ represents phase angle.

It is observed that rennet gels exhibit a lag after rennet addition while gel formation using rheometer prior to obtaining a measurable value of G' (Lucey, 2002). Dynamic moduli for rennet gels show rapid increase in the initial phase and then slowly plateau out. As the gelation progresses more and more particles are incorporated into the gel network and contact area between aggregated particles increases due to micelle fusion which is reflected in slow increase of moduli at later phase of rennet gelation. Dalglish (1983) observed that aggregation rate in rennet induced gels increases as the gelation temperature goes up and rennet gels with

highest G' values are produced at temperature around 30-35° C (Esteves et al., 2002). G' values of acid gels is higher compared to rennet gels because acid gels are almost always made from pre-heated milk (Lucey, 2002). Lucey and Singh (1997) and Anema (2008) observed that elastic modulus of acid gels increased when acid gels were prepared from pre-heated milk with denatured whey proteins interacting with casein micelles to form aggregates. Fracture strain is lower for yogurt gels and spontaneous whey separation is higher for them (Lucey, 2002). In comparison to rennet coagulated gels acid casein gels are brittle and fragile exhibiting a non-curdy and smooth texture on stirring (Lucey, 2004).

Heat stability of milk

Heat stability of milk can be defined in simpler terms as the ability of milk to withstand high temperature treatment without coagulation. The other definition of heat stability could be “the length of time which elapses between the placing of a sample in an oil bath maintained at a specific temperature and the onset of coagulation as indicated by flocculation, gelation or changes in protein sediment ability” (Fox and Morrissey, 1977). It is an important property of milk that makes treatments like pasteurization, sterilization, evaporation and dehydration possible (Fox and Morrissey, 1977). Heat stability as a property gained attention when sterilization and condensing became popular processing technique close to 100 years ago (Singh, 2004). Caseins have remarkable heat stability which is attributed to absence of secondary and tertiary structure and presence of complex quaternary structure in caseins whereas non-casein proteins in milk are more susceptible to heat

coagulation and are completely denatured on heating at 90° C for 10 min (Fox and Morrissey, 1977). Most widely accepted method to determine heat stability for research purposes involves heating of milk samples in sealed glass tubes placed on rocker and immersed in heated oil in an oil bath maintained at 140° C for milk and 120° C for concentrated milk (Rogers et al., 1921, Miller and Sommer, 1940, Cole and Tarassuk, 1946, Pyne and McHENRY, 1955, Rose and Tessier, 1959, Davies and White, 1966, Singh, 2004). Heat stability can be determined using other tests such ethanol test, a whitening test, viscosity determination and protein sedimentation test (Singh, 2004). Davies and White (1966) noticed that heat space to sample volume ratio in the glass bottle and the rocking rate influence heat coagulation time. Rose (1961a), (Rose, 1961b) studied the effect of pH and lactation on heat stability of milk and it was his research that reinforced the importance of pH in relation to heat coagulation time (HCT). Other factors that influence heat coagulation time are temperature, animal species, stage of lactation, ratio of β -LG to κ -casein, colloidal calcium phosphate and soluble salts (Fox and Morrissey, 1977). Milk generally tends to become more heat stable as lactation advances (Davies and White, 1958, Rose, 1961b). Heat stability of milk is greatly influenced by β -LG as it is the most heat sensitive of all milk proteins. β -LG to κ -casein ratio influences the heat stability of milk to a large extent, thus it both β -LG content and micelle surface characteristics that are important (Tessier and Rose, 1964). Forewarming or preheating of milk prior to concentration increases the heat stability of milk (Sommer and Hart, 1926, Webb and Bell, 1942).

Cavitation treatment of milk

Cavitation can be defined as a treatment that involves combination of microbubbles or cavities formation, growth and collapse that occurs over a fraction of second releasing localized high energy and forming localized zones of high temperature and high pressure throughout the cavitation reactor (Suslick, 1989, Gogate, 2011). Cavitation is a promising treatment that can potentially intensify physical and chemical processing while still offering energy efficiency (Gogate and Kabadi, 2009). Hydrodynamic cavitation (HC) which is also referred as mechanical cavitation can be generated by simply forcing the liquid through constrictions like a venture, orifice plate or throttling valve such that eddy currents are formed, fluid pressure is dropped and high flow velocities are obtained by kinetic energy transformation (Milly et al., 2007). The reactors used to generate hydrodynamic cavitation are comparatively simple in their design and also easier to scale-up than acoustic cavitation reactors (Gogate, 2011). One of the latest designs to generate controlled hydrodynamic cavitation consists of a mechanical rotor with radial holes in it which when rotates in a liquid chamber produces cavitation. More the number of holes more is the cavitation produced with hydrodynamic forces generating in the holes causing small bubbles to form at the bottom of the holes; these bubbles grow in size as they move out the holes and subsequently collapse generating localized high temperature and pressure along with high shear. Their other advantages over acoustic cavitators include uniform cavitation throughout the reactor, less erosion of casing metal and easy control and quantification of design parameters. When processing

large quantities of liquid food like milk hydrodynamic cavitation can serve as a more efficient alternate to acoustic cavitation (Ashokkumar et al., 2012). Application of HC have been explored in variety of areas such as inactivation of spoilage microorganisms (Milly et al., 2007), water and effluent treatment (Kalumuck and Chahine, 2000, Sivakumar and Pandit, 2002), homogenization (Crudo et al., 2014), crystallization (Moser et al., 1995, Moser, 1996), enzyme extraction, extraction of bio-components (Gogate and Kabadi, 2009), emulsification and preparation of liquid dispersions (Kozyuk, 1998). Although studies on effects on acoustic cavitation on milk properties have been studied to some extent no literature is available on effects of hydrodynamic cavitation on physicochemical properties of milk such as casein particle size, heat stability, rennet coagulation time, protein interactions.

Objectives

US manufacturers of dried dairy ingredients are looking to become preferred suppliers of NFDM globally for which they want to be more competitive in product quality, functionality and price. It is 6-10 times more economical to remove water in evaporator then in spray dryer, thus improving on manufacturing efficiencies to keep the overall cost low. However as discusses in the literature review above at higher solids the challenge is the significant increase in viscosity of concentrate. To help them achieve their goal we developed strategies in lab to increase solids of SMC in evaporator while still keeping the viscosity low. The present study aims as scaling up these strategies developed in lab to pilot plant scale and study their feasibility at the industrial scale. The other challenge with the work that was done at lab scale was the

use of inorganic acid to lower milk pH prior to concentration. Since the acid that was used in lab study do not have a GRAS status therefore during the scale up trials in pilot plant it was decided to use CO₂ injection as a method to lower milk pH. Lab trials on small scale were conducted first to study effect of CO₂ injection on viscosity of SMC and then the plant scale trials were conducted. Also as mentioned in earlier sections studies on non-thermal techniques to process milk and milk products are gaining interest and use of high pressure processing and ultrasound cavitation have been studied in quite some detail so far. However limited studies have conducted to understand the impact of hydrodynamic cavitation on milk properties. Studies so far have focused on microorganism inactivation to make the milk safe and to some extent on homogenizing effect of HC. No study so far have focused on effect of HC milk proteins, their interaction and impact on other physicochemical properties of milk.

The specific objectives of this study are as follows:

1. To study the effect of hydrodynamic cavitation on casein micelles size, heat stability, acid gelation properties, rennet coagulation properties and protein interaction of skim milk
2. To explore use of CO₂ injection as a method to lower pH of skim milk concentrate replacing HCl as a technique to lower viscosity of high solids skim milk concentrate

3. To scale-up lab scale strategies to lower viscosity of high solids skim milk concentrate to plant scale and study their feasibility

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Chapter II

Effect of hydrodynamic cavitation on particle size of casein micelles, protein interactions, heat stability, acid gelation properties and rennet coagulation properties of skim milk

ABSTRACT

Hydrodynamic cavitation (**HC**) is a process of vaporization, bubble generation followed by bubble collapse in a flowing liquid brought about by a decrease in pressure followed by a subsequent increase in pressure. The application of this new technology in the processing of milk and milk products is being investigated. Therefore, the objectives of this study were to find out the effect of HC on some important properties of milk such as particle size of casein micelles, acid gelation properties, rennet coagulation properties, interactions of proteins and heat stability of skim milk. Pasteurized skim milk (3.5% protein and 9% total solids) was preheated to 50° C and then subjected to two sets of HC treatments, namely, HC at 20 Hz, 40 Hz and 60 Hz at sufficiently high flow rate (950 liters/hour) to avoid any temperature increase during HC (**T1**) and HC at 60 Hz at low flow rates (200 liters/hour) to allow scale-free heating of skim milk increasing its temperature up to 90° C (**T2**) using APV Cavitator (supplied by SPX, Denmark) fitted with a rotor containing 4-rows of holes in 6mm housing.

The samples obtained from T1, T2, heated and unheated control skim milk samples were analyzed for changes in the particle size of casein micelles using Malvern

Zetasizer Nano ZS, heat stability using the heat coagulation time (**HCT**) test at 140° C, rennet coagulation and acid gelation properties using small amplitude oscillatory rheology (**SAOR**) at 0.5% strain, 0.1Hz frequency and 1% strain, 0.1Hz frequency respectively. The protein distribution and interactions in the skim sample and serum phase obtained from these samples by centrifugation of control, cavitated and heated milk samples at 100,000g/1h were characterized using sodium dodecyl sulfate polyacrylamide gel electrophoresis (**SDS-PAGE**).

Particle size decreased significantly for skim milk samples subjected to 40Hz (T1) and did not change for T2, however overall HC for three T1 treatments did not result in significant ($p \leq 0.05$) changes to the casein micelle size among themselves. Heat stability results indicate that HCT decreased slightly for T2 compared to T1 and control however this decrease was not significant ($p \leq 0.05$). Samples subjected to T2 exhibited significantly ($p \leq 0.05$) higher levels of whey protein denaturation compared to those subjected to T1, which was attributed to the heat generated due to cavitation during T2. The high molecular weight aggregates were also generated in samples subjected to T2 due to extensive denaturation of whey proteins and their interactions. It was observed that the storage modulus (G') of acid gels prepared with skim milk samples subjected to T1 was significantly lower ($p < 0.05$) than those prepared with samples subjected to T2. Acid gels prepared from the samples subjected to T2 with temperatures reaching 90°C had highest final G' , which was attributed to significant denaturation of whey proteins in these samples. It was also observed that this value of G' was similar to the one obtained from the conventional heating of milk,

suggesting that the cavitation treatment of milk did not adversely affect the acid gelation property of the milk. Rennet coagulation time (**RCT**) was lowest for T1 sample cavitated at 40 Hz cavitator speed and the same sample had the highest gel strength as well. Samples from T2 exhibited no rennet coagulation which is expected due to heat denaturation of whey proteins and their interaction with casein micelles which block chymosin action sites. The results of this study suggested that HC can be promising technique that can be utilized to alter some physicochemical properties of milk and can be potentially used as an alternative technology for scale-free heating of milk with minimal effect on important properties of milk.

