CONTINUOUS HIGH PRESSURE PROCESSING OF LIQUID FOODS: AN ANALYSIS OF PHYSICAL, STRUCTURAL AND MICROBIAL EFFECTS

by

GEORGE A. CAVENDER

(Under the Direction of William L. Kerr)

ABSTRACT

While the effects of high pressure on the safety and quality of food have been studied occasionally for over one hundred years, it is only recently that the widespread study and use of the technology has become feasible. One of the more interesting and least studied type of high pressure processing is continuous high pressure processing (CHPP), sometimes referred to as "high pressure homogenization" or "dynamic Pascalization." The current work examines the history and work in the field to date and goes on to investigate the effects of various CHPP systems on the microbial, physical, structural and sensory properties of liquid foods. Because the high shear conditions created in the release component(s) of a CHPP system generate significant heat, it has often been difficult to separate the anti-microbial effects of pressure and shear from those of temperature, particularly with regard to vegetative cells. By using a modified valve with inline cooling, the instantaneous temperature rise was dramatically lessened, showing that only a modest amount of inactivation results from the increased pressure and shear. The work further explains the effects of microfluidization, a type of CHPP system based upon a fixed geometry pressure release component, on ice cream mixes and

the ice creams made from those mixes. Microfluidization is shown to effect changes in the texture, melting properties and viscosity of finished ice cream and to thereby improve the sensory characteristics of the same. It is later shown that the changes are evoked on a microstructural level, with visible differences apparent in electron micrographs, and that these changes affect the particle size distribution, apparent viscosity and dynamic rheology with treated samples showing more uniform particle size, significantly higher viscosity and more solid-like behavior than untreated samples.

INDEX WORDS: High Pressure Processing, Microfluidization, Inactivation, Sensory, Rheology, Viscosity, Microstructure

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DEDICATION

Why this page is optional, I'll never know. I dedicate this work to those who struggle, those who strive, those who stumble and fall, to those who stand back up and keep on going and most importantly to those people, beings, ideas and things that help us along the way...

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Newton is credited with saying "**If I have seen further it is only by standing on the shoulders of giants.**" I agree fully with that sentiment and add that I also had a lot of help climbing up onto those shoulders. I'd like to thank my parents, not only for having rolled the dice and won my genome, but also for the things they did that led me to this point. So Dad, thanks for stressing the need to be educated, even if you didn't understand why I chose to keep at it for so long. And thanks for watching science fiction with me, so that I could be inspired by the idea of what the future could be. Mom, thanks for teaching me to read, and for all those hours spent ferrying me to school, or some program or the library. Both of you, thanks for all the sacrifices and know that I have no hard feelings about the mistakes you made- I hope you feel the same about my mistakes, after all it was the first time for both of us. I'd like to thank my girlfriend for standing by me these past *mumble-mumble* years and for all the support (both emotional and though it is embarrassing to admit, financial) you've given me. Thanks to the graduate faculty of the UGA food science department for taking a chance on my admission and master's bypass. I hope that you look back upon those decisions and feel it was worthwhile. Thanks to my advisor, Dr. Bill Kerr, for giving me the autonomy to pursue the project I wanted (and for somehow finding the funds to make it happen). Thanks to Dr. Rakesh Singh for helping with those funds, and for being so available anytime I had a problem. Thanks to Carl Ruiz, my compatriot in so many projects, for the help and advice you've given- even if I didn't always take it, it was nice of you to share. Thanks to Dr. Shields and Dr. Fan from

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CHAPTER 1

INTRODUCTION TO THE CURRENT WORK

The use of pressure and shear to effect changes in food has occurred for over 100 years. In 1899, the West Virginia Extension Service published a pamphlet outlining the groundwork of what would later be called High Pressure Processing (HPP). Utilizing steel pressure vessels and a hydraulic press, milk samples, sealed into lead or tin tubes were subjected to pressures of up to 90 tons (approx 800 kN) over an unspecified area for up to 60 hours or longer. The samples were then examined for evidence of spoilage and/or microbial growth. Despite the fact that milk could be kept "sweet" for several days without refrigeration, little further work was done with the technology until the 1970's and 80's when materials science had advanced to the point that the process was feasible. Even then, the systems produced were static systems, capable of only batch fed processes (Hite 1899; Trujillo, Capellas et al. 2002).

The use of shear to alter the properties of a fluid product was discovered at approximately the same time as Hite's pressure work, with Auguste Gaulin's development of homogenization as a means of preventing creaming in milk (APV 2008). Using high shear to disrupt and/or destroy cells came almost 50 years later with the invention of the French press. Named for its invention C. Stanley French, it consisted of a cylinder fitted with a plunger at the top and a needle valve at the bottom. This device would be filled with a suspension of cells or even organelles and then loaded into a pneumatic or hydraulic press. The downward force applied to the plunger increased the

pressure inside the cylinder and allowed the operator to thereby force the suspension through the narrow opening of the needle valve, subjecting it to extreme shearing (Milner, Lawrence et al. 1950).

While the groundwork of the development of continuous high pressure processing (CHPP) systems did occur in the first half of the 20th century, it has only been in the past 20-30 years that such systems have been the subject of much scientific inquiry. The subject of some of the earliest works in the field was cellular disruption and product recovery (Engler and Robinson 1981; Agerkvist and Enfors 1990; Keshavarz, Bonnerjea et al. 1990). The increased efficiency of the cellular disruption caused by the CHPP processes led to work on microbial inactivation, which has become one of the two general areas of inquiry into the process. The other area of inquiry is influenced more by the traditional homogenizer, that being the study of physiochemical changes.

The current work seeks to better understand CHPP, both in its ability to reduce microbial load and its ability to effect changes to the physical and sensory attributes of food. The work is divided into six chapters, including this introduction. The second chapter is a comprehensive review article devoted to CHPP, first defining the process, components and mechanisms of action and then looking chronologically at publications dealing with microbial changes or physiochemical changes.

The third chapter examines the contributions of temperature and pressure release component to microbial inactivation. While continuous high pressure processing is often though of as a non-thermal process, previous studies have shown considerable rises in process fluid temperature during CHPP (Moorman 1997; Lagoueyte and Paquin 1998; Kheadr, Vachon et al. 2002; Datta, Hayes et al. 2005; Diels and Michiels 2006; Zamora,

Ferragut et al. 2007). These authors sought to reduce the effects of this temperature rise on microbial load through the use of heat exchangers, but the product still spends several seconds at elevated temperatures, often at or above flash pasteurization temperatures. Further, little work has been done comparing the effects of different release components on inactivation. The article compares different pressure release mechanisms, both with and without active cooling of the valve body. Without cooling the temperature rises were found to be high enough to have been solely responsible for the inactivation seen. (Cavender and Kerr 2011) In the trials performed with cooling, the levels of microbial inactivation were more modest, ranging from a low of 2.04 log reductions for *Listeria innocua* in milk to a high of 5.3 log reductions for *Escherichia coli* in tryptic soy broth.

The fourth chapter examines the effects of microfluidization, a type of CHPP, on the physical properties of ice cream made with differing gum stabilizers. Previous works have shown that CHPP can change the structure and properties of various ice cream components, including stabilizers but have not examined how the changes to the properties of the mix and subsequent ice cream may differ if different stabilizers are used (Mozhaev, Heremans et al. 1996; Mussa and Ramaswamy 1997; Lagoueyte and Paquin 1998; Paquin 1999). The article looks at ice cream made with 0.2% of either xanthan or locust bean gums and how the microfluidization of the mixes will affect the sensory and physical properties of the final product. Changes were seen in sensory perception between microfluidized and untreated ice cream, as well as changes in the texture profile and mix viscosity (Cavender and Kerr 2011).

The fifth chapter further examines the changes seen in the fourth chapter by means of dynamic rheology and electron microscopy. Both the liquid mixes and ice

cream made from them were analyzed, and definite changes were seen between treated and untreated samples of each formulation. Microfluidization was found to develop a more complex structure in the mixes as measured rheologically and this is thought to be due to changes in the structure of casein-gum complexes as were seen using transmission electron microscopy. In frozen ice cream, rheological measurements showed that the microfluidization of the mixes changed the overall properties in a similar manner to the use of additional stabilizers. Cryo-scanning electron microscopy saw interesting differences in ice cream made with treated and untreated mixes, with ice cream made from microfluidized LBG mix having similar structure to those made from untreated Xanthan mixes. Further the ice cream made from treated xanthan gum showed larger voids, which implied that the treatment had increased the viscosity of the mix to a detrimental point.

The final chapter summarizes the important findings of the work and makes several conclusions based upon what was found in the above studies. It also offers insight into future areas of research opportunity and speculates as to how such areas might best be explored.

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CHAPTER 2

CONTINUOUS HIGH PRESSURE PROCESSING OF LIQUID FOODS: A REVIEW [1]

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Abstract

Continuous high pressure processing (CHPP) is subset of high pressure processing. CHPP is an emerging technology in which fluid products are pumped through a system which pressurizes them in excess of 100 Mpa by means of one or more intensifiers. The fluid is subsequently passed through a pressure release component, where, depending upon the geometry of the component, the fluid is subjected to shearing, cavitation and/or frictional effects. These effects, and the effects of the initial pressurization can contribute to microbial inactivation as well as changes in the functional properties of the fluid components. In this work we examine the body of work to date dealing with CHPP, focusing upon studies which examine the effect of the technology on either a) microbial populations or b) physiochemical properties.

Introduction:

The fact that high pressures can affect the properties of food has been known for a very long time. As far back as 1899, scientists at West Virginia University experimented with the use of high pressure as a preservative process of milk (Hite 1899). Much time was spent developing an ideal vessel for such a process, with the final design consisting of a water filled steel chamber, fitted with a deformable lead plug which was compressed using a hydraulic ram. Milk samples, sealed in tin paint tubes were subjected to treatment with extreme pressure (using up to 800 kN of force over an unlisted cross section) could be kept "sweet" for several days absent refrigeration, and by adding a moderate amount of heat this storage time was increased . Despite this early discovery, little further work was undertaken examining high pressure processing. Several other authors have commented on this phenomenon, citing the need for metallurgical and technical advances which have only recently become available (Farkas and Hoover 2000; Rastogi, Raghavarao et al. 2007; Balasubramaniam, Farkas et al. 2008). Only in the past 30 years has the field blossomed, as a simple search of online article databases clearly shows. Searching EBSCO host (http://www.ebscohost.com/) for titles containing "high pressure" and "food" shows 412 articles, excluding those which reference chromatography or blood pressure. Of those results, 400 have been published in the last 30 years, with the vast majority of those being published within the last ten years. Searches on several of the other major scientific publication databases net similar results, as can be seen in Figure 2.1.

The simplest type of high pressure processing is a static system. These systems are essentially refinements to the basic design put forward by Hite in 1899- a pressure

vessel into which food is placed (often pre-packaged) which is then filled with water or some other fluid medium and brought to pressure by mechanical means. The process applies pressure to the food isostatically and creates only a modest (approximately 3 °C/ 100 MPa) temperature rise during the pressure cycle due to adiabatic heating. Changes induced in the processed food are dependant on temperature, time and pressure, and the process itself is a batch process (Farr 1990; Farkas and Hoover 2000; Balasubramaniam, Farkas et al. 2008). While various multi-chamber or multi-unit configurations have been designed in order to make a particular high pressure process line behave in a continuous or semi-continuous manner, albeit at an increased cost due to the additional components (San Martin 2004).

While considerable scientific study and industrial innovation have been seen in recent years with regard to static high pressure systems, the design and study of continuous systems have seen less advancement. This lack of progress is surprising, considering that knowledge of the effects of pressurized pumping of liquid foods on their quality dates back almost as far as the initial work on static high pressure. One can even consider the work done by Auguste Gaulin, culminating with his demonstration of the homogenizer at the 1900 World's Fair in Paris, as the beginning of the field (APV 2008). Homogenization was quickly adopted by the dairy industry, seeing continual improvements in the intervening century; however it was only recently that scientists began looking at the modifications to those devices necessary to develop true continuous high pressure processing systems.

This review presents an overview of the common system components of continuous high pressure processing systems, including an examination of several widely

available systems and examines the microbial and physical effects of continuous high pressure processing of food, food ingredients and analogs. Static high pressure processing is not examined in this review, as it is outside the scope of this work, and it has been the subject of several excellent comprehensive review articles. The "further reading" section of this work lists a selection of publications which may be helpful for those interested in static high pressure processing.

Common Definitions:

Before any meaningful discussion can be had on an emerging field of study, the terms which will be used must be defined. When considering continuous high pressure processing, the following terms are of note:

High Pressure Processing (HPP): Processing which uses pressures in excess of 100MPa (Farr 1990; Moorman 1997; Farkas and Hoover 2000)

Continuous High Pressure Processing (CHPP): Processing of pumpable products using high pressures in a continuous or pulsed flow scheme (Farkas and Hoover 2000).

(Ultra) High Pressure Homogenization (U/HPH): Another name for *Continuous High Pressure Processing*

Microfluidization: A specific type of CHPP involving the use of a particular style of fixed geometry interaction chamber as a pressure release component. The geometry of the chamber is such that it separates fluid flow into two channels and then recombines them at a 180° angle.

Pressure Release Component: A critical part of a CHPP system taking many forms, its primary function is to serve as a point of transition between areas of high pressure and areas of lower pressure. Typical configurations are valves of various geometry or fixed geometry interaction chambers (Moorman 1997; Paquin 1999; Peck 2004; Diels and Michiels 2006; Pereda, Ferragut et al. 2007).

Single Acting Intensifier: A type of hydraulic or pneumatically driven positive displacement pump which operates by intermittently filling and emptying a fixed volume space (Hawkins 1905).

Dual Acting Intensifier: A type of hydraulic or pneumatically driven positive displacement pump which operates by intermittently filling and emptying two disparate fixed volume spaces in an alternating fashion.(Hawkins 1905)

Check Valve: A valve which permits flow in only one direction. It may be passive, and thus will permit any forward flow above a given threshold, or actively controlled, and thus only permit forward flow if opened (Parr 1998).

Components of a CHPP System

At their very core, CHPP systems are nothing more than a collection of components designed to perform the unit operation of pumping. Diels and Michiel (2006) even claim that the only required components of a CHPP system are a positive displacement pump and a valve. That said a CHPP system typically consists of a product inlet, an array of one or more intensifiers, one or more check valves and a pressure release component. A basic schematic is shown in Figure 2.2.

Product inlets fill the intensifiers and require some pressure differential across them. This pressure differential can come from gravitational potential energy, compressed air, a pump or even the action of the intensifiers. The only real requirement is that the differential is sufficient enough to allow flow to bypass the inlet check valves.

Check valves are needed to prevent backflow, either from the intensifier to the product inlet or, in the case of multiple or dual-acting intensifiers, from one intensifier chamber to another. The simplest type is a passive check valve, which will allow almost any forward flow (above a certain threshold). While simple to maintain and requiring no complex control, passive check valves nonetheless have one glaring problem- small pressure differentials across them can sustain limited flow. Thus, product could exit the system without reaching the process pressure, even if the system is disengaged. Active check valves are more complex, and require carefully timed control, but have the benefit of allowing flow only when engaged.

One of the most important parts of a system is the intensifier array. Several common configurations exist: a single single-acting intensifier, a single dual-acting intensifier and multiple single-acting intensifiers. Each configuration will have a different pressure profile; a general graphic overview of the possible profiles is shown in Figure 2.3.

Single single-acting intensifier based systems produce an intermittent or pulsed flow, which leads to a saw toothed pressure profile, where the pressure of the processed fluid goes from inlet pressure during the intake stroke to maximum pressure during compression stroke. This maximum pressure is maintained only for an instant, as the

fluid begins escaping through the pressure release component, ending at zero pressure before starting a new cycle.

In contrast, a single dual-acting intensifier based system, will alleviate this saw toothed profile somewhat, as one chamber is always filling as the other empties, but there still are pressure dips in between compression strokes. Additionally, these systems will require four check valves to isolate each compression chamber from its counterpart as well as from the product inlet.

While more complex, a system utilizing two single acting intensifiers and coupling them with active, rather than passive check valves, allows for creation of a 3-part cycle in each cylinder (intake, compression, exhaust). By adjusting the timing of the cycles, one can ensure that the actual pressure dip is negligible or even nonexistent.

The final and potentially most important component of a CHPP system is the pressure release component. Pressure release components take multiple forms, but all are designed to restrict the flow of fluid. This restriction results in an increased average particle velocity, which in turn results in high shear and potential impingement upon either the body of the component or another fluid stream (Lagoueyte and Paquin 1998; Floury, Bellettre et al. 2004). The fluid then enters an area of greater volume, which can result in cavitation, which while classically viewed as an undesirable phenomenon, is actually believed to be one of the principal mechanisms involved in the changes induced by CHPP (Shirgaonkar, Lothe et al. 1998). Individual pressure release components are typically either variable geometry valves or fixed geometry chambers. Figure 2.4 shows schematics of several popular pressure release components, and Table 2.1 presents a summary of the component configurations of some commonly used commercial systems.

Mechanisms Involved in CHPP

Continuous high pressure systems effect changes in foods through a combination of several phenomena. These phenomena are all related to the mechanics of the system, particularly the fluid mechanics, and are therefore all interrelated to some degree. The most relevant phenomena involved are shear, cavitation, pressure, adiabatic heating/cooling and fluid friction.

Shear: The definition of a shear force is one that acts tangentially to the surface of a sample (Munson, Young et al. 2002). Shear forces cause a deformation of the sample, which is measured by a property called strain. In fluid samples, this deformation is not recovered; making it is more useful to consider the change in position of a given element over time, which we call fluid velocity. Fluid velocity is not uniform, due to the properties of the fluid and the no-slip boundary condition. The gradient of this velocity across the distance from a boundary is called shear rate. In a continuous system, as the distances from a boundary change (due to constrictions or expansions in a flow path) the velocity will change inversely, as will the shear rate.

Cavitation: Cavitation is an evaporation/ condensation phenomenon. As mentioned above, constrictions and expansions in the flow path of a fluid will cause an increase or decrease in velocity, respectively. From the Bernoulli equation (1), we can see that an increase in velocity will result in a drop in pressure.

$$\frac{\rho v_1^2}{2} + gz_1 + \frac{P_1}{\rho} = \frac{\rho v_2^2}{2} + gz_2 + \frac{P_2}{\rho} \quad \textbf{(1)}$$

This drop in pressure can be sufficient to lower the boiling point of the liquid enough that it spontaneously boils, forming bubbles. A specialized form of the Euler Number, called

the cavitation number (2) is used to predict this behavior, with P_2, P_{vap} and v_{max} being the downstream pressure, vapor pressure of water and maximum fluid velocity respectively (Shirgaonkar, Lothe et al. 1998; Munson, Young et al. 2002).

$$C_v = \frac{P_2 - P_{vap}}{\frac{1}{2}\rho v_{\max}^2} \quad \textbf{(2)}$$

Systems with cavitation numbers less than or equal to one tend to favor the phenomena, although in some cases even systems with values greater than one can experience bubble formation (Shirgaonkar, Lothe et al. 1998). Should this happen and the fluid subsequently enters a sudden expansion, the resultant rapid decrease in velocity will cause an increase in pressure, which will in turn cause the bubbles to implode. The energy from these implosions create instantaneous temperature changes, high pressure acoustic shockwaves and high velocity microjets (Brennen 1995). While the effects are nearly instantaneous, they can be quite severe with localized pressures as high as 690 MPa, interface temperatures of 3400 K and microjets capable of eroding hardened steel (Brennen 1995; Munson, Young et al. 2002).

Pressure: The effects of high pressure on foods has been well studied, and they depend on the amount of time a food is subjected to elevated pressure (Balasubramaniam, Farkas et al. 2008). The typical CHPP system will subject food to elevated pressures for a relatively short time (typically measured in seconds or fractions thereof), depending upon the configuration of the system and the process conditions. While the duration of static high pressure processes are easily measured, for continuous high pressure processes, the duration of pressure must be calculated from the flow rate and volume of the high pressure region of the system (Moorman 1997). The difference in magnitude is great,

with most static processes being measured in minutes, and most dynamic processes in fractions of a second (Moorman 1997; Wuytack, Diels et al. 2002; San Martin 2004)

Adiabatic Heating and Cooling: Adiabatic heating and cooling refer to changes in the temperature which occur without heat gain or loss from the surrounding environment. In high pressure systems this change comes from the transformation of mechanical work into heat, and is manifested as an increase in temperature during pressurization and a decrease during release (Ardia, Knorr et al. 2004).

Fluid Friction: In classical mechanics, "friction" is a term given to the inherent non-inertial resistance of a system to motion due to interface conditions. The term "fluid friction" is an attempt to find analogy between classical mechanics and fluid mechanics, but is somewhat less precise due to the ability of fluids to be deformed. The term thus includes both the effects of the interface condition (the no slip boundary condition) and the cohesive effects of the fluid (viscosity). As with classical friction, fluid friction generates heat, and this heating can be quite significant, with multiple studies showing temperature increases in excess of 60°C (Moorman 1997; Peck 2004; Pereda, Ferragut et al. 2007; Sharma 2008)

Effects of CHPP

Microbial Effects: While the field is relatively new, much work has been performed using CHPP to inactivate microbes. Although much of the early work is not technically within the pressure range now typically associated with CHPP, it bears examining nonetheless, as it laid the groundwork for the use of pressure and shear to inactivate microbes. By 1990, several studies had been published, showing the destructive effect of

CHPP on vegetative bacteria, molds and yeasts. These studies all examined the technology from the standpoint of cellular disruption for cellular product recovery, and found CHPP processes were very effective at increasing the cellular product recovery and that disruption was dependent on pressure (Engler and Robinson 1981; Agerkvist and Enfors 1990; Keshavarz, Bonnerjea et al. 1990). Engler and Robinson (1981) in particular suggested that impingement was the primary mechanism of disruption, though they only used a classic style homogenization valve. In 1992 a model for the strength of the cell wall of *E. coli* subjected to high pressure homogenization (75 MPa) was developed, showing that the resistance of a cell to disruption by the process depended upon the degree of peptidoglycan cross-linkage and the length of a given cell. The authors suggest that if the degree of cross-linkage exceeds 56.8%, the strength becomes solely dependant on cell length (Middelberg and O'Neill 1993). Of course the model can really only be applied to gram-negative rod-shaped bacteria, as decades earlier, work using a cell disrupter showed that cell-wall structure had a significant influence on shear-based inactivation, with Gram-negative organisms showing a much lower disruption rate at a given pressure/flow than similarly sized/shaped Gram-positive organisms. Further, the authors found that rod-shaped bacteria were less resistant to disruption than spherical bacteria of the same Gram-type (Kelemen and Sharpe 1979).

A study by Feijoo et al. (1997) examined the ability of CHPP to inactivate *Bacillus licheniformis* spores. The authors microfluidized ice cream mixes with four different inlet temperatures (33, 36, 44 and 50ºC) at pressures up to 200 MPa, and found that with moderate product heating, they could inactivate over 50% of the spores. Temperature rises due to processing were approximately 10 ºC per 50 MPa, and in all

cases a lower exit temperature correlated with a lower inactivation level; however, the effects of the CHPP process were greater than the temperatures effects alone (Feijoo, Hayes et al. 1997).

That same year Moorman outlined the effects of a CHPP process using a micrometering valve as a pressure release component, which he termed "continuous high pressure throttling", on both the reduction of native microflora in concentrated and unconcentrated skim milk and the inactivation of pure cultures in microbial culture media. In the skim milk and milk concentrate trials, the author altered the configuration of the system by including one or more holding tubes. By altering the configuration of the system, the author was able to vary both the total time the fluid was held at pressure before release and allow for extended holding at an elevated temperature after release. Unconcentrated skim milk processed at 310 MPa with either a 1 s or 0.3 s pressure dwell showed a 3.1 log reduction of native microflora, and this value increased to as high as 3.83 log with the addition of a 10 s temperature hold. Skim milk concentrates showed a reduced microbicidal effect with increased pressure dwell, which the author attributed to the variability of initial microflora levels. The microbial broth trials offered more control over initial microbial count, and allowed the author to examine the effects of two different pressure release components and two different intensifier arrays. The different component configurations produced different flow rates, and different levels of inactivation. For the Gram-negative *Lactobacillus sake*, no difference was seen in inactivation levels (8.32 log reduction) between an interaction chamber and a micrometering valve using the same intensifier array. The results were not mirrored for the inactivation of the fungal *Zygosaccharomyces bailii*, which showed the interaction

chamber was more effective at inactivation than the micrometering valve (3.44 vs 2.62 log reductions) (Moorman 1997). This work lead to US Patent #6120732 for the process in 2000 (Toledo and Moorman 2000).

Shirgaonkar et al (1998) revisited the question of the mechanism involved in cellular disruption and inactivation, and concluded that the formation and collapse of cavitation bubbles was most responsible. They first measured cavitation by the liberation of iodine from potassium iodide solution, and then repeated the experiment with cultures of *Sacchromyces cervisiae*. Though their system was operated below the 100 MPa threshold for high pressure (approximately 75 MPa) and they used a single eukaryotic organism, they were able to show that cellular disruption, as measured by product recovery, was greater under conditions which had higher levels of cavitation (Shirgaonkar, Lothe et al. 1998).

Static and continuous high pressure processes were compared in a 2002 study, showing that the two types of process had different patterns of inactivation (Wuytack, Diels et al. 2002). Using 11 different organisms CHPP using a single intensifier CHPP system fitted with a homogenizing valve was less likely to leave sub-lethally injured cells than a static. Further, while some Gram negative species were shown to be more resistant to inactivation by static HPP than some Gram-negative species, in CHPP Gram-stain status was always an indicator of resistance, with Gram-positive organisms having the greater resistance.

Work by Vachon et al. (2002) similarly compared CHPP to static HPP, this time with the food borne pathogens *Escherichia coli* O157:H7, *Salmonella enterica* and *Listeria monocytogenes*. Using a CHPP system with a single single-acting intensifier and

a homogenizing valve as the pressure release component, milk and phosphate buffered saline solution were treated at three different pressures (100, 200 and 300 MPa) for up to 5 passes. The inactivation results for the saline solution trials were compared to a 10 minute static HPP at 200 MPa. Overall, CHPP at a given pressure was considerably more effective (2 log reduction difference) than static HPP. For both the milk and saline trials, inactivation by CHPP was found to be influenced by both increases in initial temperature, pressure and number of passes, as well as showing variation based upon organism, with the Gram-positive *Listeria monocytogenes* being the most resistant. The trials using milk showed lower levels of inactivation compared to the saline solution trials at a given processing condition, with the authors speculating that this difference was due to some protective action by a milk constituent. Overall, the authors suggested that the inactivation of the microoganisms was due to the rupture of bacterial cells, a suggestion supported by the electron microscopy.

That same year, multiple CHPP passes were found to improve the microbial quality of milk and the cheese made from it (Kheadr, Vachon et al. 2002). Using a multi-intensifier system with a homogenization valve operated at 200 MPa the authors reduced the level of *Listeria innocua* by 3-4 log in the milk which carried over into the cheese, with a predictable time-dependant reduction in the aged cheese. These reductions were not only significantly inferior to those achieved by thermal methods, but were also unable to meet the legal requirements for pasteurization.

In 2006, Diels and Michiels reviewed the microbicidal applications of CHPP. The authors placed significant emphasis on the various, often conflicting or even interdependent proposed mechanisms of inactivation, and the effect of different

experimental parameters on inactivation. These parameters included both processing parameters, such as flow and pressure, as well as fluid properties such as viscosity, cell density, buffer capacity and additives. They concluded that all evidence thus far presented supported an “all or nothing” inactivation method, and that, as the process had a very limited number of parameters which influenced microbial inactivation, CHPP was particularly suited to application in the industry (Diels and Michiels 2006).

The year 2007 saw two papers on shelf life and general microbial quality of CHPP milk. Pereda et al. (2007) used a dual single acting intensifier system with pilot operated check valves and a conical disruption valve to determine changes in native microflora over several storage days compared to HTST pasteurization. They found that for coliforms, lactobacilli and enterococci processing at or above 200 MPa gave the same results as the HTST process- namely the undetectable levels of those organisms. For psychotrophs, bacterial spores and lactococci, there was some residual activity at day zero after processing at or above 200 MPa, but similar day zero results were seen in the HTST samples. However, after prolonged (21 days) storage, differences did evolve between the CHPP and HTST samples, some positive and some negative. In all cases, CHPP at 200 MPa and a 30 ºC inlet temperature performed as well or better than the HTST pasteurization. The samples processed at 300 MPa and those processed at 200 MPa, but with higher inlet temperature (40 °C) actually showed higher counts of spores, psychotrophs and Lactococci than the pasteurized samples. The authors suggested that the differences observed were possibly due to differences in the levels of inactivation of native antimicrobial systems between the different process conditions (Pereda, Ferragut et al. 2007).

Smiddy et al. (2007) conducted a similar study, using similar equipment, but with higher inlet temperatures (55 and 75 °C), and did not compare the results to thermal pasteurization. They saw a correlation between higher temperatures and pressures and greater inactivation, and that despite undetectable levels on day one of storage, significant growth occurred over the three weeks of storage. This result led them to conclude that unless very high pressures or multiple passes were used, CHPP could not replace traditional pasteurization (Smiddy, Martin et al. 2007).

While many studies have looked at replacing pasteurization with CHPP, in 2008, the potential for hybrid processes involving post pressurization heating was examined as a potential replacement for sterilization by Sharma (2008). He used a dual single-acting intensifier system with pilot actuated (active) check valves which had been fitted with a steam fed heat exchanger between the exit check valves and the pressure release component. This design allowed him to increase the temperature of the pressurized soymilk by 20-60 °C before passing it through a micrometering valve. The heating generated by the high shear in the valve further raised the temperature to either 121, 135 or 141 °C, which was held for a short time before cooling to 4C. This process was able to effect inactivation of *Clostridium sporogenes* spores as high as a 5.8 log reduction with the highest pressure, temperature and hold time. Data for the less extreme processing conditions led him to conclude that the rate of inactivation of said spores was dependant upon pressure, retention time and temperature (Sharma 2008).

The effect of fluid compositions was again examined in 2009, when scientists reported a correlation between higher fat content in milk and increased inactivation of *Listeria monocytogenes.* The authors used a twin single-acting intensifier system with a

ceramic disruption valve to do the study, and used pressures from 200-400 MPa. Temperature rise was seen to increase with both pressure and fat content, with the highest temperature rise (84 °C) occurring at 400 MPa and 15% fat. The authors concluded that while this increase in frictional heating contributed to the increased inactivation in higher fat samples, it was not the only mechanism, as samples of different fat content at different pressures, but with similar temperature rises showed as much as a 3 log difference in inactivation levels (Roig-Sagués, Velázquez et al. 2009).

Physical and Sensory Effects : One of the primary forerunners of CHPP is homogenization, which was adopted due to the physical and sensory changes induced by the process. CHPP, of course operates at much higher pressures and produces much higher shear rates. These high shear rates can actually alter the molecular structure of various polymers, which in turn effects their properties (Harrington and Zimm 1965). Since most foods are complex systems which include various polymeric compounds such as proteins and complex carbohydrates, it follows that subjecting them to CHPP would induce profound, marked effects on the physical and sensory properties of various foods.

Conventional homogenization is used to disperse two discontinuous phases into a stable product, performing such tasks as preventing the separation of whole milk or allowing flavoring oils to be added to aqueous products. Some of the earliest works on CHPP therefore predictably focus on its dispersive effects. Perhaps the earliest study was a two part study, published in 1987, which examined the effects of different valve geometries on the creation of oil-water dispersions via high pressure homogenization (Mohr 1987; Mohr 1987). While the author did not include any pressure ranges the

conclusions were still insightful. In the first part of the study, the author concluded that dispersion was the result of a two stage process caused by the formation of turbulent eddies, which were dependant on the geometry of a given valve. The author found that the primary stage of dispersion was influenced by the formation of, and flow through large high-energy eddies. The properties of these eddies depended upon one aspect of valve geometry in particular: the size of a chamber located on the low pressure side of the valve, which the author referred to as the "turbulence chamber". Further, the secondary stage of disruption depended upon smaller eddies and was caused either by high shear between them or pressure fluctuations within them, depending upon particle size (Mohr 1987). For the second part of the study, the author examined several different common valve geometries and process conditions to determine the effects of cavitation on particle size. By controlling the pressure drop in the valves, the author was able to limit cavitation, and thereby discovered that that cavitation had a negative effect on both the average particle size and the particle size distribution. He went on to describe the design features of a homogenizing valve which would produce the greatest turbulence and lowest cavitation, settling on a nozzle-based design with a turbulence chamber located just after the nozzle (Mohr 1987).

Further work on the use of microfluidization as means of dispersing discontinuous phases was completed by Washington and Davis (1988) who used the technology to create parenteral feeding emulsions from soy oil, water and lecithin. Using pressures of 3000-10000 psi (20.7-68.9 MPa) and up to 6 cycles, they were able to create a product with particle size distributions better than the commercially available product. Further, when compared with ultrasonic homogenization, the technology was found to produce

vastly superior products – not only were the microfluidized emulsions finer and more uniform, but also the time required to achieve dispersion was shorter, and the product was subject to a much lower temperature rise.