Key Words: cavitation, elastic modulus, denaturation

INTRODUCTION

Currently the primary method for milk processing at industrial scale is thermal treatment and it is widely accepted to make the product safe for consumption as well as to improve product shelf life (Shanmugam et al., 2012). However the intense heat involved in thermal treatment of milk have its drawbacks like it lowers the nutritive value of milk proteins (Alkanhal, 2001, Lacroix et al., 2008), modifies and denatures them (Oldfield et al., 1998a, Oldfield et al., 1998b, Anema, 2009), alters mineral balance (Hansen and Melo, 1977, Seiquer et al., 2010), deteriorates vitamin content (Burton, 2012) and produces undesirable color and flavors in the milk (Fellows, 2009, Barbosa-Canovas et al., 2010). Keeping in mind these disadvantages of thermal treatment there is a latent need to explore non-thermal processing techniques that cause minimum damage to milk constituents and still provide safety and improved shelf life. Non-thermal techniques such as ultrasound, pulsed electric field, ozone treatment, gamma radiation, high pressure processing, microwave and ohmic heating are being explored for this purpose. High pressure processing and ultrasound (**US**) have received wide attention over past few years compared to gamma radiation, pulsed electric field and microwave mainly because they are perceived to be safer and non-toxic by the general consumers (Barbosa-Canovas et al., 2010, Bermúdez-Aguirre et al., 2011). Similar to US Hydrodynamic cavitation (**HC**) is an emerging technology which is relatively new to dairy manufacturing. Cavitation can be described as a combined phenomenon of microbubble formation, growth and finally collapse occurring over an extremely

small span of time (Gogate, 2011). Hydrodynamic cavitation is brought about by a difference in vapor pressure at the bottom of the hole and the flowing liquid in the housing. The spinning rotor generates internal liquid friction while the holes on the rotor generate hydrodynamic cavitation.

Hydrodynamic cavitation is known to induce microparticulation involving denaturation and creation of ideal particle size distribution along with scale free heating. There is very limited literature available on application of hydrodynamic cavitation in dairy manufacturing. Crudo et al. (2014) found HC to be an effective technique for microorganism inactivation and homogenization of fresh untreated milk. Milly et al. (2007) used HC in combination with UV systems for minimal processing of foods. There are other applications of HC that have been reported in literature like, waste water treatment (Sivakumar and Pandit, 2002), selective release and purification of intracellular enzyme (Balasundaram and Pandit, 2001). Other reported applications of HC in food processing are scale-free heating, mixing, gum hydration, emulsification, and aeration (Mancosky and Milly, 2011). Due to a lack of research on HC in dairy manufacturing and its similarity with ultrasonic cavitation (UC), literature on UC is usually taken into account. Ultrasonic cavitation has also been shown to improve the gelation properties of whey protein concentrate systems (Zisu et al., 2011). Ultrasonic treatment along with preheat treatment was found to improve heat stability of whey proteins (Ashokkumar et al., 2009). Nguyen and Anema (2010) studied the effect of ultrasonic cavitation on acid gels formed from cavitated skim milk. UC did not have any effect on stability of casein micelles nor

was there any change observed in the size of casein micelles (Chandrapala et al., 2012). Other studies have reported a small decrease in the size of casein micelles when skim milk is processed using UC (Villamiel and de Jong, 2000, Shanmugam et al., 2012). Ultra-sonication delays the gelation point and increases contact points between the gelling particles making the gel network more interconnected thus improving firmness of acid gels made from casein solutions (Madadlou et al., 2010). Ultrasound treatment of reconstituted skim milk along with pH cycling was found to improve renneting properties which include rennet coagulation time and cur firmness (Liu et al., 2014). However it has very little effect on renneting properties when reconstituted skim milk was treated at pH 6.7.

Basis literature review it is evident that application of ultrasonic treatment on milk have been studied in quite detail with studies done to understand impact on various physicochemical properties of milk, however hydrodynamic cavitation is a relatively new technology as far as application in dairy is concerned. So far studies on application of HC in dairy include inactivation of microorganisms and homogenization of milk. Considering the need to explore non-thermal technologies to process milk and milk products ensuring minimal damage from heat to heat sensitive milk components it is important that effect of HC on basic physicochemical properties of milk is studied in detail. With these thoughts and with a view to fulfill this knowledge gap the main objectives of this study were: 1) to find out the impact of HC on protein interactions in skim milk and study the impact on casein micelles by observing the change in mean particle size of casein micelles 2) To find out how

these changes affect properties like rennet coagulation properties, acid gelation properties and heat stability of skim milk treated with HC. It was hypothesized that hydrodynamic cavitation will induce slight disintegration of casein micelles and the heat generated due to shearing action will affect the protein interaction thus affecting rennet coagulation and acid gelation. HC with scale-free heating will produce whey protein complexes involving self-aggregation rather than covalently bonding with κ -casein, thus not affecting the size of casein micelles upon heating. Skim milk subjected to HC will produce rennet gels with lower or comparable rennet coagulation time.

Materials and methods

Materials and experimental design

For this study an incomplete 3 X 2 factorial experimental design was used with factors being cavitator speed and flow rate as shown in Table 1. Cavitator speed as factor had three different levels 1162 RPM (20Hz), 2326 RPM (40Hz) and 3490 RPM (60Hz) while flow rate has levels high or low allowing for shorter and longer residence time in cavitator respectively. So the experimental plan involved cavitating skim milk samples either at low flow rate along with cavitation at 60Hz cavitator speed or at high flow rate with cavitation at 20Hz, 40Hz and 60Hz cavitator speed. There were two control samples; one was skim milk without cavitation or heating called cold control and the other was skim milk heated conventionally in the lab at 90° C for 10 min called heated control. Skim milk with 9% total solids (TS) and

3.5% protein was obtained from Davis Dairy Plant (South Dakota State University, Brookings, SD, USA) warmed to 50° C before being subjected to various HC treatments.

Hydrodynamic cavitation

Hydrodynamic cavitation was carried out using an 8 inch APV hydrodynamic cavitator (SPX Flow Technology, Pasteursvej, Silkeborg, Denmark) was attached with a 4 row rotor with 6 mm gap between rotor and stator. The extent of cavitation is determined by speed of the rotor that is attached to a 10HP motor and the speed is controlled by a variable frequency drive (VFD) attached to the motor. Two sets of treatments were designed. The first set of treatments (T1) was carried out at 20, 40 & 60 Hz of VFD frequency which corresponds to 1162, 2326 and 3490 rpm of rotor speed respectively with a flow rate of ~950 L/h and second treatment (T2) was carried out at 60 Hz or a rotor speed of 3490 rpm maintaining a flow rate of ~200 L/h. T1 involved no temperature rise, T2 sample was heated to 90° C. Heated control sample was heated to 90° C for 10 min without any cavitation treatment. All samples were collected and stored below 5° C prior to analysis.

Particle size analysis

Samples for particle size measurement were prepared by diluting original milk samples in calcium imidazole buffer in a ratio 1:67. Dynamic light scattering method was used to analyze particle size (Z-average particle diameter) as per method

described by Meletharayil et al. (2015) using a Malvern Zetasizer Nano-ZS instrument (Malvern Instruments Ltd., Malvern, UK).

Heat coagulation time (HCT) test

Heat coagulation time is a direct representation of heat stability with higher values of HCT depicting higher heat stability of a sample. Heat stability of cavitated and control milk samples were studied by measuring heat coagulation time (HCT) of the milk samples in an oil bath fitted with rocker and maintained at 140° C using a method described by Dissanayake and Vasiljevic (2009). 3 ml milk samples each were poured in 8 ml autoclavable glass tubes (Wheaton, 17 mm outer diameter, 63 mm height), tubes were screw capped airtight and clamped on the rocker. Roker was switched at speed 3 and the tubed were allowed to submerge in heated oil maintained at 140° C while constantly rocking. Tubes were continuously observed for development of first signs of visible precipitation or coagulation with total time between placing the samples in oil batch and onset of precipitation or coagulation representing HCT.

Acid gelation properties using small amplitude oscillatory rheology

Acid gelation properties such as acid gelation time and gel strength were studied using Stresstech rheometer (ATS rheosystems, Rheological Instruments Inc., NJ, USA) by following the method outlined by Meletharayil et al. (2015). A predetermined quantity of glucono- δ -lactone (GDL) was added to 30 ml milk sample which was then mixed for 5 minutes. Out of this 30 ml quantity, 13 mL was

transferred to the cup using a bob and cup geometry (CC25 CCE) of the rheometer for the gelation to occur in the geometry while it was continuously monitored for change in elastic modulus (G') for 4 hours. The milk sample in cup was covered with a layer of vegetable oil in order to avoid any loss of moisture during the period of 4 hours. The onset of gelation was studied using strain controlled oscillatory measurement profile (0.1 Hz frequency and 1% strain) with G' data being logged every 5 min for 4 hrs. The remaining 17 ml of milk sample was maintained at 30° C and simultaneously measured for pH change with the data continuously logged to a computer during the gelation process.

Rennet gelation properties

Similar to acid gelation time, rennet coagulation time was measured using Stresstech rheometer (ATS rheosystems, Rheological Instruments Inc., NJ, USA) employing strain controlled oscillatory profile of 0.5% strain and 0.1 Hz frequency at 34°C. Double strength rennet CHY-MAX® supplied by Chr. Hansen was diluted to appropriate strength and added in calculated amount to the milk samples of which 13 ml was transferred to bob and cup geometry (CC25 CCE) of the rheometer. Onset of coagulation was considered to be a point where G' (elastic modulus) became more than 1 for the first time.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis was used to study protein distribution and their interaction in cavitated and control milk samples. Gels

containing 4% bis/acrylamide concentration in stacking gel and 15% bis/acrylamide concentration in resolving gel were prepared as per the method outlined by Patel (2007) with slight modifications. 15 μ L of milk and serum phase samples after appropriate dilution with SDS sample buffer (sample to SDS-buffer ratio of 1:20) were loaded on the gel and the gels were run at 200V for an hour. This was followed with staining and destaining of gels which were scanned using a Bio-5000 Microtek Scanner (Taiwan) and then analyzed using the Bio Image Intelligent Quantifier v 3.3.7 system (Jackson, MI). Soluble phases (serum phases) were collected by centrifugation of control and treated samples at 100,000 g in an Eppendorf 5403 centrifuge (Brinkmann Instruments, Westbury, NY, USA) for 1 hour at 20 °C.

Statistical Analysis

Statistical analysis of data was done using SAS (Version 9.3, SAS Institute Inc. Cary, NC, USA). Data were tested for ANOVA and differences among samples were considered significant with $p \leq 0.05$.