Microfluidized emulsions of soy oil and water were also used by Silvestri and Lostritto (1989) in order to develop a mathematical model to predict the mean oil droplet diameter in sub-micron emulsions. The single single-acting intensifier system was assumed by the authors to produce a uniform emulsion after five passes through the interaction chamber, an assumption validated by the analysis of the resultant emulsions by dynamic laser light scattering. The 18 different formulations tested differed in oil content, surfactant type and surfactant level. Even with those variations, the average polydispersity of all the samples ranged from 1.2-1.57, with an average of 1.38±0.03, and no changes in these values were seen over the eight days of testing. These fine dispersions and their prolonged stability allowed the authors to validate their mathematical model, which was shown to predict the size of droplets in a given emulsion with a mean error of 2.8 ±0.48%.

The previously referenced studies deal with the reduction of physical particle sizes, essentially treating the CHPP process solely as a means of homogenization. And while CHPP excels in that application, it has the ability to do so much more. Since the 1960's high shear has been known to alter the molecular structure of polymers. In 1991 the effects of microfluidization on gum tragacanth were studied (Silvestri and Gabrielson 1991). Gum tragacanth solutions were passed through a single single-acting intensifier four times at each of three operating pressures- 52, 86 and 121 MPa. Samples were taken after each pass and tested by capillary viscometry to determine absolute viscosity of the

solution, which was then used to calculate intrinsic viscosity and average molecular weight of the gum. Calculated reductions in average molecular weight after treatment ranged from 15% to 91% , with greater reductions correlating with those treatments that involved higher pressures and more passes. After validating the assumptions of particle size and shape by dynamic laser light scattering spectrometry, the authors concluded that the changes in average molecular weight of microfluidized gum tragacanth were consistent with the random scission model of polymer degradation under shear previously proposed by Harrington and Zimm (1965).

McCrae (1994) compared the changes in particle size resulting from microfluidization at 35 or 103 MPa to those of traditional homogenization at pressures (≤ 35 MPa), and found that the microfluidizer consistently created smaller particle sizes than the homogenizer, even when the two devices operated at the same pressure (McCrae 1994). Additionally, the fat globules in the microfluidized samples showed greater adsorption of serum protein than those in the homogenized samples and were more resistant to clustering. These observations led the author to speculate that there might be some correlation between the two phenomena, and to propose that the mechanism of homogenization seen in the microfluidizer differed from the mechanism seen in traditional valve homogenization.

A study by Adapa et al. (1997) found changes in the functional and physical properties of skim milk and skim milk concentrate after CHPP. Samples of both skim milk and skim milk concentrate were processed at 310 MPa in a continuous high pressure throttling system consisting of a single dual-acting intensifier, requisite check valves and a micro-metering valve. Samples were evaluated for overrun capacity, foam stability,

viscosity, emulsion stability index, feathering, interfacial and surface tensions as well as color changes. With the exception of interfacial tension and feathering, all of the physical properties examined were altered by CHPP, though the relative changes differed between concentrated and unconcentrated samples. The most drastic example was that the process improved the emulsion stability index of the unconcentrated sample, but decreased it in the concentrated one. Both overrun capacity and foam stability increased from non-existent levels in both concentrations, with the unconcentrated samples showing markedly greater improvements in foam stability and the concentrated sample showing similar improvements in overrun capacity. Viscosity and surface tension increased similarly within a given concentration, but the color changes induced by the process differed based upon the concentration level.

In addition to the microbial effects mentioned in the previous section, Moorman (1997) observed rheological changes in skim milk and concentrates processed with CHPP which he believed could increase consumer appeal, not only in the fluid milk but also in concentrates and yogurt made from the milk. The study treated unconcentrated skim milk (9% soluble solids) and three concentrates (15, 19 and 24% soluble solids) in a continuous high pressure throttling system consisting of a single dual-acting intensifier, requisite check valves and a micro-metering valve. By altering the configuration of the system, the author was able to vary both the total time the fluid was held at pressure before release and allow for extended holding at an elevated temperature after release. After processing at 310 MPa with either a 1 s or 0.3 s pressure dwell time all samples saw an increase in viscosity, roughly 2-fold for all samples except the most concentrated one which saw an almost 3-fold increase. Additionally, the treated and control samples were

evaluated for gel formation during refrigeration and the CHPP treated 24% soluble solid samples were found to be unique among all of the other concentration/ treatment groups in their ability to form stable, reversible gels during refrigeration. The author also found that yogurt made from CHPP treated skim milk was more viscous and had greater water holding capacity than those prepared from untreated milk.

Lagoueyte and Paquin (1998) examined the effects of microfluidization on the physical and chemical properties of xanthan gum. One percent solutions of the gum were treated at 75 MPa using a single single-acting intensifier system for up to 20 passes. Microfluidized samples were either left as a solution or spray dried to form a powder which was then rehydrated in distilled water. All were examined for changes in flow characteristics, water uptake and molecular weight. The number of passes affected both the viscosity and pseudoplastic behavior of the solutions, with both decreasing as the number of passes increased. The flow behavior indices also increased with additional passes, but became steady after either the 12^{th} pass for the solutions made from rehydrated dried samples or the 8^{th} pass for the original solutions. Similar plateauing was seen in the decrease of the consistency indices. Molecular weight measurements by size exclusion chromatography showed that as the number of passes increased, the average molecular weight of the gum decreased in a non linear fashion, plateauing again after the 12^{th} pass. The authors postulated that the changes seen came from two phenomena: the first a disruption/change in inter molecular forces and the second a breaking of intramolecular bonds, potentially from the backbone, but also possibly from the side chains.

One of the first review articles to address the physical effects of CHPP focused on dairy products and ingredients, particularly the effects on the fat, proteins and polysaccharides (Paquin 1999). Preference was given to studies involving functional applications, including increased emulsion stability, denaturation of macromolecules and one study previously published by the author which dealt with creating increased yield and acceptability in cheese. The author's conclusion on the work at that point was that the effects seen offered promise, but that more work was needed to better understand the interactions which might happen in CHPP treated complex dairy products and how the changes seen could best be applied in the industry (Paquin 1999).

The first few years of the new millennium saw scientists beginning to examine potential interactions of ingredients processed by CHPP to better understand the effects on complex systems. Laneuville et al. (2000) used a microfluidizer operating at 75 MPa to produce complexes of whey protein and xanthan gum for potential use as fat replacers. Solutions of the two ingredients with four different protein to polysaccharide ratios were passed through the microfluidizer 4, 8 or 12 times and examined for changes in structure, rheology and particle size. Structurally, the authors found that microfluidization prevented the formation of fibrous complexes which are unsuitable for use as a fat replacer, while the flow behavior of the complexes were altered significantly by the process, and varied based upon both the mixture composition and number of passes. Particle sizes and shapes were affected by the process, with some having analogy to commercially available protein and protein-polysaccharide based fat replacers.

Tunick et al. examined the ultra-structural differences in cheese made from non-homogenized milk and that made from microfluidized milk (Tunick, Van Hekken et al.

2001). Using a single dual-acting intensifier system, pasteurized milk at either 10 or 54°C inlet temperature was treated at either 34 or 172 MPa and then used to make mozzarella cheese. The authors found that microfluidization reduced the fat globule size, and re-arranged the pattern of electron dense regions surrounding fat globules. Higher pressures and temperatures led to changes in the nanostructure, with the full fat cheese made from the highest pressure/ initial temperature combination having dispersion so great that it was difficult to see via TEM. In all of the cheeses, except the 54°C/172MPa full fat cheese, similar trends of structural rearrangement were seen after six weeks of storage.

In 2004, a formulated blueberry beverage containing 20% juice and 7% whey protein were processed by CHPP at 300 MPa and evaluated with sensory and non sensory means (Peck 2004). Experimental samples were prepared using a dual single-acting intensifier system fitted with a micro-metering valve and a post valve cooling coil, while the control samples were subjected to a 6 s HTST pasteurization process at 125 °C. The CHPP system produced an instantaneous 75 °C rise in product temperature due to the flow properties of the system, however the cooling coil reduced the product exit temperature to 20 °C. The product processed with high pressure was found to have equivalent acceptability initially, but increased acceptability after prolonged (35 days) storage. CHPP also resulted in an overall reduced particle size and a difference in sedimentation character when compared to the HTST treated beverages. Interestingly, both the amount and type of sediment differed between the two with the CHPP product having less cohesive sediment which was easily re-dispersed by agitation.

The high shear rate obtained in the pressure release mechanism of a CHPP system results in instantanteous heating of the product, the significance of which was examined

by Datta et al. (2005). Using a dual single-acting intensifier system with active check valves and a conical disruption valve as the release component, raw milk of several different inlet temperatures (10-50 °C), was processed at 200 MPa. At this pressure, the authors noted a temperature increase of 0.5885 degree per degree of inlet temperature, leading to an outlet temperature range of 56-80 °C. For comparison, aliquots of raw milk were also subjected to thermal processes which mirrored the temperature changes seen by samples during CHPP. The authors examined both the size of fat globules, denaturation of β-lactoglobulin and the residual activities of 4 enzymes: plasmin, alkaline phosphatase, lactoperoxidase and lipase. Fat globule size was shown to be reduced by CHPP, and the only difference seen was attributed to the melting point of milkfat, with those samples having an inlet temperature above 35°C showing smaller sizes than those below 35°C, while the denaturation of β-lactoglobulin by CHPP appeared to have a synergistic relationship with exit temperatures greater than 65°C. The effect of CHPP on the activity of each enzyme differed: lipase activity was greater in the CHPP samples, the lactoperoxidase and plasmin activities were greater in the thermally treated samples, and the activity of alkaline phosphatase showed no differences between the CHPP and the thermally treated samples at a given temperature. This result lead them to conclude that while some of the changes caused by CHPP were due to thermal effects, others were due to the combination of heat and shear.

Pereda et al. (2007) assessed the physiochemical changes over 21 days in whole milk processed with CHPP, using a system with dual single-acting intensifiers and a two stage release valve. Properties examined included temperature rise during processing, viscosity, color, pH, acidity, rate of creaming, and particle size as well as the residual

activities of two enzymes. Two different inlet temperatures (30 °C and 40 °C) and three different pressures (100, 200 and 300 MPa) were examined, and a temperature rise of 19.5 °C per 100 MPa was observed, giving a range of exit temperatures from 64.7 to 103 °C. Visocisty effects followed an interesting pattern, with milks treated at 200 MPa always having the lowest viscosity and the 300 MPa samples tending to have higher viscosities. These differences in viscosity were attributed to differing particle sizes between the two highest pressure treatments, namely that the 200 MPa samples showed evidence of disrupted casein micelles and the 300 MPa showed the formation of large complexes and/or fat aggregates. Reductions in the lightness values (L*) of milk after the 200 MPa treatment were also attributed to the disruption of the casein micelles. Changes in pH at day zero, while significant, were quite small. Over several days of storage, pH began to change differently depending on the treatment conditions. This result was attributed to differences in microbial growth due to the incomplete inactivation of lactoperoxidase at 200 MPa, as the lactoperoxidase system is well known to have natural antimicrobial effects. Finally none of the CHPP processed samples showed any creaming, despite different particle size distributions.

Zamora et al. (2007) evaluated the effects of CHPP on the cheese making properties of milk. A CHPP system with dual single acting intensifiers and either a single stage or two-stage release valve was operated at pressures of 100, 200 and 300 MPa. The milk was then used to determine coagulation time, curd properties, cheese yield, milk particle size and curd microstructure. The authors found that for a single stage valve, higher pressures led to higher yields, greater moisture content and a change to the ionic balance of the whey byproduct. Additionally, most of the CHPP treatments, particularly

the single stage ones, increased the divalent calcium ion concentration in the whey and lowered the ph, a phenomenon thought to aid in lowering the coagulation time. The two-stage valve process reduced fat globule size more than the single stage valve, however overall it was detrimental to the cheese making properties of the milk (Zamora, Ferragut et al. 2007).

Sivanandan (2007) examined the effects of CHPP on whole-bean soy milk, particularly particle size, structure and consumer acceptability. Using a dual single-acting intensifier system, fitted with an in-line heat exchanger and a micro-metering valve, soymilk was processed at 5 different pressures, from 68.95 to 275.79 MPa with inline heating to 80 °C, which resulted in an exit temperature in excess of 121 °C. The CHPP process reduced particle size, reduced or eliminated product separation and created a smoother product. Consumer acceptability scores of both the CHPP and non-CHPP samples were very low when compared to those of a commercially available product. The author attributed these results to a lack of consumer familiarity with the product and the commercial samples use of adjunct ingredients.

More recently Innocente et al. (2009) examined the effects of CHPP on reduced fat ice cream mixes. Using a single single-acting intensifier system fitted with a two stage homogenization valve, and operating at 100 MPa, the authors processed mixes with 5 and 8% milkfat. These products were compared to identical formulations which had been conventionally homogenized (18 MPa) using the same equipment. Compared to the conventionally processed product, the CHPP mix had a smaller average droplet size and a more narrow distribution, with greater viscosity and increased visco-elastic behavior. Of particular note was the observation that the 5% fat mix which had been subjected to

CHPP had an apparent viscosity statistically indistinguishable from the conventionally homogenized 8% fat mix, leading to the speculation that this technology may be applicable in formulating reduced fat products with similar characteristics to the higher fat product.

Cavender and Kerr (2011) investigated the effects of CHPP on full-fat ice cream mixes with two different gum stabilizers. Using microfluidizer with a single dual-acting intensifier, and operating at 220-250 MPa they examined the changes in viscosity of the mix, as well as textural and sensory properties of the ice cream. They found an 8.5-17 fold increase in mix viscosity after processing depending upon the gum stabilizer, as well as changes in textural properties. Consumer acceptability increased due to processing and in one formulation, the changes in consumer opinions were found to be analogous to those in textural properties.

Conclusions

Continuous high pressure processing has seen much interest by the food industry in recent years. The process inactivates microbes, modifies the functional properties of existing ingredients and shows promise in the area of creation of novel ingredients. The effects of a given process are due to a complex interaction of pressure, shear rate, frictional heating and other flow phenomena, and it is often difficult to isolate the contributions of each. This difficulty is further confounded by the effects of the many different system configurations available in the market on those phenomena. Despite the numerous studies on the process and marked differences in results seen between studies, inquiries into just how these differing equipment configurations affect the outcome of the

process remain, for the most part, undone. Until such studies are completed, the standardized guidelines necessary for widespread industry use will be difficult to develop.

Further Reading

Readers interested in static high pressure systems are encouraged to consult the following papers, organized by topic:

General Review Articles:

D. Farr, 1990. "High pressure technology in the food industry". *Trends in Food Science and Technology*, volume 1.

D.F. Farkas& D.G Hoover, 2000. "High Pressure Processing" *Journal of Food Safety*. Volume 65, pages 47-64.