Results and discussion

Particle size analysis and heat coagulation time (HCT)

Casein micelles size measured as Z-average particle diameter is for control sample, samples from treatment 1 (T1) and treatment 2 (T2) is shown in Figure 1. Casein particle size decreased slightly for all samples subjected to T1 with the largest decrease observed for samples treated at 40 Hz where size decreased from 171 nm in cold control to 166 nm, which was statistically significantly significant. Samples

subjected to treatment T2 that involved simultaneous heating along with cavitation showed almost no particle size increase with size averaging at 172 nm compared to 171 nm for cold control. The other control sample that was heated conventionally in lab to 90 °C for 10 min had the largest particle size of 177 nm which was significantly different ($p \leq 0.05$) in comparison with all other samples. This increase in size of casein micelles in conventionally heated control sample is attributed heat denaturation of whey proteins and then formation of complexes between denatured whey proteins and κ -casein (Corredig and Dalgleish, 1999). No previous literature is available of effects of HC on casein micelle size however similarities are drawn between HC and US cavitation therefore reported results from US cavitation can be discussed. Similar observations on effect of US cavitation on casein particle size have been made by Villamiel and de Jong (2000) and Shanmugam et al. (2012) who observed a small decrease in casein particle size, although some of the results were from studies involving treatment of milk at high pH with milk treated at normal pH showing no change in casein particle size. Also Chandrapala et al. (2012) found that US did not affect size of casein micelle and the micelles held their integrity after the treatment. The slight decrease in size of casein micelles subjected to treatment T1 can be attributed to localized high shear forces created by HC. These shear forces can possible alter casein micelle size and also affect their interaction with whey proteins (Chandrapala et al., 2012). Depending on the degree of induced cavitation which is determined by the speed of rotor, the shear forces can break heat induced protein aggregates (Ashokkumar et al., 2009). Breakdown of heat induced protein

aggregates by high shear forces from HC possibly explain no increase in casein micelles size in treatment T2 although it involved simultaneous scale free heating along with cavitation.

Heat coagulation time (HCT) test

Heat coagulation time for skim milk samples subjected to HC and control samples measured in seconds is depicted as bar graph in figure 2. HCT increased slightly for treatment T1 at 40 Hz and then decreased slightly for treatment T2 at 60 Hz, although overall HCT was not affected by different cavitation treatments and there was no significant difference ($p \leq 0.05$) observed in HCT among samples from treatments T1, T2 and control. So far there are no studies done to find out the impact of cavitation on heat stability of milk although people have tried using cavitation, high shear mixing to improve heat stability of whey protein systems. There are conflicting reports on whether combination of heat and high pressure, high shear treatments like homogenization or micro-fluidisation or for that matter sonication can improve heat stability of whey protein solutions or not. Dissanayake and Vasiljevic (2009), (Zisu et al., 2010) and Koh et al. (2014) reported improvement in heat stability of whey protein systems using sonication and high shear mixing/micro-fluidisation alone or in combination. They attributed this increase in heat stability to disruption and breakage of whey protein aggregate formed by denaturation and aggregation on heating. Others reported a decrease in heat stability of whey proteins when heat and micro-fluidisation were used (Iordache and Jelen, 2003). Also condensed milk heat stability decreased when heat and homogenization were used in

combination (Deysher et al., 1929). Although not significantly lower, the decreased heat stability of samples subjected to treatment T2 may be due to the fact that disrupted whey protein aggregates are sensitive to secondary heat induced coagulation (Iordache and Jelen, 2003).

Acid gelation properties

Heat treatment prior to acid gelation is an important factor that determines the final elastic modulus (G') of the acid gels. HC with simultaneous scale free heating can be used to produce acid gels with the final G' of gels comparable to the G' of gels obtained from conventionally heated sample. Figure 3 demonstrates elastic modulus (G') vertical axis against time on the x-axis for acid gels prepared from skim milk subjected to different HC treatments. A steady increase in gel strength of acid gels obtained from heated control sample and treatment T2 can be observed. Also evident is the shorter gelation time from these two samples and their higher pH of gelation from Figure 4 as compared to gels from samples subjected to treatment T1 and non-heated control sample. Samples from treatment T1 where no heating was involved during the cavitation process formed very weak gels as indicated by their low final G' which was significantly ($p \leq 0.05$) lower than the final G' of gels obtained from milk from treatment T2 and heated control, their gelation time is longer and gelation started at much lower pH (Figure 4). It is only sample from treatment T2 where skim milk was scale-free heated along with cavitation that had G' comparable to the one obtained from gel formed from conventionally heated sample. Acid gels obtained from samples subjected to HC treatment T2 where

treatment temperature was above the denaturation temperature of whey proteins higher final gel strength indicated by higher G' which was very close to G' of gels obtained from skim milk samples heated conventionally, whereas weak acid gels were obtained from treatment T1 samples where milk was not heated along with cavitation and the treatment temperature remained well below denaturation temperature of whey proteins. These results show that most of the effect of HC on acid gelation can be attributed to the heat generated during the cavitation process. The gels strength of samples from treatment T1 were higher than the control sample with no heating involved indicating that HC with temperature control did improve acid gelation properties slightly. To summarize it can be said that most of the effect of HC on acid gelation properties of skim milk is due the heat generated during cavitation which was responsible for whey protein denaturation and influenced their interaction with casein micelles and Nguyen and Anema (2010) reported similar results from ultrasonication treatment on acid gelation properties of skim milk. Other study involving thermosonication of milk where process temperature reached 72°C causing denaturation of whey protein also showed improvement in G' of acid gels made therefrom (Riener et al., 2009).

Rennet gelation properties

Impact of HC on rennet coagulation time (RCT) and strength of curd formed from rennet coagulation was evaluated. Figure 5 shows the time it took for the onset of coagulation after rennet was added to skim milk as measured by small amplitude oscillatory rheology. Onset of gel formation is indicated by value of elastic modulus

(G') attaining the value 1. Figure 5 shows RCT measured in seconds on the primary y-axis along with casein micelles particle size on the secondary y-axis and various HC treatments on the x-axis. Figure 6 and 7 show G' and \log_{10} values of G' respectively for rennet gels on y-axis and time on x-axis to highlight gel strengths and the rennet gelation rate. The time taken between addition of rennet to milk samples and value of G' to reach 1 is referred to as rennet coagulation time. Figure 6 shows that gel strength of rennet gels from treatment T1 for 40 & 20 Hz cavitation speeds was higher than the control sample. In Figure 7 \log_{10} values of G' are used to clearly show which sample coagulated faster and the graph suggests that skim milk sample for 40 Hz (T1) treatment were the first to coagulate after the rennet is added. HC treatment reduced RCT for 40 & 20 Hz cavitation speeds of treatment T1, and RCT was lowest for samples treated at 40 Hz. This coincided with lowest casein micelle size observed from the same treatment. This decrease in RCT is in line with finding from earlier research suggesting decrease in RCT with decrease in size of casein micelle (Gaucheron et al., 1997, Needs et al., 2000, Huppertz et al., 2004). Other studies have also shown that as the casein micelle size decrease, rennet gelation rate and gel strength goes up (Ford and Grandison, 1986, Niki et al., 1994a, Niki et al., 1994b). Samples subjected to treatment T2 and conventional heating exhibited no rennet coagulation which is because of the heat denaturation of whey proteins and their association with casein micelles (Singh and Waungana, 2001, Hyslop, 2003, Vasbinder et al., 2003).

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Figure 8 shows non-reduced SDS-PAGE electrophoretograms of skim milk and milk serum phase samples from various HC treatments highlighting the protein distribution pattern for these samples. Gel A in Figure 8 depicts pattern of the proteins in milk samples while gel B shows protein distribution pattern from milk serum obtained from these skim milk samples which were subjected to different HC parameters. Significantly reduced intensities in protein bands for α -LA and β -LG were observed for treatment T2 lane 5 & 6 that involved heating along with cavitation to temperatures above the denaturation temperature of whey proteins and heated control samples lane 7. Also visible for these lanes are the aggregates held in wells in gel A. However one thing to be noticed is the intensity for κ -casein band did not change much suggesting that these aggregates held in well are whey protein/whey protein aggregates and that also explain a non-significant increase in casein micelle for these treatments. A similar decrease in intensity of protein bands representing α -LA and β -LG is also observed in gel B for serum phase samples. The decrease in band intensities of whey proteins only in HC treatments involving simultaneous scale-free heating suggests that HC in itself does not have effect on protein denaturation and distribution and most of the effect seen in SDS-PAGE study is due to simultaneous heating along with cavitation.

CONCLUSION

Hydrodynamic cavitation was able to significantly alter ($p \leq 0.05$) the particle size of casein micelles in 40 Hz (T1) treatment in comparison to cold control, however the size did not differ statistically among three treatments grouped as treatment T1. On the other end even with treatment T2 involving simultaneous heating alongside HC where higher β -lactoglobulin denaturation and aggregation were observed the particle size did not increase much because of the shearing action from cavitation. The treatment at 40 Hz decreased the casein particle size the most and it was for this treatment that we observed lowest RCT and highest rennet gel strength which is in line with earlier findings of decrease in RCT as the size of casein micelles decrease. In case of acid gels cavitation treatment which involved heating produced gels with final gel strength almost similar to the gels from conventionally heated milk samples. Although most of effect from this treatment on gel strength was attributed to the heat generated during cavitation, however this proved the fact that HC is promising technology that has a potential to be used as an alternate for scale-free heating of milk for yogurt making with minimal effect on other important milk properties. Effect on renneting properties is positive with potential benefits in cheese manufacturing and can be further explored with various pH adjustments. Future research can focus on cavitation of milk at various pH profiles, with different preheat treatments and with fortifications.

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Table 1. An incomplete 3 X 2 factorial experimental design showing treatment T1 and T2 that was used for this study with factors being cavitator speed and flow rate. Cavitator speed as factor had three different levels 20Hz, 40Hz and 60Hz and flow rate has levels low or high.

		Cavitation Speed		
		1162 RPM 20 Hz	2326 RPM 40 Hz	3490 RPM 60 Hz
Flow Rate	Low	X	X	√ (T2)
	High	√ (T1)	√ (T1)	√ (T1)

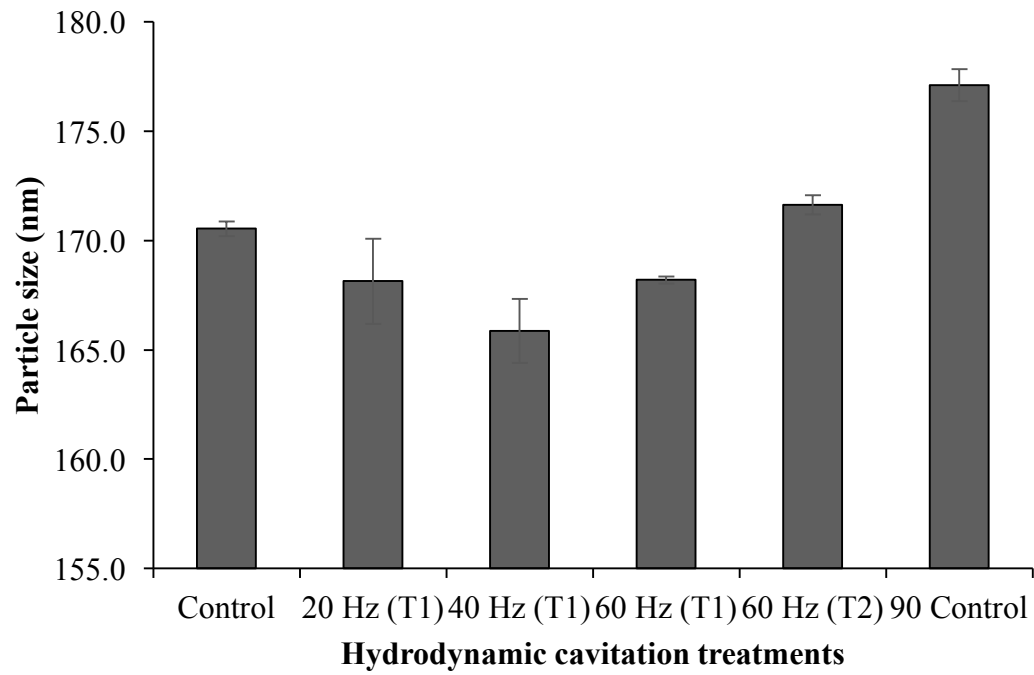


Figure 1. Z-average particle diameter of casein micelles of skim milk subjected to various hydrodynamic cavitation treatments along with two control samples. Values indicate means of data from triplicate analysis and vertical error bars indicate standard deviation.

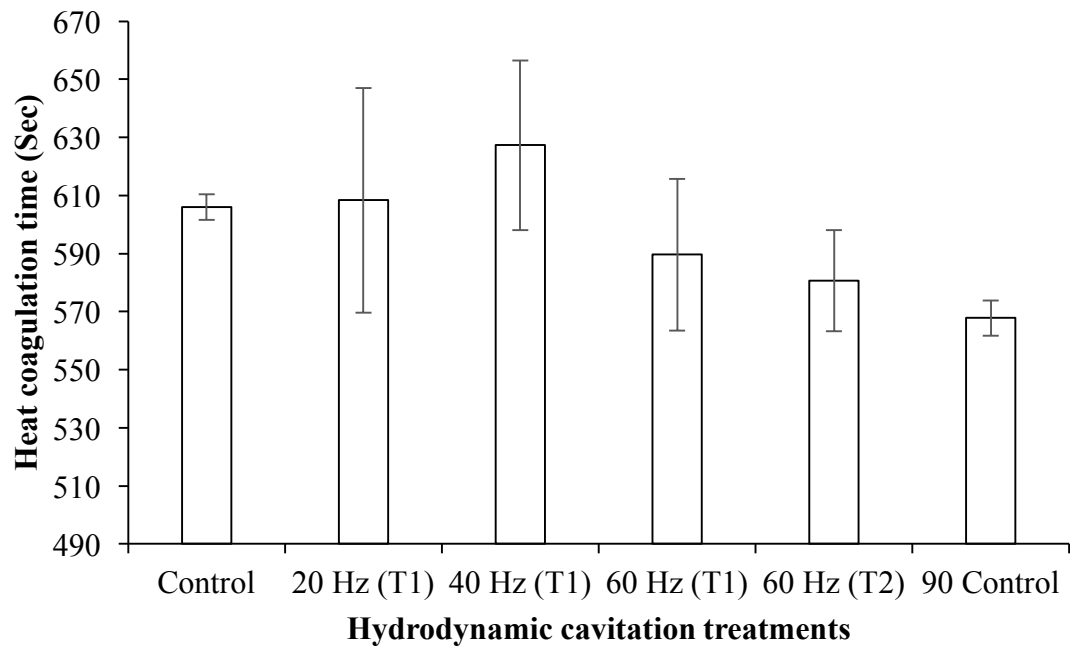


Figure 2. Heat coagulation time of skim milk samples represented by bars. Y-axis represents HCT in seconds and X-axis represents various HC treatments. Bars depict mean values from triplicate analysis with error bars depicting standard deviation.

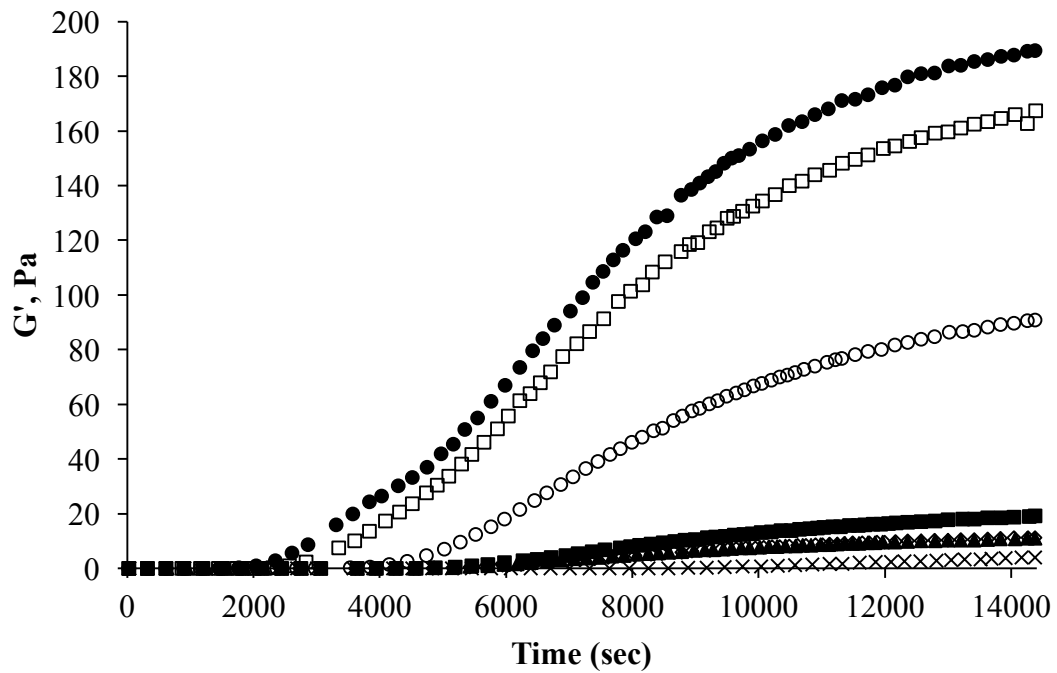


Figure 3. Elastic modulus (G') of acid gels as a function of time during the acid coagulation of skim milk at 30°C of control samples, (●) 90 control, (x) control and samples subjected to various hydrodynamic cavitation treatments, (□) 60 Hz (T2), (○) 60 Hz 80, (◇) 60 Hz (T1), (▲) 40 Hz (T1), (■) 20 Hz (T1) as measured by small amplitude oscillatory rheology.

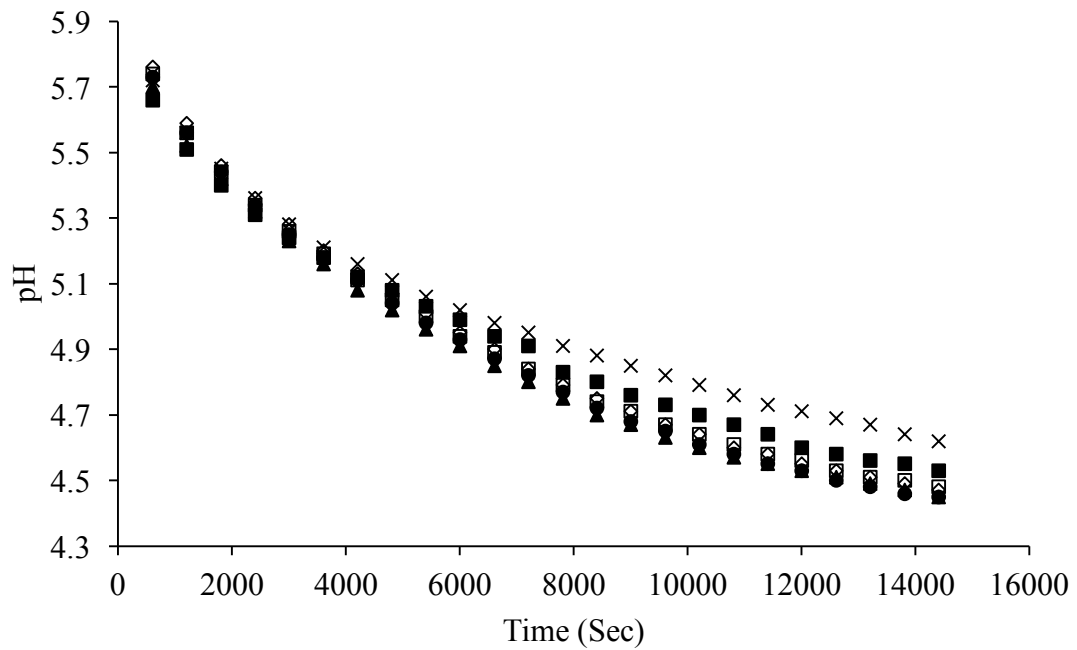


Figure 4. pH profile of acid gels as a function of time during the acid coagulation of skim milk at 30° C of control samples, (●) 90 control, (x) control and samples subjected to various hydrodynamic cavitation treatments, (□) 60 Hz (T2), (◇) 60 Hz (T1), (▲) 40 Hz (T1), (■) 20 Hz (T1) measured and logged continuously during the acid gelation process.

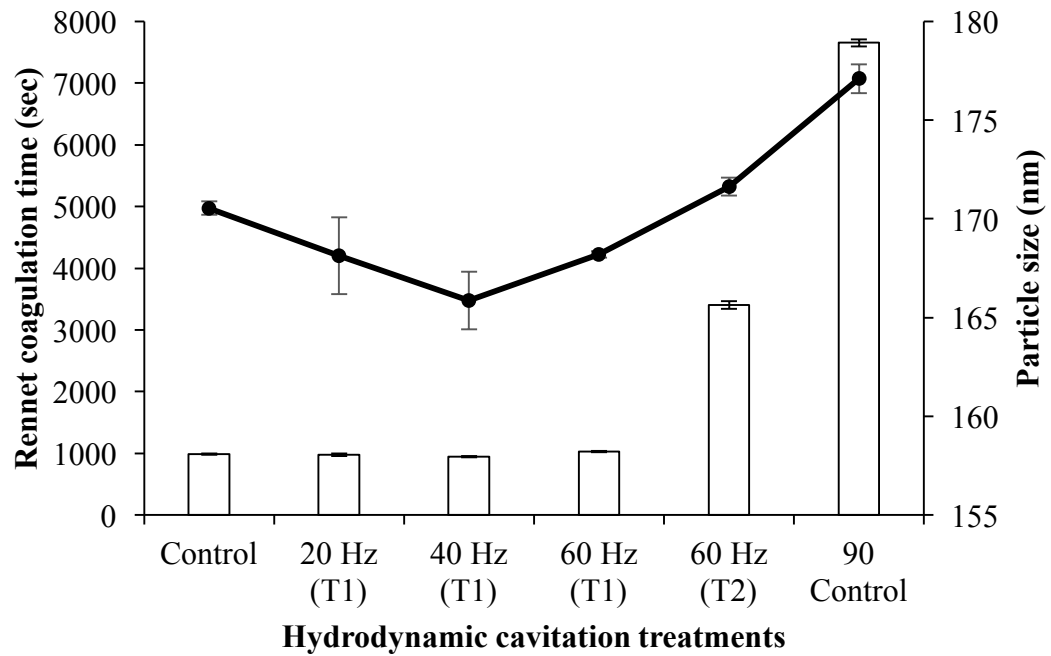


Figure 5. Rennet coagulation time of skim milk samples represented by bars on primary Y-axis measured in seconds and casein micelle size represented by line graph on secondary Y-axis measured as average diameter in nm. X-axis represents various HC treatments and vertical error bars represent standard deviation.