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Figures and Tables

Table 2.1: Brief Overview of Available CHPP Systems

System Name	Intensifier Array Type	Check Valves (#)	Check Valves (type)	Pressure Release Component	Manufacturer
Emulsiflex C-5	Single single-Acting	1	Passive	Valve (Homogenizing)	Avestin, Inc [A]
Emulsiflex C-50	Single Dual-Acting	4	Passive	Valve (Homogenizing)	Avestin, Inc [A]
Emulsiflex C-160	Triple single acting	6	Passive	Valve (Homogenizing)	Avestin, Inc [A]
Microfluidics M140-K	Single dual acting	4	Passive	Fixed Geometry chamber	Microfluidics, International [B]
Microfluidics M-700 Series	One or two single acting	1-4	Passive	Fixed Geometry chamber	Microfluidics, International [B]
Stansted Hyd-Lok Series	One or two single acting	4	Active or passive	Valve (Conical)	Stansted Fluid Power [C]
Stansted NM-Gen 7900	Two single Acting	4	Active	Valve (conical)	Stansted Fluid Power [C]
DeeBEE-2000	Two single Acting	4	Unknown	Homogenizing cell	BEE International [D]

Company Websites:
A: http://www.avestin.com/English/products.html
B: http://www.microfluidicscorp.com
C: http://www.homogenizersystems.com/index.html
D: http://www.beei.com/index.html

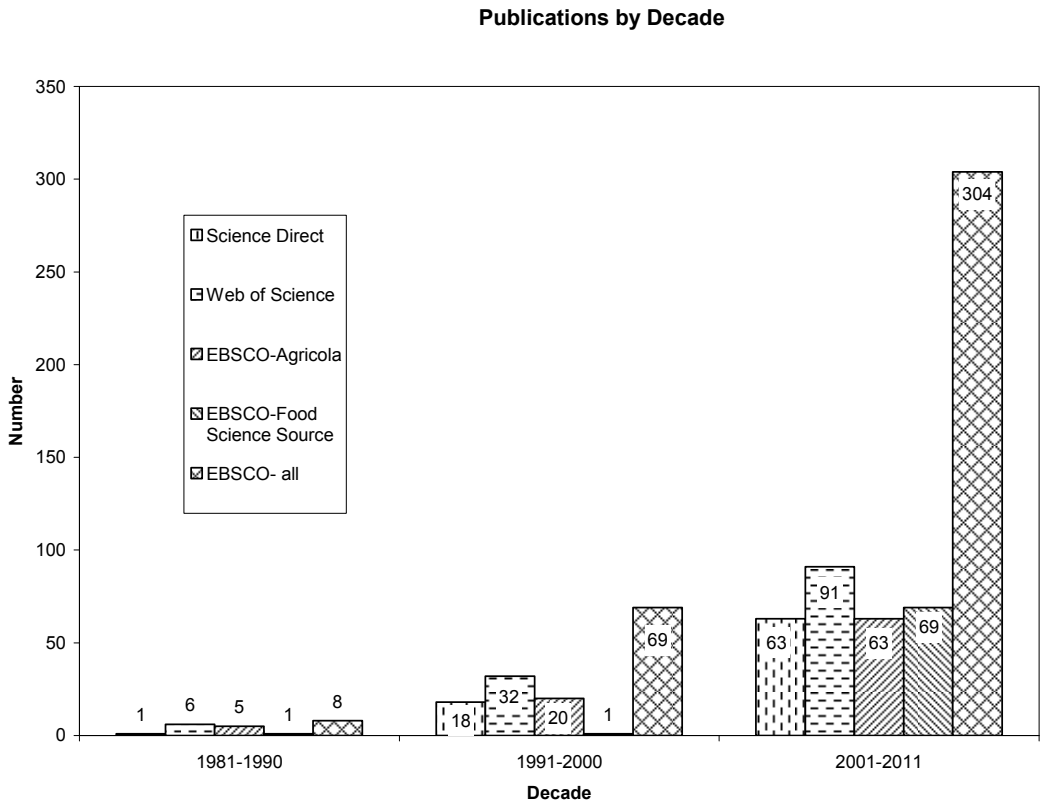

Figure 2.1: Scientific Publications Involving High Pressure and Food by Decade

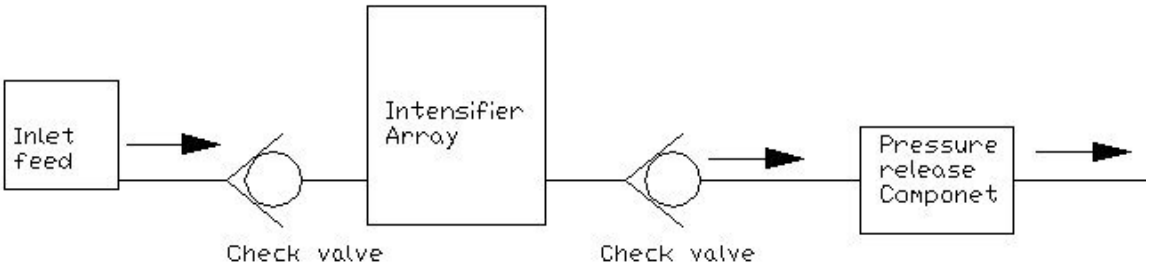

Figure 2.2: Basic Layout of a CHPP System

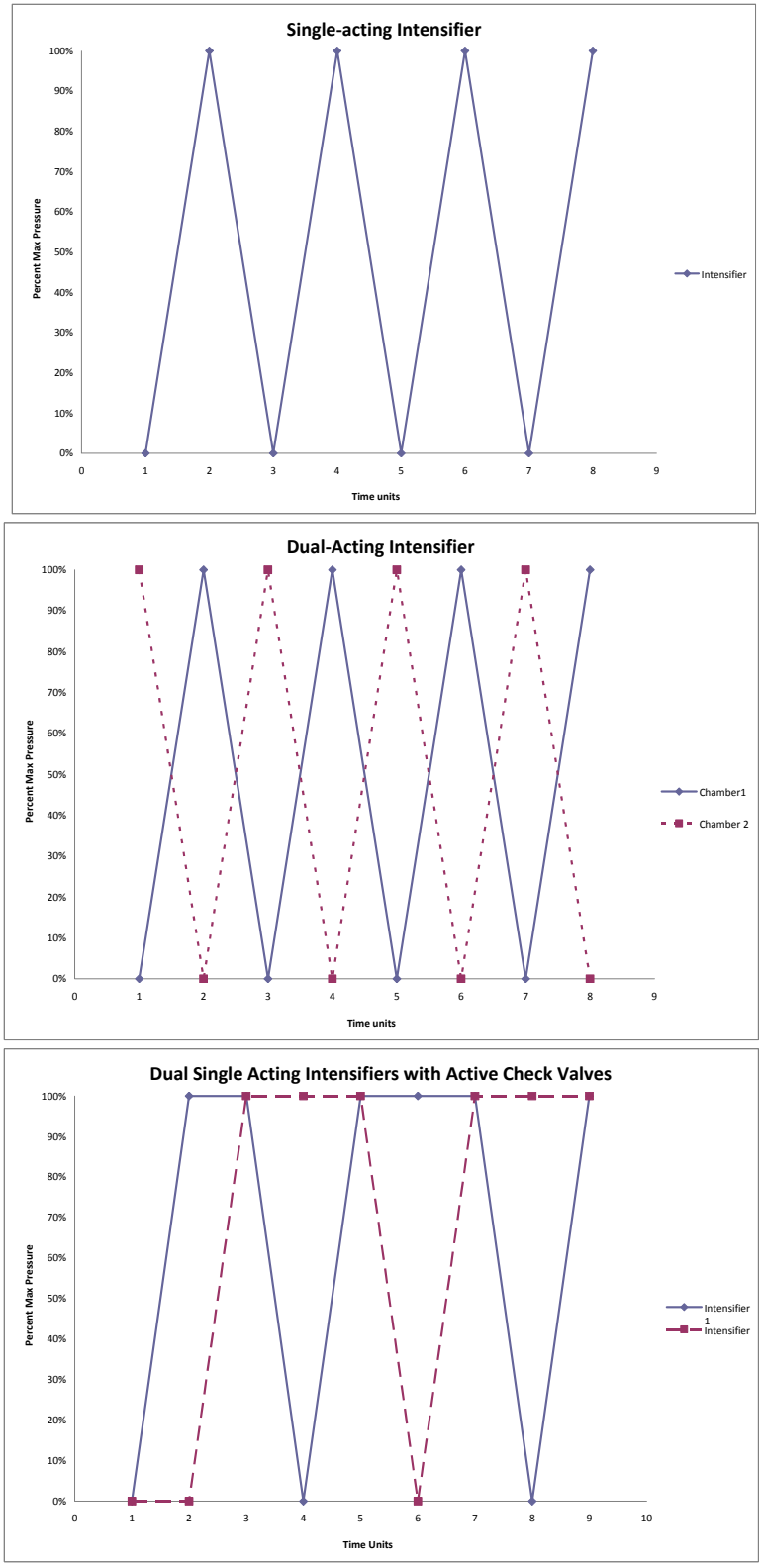

Figure 2.3: Comparison of Pressure Profiles Generated by Various Intensifier Configurations.

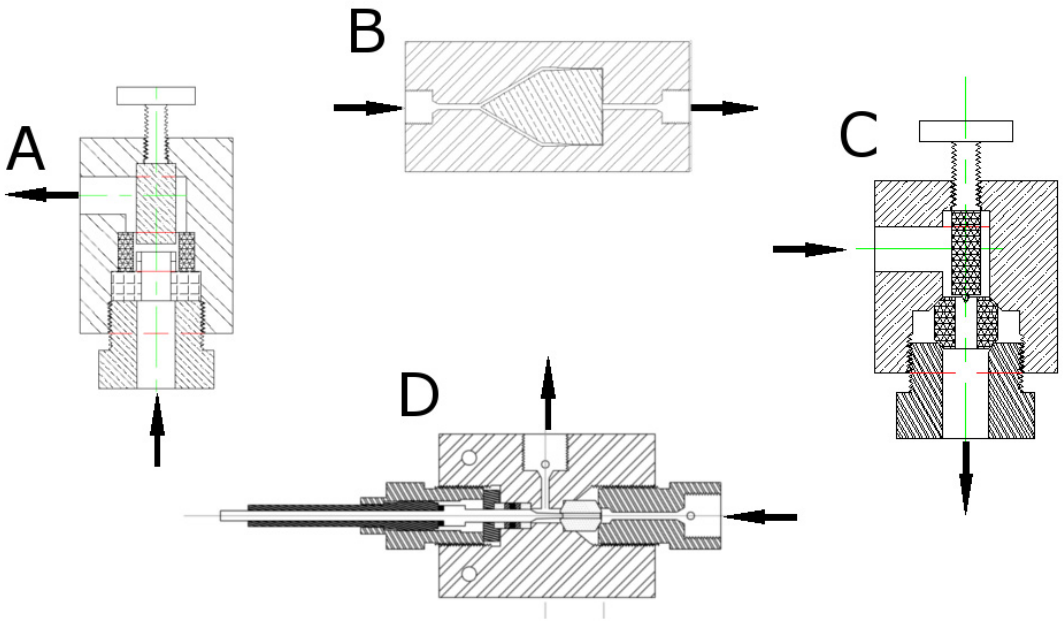

Figure 2.4: Four Common Presssure Release Components- A) Homogenizing Valve, B) Interaction Chamber, C) Conical Disruption Valve, D) Micrometering Valve. Arrows indicate fluid flow.

CHAPTER 3

INACTIVATION OF VEGETATIVE CELLS BY CONTINUOUS HIGH PRESSURE PROCESSING: NEW INSIGHTS ON THE CONTRIBUTION OF THERMAL EFFECTS AND RELEASE DEVICE [1]

CAVENDER, G.A. AND W.L. KERR. Accepted by *The Journal of Food Science.* Reprinted here with the permission of the publisher, 6/23/2011

Abstract

Dynamic or continuous high-pressure processing of fluid foods has drawn significant interest as a microbial reduction process in the past decade, and many attempts have been made to better understand the mechanisms involved in that reduction. This study was intended to provide insight into the contributions of thermal effects and differences in pressure release components in the inactivation of two vegetative pathogen surrogates- the Gram-positive *Listeria innocua* and the Gram-negative *Escherichia coli*. Fluids containing microbial loads of 10^8 or greater were subjected to continuous high pressure processing at 200-210 MPa. Without active cooling of the release components, all fluids experienced a temperature rise in excess of 70ºC, thus occluding any pressure-related effects for all release components. Active cooling of the valve bodies of the two valve-style release components (a conical disruption valve and a micro-metering valve) allowed the temperature rise to be abated enough to isolate the effects unique to a given valve. In Tryptic soy broth trials, the mean inactivation levels of E. *coli* between valves were similar- 5.16 log and 5.33 log for the micro-metering and conical disruption valves, respectively. When repeated with *L. innocua* a similar inactivation level was observed in the conical disruption valve (5.1 log), but not the micro-metering valve (3.02). *L. innocua* trials were also repeated using fluid whole milk, which showed a lower levels of inactivation- 2.04 log for the micro-metering valve and 2.51 log for the conical valve.

Introduction

Thermal processes, while effective at reducing microbial load, can impart undesirable changes in foods. This phenomenon has led to an increased awareness of "non-thermal" processes, that is processes for which the primary processing effect is due to something other than heat (Raso and Barbosa-Cánovas 2003). High pressure processing is often touted as one such process. It is useful to separate more traditional static high-pressure systems from newer continuous systems. Static high pressure systems are essentially large pressure vessels, which are filled with product (often pre-packaged). The product is then brought to a very high pressure (700-900 MPa) via some working fluid and held for a length of time (Farkas and Hoover 2000). By their design, these are batch processes and any thermal effects occur only due to adiabatic heating, which can typically be well managed with proper operation (Peck 2004; Rastogi and others 2007).

Continuous high pressure systems operate on a different principle. Liquid product is fed into one or more intensifiers which quickly bring the pressure of the fluid to the target value. The product then exits a pressure release component, typically a valve or a fixed geometry chamber. Working pressures tend to be lower than static systems (300-400 MPa), and the total time at a given pressure can be quite short compared to static systems (Datta and others 2005). In addition, the flow of the fluid through the pressure release mechanism results in high shear flow which causes frictional heating of the product (Datta and others 2005). Previous works have shown the rise in temperature of the product to be quite large, and typically have attempted to reduce the contribution of thermal effects through post valve cooling (Moorman 1997; Lagoueyte and Paquin 1998; Kheadr and others 2002; Peck 2004; Datta and others 2005; Zamora and others 2007).

While this approach can reduce the temperature of a heated liquid fairly quickly, the product will still experience extreme temperatures for short times (Moorman 1997). As inactivation of pathogenic vegetative cells increases exponentially with temperature, even short times can significantly contribute to microbial death (Moats 1971; Jay 2000). Further, several different pressure release components are used in high pressure systems, with the fixed geometry interaction chamber, conical ceramic valve and the micro-metering valve being among the most common. Each exhibits differences in geometry and flow pattern which can affect the type and intensity of forces to which the process liquid is exposed (Diels and Michiels 2006). Because of the extreme heating, the different intensifier configurations and the different pressure release mechanisms, comparing different bodies of work on dynamic high pressure processing can pose difficulty, particularly if one is attempting to develop a series of guidelines for the use of the technology as a replacement for thermal pasteurization.

The thermal pasteurization of foods aims to destroy vegetative pathogens in a given food, seeking to reduce any populations by five logs (Jay 2000). This level of inactivation is ensured by targeting the most thermally resistant species in a given food. Similarly, in order to replace thermal processing with a non-thermal treatment, care must be taken to base the total treatment upon the pathogens that are most tolerant to the non-thermal aspects of a given process.

Broadly speaking, bacteria can be classed as either Gram-positive or Gram-negative based upon their response to the Gram-staining procedure, which in turn is based upon the structure of their cell wall (Jay 2000). In general, Gram-positive bacteria are more resistant to high pressure processing. This resistance is attributed to the

increased level of cross-linking within the peptidoglycan layer of the cell wall (Middelberg and O'Neill 1993; Wuytack and others 2002; Diels and Michiels 2006). Many pathogenic bacteria potentially exist in food with the most common being the Gram-negative *Campylobacter jejuni, Escherichia coli* and *Salmonella enterica* (CDC 2010). While Gram-negative bacteria are of concern, there are also several Gram-positive bacteria of concern, including *Listeria monocytogenes* (Jay 2000).

This study aimed to examine the differences in temperature rise and microbial inactivation between three of the common pressure release devices used in continuous high pressure processing. Further, we aimed to better isolate the thermal effects from the pressure and flow effects of a high pressure process as well as determine the effectiveness of multiple pressure release mechanisms on surrogates of two vegetative pathogens of concern.

Materials and Methods

Processing Equipment: All liquids were processed using a continuous high pressure system (Stansted nm-gen 7900, Stansted Fluid Power, Stansted, England) which had been fitted with a thermocouple array and modified to feed from a 2-liter vessel which was pressurized with compressed air at ~550 kPa. One of the two factory outputs was fitted with a jacketed ceramic disruption valve (Stansted FPG4755) while the other was fitted with either a fixed geometry interaction chamber (Model G10Z, Microfluidics, Newton, MA) or a stainless steel metering valve (Model 60vrmm4882, Autoclave Engineers, Fluid Components, Erie, PA) depending on the experiment. Two metering valves were used, one modified by the authors to provide for the circulation of coolant over the actual valve

seat and the other unmodified. A summary of all modifications is listed in Table 3.1, and the general schematic of the processing system is shown in Figure 3.1. Figure 3.2 details the changes made to the metering valves. Of particular note in Figure 3.1 is the intensifier layout. The Stansted system uses two single acting pistons and four pneumatically controlled check valves. This setup ensures the fluid pressure immediately prior to the release mechanism is more consistent than systems which utilize manual check valves or single pistons. The former allow for partial fluid flow during pressurization, and the latter, whether single or dual acting, suffer from a significant drop in pressure partway through a given compression cycle

Microbial cultures: *Escherichia coli* (ATCC25922) and *Listeria innocua* (ATCC51742) were chosen due to their non-pathogenic nature and similarity to pathogens of concern (respectively hemorrhagic *E. coli* and *L. monocytogenes*). Both bacterial strains used in this study have a well established history of use as pathogen surrogates for thermal and non-thermal processes. *E. coli* ATCC25922 is marketed as an O157:H7 analog and has been previously used in inactivation studies involving a wide range of technologies, including high pressure, thermal treatments, UV light and others (Chiruta and others 1997; Koseki and Yamamoto 2006; Milly and others 2007). *Listeria innocua* has also been recommended as a pathogen analog and has seen widespread usage, particularly in high pressure studies (Kheadr and others 2002; Wuytack and others 2002; Black and others 2007; Ferrante and others 2007). Pure lyophilized cultures of each were obtained from American Type Culture Collection **(**Manassas, VA). Samples of each were rehydrated using sterile Tryptic Soy Broth (Becton, Dickinson and Company, Franklin

Lakes, NJ) and then aliquots (1 mL) of the rehydrated cultures were transferred to individual flasks containing 100 mL Tryptic Soy Broth (TSB) and incubated for 48 hours at 37 ºC. These cultures were used to propagate the bacteria used in each experiment. Aliquots of the *E. coli* culture were transferred serially into TSB which had an increasing levels of naladixic acid (MP Biomedicals, Solon, Ohio) added until the final concentration of 50 mg/L was reached. Aliquots of the *L. innocua* were similarly transferred into TSB with increasing levels of lithium chloride (Fisher Chemical, Fair Lawn, NJ), sodium chloride (Fisher Chemical, Fair Lawn, NJ) and naladixic acid (MP Biomedicals, Solon, Ohio) until the final concentrations were 30 g/L, 15 g/L and 20 mg/L, respectively. The resultant cultures were used to propagate bacteria for each processing. For experimental runs which required centrifugation, cultures were centrifuged twice at 5000 g first for ten minutes then for twenty minutes. After each centrifugation, each tube was decanted and rinsed with sterile 0.1% peptone solution (Bacto Peptone; Becton, Dickinson and Company). Final pellets were stored under 100 mL of sterile peptone at 4 ºC until the day of use.

Processing Fluids: For this experiment, preliminary studies were conducted utilizing Tryptic soy broth in order to establish a baseline level of inactivation as well as determine which of the two organisms was more resistant to processing. Once that had been determined, trials were conducted in commercially pasteurized whole milk (The Kroger Company, Cincinnati, Ohio)

Processing conditions: All fluids were stored at 4 ºC prior to processing. All fluids were processed at 200-210 MPa pressure. Those experiments which called for valve cooling used a circulating water bath (Neslab HTC 1200D Thermo Fisher Scientific ,Waltham, MA) to pass a 60% propylene glycol solution at -20 ºC over the valve bodies for at least 3 hours prior to and throughout processing. Temperature measurements of the processing fluid were taken automatically while volumetric flow rates were measured manually. All samples were taken in sterile 50 ml collection vessels which were immediately transferred to an ice bath to cool prior to enumeration.

Enumeration: Microbial counts were performed using three-tube Most Probable Number (MPN) methodology. *E. coli* were enumerated per the US FDA's Published Procedure (Feng and others 2009) using the standard Lauryl Tryptose Broth (Becton, Dickinson and Company) to which 50 mg/mL naladixic acid had been added. Tubes were incubated at 37 °C and examined at 24 and 48 hours for gas production. *L. innocua* were enumerated using Fraser Broth (Becton, Dickinson and Company). No additional selective agents were added to the broth, as the formulation of the broth contains several such agents. Tubes were incubated at 35 ºC and examined at 24 and 48 hours for a positive esculin hydrolysis response. In both cases, an automated computer spreadsheet, available on the FDA's website was used in lieu of printed tables to determine a more accurate count. No enumeration received a separate recovery step as previous research indicates that continuous high pressure processes do not produce sub-lethally injured vegetative cells, but rather inactivates in an "all or nothing" fashion (Wuytack and others 2002). Further, the FDA Bacteriological Analytical Manual offers no procedure for a

liquid media recovery step, and only recommends the solid media recovery steps be performed when the presence of sub-lethally injured cells are expected (Feng and others 2009).

Results and Discussion

Trials without Cooling: Table 3.2 presents the temperature and flow data for un-cooled release components. Despite microbial populations in excess of 8 log in the process fluid, no viable microbial populations were detected in any post- processing sample. This result is not surprising given the elevated temperatures observed. For simple comparison, previous studies have shown that *E. coli* bacteria have mean thermal decimal reduction times of 2.0 seconds at 62 ºC and 0.117 seconds at 75.6 ºC. (Read and others 1961; Chiruta and others 1997) These figures allow us to calculate the holding time necessary to effect an 8 log reduction in the *E. coli* population at 75.6 ºC as 0.94 seconds. Thus, any lethality effects which might be due to pressure would be totally masked by the thermal effects experienced in these trials.

Trials with Cooling: Table 3.3 presents the temperature, flow and inactivation data of both *E. coli* and *L. innocua* for the cooled release mechanisms. While the conical valve and the micro-metering valves experienced no difference in process temperature during the un-cooled studies, a 15 to 20 ºC temperature difference was noted in the cooled studies, which implies that the cooling mechanism for the conical valve was less efficient than the modified micro-metering valve, even though both valves had the same coolant flow rate. This result implies that the rate of heat transfer through the valve assembly is lower in the conical valve than in micro-metering valve, which is not surprising given

that the conical valve is of composite construction, having a ceramic needle and valve seat, and the micro-metering valve has valve seats and needles of stainless steel, the latter being a better conductor of heat than the former.

More interesting are the differences in inactivation levels between the two valves. No significant difference was discovered between the inactivation levels of *E. coli* for either valve, but an approximately 2 log difference was noted in *L. innocua* inactivation in TSB, with the conical valve being more efficient at inactivation. This observation might be related to the temperature differences, as Fairchild and Foegeding (1993) report a D-value for *L. innocua* of 18.1 s at 63.8 ºC and a z-value of 5.1 ºC to 5.6 ºC. That said, one would expect to have seen an even more pronounced difference in the inactivation of *E. coli*, given the shorter thermal decimal reduction times of *E. coli*. This result implies that the total time the samples spent at elevated temperatures prior to ice bath cooling were negligible when compared to the decimal reduction times, and allows us to conclude that some difference in valve geometry and or flow behavior makes the conical valve more efficient for inactivating Gram-positive organisms. This difference in behavior is of some interest, particularly if one is attempting to compare similar studies using different valve geometries.

Table 3.4 presents the data for trials on milk. Interestingly, the temperature rises due to processing were essentially the same as were observed in the TSB, despite the difference in the fluids. Further, no difference was noted between the inactivation levels of *L. innocua* between the two valves, and despite the similar thermal and flow measurements, these inactivation levels were lower than those observed in TSB. This is unsurprising as milk has long been known to provide protective effects against thermal

inactivation when compared to other less complex fluids, and more recently it has been shown that the various constituents of milk, particularly the minerals calcium, magnesium, phosphate and citrate, provide baroprotective effects in static high pressure systems (Doyle and others 2001; Black and others 2007). The individual contributions of the other potential mechanisms of inactivation, namely shear and cavitation are more difficult to quantify. Recently Floury and others (2004) have used computer modeling to offer great insight into the specifics of the conical valve (particularly how and where shear/ cavitation phenomena are likely to occur) but unless similar work is done on the micro-metering valve, we can only speculate as to any differences between the two.

Conclusions

Differences between the pressure release components of a continuous high pressure processing system produce an interesting pattern of process temperature and microbial inactivation levels, which vary based upon process fluid and microbial species. Further, while continuous high pressure processing has been suggested as a potential "non-thermal" replacement to traditional processes, this study shows that the different release components commonly used are responsible for significant amounts of heat generation. This heat interacts with the food, and can occlude any effects contributed by the pressure and flow of the fluid through the release component. In the case of valve-based components, these thermal effects can be abated through active cooling of the valve body. By removing or lessening the thermal effects of the process, the actual effects of the pressure and shear components of the process can be better understood, particularly as it applies to microbial inactivation. This study found that the inactivation levels of *L.*

innocua and *E. coli* were greatly affected by the thermal component of the process, and when that component was abated, the inactivation levels were more modest. As always, caution must be exercised in translating the effects of the process on surrogates to actual pathogens, and further study should be undertaken to determine how predictive these surrogates are in relation to the actual pathogens.

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Figures and Tables

Table 3.1: Summary of Modifications to the Stansted Model nm-gen 7900 High Pressure Processing System

Modification	Description	Purpose
Thermocouple Array	Installed in-line thermocouples at the inlet, after pressurization and at the point of exit	Allows for recording and analysis of temperatures at various points
Pressurized feed	Installed a 2L stainless vessel and fittings for compressed air	Reduces minimum run volume and simplifies cleaning process
Micro-metering Valve	Significantly modified valve to provide in-line cooling at the point of pressure release.	Allows abatement of thermal effects

Table 3.2: Thermal Effects of Various Pressure Release Devices on Tryptic Soy Broth [A]

Mechanism	Flow Rate mL/min	Fluid Temperature ºC
Micro-metering Valve	732.5 ± 10.6a	79.54 ± 1.18c
Conical Valve	770 ± 14.1a	76.70 ± 1.46c
Interaction Chamber	580 ± 28.3b	74.58 ± 5.94c

A: Mean Values ± SD. Values in the same column followed by the same letter are NOT statistically different at α=.05 per ANOVA and Tukey's Post-Hoc testing

Table 3.3: Microbial Reduction in Tryptic Soy Broth [A]

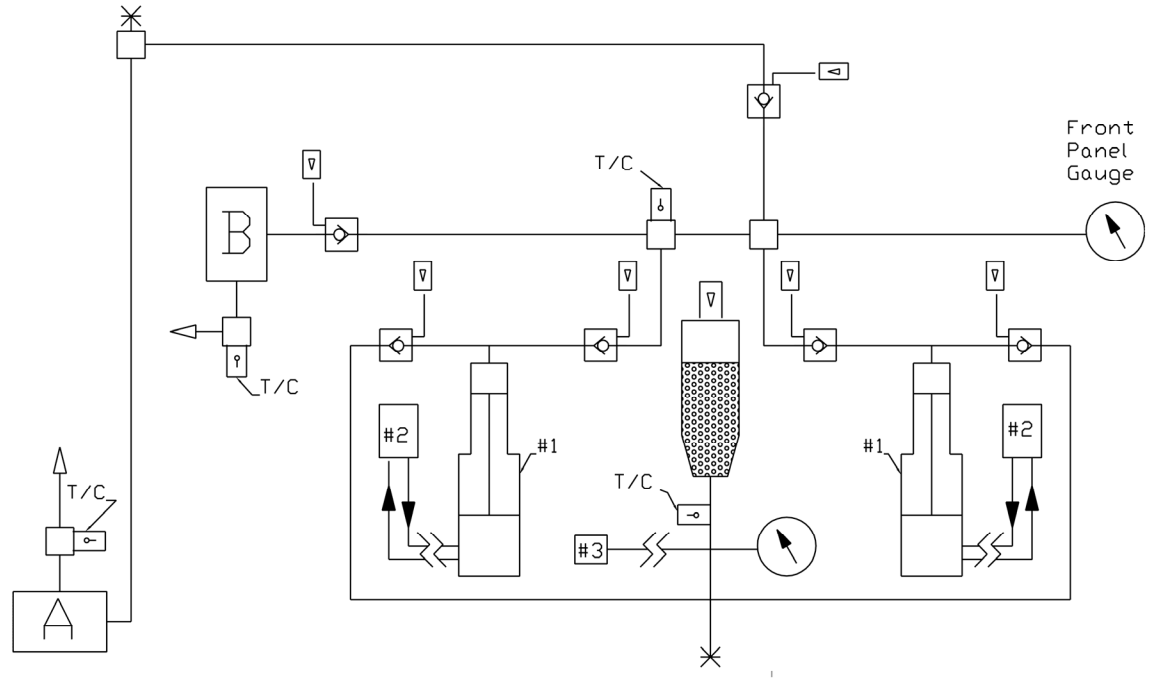
Organism	Valve	Flow Rate mL/min	Fluid Temperature ºC	Reduction Log MPN
Escherichia coli	Micro-metering	1020 ± 58.3a	43.02 ± 6.72b	5.16 ± 1.30d
	Conical	1020 ± 23.1a	59.60 ± 2.74c	5.33 ± 0.88d
Listeria innocua	Micro-metering	1030 ± 94.5a	46.17 ± 4.87b	3.02 ± 0.52e
	Conical	965 ± 47.3a	64.10 ± 0.94c	5.10 ± 0.91d

A: Mean Values ± SD, n=4. Values in the same column followed by the same letter are NOT statistically different at α=.05 per ANOVA and Tukey's Post-Hoc testing

Table 3.4: Microbial Reduction of *Listeria inocua* in Whole Milk [A]

Valve	Flow Rate mL/min	Fluid Temperature ºC	Reduction Log MPN
Micro-metering	930 ± 73.9a	43.09 ± 4.93b	2.04 ± 0.64d
Conical	893 ± 46.2a	63.16 ± 1.70c	2.51 ± 1.21d

A: Mean Values ± SD, n=4. Values in the same column followed by the same letter are NOT statistically different at α=.05 per ANOVA and Tukey's Post-Hoc testing



Legend

A- Release mechanism position "A"
B- Release Mechanism position "B"
T/C- Thermocouple position
1- Hydraulically Actuated Intensifier
2- Main Hydraulic pump
3- Feed Pressure transducer

Figure 3.1: Schematic of High-Pressure System

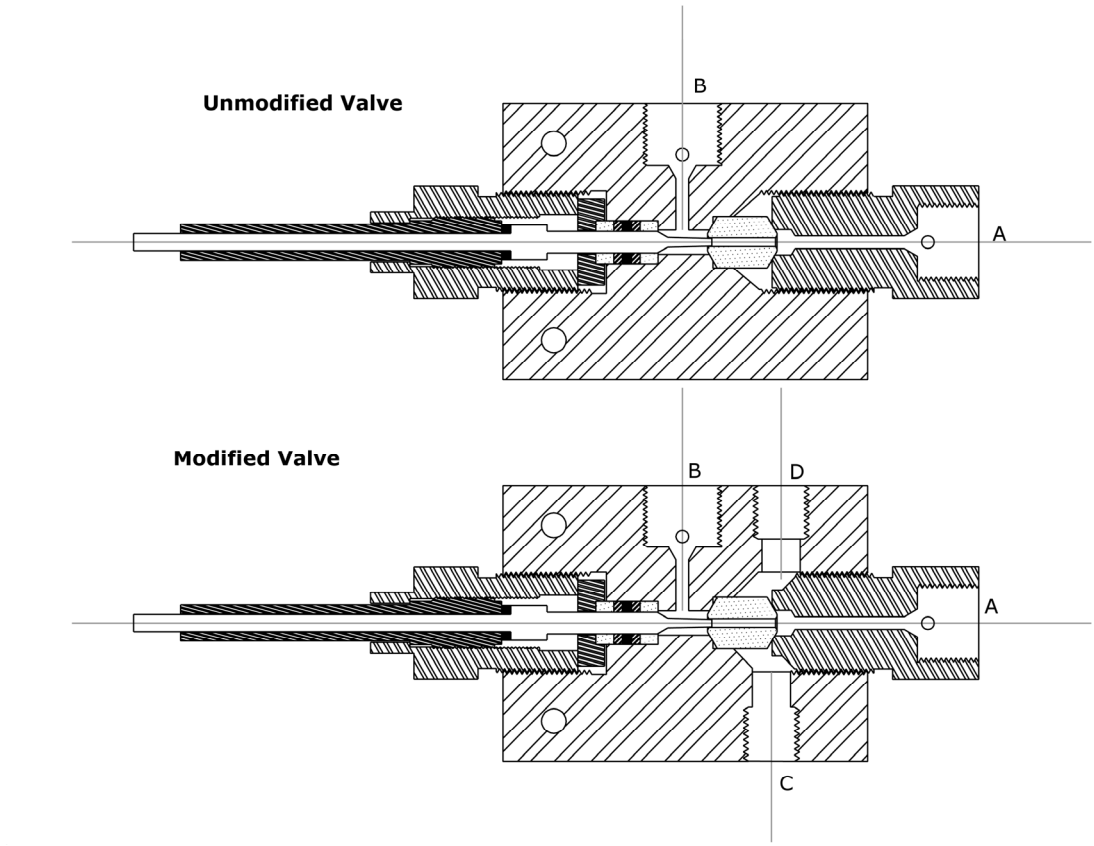


Figure 3.2: Micro-metering Valve Modifications (Cross-Sectional View)

CHAPTER 4

MICROFLUIDIZATION OF FULL-FAT ICE CREAM MIXES: EFFECTS OF GUM STABILIZER CHOICE ON PHYSICAL AND SENSORY CHANGES [1]

CAVENDER, G.A. AND W.L. KERR. Accepted by *The Journal of Food Process Engineering*. Reprinted here with the permission of the publisher, 4/4/2011

Abstract: In this study, we examined the changes in ice cream induced by microfluidization at 220-250 MPa of their respective mixes. Two full-fat ice cream mixes were formulated with either xanthan or locust bean gum and either frozen into ice cream using a batch freezer or microfluidized and then frozen. The resultant products were subjected to modified texture profile analysis, meltdown testing and sensory testing for difference and preference. Increases in hardness and adhesiveness observed between treatment groups in both formulations, as well as an increase in gumminess in the locust bean gum formulation. No difference was observed in meltdown rate between any of the treatment/ formulation combinations. Sensory difference tests showed that panelists could distinguish differences between ice cream prepared from low or high pressure treated mix. Acceptability tests indicated that consumers preferred the firmness and creaminess of ice cream made from microfluidized mixes with LBG.