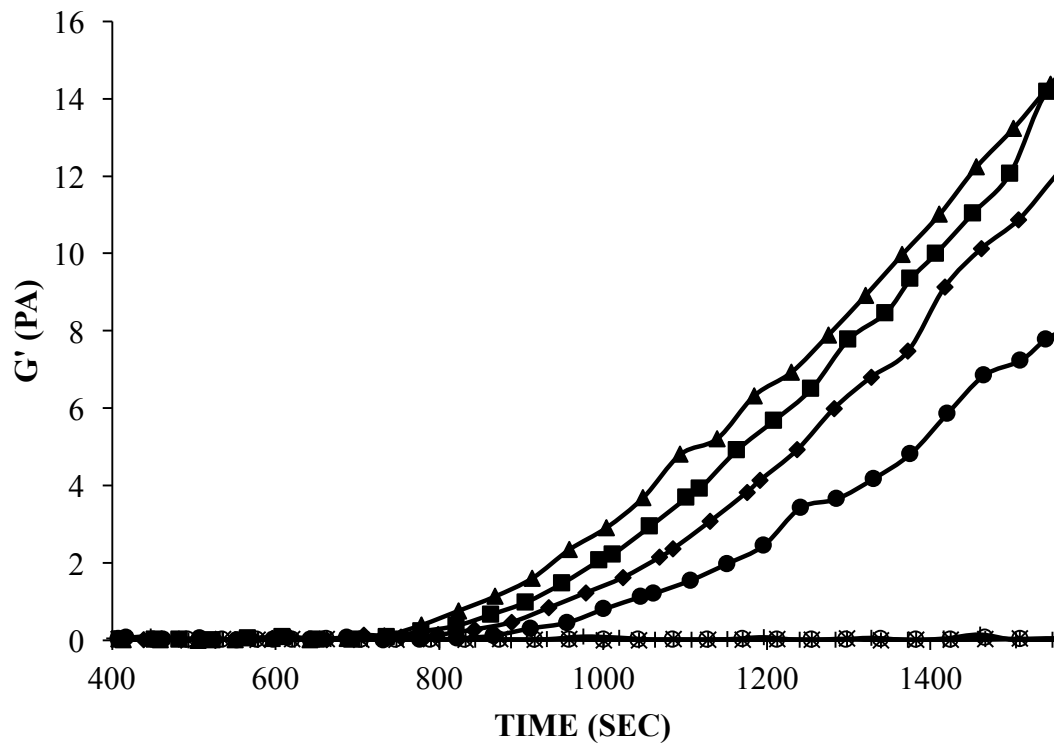


Figure 6. Elastic modulus (G') of rennet gels as a function of time during the rennet coagulation of skim milk at 38°C of control samples \blacklozenge -Control \blackcross -90 H and samples subjected to various hydrodynamic cavitation treatments, \blacksquare -20 Hz \blacktriangle -40 Hz \bullet -60 Hz \circ -60 Hz 90 C measured by small amplitude oscillatory rheology.

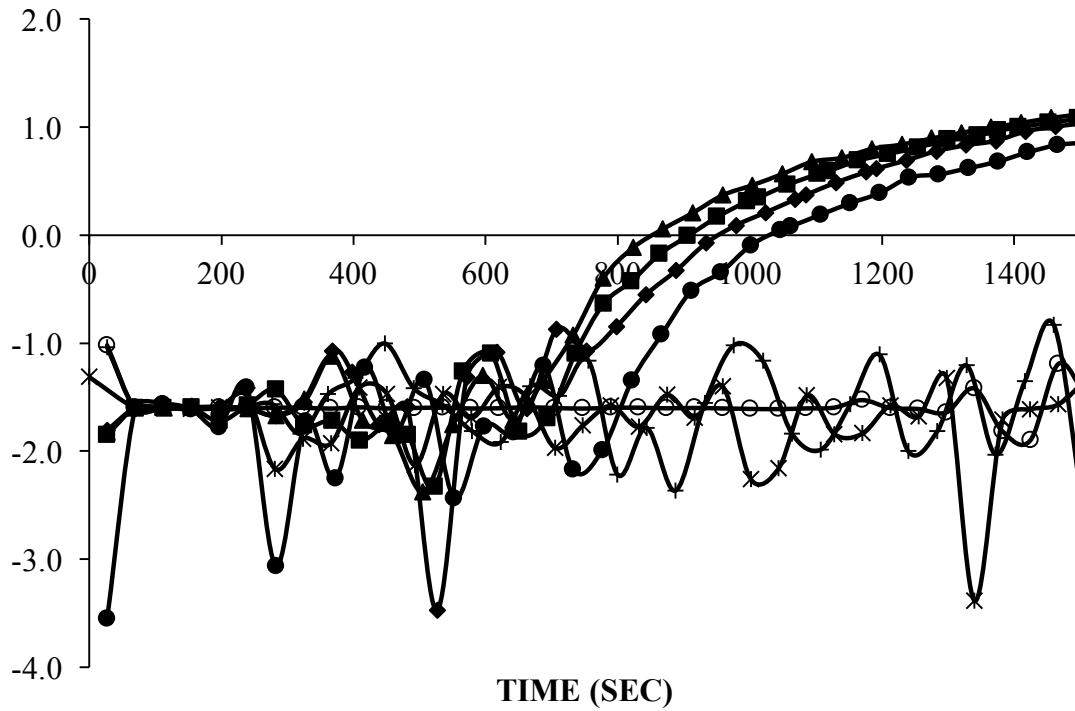


Figure 7. Log 10 of elastic modulus (G') of rennet gels as a function of time during the rennet coagulation of skim milk at 38° C of control —◆— Control —+— 90 H and samples subjected to various hydrodynamic cavitation treatments, —■— 20 Hz —▲— 40 Hz —●— 60 Hz —○— 60 Hz 90 C as measured by small amplitude oscillatory rheology.

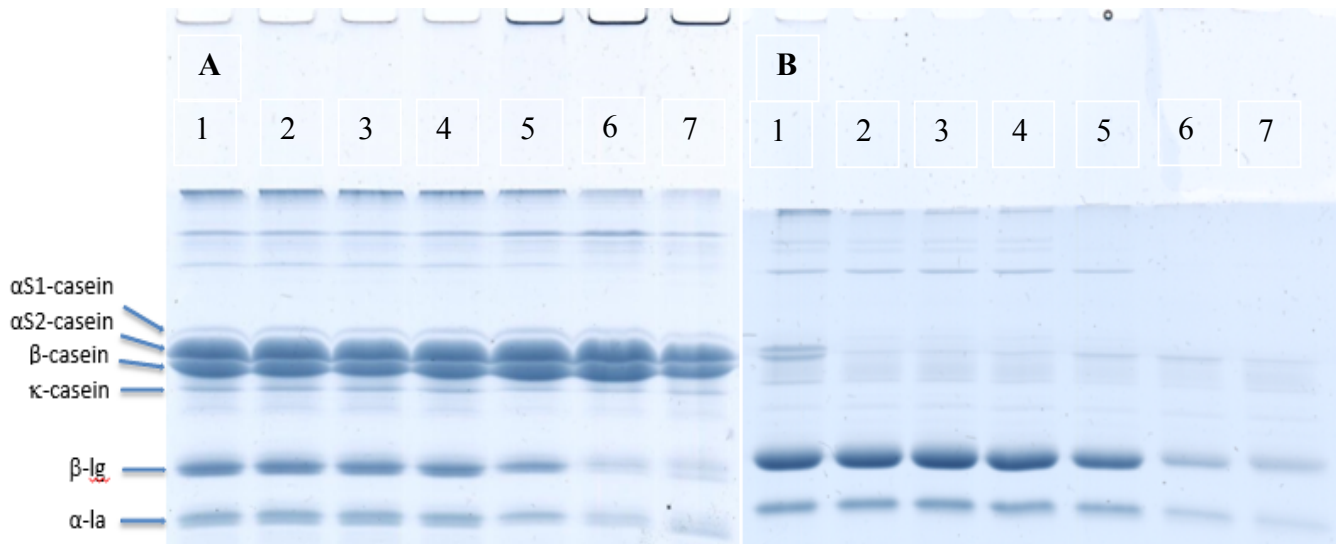


Figure 8. Non-reduced SDS-PAGE electrophoretograms of skim milk (A) and serum phase (B). Lane 1, 2, 3, 4, 6 and 7 in gel A represent unheated control, 20 Hz (T1), 40 Hz (T1), 60 Hz (T1), 60 Hz (T2) and heated control of skim milk samples respectively. Similarly lane 1, 2, 3, 4, 6 and 7 in gel B represent unheated control, 20 Hz (T1), 40 Hz (T1), 60 Hz (T1), 60 Hz (T2) and heated control of serum phase from skim milk samples respectively.

Chapter III

Effect of CO₂ injection on protein interaction to reduce viscosity of high solids skim milk concentrate

ABSTRACT

Viscosity of skim milk concentrates (SMC) is an important property that constraints the extent of concentration obtained during evaporation and leads to problems like increased tube fouling, reduced heat transfer, and shorter run time. The overall process to manufacture nonfat dry milk (NFD) can be made more efficient if more water is removed in evaporator rather than spray dryer and to make that feasible it is important that the viscosity of skim milk concentrate (SMC) is controlled when total solids increase beyond 50%. Therefore the objective of the present experiment was to investigate the effect of carbon dioxide (CO₂) injection as an intermediate processing step to lower the pH of skim milk concentrate and decrease the viscosity of higher solids SMC. In the pilot plant scale experiment skim milk was concentrate to 30% total solids (TS) SMC and stored overnight at 4 °C or below before being injected with CO₂ next day to bring down the pH to 6.05 from and initial pH of 6.5. Following carbonation the SMC was further concentrated in a pilot plant scale single effect falling film evaporator to 55-57% TS and a portion of this high solids concentrate was dried on pilot plant scale Niro dryer to obtain nonfat dry milk which was analyzed for various functional properties. Prior to pilot plant scale experiment a lab scale experiment was conducted to prove the concept. SMC

was with 30 % TS was treated with CO₂ to decrease pH from 6.5 to 6.05 and then was concentrated to 55% TS using a rotary evaporator under vacuum at 70 °C. Apparent viscosity of the final concentrate in both the experiments was measured immediately after concentration using an ATS Stresstech Rheometer at 55 °C with shear rates from 50 s⁻¹ to 1050 s⁻¹ with 50 s⁻¹ ramp. NFDM obtained from control (untreated) concentrate and from CO₂ treated concentrate were analyzed for loose, packed and final bulk density, solubility index and heat stability of reconstituted powder solutions. The experiments were repeated at least twice and all results were analyzed for statistical significance (at $p \leq 0.05$) using SAS software. Significant difference ($p \leq 0.05$) in apparent viscosity was observed between CO₂ treated and control SMC samples. Concentrate obtained from CO₂ treated SMC sample had significantly ($p \leq 0.05$) lower viscosity compared to that obtained from untreated control sample. NFDM produced from concentrate that was CO₂ treated had higher loose, packed and final bulk densities, improved solubility, better heat stability on reconstitution compared to NFDM obtained from control concentrate with no CO₂ treatment. In conclusion CO₂ injection can be used as a clean label treatment to control viscosity of highly concentrated SMC to eventually improve efficiency of drying process while producing NFDM with optimal functional properties.