Introduction:

Ice cream is a complex product , consisting of a blend of ingredients including milk, cream, sweeteners, emulsifiers, flavors, colors and stabilizers (Marshall et al., 2003). One of the most common types of stabilizers used are polysaccharide gums (Marshall et al., 2003). While there is great variety in the structure of the various polysaccharide gums, all share a common motif- long chains of monosaccharides (called a backbone), with differing side chains branching off of that backbone (BeMiller and Whistler, 1996). Some, like xanthan gum have orderly placed, long side chains, while others, like locust bean gum (LBG) have more irregularly placed short side chains (BeMiller and Whistler, 1996). Both xanthan gum and LBG are routinely used in the dairy industry ,not only for stabilization, but also for emulsification, viscosity enhancement and fat replacement (BeMiller and Whistler, 1996). Fat replacement is particularly important to the frozen dessert industry, as while US regulations require a product labeled as “ice cream” contain a minimum of 10% milk fat, research has shown that consumers tend to find ice cream with higher fat contents more appealing (Prindiville et al., 1999, Roland et al., 1999a, FDA, 2008). Since milk fat can be an expensive ingredient, and manufacturers are always looking for ways to reduce that cost (Halliday, 2007).

To improve the quality and value of a frozen dairy product, several methods are available including both formulation changes (e.g. using fat mimetic or stabilizers like vegetable gums) and the use of emerging processing technology (Marshall et al., 2003). Microfluidization is one such emerging processing technology, and one that has particular promise. During microfluidization, liquid foods are forced by means of

extreme pressure (150-250 MPa) through a fixed geometry interaction chamber consisting of two parallel channels which make an abrupt 90 degree turn and feed into a single channel (Microfluidics, 2008). Products subjected to this process experience extreme shearing forces which cause instantaneous heating, microbial inactivation and conformational changes in the product's macromolecules (Microfluidics, 2008). Previous research has shown that the changes to milk components due to microfluidization and other similar high pressure processes can have fat mimicking properties (Paquin, 1999), may provide a richer color and may decrease microbial populations (Adapa et al., 1997).

A small number of studies dealing with the microfluidization of ice cream mix have been published. Olson et al. (2003) microfluidized mixes of various fat contents formulated with a blend of locust bean gum and carrageenan as stabilizers/ fat replacers and determined meltdown rates and sensory preference of the frozen dairy desserts, while Feijoo et al. (1997) focused on the inactivation of bacterial spores. Both studies involved varying the pressure of samples from 50-200 MPa, and both showed limited success, namely a slight change in meltdown characteristics and a less than 70 percent spore lethality respectively (Feijoo et al., 1997, Olson et al., 2003). Work utilizing microfluidization (and other similar high pressure treatments) on other dairy products has had much better success. Mussa and Ramaswamy (1997) noted an increase in viscosity in milk which had been subjected to high pressure homogenization at 200-400 MPa, while Paquin (1999) described the augmentation of cheesemilk by a microfluidized (50-200 MPa) whey protein which resulted in a cheese with 15% less fat than the traditional product, but similar sensory attributes.

At least one study of xanthan gum solutions subjected to high pressure processing has been published, showing that changes occurred to both the molecular weight and water holding capacity as a result of processing, and postulating that the changes were either due to a shortening of the side chains, or a cleavage of the backbone (Paquin, 1999) . While the above study looked at simple suspensions of xanthan gum, little or no work examining how those changes might differ among different gums, and how they might affect a more complex product like ice cream have been published. As the two gums differ in both side-chain length and regularity, (BeMiller and Whistler, 1996) we hypothesize that the microfluidization process will affect them differently, which in turn will cause them to exhibit different behavior and potentially result in a marked difference in their interactions with other ingredients. Therefore, the aim of this study is to determine how microfluidization affects the physical, sensory and hedonic properties of ice cream made from mixes which contain either xanthan gum or LBG .

Materials and Methods

Production: Three batches of ice cream mix (11% milk fat) were formulated per Table 4.1, using either LBG or xanthan gum as a stabilizer. The batches were mixed with a Waring blender on low until smooth and then allowed to rest at 4 ºC for 18-24 hours. After resting, the mixes were stirred for one minute, and approximately one half (volumetrically) of each batch was processed at 220-250 MPa using a high pressure processing system (Model M140-K, Microfluidics, Newton, MA) fitted with a diamond interaction chamber (Model G10Z, Microfluidics, Newton, MA). The four resultant mixes were labeled and stored at 4 ºC for up to five days before freezing. On the day of

freezing, identical amounts of each formulation were transferred to a batch freezer (Taylor Model 104-12, The Taylor Company, Rockton, IL) and underwent an 8-10 minute freezing cycle. The resultant ice cream was transferred into lidded polypropylene containers, hardened for a minimum of four hours at -20ºC and then transferred to another freezer to temper at -12 ºC to -10 ºC prior to sample preparation.

Sensory Testing: Sensory tests for difference and acceptability were performed on all samples. Tests were performed over five different days with only one type of test (difference or acceptability) occurring on a given day. Each formulation/treatment combination was assigned one or more unique randomly generated three-digit identification codes. Samples were dispensed into lidded custard cups with a number 60 portioning scoop, and each cup was labeled with the appropriate identification code. Individual samples were stored at -11 (±1) ºC in a freezer (Frigidaire FFU12C2AW0, White Consolidated, Cleveland, OH) prior to presentation. The temperature of the storage freezer was periodically monitored to ensure all samples on a given day were of the same temperature range. Samples were removed from the storage freezer only after a panelist had been seated in a booth. All evaluations were carried out under sodium vapor light due to the potential of processing induced color changes. Panelists also received a spoon for each sample as well as water and unsalted top saltine crackers for use in palette cleansing.

Difference was evaluated via triangle testing of each formulation per ASTM E1885 – 04. These tests were conducted on three separate days. On each sampling day, each formulation/treatment combination was assigned two randomly generated three-digit identification codes. Identification codes were not repeated on different sampling days.

Panelists were presented with a triad of samples from either the LBG (n=82) or xanthan gum (n=76) formulations, and asked to determine which one was different. Panelist responses were analyzed for statistical significance via binomial probability using statistical software (SAS v9.1, The SAS Institute, Cary, NC). Results were considered to be different if $\alpha<0.05$.

Ice cream samples were evaluated for consumer acceptability of four criteria- Firmness, Creaminess, Vanilla Flavor and Sweetness using an unstructured 145 mm line scale marked with labels "unacceptable", "acceptable" and "ideal". These labels were a modified version of the three point hedonic system developed by Shewfelt et al. (1997) and elaborated by DuBost et al. (2003). Panels were conducted on two separate days, and an individual panelist was not allowed to participate on both days. In order to provide temperature control during assessment, each sample cup was placed inside an insulated double walled container which had previously been filled with water and frozen. These containers were stored in the above mentioned freezer to ensure homogeneous temperature. Panelists (n=48) were presented with four samples- one of each treatment/ formulation combination formulations, and asked to indicate on the line their opinion of a given samples characteristic. A balanced order of presentation was used. The ballots were collected and the distance between the leftmost end point and the panelist's mark was measured in centimeters to arrive at a numeric value. Data thus generated were analyzed for statistical significance via multi-way analysis of variance and Tukey's HSD post hoc testing as appropriate using statistical software (SAS v9.1, The SAS Institute, Cary, NC). Results were considered to be different if $\alpha<0.05$.

Texture Analysis: Samples were subjected to a modified "two-bite" TPA analysis (after Prindiville et al., 1999), using a TA-XT2i universal testing machine (Stable Microsystems Ltd, Godalming, Surrey UK). The machine was fitted with a 25 kg load cell and a 3 mm cylindrical stainless steel probe. Cross arm speed was 3.0 mm/s pre and post testing and 3.3 mm/s during testing. Trigger force, penetration depth and collection rate were 5 g, 22.5 mm and 200 pps respectively. Three readings were taken from different locations on each sample and data were collected and analyzed with the Texture Expert Exceed software package (v 2.61, Stable Microsystems Ltd, Godalming, Surrey UK) to obtain hardness, cohesiveness, gumminess and adhesiveness values. Data thus generated were analyzed for statistical significance via multi-way analysis of variance and Tukey's HSD post hoc testing as appropriate using statistical software (SAS v9.1, The SAS Institute, Cary, NC). Results were considered to be different if $\alpha<0.05$.

Viscosity of Ice Cream Mix: Dynamic viscosity measures of each formula/treatment combination were taken using a Brookfield Viscometer (Model LVDVE115, Brookfield Engineering, Middleboro MA) which had been fitted with either an RV4, RV5 or RV6 spindle (Brookfield Engineering, Middleboro MA). Spindle size was chosen by selecting the largest spindle which would give a reading that was not out of range. Prior to testing, each sample was allowed to age for 24-26 hours at 9-10 ºC and was shaken briefly to prevent separation immediately prior to measurement. All measurements were taken in triplicate and collected data were then analyzed for statistical significance via analysis of variance and Tukey's HSD post hoc testing as appropriate using statistical software (SAS v9.1, The SAS Institute, Cary, NC). Due to the large differences observed between

formulations, results were examined only within a given formulation and were considered to be different if $\alpha<0.05$.

Meltdown: An apparatus for meltdown testing was constructed after Marshall et al. (2003) consisting of a stainless steel mesh (three 0.8 mm holes/cm) on top of a small funnel. The funnel was suspended by a laboratory ring stand over a collection container which rested on a balance. All meltdown experiments were conducted at room temperature (19±2 ºC). Cylindrical cores of 2.54 cm diameter were excised from the samples used in texture analysis using a purpose-built coring device. These cores were sliced radially to produce three meltdown samples for each treatment/formulation combination. Mean mass of samples across all replications was 12.70 g (±2.35 g) and samples were stored in a storage freezer (-11 ±1 ºC) until needed. Samples from a given replication were tested in a random order on the day of sampling. Each sample was placed onto a clean, dry screen, at which time start time was recorded. The mass of melted ice cream that dripped down into the collection vessel was recorded at regular intervals and after collection, a plot of percent mass melted versus elapsed time constructed. These plots are characteristically sigmoid, with the slope of the linear region defining the meltdown rate (Marshall et al., 2003). Meltdown rate was calculated using Microsoft Excel. A representative example graph is shown in Figure 4.1. Data were analyzed for statistical significance via multi-way analysis of variance using statistical software (SAS v9.1, The SAS Institute, Cary, NC). Results were considered to be different if $\alpha<0.05$.

Results and Discussion

Sensory: Sensory difference data are presented in Table 4.2 and indicate that panelists were able to discern a difference in microfluidized and non-microfluidized samples, both for LBG and xanthan gum. These results appear to be contrary to a previous study in which no difference in sensory scores (flavor, body and texture) was found between microfluidized and non-microfluidized samples (Olson et al., 2003). However, the previous work and this one differed in several key factors. First Olson's study dealt with reduced fat ice cream, while this study deals with full fat ice creams. Second, in Olson's study an experienced panel consisting of only seven panelists was used, potentially increasing accuracy but also greatly limiting statistical degrees of freedom. Finally, the previous work dealt with descriptive analysis rather than difference testing. We cannot ascertain whether textural, gustatory or aromatic characteristics provided our panelist with the ability to discern samples. While textural differences were observed instrumentally (see below) , certain compounds can affect the perception of flavor in frozen desserts such as reduced fat ice cream (Roland et al., 1999b) and further that changes in the structure of certain molecules affect how flavor is perceived (Abd El-Rahman et al., 1997). Therefore one or more of these phenomena are likely responsible for the sensory differences detected by panelists in this study.

Acceptability data are presented in Table 4.3. For all four criteria each formulation/treatment combination had a mean score above the midpoint of the line (7.25 cm) indicating the samples were not only acceptable, but were approaching ideal (14.5). Further, for the LBG samples, a significant difference was noted in consumer opinions of the firmness, with the microfluidized samples showing scores closer to ideal than the

control samples. If we consider firmness (as perceived by panelists) and hardness (as determined instrumentally) to be analogous, this seems to indicate that, at least for the LBG formulation, the differences in hardness treatment groups formulations were above the threshold of detection for the average consumer. No difference was observed among any of the formulations with regard to creaminess. This lack of difference is not surprising as creaminess is a very complex descriptor, subsuming texture, flavor and fat content (Kirkmeyer and Tepper, 2003). Thus even if the textural differences which have been determined instrumentally were noticeable and contributed to the perception of creaminess, the fat content and vanilla flavor could have easily masked it from an untrained panelist. Finally, no difference was seen in either sweetness or vanilla flavor, implying that the changes induced by processing did not affect the simple carbohydrates responsible for sweetness, nor did the process create complexes that either augmented or masked the vanilla flavor, which was added after processing.

Texture Profile: The aggregate data from the texture profile analysis, presented in Table 4.4, indicate that the LBG samples made from microfluidized mixes were harder, more adhesive and gummier than those that had not. Samples made with xanthan gum also showed an increase in hardness and adhesiveness (but not gumminess) between samples made with microfluidized mixes and those in the control group. The microfluidization process also had an interesting effect on cohesiveness. While no difference was observed between the cohesiveness of the non microfluidized LBG and xanthan formulations, the microfluidized samples showed a marked difference, with the xanthan formulation showing both a higher mean cohesiveness and an increased variability.

The increase in hardness of the xanthan formulation tends to agree with earlier research which found that microfluidized xanthan gum solutions formed stronger gels than non microfluidized solutions (Lagoueyte and Paquin, 1998). Later work determined that xanthan gum actually undergoes changes at the molecular level during high pressure processing, showing a decreased molecular weight, and the author hypothesized that the physiochemical changes which were observed, stem from said changes (Paquin, 1999). Similar changes likely occur in the LBG formulations during microfluidization, which explain their hardness increases as well. The changes in adhesiveness, gumminess and cohesiveness observed in this study imply that either some property of the individual gums is altered differently by the process, or that the gums interact differently with the other components (primarily lipids and proteins) post processing or some combination of the two. All are potentially likely scenarios given previous work. High pressure processing causes structural changes on the molecular level to xanthan gum (Paquin, 1999), and that the gum's structure is known to be quite different than LBG (BeMiller and Whistler, 1996). Further, non-polysaccharide ingredients undergo changes during high pressure processing which can increase the solubility of divalent ion containing compounds by altering secondary and tertiary structures, which in turn makes the formation of inter molecular bonds more likely (Huppertz et al., 2006). Finally, previous work has shown a synergistic effect between microfluidized whey proteins and microfluidized xanthan gum with regard to the size of protein-polysaccharide complexes (Paquin, 1999).

Viscosity: Viscosity data are presented in Table 4.5. In both formulations viscosity increases were observed between the control samples and microfluidized samples of a given formulation. In the case of xanthan gum, the increase was over 17 fold, while the LBG showed an increase of 8.5 fold. Both increases were significant when examined within a given formulation. These increases in viscosity are most likely responsible for some of the textural changes, as an increase in the viscosity of a bulk fluid slows the movement of particles (such as air bubbles) passing through it. Thus, when being frozen, the air bubbles which might normally coalesce and form larger bubbles do not flow as freely and thus may not be able to encounter another bubble before becoming trapped within the freezing matrix. Such a phenomenon would create a more evenly distributed gaseous phase within the ice cream, and such fine distributions of air are thought to improve the perception of smoothness among consumers (Goff, 2002). Additionally, these smaller bubbles could also result in an improved shelf life, as smaller air bubbles are known to increase the long term stability of ice cream (Barfod, 2001) .

Meltdown: Meltdown data are presented in Table 4.6. No significant differences were determined for any of the formulation/treatment combinations. These results were unexpected, given that previous work on reduced fat ice cream had indicated a reduced meltdown rate due to microfluidization (Olson et al., 2003). We do acknowledge that Olson's work also showed that traditionally homogenized full fat ice cream showed a different meltdown rate from traditionally homogenized low fat formulations (Olson et al., 2003), thus perhaps the presence of higher fat content somehow effected the changes we observed. Another study showed microfluidization reduced the size of fat globules

and casein micelles, caused the formation of encapsulated fat particles as well as extensive protein-fat complexes (Olson et al., 2004). As our samples had a significantly higher percentage of fat than Olson's it is very likely that these complexes helped to mask differences which might have been seen had we used a lower fat formulation.

Additionally, though it did not significantly affect the rate of meltdown, it should also be noted that several control samples of our LBG formulation exhibited the "foamy" defect during melt down, while none of the other treatment/formulation combinations did. This type of defect is typically associated with the presence of surface active substances like emulsifiers (Marshall et al., 2003). While no such surface active substances were used in any formulation in this study, it is possible that native compounds in the dairy ingredients can act in that role, particularly proteins with significant hydrophobic and hydrophillic regions. The previously mentioned formation of encapsulated fat particles (Olson et al., 2004), offers the very likely possibility of a reduction in available oil-water interfaces, which could very well interfere with the native surface active compounds in the LBG formulation.

Conclusions

By treating full fat ice cream mix with microfluidization, one can affect both sensory and physical properties of the finished product, and further those changes differ based upon the gum used. Perhaps more importantly, the changes induced by microfluidization can create a significant increase in consumer acceptability in formulations made with either xanthan or locust bean gum. While these changes are quite promising, additional work is needed to better characterize the mechanisms of the changes, how they act differently on

the various stabilizers, and how this knowledge can be used to produce a more desirable product.

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Figures and Tables

Table 4.1: Ice Cream Formulation

Ingredient	Amount (%w/w)
Half and Half [A]	64.3%
Heavy Cream [A]	11.0%
Non-Fat Dry Milk [A]	4.5%
Sucrose [A]	12.0%
Corn Syrup Solids (DE:20-23) [B]	5.0%
Gum [C]	0.2%
Vanilla Extract [D]	3.0%

A: The Kroger Company, Cincinnati, OH
B: Maltrin 200, Grain Processing Corporation, Muscatine, IA
C: Either xanthan gum: TIC Gums, White Marsh, MD or locust bean gum: Degussa Texturant Systems, Atlanta, GA.
D:Tone's-ACH Food Company, Memphis, TN.

Table 4.2: Sensory Difference Results

	Response [A]			
Formulation	Total Panelists	Correctly Identified	Incorrectly Identified	p-value [B]
LBG	84	40	44	0.0016
Xanthan	76	38	38	0.0044

A: Indicates ability or inability to correctly identify the different sample in a triad
B: Determined by binomial probability testing Values less than 0.05 are considered statistically significant.

Table 4.3: Consumer Acceptability Results

	Response[A]			
	Firmness	Creaminess	Vanilla Flavor	Sweetness
LBG Control	9.00 ± 3.57ab	9.74 ± 3.21c	8.85 ± 3.19d	9.62 ± 3.21e
Microfluidized LBG	10.73 ± 3.24a	10.58 ± 2.99c	8.85 ± 3.789d	10.19 ± 3.221e
Xanthan Control	8.83 ± 3.65b	9.94 ± 2.97c	9.59 ± 3.31d	9.76 ± 3.501e
Microfluidized Xanthan	9.88 ± 3.01ab	9.09 ± 3.47c	8.33 ± 3.77d	9.29 ± 3.641e

A: Mean + SD of the distance to the panelist's mark in centimeters. Values in a given column with the same letter following are not statistically significant via ANOVA and Tukey's Post-Hoc testing

Table 4.4: Textural Properties of Ice Cream

Formulation	Property [A]			
	Hardness (g)	Adhesiveness (g)	Gumminess	Cohesiveness
LBG Control.	64.73 +/- 22.49**a**	-135.04 +/- 34.18**c**	31.56 +/- 7.91**e**	0.51 +/- 0.10**gh**
Microfluidized LBG	96.57 +/- 49.10**b**	-201.55 +/- 88.45**d**	44.19 +/- 19.72 **f**	0.49 +/- 0.09**h**
Xanthan Control	76.47 +/- 21.13**a**	-159.67 +/- 21.20**c**	38.02 +/- 5.71**ef**	0.52 +/- 0.12**gh**
Microfluidized Xanthan	96.14 +/- 40.71**b**	-201.78 +/- 48.44**d**	47.43 +/- 12.32 **f**	0.57 +/- 0.23**g**

A: Mean +/- SD, n=9.Values in a given column with the same letter following are not statistically significant via ANOVA and Tukey's Post testing

Table 4.5: Viscosity of Ice Cream Mix

		Control Samples [A]	Microfluidized Samples [A]
	n	cP	cP
LBG	3	76.8 ± 2.1	654.7 ± 14.2
Xanthan	3	795.7 ± 19.5	13816.7 ± 1329.1

A: Mean Viscosity +/- SD. Values in the same row with the same letter following are not statistically significant via ANOVA and Tukey's Post testing within a given formulation.

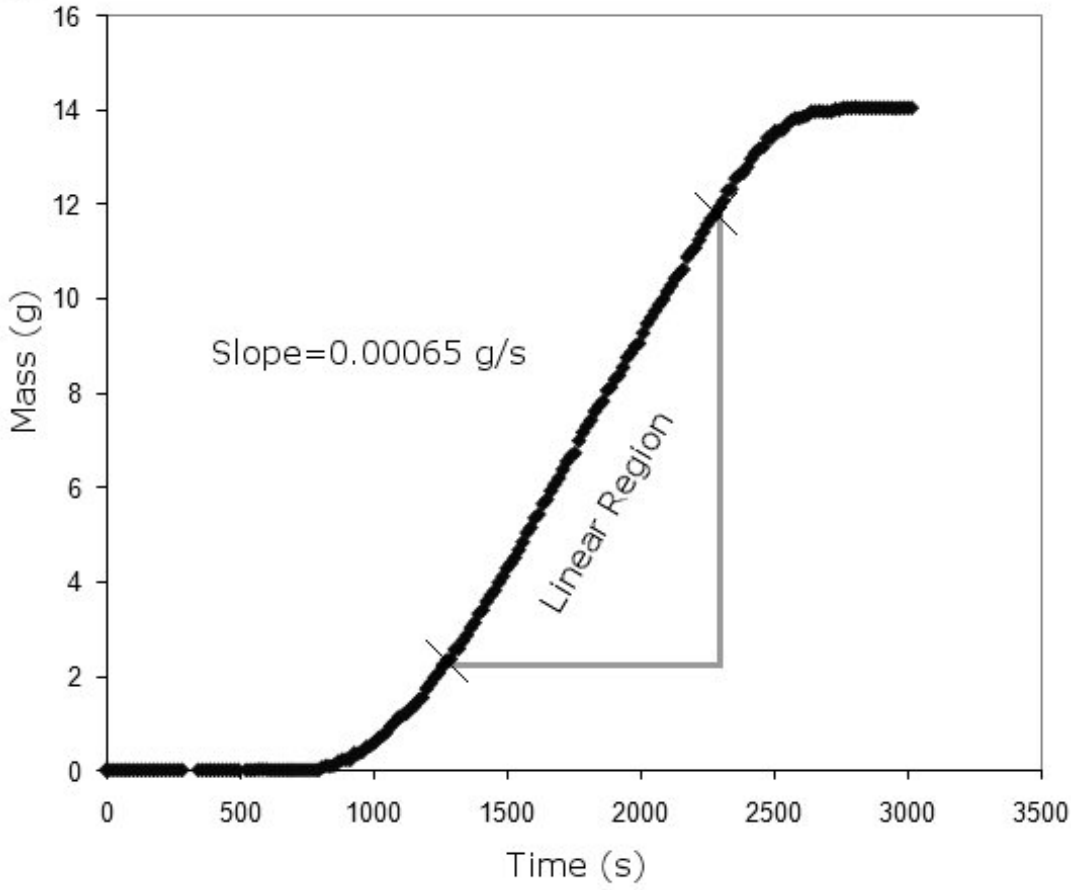
Table 4.6: Meltdown Characteristics of Ice Cream

			Property	
	Rep.	n	Mean Meltdown Rate (grams per second) [A]	Mean Initial Sample Mass (g)
LBG Control	1	3	0.102 ± 0.004	9.973 ± 1.259
	2 [B]			
	3	3	0.075 ± 0.004	13.337 ± 1.301
	all	**6**	**0.089 ± 0.015**	**11.655 ± 2.169**
Microfluidized LBG	1	3	0.107 ± 0.005	11.860 ± 0.502
	2	2	0.069 ± 0.008	16.105 ± 5.551
	3	3	0.070 ± 0.010	13.737 ± 2.754
	all	**8**	**0.083 ± 0.021**	**13.626 ± 3.121**
Xanthan Control	1	3	0.121 ± 0.001	9.057 ± 0.309
	2	2	0.092 ± 0.005	13.480 ± 1.428
	3	3	0.078 ± 0.010	14.047 ± 2.843
	all	**8**	**0.097 ± 0.021**	**12.034 ± 2.960**
Microfluidized Xanthan	1	3	0.125 ± 0.012	11.300 ± 1.411
	2	2	0.096 ± 0.001	11.760 ± 0.693
	3	3	0.073 ± 0.004	14.410 ± 1.161
	all	**8**	**0.098 ± 0.025**	**12.581 ± 1.831**

A: No significant difference exists between values in this column at α=.05 per ANOVA and Tukey's Post-Hoc testing

B: Data for this replication and formulation combination was lost due to equipment error.

Figure 4.1: Representative Meltdown Graph



CHAPTER 5

MICROFLUIDIZATION OF FULL-FAT ICE CREAM MIXES: RHEOLOGY AND MICROSTRUCTURE [1]

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Abstract: In this study, we examined the changes in ice cream induced by microfluidization at 220-250 MPa of their respective mixes. Two full-fat ice cream mixes were formulated with xanthan or locust bean gum and subjected to dynamic rheology, particle size analysis and transmission electron microscopy. Fitting the mix rheology data to a power-law model showed that microfluidization increased the shear-thinning behavior of the mixes marginally while dramatically increasing the consistency of both formulations. Goodness of fit was lower in the treated samples, and it is believed that this was due to the more solid-like behavior of the treated samples. Thus the data were fitted using a modified cross model, which includes a term for polymer relaxation. This fitting was found to better predict the behavior of the microfluidized samples, reinforcing the hypothesis that treated samples were more solid-like. Particle size distribution predictably became more uniform after treatment, but average particle size increased slightly. Micrographs revealed easily visible changes in the ultra-structure of casein complexes in both formulations. Mixes were also frozen into ice cream which was subjected to dynamic rheology and scanning electron microscopy. The ice cream rheology data displayed showed that the changes seen in the mixes carried over into the frozen product and the SEM images showed changes in the air bubble size due to treatment in both formulations. Overall the changes observed in all tests were more pronounced in the locust bean gum formulation, which we believe is due to the differences in structure between the two gums.

Introduction:

Ice cream is a complex product formulated with several ingredients including milk, cream, sweeteners, stabilizers, emulsifiers, flavors and colors (Marshall et al., 2003). Formulations vary from product to product, depending on cost, consumer demand and applicable regulations. In the US, the standard of identity of ice cream requires a minimum of 10% milk fat, and some manufacturers use even higher amounts, as researchers have shown that higher fat contents correlate with increased consumer appeal (Prindiville et al., 1999, Roland et al., 1999, FDA, 2008) .

The increase in appeal of higher fat ice cream is offset by the fact that milkfat is an expensive ingredient, and manufacturers are always looking for ways to decrease that cost without appreciable loss of quality (Halliday, 2007). In addition, concerns over obesity and related health issues have driven demand for lowfat products. Among the ways of reducing fat and increasing quality are the use of stabilizers, fat mimetics, vegetable gums, and emerging technology (Marshall et al., 2003). One such emerging technology is microfluidization, wherein fluid foods are forced through a fixed geometry interaction chamber by means of extreme pressure (150-250 MPa). Inside the interaction chamber the inlet stream is divided into two parallel channels which then make abrupt 90 degree turns to rejoin in a single outlet stream (Microfluidics, 2008). This conformation subjects the fluid to high shear, instantaneously raising the temperature and inducing conformational changes in the fluid's macromolecules (Microfluidics, 2008).

High shear processes have been shown to effect changes in the physical and structural properties of dairy products. McRae (1994) observed reductions in fat globule size compared to traditional homogenization and also suggested an inhibitory effect on

fat clustering. Mussa and Ramaswamy (1997) saw an increase in the viscosity of milk after high pressure (200-400 MPa) homogenization. Paquin (1999) microfluidized (50-200 MPa) whey protein to create a cheese with 15% less fat than the traditional product, but similar sensory attributes. Olson et al. (2003) showed changes in the meltdown rate of reduced fat ice cream produced from microfluidized mix. Cavender and Kerr (2011) examined the sensory and physical attributes of ice cream made from microfluidized mixes and found significant changes in both texture properties and sensory scores.

In addition to dairy products, several studies have examined the effects of high pressure- high shear processes on stabilizer ingredients commonly used in dairy foods. Lagoueyte and Paquin (1998) observed changes in water uptake, viscosity, flow behavior and molecular weight of xanthan gum while Laneuville et al. (2000) found that mixtures of whey protein and xanthan gum, when microfluidized had increased viscosity and other desirable properties. These changes are thought to be from alterations to the structure of the polymers, based on earlier work done on the effects of high shear on non-food polymers (Harrington and Zimm, 1965, Paquin, 1999).

Both xanthan and locust bean gum (LBG) are commonly used stabilizers in the frozen dessert industry, and previous studies have shown that frozen dessert mixes made with those stabilizers and processed in high-shear conditions undergo measurable changes in physical and sensory properties (Olson et al., 2003, Marshall et al., 2003, Cavender and Kerr, 2011). The nature of these changes is not well understood. Thus, we examined in greater depth the changes imparted by the microfluidization of full-fat ice cream mixes made with either xanthan or locust bean gums through rheology and electron microscopy of both the mixes and the frozen ice creams.

Materials and methods

Production: Ice cream mix containing 11% milk fat and stabilized with 0.2% of either xanthan gum (TIC Gums, White Marsh, MD) or locust bean gum (Degussa Texturant Systems, Atlanta, GA) was prepared after Cavender and Kerr (2011). To prevent clumping, gum stabilizers were first dispersed into the dry ingredients prior to addition to the dairy ingredients. Vanilla extract was omitted from all batches until the day of freezing. Dry and wet ingredients were mixed in a Waring blender set on low speed. Dry ingredients were added incrementally as mixing occurred. After all dry ingredients had been added, the mixing was continued for one minute to ensure smoothness. The mix was transferred into lidded containers and allowed to rest at 4 ºC for 48 h. After resting, the mixes were stirred for one minute and approximately one half (volumetrically) of each batch were processed at 220-250 MPa using a high pressure processing system (Model M140-K, Microfluidics, Newton, MA) fitted with a diamond interaction chamber (Model G10Z, Microfluidics, Newton, MA). The four resultant mixes were labeled and stored at 4 ºC until the day of freezing. On the day of freezing, 1455 g aliquots of each formulation were portioned out for the production of frozen ice cream and flavored with 45 g of vanilla extract. These aliquots were shaken vigorously to disperse the extract and then transferred to a batch freezer (Taylor Model 104-12, The Taylor Company, Rockton, IL) in a random order for freezing. Each aliquot was subjected to a 7-8 minute freezing cycle and the resultant ice cream was transferred into 1-liter lidded polyethylene containers by hand prior to hardening. Resultant samples were hardened for a minimum of 4 h at -30ºC and then transferred to another freezer to temper at -12 ºC to -10 ºC prior to frozen sample preparation.

Particle Size Analysis: Aliquots (~50 mL) of each formulation were taken prior to the addition of vanilla extract and subjected to particle size analysis by a Mastersizer S laser light scattering device (Malvern Instruments, Southborough, MA) fitted with a 300 mm lens and a liquid dispersion cell. De-ionized water was used as a dispersant, and samples were added drop-wise until obscuration fell between 14 and 16 %. An impeller speed of 2100 RPM was maintained for all samples. Each measurement consisted of 2000 sweeps and was repeated in duplicate for each formulation.

Mix Rheology: Aliquots (~50 mL) of each mix were taken and subjected to dynamic rheology using a stress-controlled rheometer (Model SR-5000, Rheometric Scientific, Piscataway, NJ) fitted with Couette geometry consisting of a 32 mm ID cup and a 29.5 mm diameter bob. The bottom gap was set at 1.5 mm and sample volume was 5.5 mL For all experiments, temperature was maintained at 4 ºC by means of a cooling bath,. Shear rate ramp tests were performed from 0-100 s^{-1}. Experiments were repeated after 3 weeks of storage at 4 ºC to determine if storage time influenced mix rheology. The data of shear stress (τ) versus shear rate ($\dot{\gamma}$) were fit to the power law model:

$$\tau = K\dot{\gamma}^n \tag{1}$$

where K is the consistency index and n the power law index. K approaches the Newtonian viscosity of the fluid at zero shear, while n measures the shear-rate dependence. Data of the apparent viscosity (η) versus shear rate were fit to a modified Cross model which neglects the infinite shear viscosity as suggested by Rao (1999a):

$$\eta = \frac{\eta_0}{1 + (\alpha_c \dot{\gamma})^m} \tag{2}$$

where "η_0" is the viscosity near zero shear and γ is the shear rate. The Cross rate constant "m" measures the dependence of viscosity on shear rate in the shear-thinning region and the time constant "α_c" is related to the relaxation time of the sample.