Key words: Skim milk concentrate, viscosity, CO₂ injection

INTRODUCTION

Production and export of US nonfat dry milk (NFDM) continues to increase year on year with US accounting for nearly a quarter of world's NFDM production (USDEC). Roughly 50% of NFDM produced in US is exported with an aim to fulfill ever increasing demand of US produced NFDM in global export market. However there are challenges as well with this huge opportunity which include consistency in quality and functional properties of NFDM at a competitive price point. Apart from big use of NFDM in making recombined milk and infant formula other uses include bakery, confectionary, beverage formulations, meat products, soup, sauces, prepared mixes and other food dishes. The terms nonfat dry milk and skim milk powder (SMP) are used interchangeably with the two products being very similar, however their compositional definition is regulated by two separate authorities (USDEC). SMP is defined by CODEX Alimentarius and NFDM is defined by US Food and Drug Administration (FDA). Both NFDM and SMP are manufactured by removing water from pasteurized skim milk and should contain 5% or less moisture by weight and 1.5% or less milk fat by weight, the difference being that SMP should have a minimum of 34% milk protein while there is no such standard for NFDM.

Two basic unit operations involved in manufacture of NFDM are evaporation and drying. Evaporation remains most common operation for water removal in dairy industry with multiple effect falling film evaporators being the most popular as they are easy to maintain, provide superior energy efficiency and can be operated at lower temperatures (Caric, 1994). Evaporation is far more energy efficient process to

remove water and can be 6-12 times more efficient in removing same amount of water in comparison to spray drying depending on number of effects of falling film evaporator and use of thermal vapor recompression or mechanical vapor recompression. Milk is a Newtonian liquid and casein micelles are the main contributors to viscosity of skim milk (Walstra and Jenness, 1984). With removal of water and increase in total solids (TS) of skim milk the viscosity increases nonlinearly. At concentrations higher than 45% the viscosity increases many folds with a small increase in TS and with further increase in concentration the flow behavior of skim milk concentrate (SMC) transitions from Newtonian to non-Newtonian (Walstra and Jenness, 1984, Prentice, 1992, Bienvenue et al., 2003, ENRÍQUEZ-FERNÁNDEZ et al., 2013). Increased viscosity limits the extent of water removal in evaporators as it causes several problems like decreased flow rate and heat transfer leading to tubes fouling thereby decreasing machine uptime. Therefore reducing the extent of increase in apparent viscosity of SMC can enable manufacturers to remove more water in evaporators while still maintaining quality of product thus helping them to become more efficient in producing NFDM. Bloore and Boag (1981) stated following to be main factors influencing SMC viscosity, total solid, temperature, protein content, preheat treatment and holding time. Therefore controlling these factors can serve as a tool to control overall viscosity of SMC. Casein micelles are the main contributors to viscosity in skim milk systems (Walstra and Jenness, 1984). Snoeren et al. (1981) also reported that it is the volume fraction of casein micelles that has major effect on viscosity of SMC and viscosity of

continuous phase has minor effect on viscosity. Hence any strategy that can control the voluminosity of casein micelle would work to reduce the viscosity of high solids SMC. So far researchers have used several techniques to decrease viscosity of SMC which include decreasing mineral content of milk (Bienvenue et al., 2003), use of ultrasound treatment (Zisu et al., 2013), adjusting pH of milk prior to heating (Anema et al., 2014, Sutariya, 2015) and treatment with CO₂ followed by decarbonation (Lee, 2014), however most of these studies are not done on high solids concentrates. This study is an extension of our previous work done in lab by Sutariya (2015) to demonstrate the effect of pH of milk at preheating on the viscosity of high solids concentrate and was aimed at scaling up of those strategies developed at lab scale to pilot plant scale and study their feasibility. The other objective of the study was to explore use of CO₂ injection replacing use of hydrochloric acid as a method to modify pH of skim milk concentrate to decrease viscosity of high solids skim milk concentrate.

Materials and Methods

Preparation of skim milk concentrate

12000 pounds of pasteurized skim milk with ~10% totals solids (TS) was sourced from Davis Dairy Plant, South Dakota State University (SDSU), Brookings, SD for each experiment and the experiments were conducted using the evaporator in Davis Dairy Plant. The first step involved concentration of skim milk to obtain SMC with 30% TS which was then concentrated further in the next step to high solids

SMC with 56-57% TS and served as control or CO₂ was injected in the concentrate obtained in the first concentration to lower its pH to 6.05 and then it was concentrated further in the second concentration to high solids SMC with 56-57% TS and served as treated sample. 4 liters of high solids SMC was collected and dried on the NIRO dryer to obtain NFDM from high solids concentrate which was further analyzed for bulk density, solubility index, heat stability on reconstitution and WPNI.

CO₂ injection to lower the pH

Skim milk concentrate with 30% TS was stored overnight at 4 °C or below in a jacked tank cooled with glycol supply. CO₂ was injected using a sparger connected to a carbon dioxide cylinder through a flow meter, fitted in a line that formed a closed loop with a centrifugal pump and tank in which SMC was continuously recirculated. CO₂ was injected at an injection pressure of 5.5×10^5 N/m² maintaining a flow rate of 3.40 cubic meter/hour till the pH was lowered to 6.05 from an initial pH of 6.5. Following this treatment SMC was concentrated to 55% TS in a single effect falling film with four passes under 71114 Pa vacuum at 80 °C. A portion of high solids SMC was spray dried into NFDM using a pilot scale dryer supplied by Niro (ASO412E, Niro Inc., Columbia, MD, USA) keeping 195 °C inlet air temperature and 90 °C outlet air temperature. SMC samples were analyzed immediately after evaporation for viscosity and NFDM samples were stored in sealed bags before further analysis.

Lab scale experiments

Before plant scale experiments were conducted to investigate the effect of carbon dioxide (CO₂) injection as an intermediate processing step to lower the pH of skim milk concentrate and decrease the viscosity of higher solids SMC, lab scale study was done to prove this concept on a small scale. 20 liters of SMC with 30% TS at pH 6.5 was warmed to 55 °C and CO₂ was injected using a sparger fitted in a line that formed a closed loop with a positive displacement pump and a conical hopper for recirculation. CO₂ was injected at an injection pressure of $5.5 \times 10^5 \text{ N/m}^2$ maintaining a flow rate of 0.09 cubic meter/hour till the pH was lowered to 6.05. Following this treatment SMC was concentrated to 55% TS using a rotary evaporator under vacuum at 70 °C.

Viscosity

Viscosity analysis was performed on all SMC samples using a Stresstech rheometer (ATS rheosystems, Rheological Instruments Inc., NJ, USA) employing the method described by Zisu et al. (2013) with some modifications. Analysis was carried out at 55° C a temperature that is very similar to the temperature of last effect or finisher of the evaporator using a shear rate profile from 50 – 1050 s⁻¹ increasing shear rate by 50 s⁻¹ every 10 seconds in a ATS Stresstech rheometer selecting CC25 CCE SS geometry setting while using cup and bob attachment. Samples were tested for viscosity as soon as they are drawn for accurate results and to avoid cooling of samples or to allow age-thickening to set in.

Loose, packed and final bulk density

Bulk density cylinder with a known volume of 100 ml was weighed for the empty weight. The cylinder is then filled up to the rim with powder and weighed again. This weight divided by the cylinder volume gives the loose bulk density of the powder. The cylinder top is sealed using a paraffin film to make sure no powder falls out when the cylinder is tapped and then tapped on a rubber mat for 10 times. The new volume that the powder occupies after 10 tapping is noted and initial weight of powder divided by this volume gives packed powder bulk density. The cylinder is further tapped 90 times and the new volume that the powder occupies now is noted. Final powder bulk density was calculated by dividing initial weight of powder by the final volume after tapping 100 times total.

Solubility index

The American Dairy Products Institute (ADPI) method to determine insolubility index was used to test the solubility of NFDM obtained from CO₂ treated and control high solids concentrates. 8 g of sample was weighed and added to 200 ml of distilled water at 70° C in the mixing bowl of KitchenAid 10 speed mixer. 4 drops of anti-foaming solution were also added to avoid excessive foam formation. Mixer was switched on at #2 speed setting and power was mixed for 90 seconds. The solution was allowed to stand for 5 minutes and then thoroughly mixed with spoon before transferring in conical centrifuge glass tubes for solubility index measurement. Tubes were filled up to the 50 ml mark and centrifuged 980 rpm for 5 minutes. After centrifugation for 5 minutes supernatant liquid was poured off till 5

ml above the sediment level making sure that the sediment is not disturbed. 25 ml of distilled water at 70° C was added to the tubes and tubes were shaken to disturb and mix the sediment. Wires were used in case sediment was not dislodged by shaking. Tubes were filled up to 50 ml mark by adding more distilled water at 70° C. Contents were shaken, mixed thoroughly and tubes were again centrifuged at 980 rpm for 5 minutes. Tubes were taken out of centrifuge and read for milliliters of sediment, with upper level of sediment read to the nearest graduation on tube and solubility index expressed as milliliters of sediment.

Heat coagulation time (HCT) test

Heat stability of the reconstituted NFDM solution can be determined by measuring the heat coagulation time (HCT) as it is directly correlated with heat stability, with a higher value of HCT representing higher heat stability of a sample and vice versa. Heat stability of freshly reconstituted solution prepared from NFDM from CO₂ treated SMC and that from control SMC was determined by measuring HCT of the reconstituted skim milk samples in an oil bath maintained at 140° C using the method reported by Dissanayake and Vasiljevic (2009). 3 ml reconstituted skim milk samples were poured in 8 ml autoclavable glass tubes (Wheaton, 17 mm outer diameter, 63 mm height), tubes were screw capped airtight and clamped on the rocker. Roker was switched on and set at speed 3 and the tubes were allowed to submerge in heated oil maintained at 140° C while constantly rocking. Tubes were continuously observed for development of first signs of visible precipitation or

coagulation and the total time elapsed between placing the samples in oil bath and onset of precipitation or coagulation represented HCT.

Statistical Analysis

Statistical analysis of data was done using SAS (Version 9.3, SAS Institute Inc. Cary, NC, USA). Data were tested for t-test and results from samples were considered significant with $p \leq 0.05$.

Results and discussion

Viscosity

At 50% solids and above the skim milk concentrate exhibits shear thinning behavior and follows power law model i.e. as the shear rate increases viscosity decreases (Velez-Ruiz and Barbosa-Cánovas, 1998). Figure 1 shows the apparent viscosity of 57% TS skim milk concentrate for both CO₂ treated and control samples measured at 55 °C as a function of shear rate. The apparent viscosity decreased for both the samples as the shear rate increased however the rate of decrease initially was higher for control SMC samples which also had significantly ($p \leq 0.05$) higher initial viscosity compared to CO₂ treated SMC samples. CO₂ treated SMC samples exhibited a significant ($p \leq 0.05$) decrease in viscosity compared to control SMC samples at all shear rate points. Figure 2 shows apparent viscosity results for 55% TS concentrate from both CO₂ treated and control samples. Intermediate injection of CO₂ to lower the pH of SMC was able to reduce the viscosity of SMC at this solids percentage as well proving that it is an efficient method to keep the viscosity of high

solids SMC in check. As discussed in the above section that lab scale experiments were conducted to prove this concept before studying its feasibility at the plant scale level. Figure 3 exhibits the apparent viscosity of SMC from control and CO₂ treated concentrates and a significant ($p \leq 0.05$) decrease in apparent viscosity of high solids (57% TS) SMC after CO₂ treatment is observed at all shear rates values. Sutariya (2015) worked in lowering the pH of skim milk before concentration and reported similar decrease in viscosity of SMC although in his study it was skim milk pH that was decreased to 6.5 from 6.7 and hydrochloric acid was used as an acidulant to lower the milk pH. Use of CO₂ as an acidulant has been tried by some researchers as there are advantages in using CO₂ because residual CO₂ can be easily removed by applying heat or vacuum (Marella et al., 2015). Lee (2014) reported reduction in viscosity of SMC from obtained by reconstitution and ultra-filtration (UF) after CO₂ was injected and subsequently removed. Similarly UF retentate viscosity was reduced using CO₂ injection during production of milk protein concentrate (Marella et al., 2015). Hence the technique to inject CO₂ in skim milk concentrate to reduce the viscosity can be crucial method to remove more water in evaporator thus making the manufacturing process of NFDM more efficient.

Loose, packed and final bulk density

Bulk density is an important property of milk powders and plays an important role in storage, handling and transportation of the product, therefore it becomes even more important if the powders are to be exported across the globe. Factors that determine bulk density are particle size of powder, internal porosity of powder

particles and arrangement of particles (Sharma et al., 2012). Figure 4 shows loose, packed and final tapped bulk density of NFDM samples from high solids SMC produced by CO₂ injection and NFDM made from control concentrate. CO₂ injected SMC produced NFDM with higher loose, packed and final bulk density. The difference between the bulk densities for two powders was highest for packed bulk density and the difference was least for final bulk density. Lee (2014) also reported increase in bulk density of powder made from CO₂ treated concentrate compared to powder made from control concentrate.

Solubility index

Solubility is an important functional property of all dried dairy ingredients that determines the ease of reconstitution thereby affecting the processing time and processing losses (Sharma et al., 2012). Figure 5 shows that solubility index of powder samples from high solids SMC produced by CO₂ injection and powder made from control concentrate. Powder made from concentrate that was CO₂ treated had lower solubility index compared to skim milk powder made from control concentrate which indicates better solubility of these powders. Similar reports on improvement of solubility of milk protein concentrate powders that were made from retentate treated with CO₂ was reported by Marella et al. (2015). Lee (2014) also reported improvement in wettability in powders made from carbonated concentrates compared to powder from control or untreated concentrate. One possible explanation of improvement in functional properties of NFDM produced from carbonated SMC is that CO₂ injection at low temperature increases the non-micellar casein content of

the milk (Kosasih et al., 2016) and this increase improves properties like solubility (Schokker et al., 2011). Increased porosity of powder particles when produced from skim milk concentrate that has been injected with CO₂ may be another reason that improves various functional properties of such powders. Kosasih et al. (2016) found that unlike some other researcher CO₂ injection did not improve wettability of whole milk powders. One difference here was it was whole milk powders and not SMP as reported by (Lee, 2014), the other factor that was highlighted was the temperature at which CO₂ injection was carried out also played an important role with low temperature of injection being more effective in improving functional properties of resultant powders.

Heat coagulation time (HCT) test

Heat stability of NFDM is an essential attribute especially important for applications that involve high heat treatment such as hot beverages, soups, sauces, bakery and recombined evaporated milk (Oldfield and Singh, 2005, Sharma et al., 2012). Figure 6 depicts heat coagulation time (HCT) values of powder samples from high solids SMC produced by CO₂ injection and powder made from control concentrate. NFDM made from concentrate that was CO₂ treated had higher HCT values compared to skim milk powder made from control concentrate which indicates better heat stability of NFDM produced from carbonated skim milk concentrate. There is no literature available on improvement of heat stability of milk powders by carbonation of concentrate.

CONCLUSIONS

Carbon dioxide injection as an intermediate step to reduce pH of skim milk concentrate is an effective way to significantly reduce viscosity of high solids skim milk concentrates. It is feasible to use CO₂ injection as a technique to produce high solids SMC up 57% TS in falling film evaporators while keeping the viscosity low thereby removing more water in evaporator and making the operation to manufacture NFDM more efficiently at lower cost. Apart from controlling the rise of viscosity in high solids SMC as the solids increase beyond 50% TS, the subsequent NFDM produced from carbonated high solids concentrate also had improved functional properties. NFDM from carbonated SMC had higher bulk density, better solubility and improved heat stability in comparison to NFDM made from control SMC. Therefore it can be summarized from the results that CO₂ injection not only helps control viscosity of SMC but the powder from such concentrated have improved functional properties.

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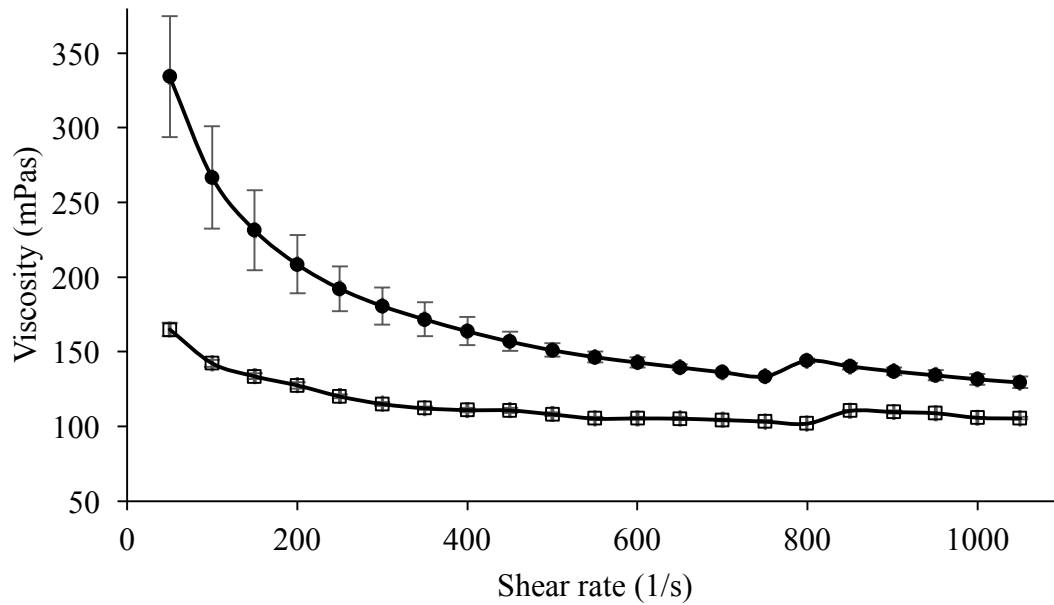


Figure 1. Viscosity of 57% total solids skim milk concentrate (SMC) measured at 55 °C immediately after concentration in plant evaporator. (—●—) represents SMC from control concentrate and (—□—) represents SMC obtained from concentrate treated with CO₂. Values are mean from four observations and vertical error bars represents standard deviation.

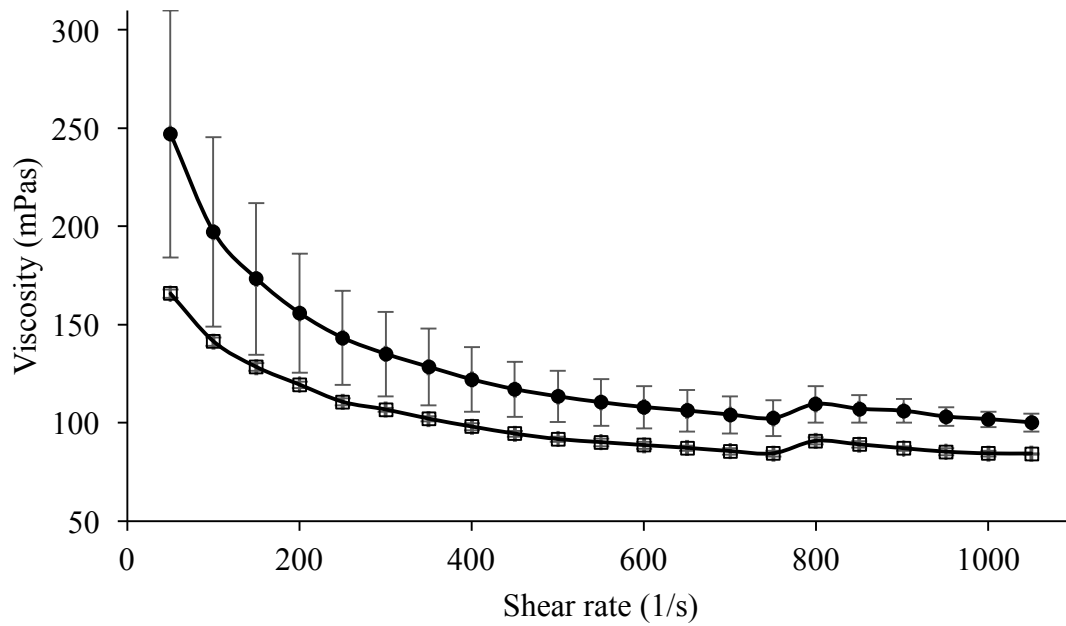


Figure 2. Viscosity of 55% total solids skim milk concentrate (SMC) measured at 55 °C immediately after concentration in plant evaporator. (—●—) represents SMC from control concentrate and (—□—) represents SMC obtained from concentrate treated with CO₂. Values are mean from four observations and vertical error bars represents standard deviation.

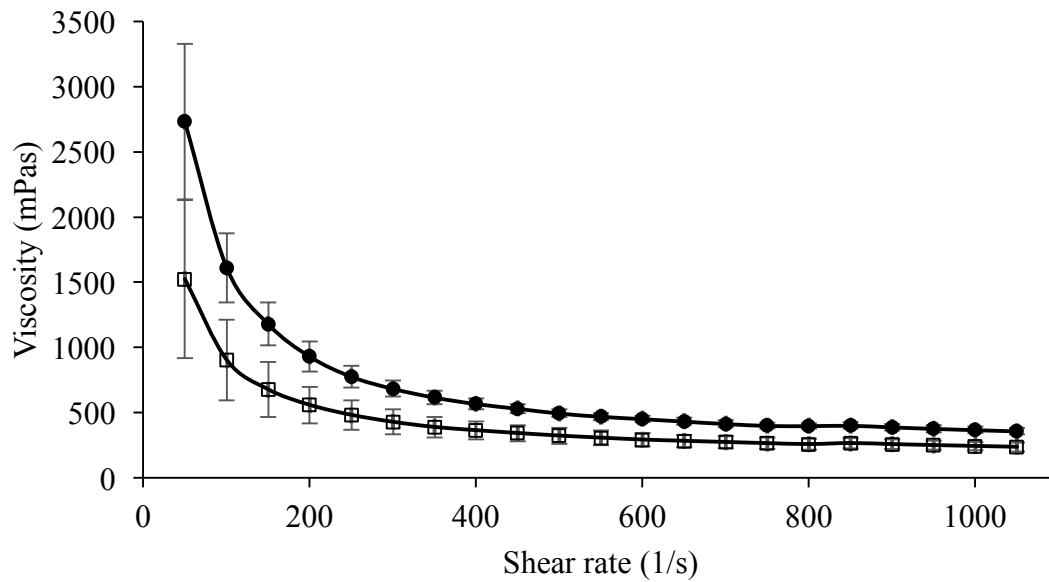


Figure 3. Viscosity of 57% total solids skim milk concentrate (SMC) measured at 55 °C immediately after concentration in rotary evaporator in lab. (—●—) represents SMC from control concentrate and (—□—) represents SMC obtained from concentrate treated with CO₂. Values are mean from five observations and vertical error bars represents standard deviation.

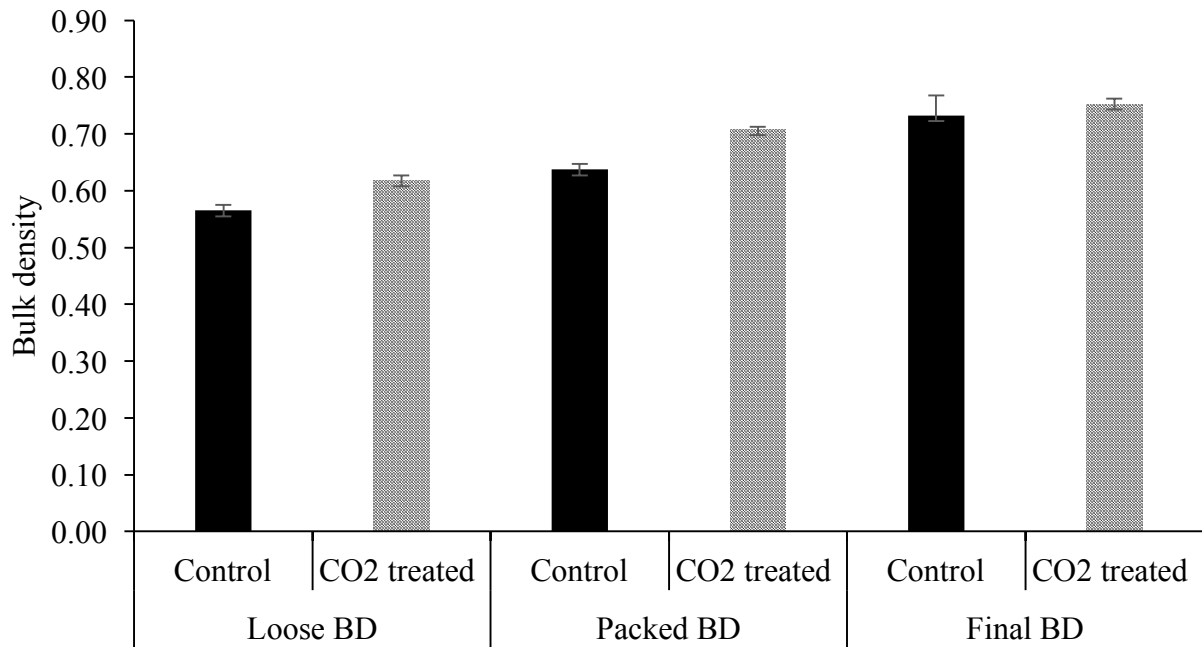


Figure 4. Loose, packed and final bulk densities of NFDM produced from control concentrate represented by (■) bar and NFDM produced from concentrate that was injected with CO₂ represented by (▨).

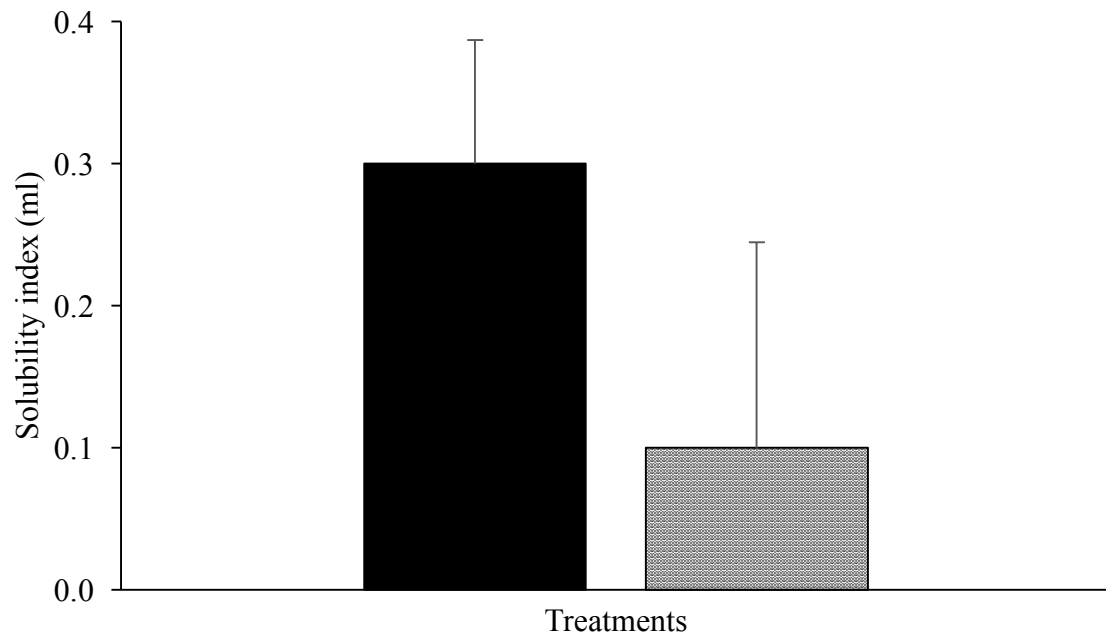


Figure 5. Solubility index of NFDM produced from control concentrate represented by (■) bar and NFDM produced from concentrate that was injected with CO₂ represented by (▨). Column height represents mean of solubility index values and the vertical error bars represents standard deviation

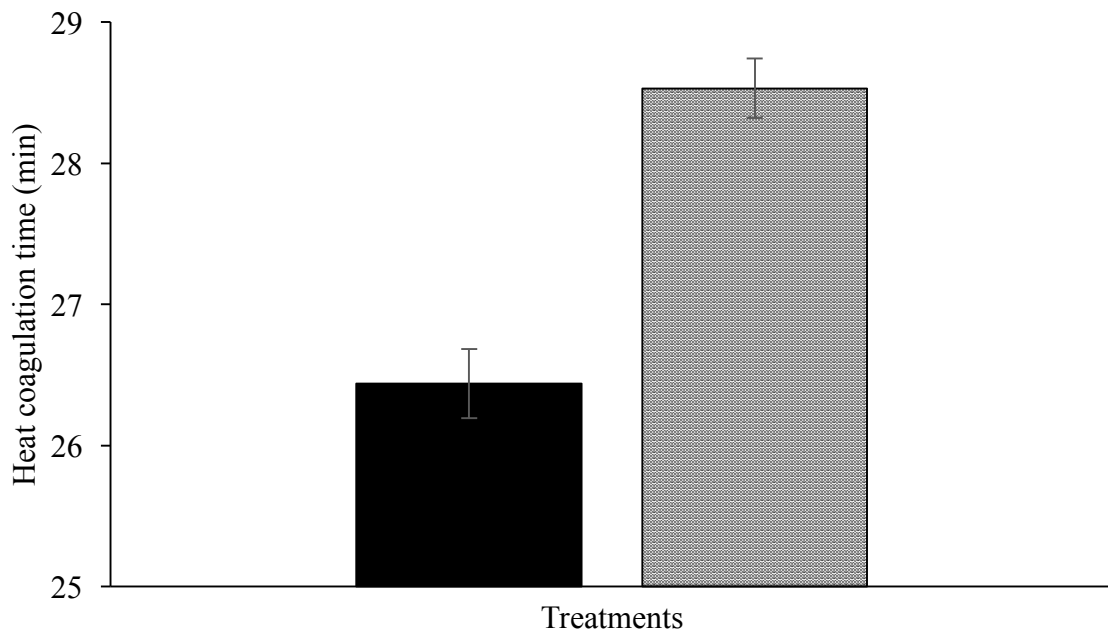


Figure 6. Heat coagulation time (HCT) reconstituted powder solutions made from NFDM produced from control concentrate represented by (■) bar and NFDM produced from concentrate that was injected with CO₂ represented by (▨). Column height represents mean of HCT values and the vertical error bars represents standard deviation.

Overall conclusions

The study highlights that hydrodynamic cavitation can be an useful alternative to conventional heating with minimal deteriorating effect on milk constituents while positively affecting physicochemical properties of milk. HC had little effect on the particle size of casein micelles, although cavitation treatment with no temperature rise a small decrease in the size was observed. Higher level of β -lactoglobulin denaturation and aggregation were observed in treatment that involved simultaneous heating along with HC, however even in this case the particle size did not increase much because of the shearing action from cavitation. Lowest rennet coagulation time and highest rennet gel strength was observed in samples treated at 40 Hz, also these milk samples had the smallest casein particle size as well. The correlation between decrease in casein micelle size and lower rennet coagulation time has been documented in earlier research findings. Acid gels made from skim milk subjected to cavitation treatment which involved heating had final gel strength comparable to the gels from conventionally heated milk samples. Most of effect from HC on gel strength was attributed to the heat generated during cavitation, however this proved the fact that HC is promising technology that has a potential to be used as an alternate for scale-free heating of milk for yogurt making with minimal effect on other important milk properties.

In the second experiment carbon dioxide injection as an intermediate processing technology to modify pH of skim milk concentrate was observed to be an effective way to significantly reduce viscosity of high solids skim milk concentrates and

therefore remove more water in the evaporator. It was found to be feasible to use CO₂ injection as a technique to produce high solids SMC up 57% TS in falling film evaporators while maintaining lower viscosity hence bringing in more efficiency in manufacturing NFDM by removing more water in evaporator and less in dryer.

Additionally the NFDM produced from carbonated high solids concentrate during subsequent spray drying had better functional properties compared to NFDM made from control concentrate. NFDM produced from carbonated SMC had higher bulk density, improved solubility and heat stability in comparison to NFDM made from control SMC. Therefore it can be concluded that new processing technologies of hydrodynamic cavitation and CO₂ injection can be used to not only improve various physical properties of skim milk and skim milk concentrate respectively but also NFDM from such concentrate have improved functional properties.

Recommendations for future studies

- Application of hydrodynamic cavitation can be further explored on milk subjected to various different pretreatments, at different pH profiles and adjustment especially for rennet coagulation properties and on milk with various fortifications.
- Hydrodynamic cavitation can also be explored to study its effect on properties of skim milk concentrates and various other filtration streams.
- Viscosity reduction using carbon dioxide injection as a tool can be studied on other dairy streams obtained from filtration.