Rheology of Frozen Ice Cream: Using a modified procedure developed by Goff et al. (1995), samples of ice cream were taken immediately after batch freezing and placed into a pre-chilled sampling device consisting of a solid bottom plate and a 3 mm thick top plate with three 19 mm diameter circular wells cut into it, leveled off using a pre-chilled flat stainless scraper, wrapped in foil and placed into a -35 ºC freezer to harden for at least 24 h. On the day of testing, each sample was removed by separating the two plates and pressing the individual samples out using a pre-chilled cylinder. Samples were then subjected to dynamic rheology using the above stress-controlled rheometer, fitted this time with a parallel plate geometry. Components were pre-chilled to -8 ºC before sample loading and gap was set at 1.3 mm to ensure complete contact. Each formulation was first subjected to a dynamic stress sweep at 1 Hz to determine the linear visco-elastic region (LVR) and the subsequent dynamic frequency sweep testing of a given formulation was performed within that LVR.

Transmission Electron Microscopy of Ice Cream Mix: Aliquots of each mix, taken prior to the addition of vanilla extract were examined with a 200 kV TEM (Philips/FEI Technai 20 -FEI Co., Eindhoven, Netherlands) to observe the effects of the process on casein and macromolecular structure. Samples were prepared using a modified version of a procedure described by McMahon and Oommen (2008). Individual copper TEM grids

were prepared by first coating them in dissolved paralodion (SPI Chemicals, West Chester, PA), and allowing them to dry. Then each grid was coated in poly-L-lysine (Electron Microscopy Sciences, Hatfield, PA) to create a net positive charge on the grids to aid in protein adherence. Mixes were diluted 1:100 and then applied to each grid. After several seconds, grids were dipped into de-ionized water twice to rinse off non-adhered materials. Grids were then allowed to dry before negative staining with uranyl acetate (Fisher Scientific, Fair Lawn, NJ). The resulting grids were stored in a desiccator under refrigeration for up to 2 weeks before observation.

Scanning Electron Microscopy of Frozen Ice Cream Mix: One-liter lidded polyethylene containers of each formulation, which had been previously prepared as above, were frozen to a temperature of -78.5 ºC to facilitate sampling by fracture. A stainless steel blade was then used to strike the surface of the frozen ice cream, producing small samples with a freshly fractured surface which were then loaded onto a pre-chilled sample holder fitted with a standard SEM stub. The sample holder was then transferred into a variable pressure Field Emission SEM (Hitachi SU6600, Hitachi High Technologies America, Schaumburg, IL) for imaging. Imaging was performed in backscatter mode at an excitation voltage of 20 kV and a pressure of 30 Pa.

Results and Discussion

Particle Size analysis: The changes in particle size distribution of the LBG and xanthan gum formulations resulting from microfluidization are shown in Figure 5.1. As expected, the process of microfluidization (itself a homogenization process) decreased the overall

distribution of the particle sizes in both formulations. Additionally, both formulations also showed an increase in average (50^{th} percentile) particle size. This phenomenon is most likely due to interactions between what were previously smaller particles due to the effects of the high pressure/shear, and was not totally unexpected, as one previous study saw similar similar changes in microfluidized whey protein and xanthan mixes. In that study, the authors suggested that such changes were predicated by disruptions in the structure of the xanthan gum, which allowed for an increased number of bonds (Laneuville et al., 2000). Finally, the LBG formulation showed an increase in the size of the 90^{th} percentile particle size after microfluidization, while the xanthan formulation did not. The untreated sample has significant "tailing" in the region of greater particle size (<20 μm). As this tailing is not present in the microfluidized LBG sample, and similar tailing is not seen in either xanthan sample, it follows that the phenomenon is related to some facet of the structure of the unmodified LBG that is not shared with xanthan gum. There are three such structural properties- the backbone sugar, side chain length and regularity. LBG is a galactomannan, with a mannose backbone and side chains of galactose; these side chains are attached at a ratio of approximately 4:1 in a disorderly fashion (BeMiller and Whistler, 1996). In contrast xanthan has a glucose backbone and tri-saccharide side chains attached in a ratio of 2:1 (BeMiller and Whistler, 1996). As the backbone sugar of LBG could not have been changed by microfluidization, possible changes in the sidechain are the most likely cause of the differences in behavior. Sidechain length could only have been shortened, and as LBG has a monosaccharide sidechain, this would mean the side chain were totally removed. Thus, any individual side chains which remain on LBG after microfluidization must have the same structure as the

native LBG. Changes in regularity are therefore the most likely cause of the different behaviors seen. Reduction of the presence of long regions of unsubstituted backbone molecules between individual side chains would easily explain the behavior, and such a modification could easily occur if the backbone is broken in a region of extended unsubstituted backbone units lying between two more heavily substituted regions. The products of such scission would then be two molecules which were smaller, but did not have the interruption of their more heavily substituted regions. This type of modification is likely, given the fact that random-scission effects have been proposed as a mechanism of polymer degradation by several studies on non-food and food polymers (Paquin, 1999, Silvestri and Gabrielson, 1991, Harrington and Zimm, 1965).

Mix Rheology: Figure 5.2 shows the plots of stress versus shear rate for the xanthan and LBG formulations. A power-law model fitting of the data was attempted. This model is often referred to as a good general purpose model which allows one to determine how close to Newtonian a given fluids behavior is. It involves two terms- a consistency index and a power law index. Power law indices quantify how Newtonian a fluid is: perfect Newtonian fluid will have a power law index of one, while values less than one indicate shear thinning fluids and values greater than one indicate shear thickening fluids (Rao, 1999a). Thus, according to the model fitting, both formulations showed a slight increase in shear thinning behavior and a sizable increase in consistency index after microfluidization. The legend lists the resultant indices and correlation (r^2) values for both mixes after 3 and 27 days of storage. The day 3 results are analogous to those reported by Lagoueyte and Paquin (1998) for 1% xanthan gum solutions. Despite these

similarities, the microfluidized samples were less well predicted by the power-law model, a stark contrast to the pure solutions in the previous work. Of further interest is the effect of prolonged storage on the mixes. The microfluidized samples showed a marked decrease in consistency index after storage, while the non- microfluidized samples of the same formula did not. The storage time examined was excessive for most freshly made ice cream mixes, however, there has recently been an interest in shelf stable ice cream mix, and if aseptically packaged, the mix may have a shelf life far in excess of the storage time examined here (Scott, 2008, Anonymous, 2006).

The inability of the power law model to accurately predict the behavior of the microfluidized samples, is contrasted by the fit determined using the modified Cross model. As can be seen in Figure 5.3, the Cross model does a much better job of predicting their behavior. This improvement of fit is due to the inclusion of the time constant, which relates to the relaxation time of the sample. Relaxation time can be defined as the amount of time for a given disrupted complex structure to become disentangled (Strobl, 1997). The need to account for structural disentanglement is indicative of an overall increase in structure complexity in the mixes due to microfluidization. Further the relaxation constant for the microfluidized xanthan formulation is approximately 67% greater when compared to the LBG formulation (0.104 vs 0.0621) which may help explain the differences in the properties of the frozen products seen between the two formulations in the current and previous works.

Rheology of Frozen Ice Cream: Figure 5.4 shows the dynamic stress sweep data of all four treatment/ formulation combinations. G' is the storage modulus, an indicator of how

solid-like a substance behaves, while G'' is the loss modulus, which indicates how liquid-like a substance behaves (Goff et al., 1995, Rao, 1999b). While overall the LBG samples (control and microfluidized) had higher storage and loss moduli compared to the xanthan samples, both formulations showed reductions in storage and loss moduli after microfluidization. Additionally, the microfluidized xanthan sample showed a smaller linear visco-elastic region than all of the other samples. Figures 5.5 and 5.6 show the results of dynamic frequency sweeps of the LBG and xanthan formulations, respectively. The general trend of decreased moduli in the microfluidized samples which was seen in the dynamic stress sweeps was repeated in the frequency sweeps. The similarities in behavior of the samples made from microfluidized samples when compared to the non microfluidized samples is not surprising, as our previous work also showed similarity in the changes due to microfluidization in mix viscosity, texture and even some sensory tests (Cavender and Kerr, 2011). Further, data reported by Goff et al. (1995) show that stabilizers tended to reduce both storage and loss moduli in ice cream. This result suggests that the microfluidization of stabilized mixes amplifies the effect of the stabilizers.

While some similarities were seen in the general trends of the rheology data of the two formulations, there were some marked differences. Dynamic frequency data shows that at -8 °C only the xanthan formulations experienced G'/G'' crossover , $G_{c,}$ which is the point at which the samples moves from predominately solid-like to predominantly liquid-like behavior (Rao, 1999b). Thus, at the given temperature, the LBG formulations display predominantly liquid-like behaviors throughout the frequency range, while the xanthan formulations have solid-like behavior at lower frequencies but transition into

liquid-like behavior as frequency increases. This behavior is not surprising, given that xanthan gum is known to form stronger gels compared to LBG, and thus mixes containing xanthan gum should have more solid like properties (Yaseen et al., 2005, Higiro et al., 2006). Looking further, in the samples made from untreated mix, the difference between G' and G'' increases dramatically as frequency increases, while the samples made from treated mix have much closer values. While this increase is more marked in the xanthan samples, the LBG samples also show a similar trend, albeit to a lesser degree.

Transmission Electron Microscopy of Ice Cream Mix: Figures 5.7 and 5.8 show typical structures found in the two mix formulae before and after microfluidization. Of particular note are the hair-like structures present in both control samples, but absent in the microfluidized samples. McMahon and Oommen (2008) noticed similar structures in their micrographs of casein micelles, and identified them as chains of proteins extending outward, terminating in either single or double (disulfide-linked) κ-casein. Their micrographs did show these hair-like structures all around the micelles, while ours did not but this is likely due to interactions between the micelles and other ingredients of the ice cream mix, particularly the gums. In fact, both xanthan and LBG can be used to facilitate the concentration/ separation of casein (Ambrose, 1935, Hemar et al., 2001). Schorsch et al. (1999) explain this phenomenon as being related to the thermodynamic incompatibility, wherein the polymer (in his study LBG) interacts more favorably with the solvent than the protein, causing agglomeration of protein which will lead to phase separation. This effect was readily observable after only a few days of storage, as is

shown in Figure 5.9. Interestingly, separation was not seen in the microfluidized LBG sample nor was any separation observed in either of the xanthan formulations, even after weeks of storage.

Scanning Electron Microscopy of Frozen Ice Cream : Figures 5.10, 5.11 and 5.12 are representative images taken from the SEM of each frozen ice cream at 800, 1200 and 2000X magnification, respectively. Overall, great similarity can be seen between the samples made from untreated mix containing xanthan gum and those made from microfluidized mix containing LBG, particularly as it relates to pore size. Previous work has speculated that mix viscosity increases could be responsible for the formation of smaller bubbles, and thus creamier texture, and that is likely the phenomenon responsible for these similarities (Cavender and Kerr, 2011, Goff, 2002). Cavender and Kerr (2011) found that the viscosities of microfluidized mix containing LBG and non-microfluidized mixes containing xanthan gum were similar (795.7 cP and 654.7 cP, respectively) thus it could be expected that the dynamics of bubble inclusion and coalescence would be similar between them. The differences between the xanthan samples are more difficult to explain. Both the previous work by Cavender and Kerr (2011) and this work show significant increases in the viscosity of xanthan-containing mixes, yet the SEM images of the frozen product appear to show an overall increase in pore size between the control and treated samples. We believe this phenomenon to be the result of the extreme viscosity of the microfluidized xanthan mix interfering with the ability to form small bubbles via agitation of the mix during freezing. The logical extension of the proposed explanation is that for a given freezing process there exists a range of viscosity which

will have a positive effect on the distribution and size of air pockets in ice cream, and values outside of that range will produce less desirable distributions, either by allowing small bubbles to coalesce into larger ones or by preventing the formation of small bubbles by cohesion.

Conclusions: All of the evidence collected in this study points to microfluidization as a means of inducing profound changes to the structure and thus the behavior of the components of ice cream mix. While the effects differ depending on the gum stabilizer used, these structural effects can result desirable changes in product viscosity, stability, particle size and other properties. Further, these changes can be seen both in the treated mixes and in frozen ice cream made from said mixes. Given the significant change in mix viscosity, this technology could be used to reduce stabilizer usage/ cost, as well as offering the opportunity to produce higher quality products.

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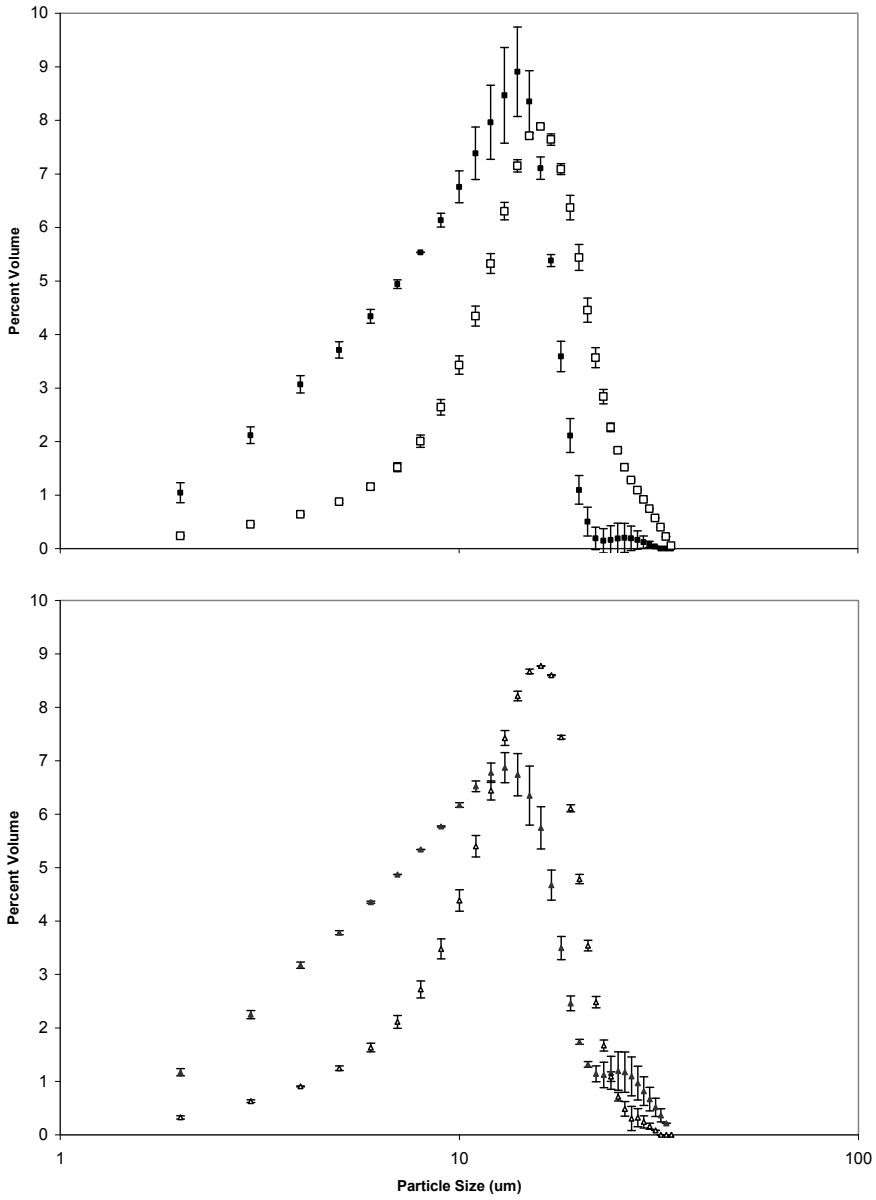
Figures and Tables

Table 5.1: Rheology Data

	Age (days)	Consistency Index ($Pa \cdot s^n$)	Power Law Index	Model Correlation (r^2)
Xanthan Control	3	1.3436	0.5494	0.9533
Xanthan Microfluidized	3	13.213	0.52	0.8759
LBG Control	3	0.1269	0.7765	0.9963
LBG Microfluidized	3	10.644	0.6129	0.9161
Xanthan Control	27	1.3807	0.5049	0.9533
Xanthan Microfluidized	27	1.0091	0.7526	0.8759
LBG Control	27	0.1008	0.7876	0.9963
LBG Microfluidized	27	2.1592	0.7549	0.9161



		10^{th} Percentile	Average Size	90^{th} Percentile
▲	Xanthan Control	0.895±0.0071	2.640 ± 0.0282	11.570 ± 4.0022
Δ	Xanthan Microfluidized	1.470±0.0141	3.935 ± 0.1202	9.250 ± 0.4525
■	LBG Control	0.915±0.0212	2.505 ± 0.0212	7.020 ± 2.6445
□	LBG Microfluidized	1.715±0.0212	4.725 ± 0.0919	15.060 ± 0.1272

A: Mean value ± S.D, n=2

Figure 5.1: Effect of Microfluidization on the Particle Size Distribution of Ice Cream Mixes

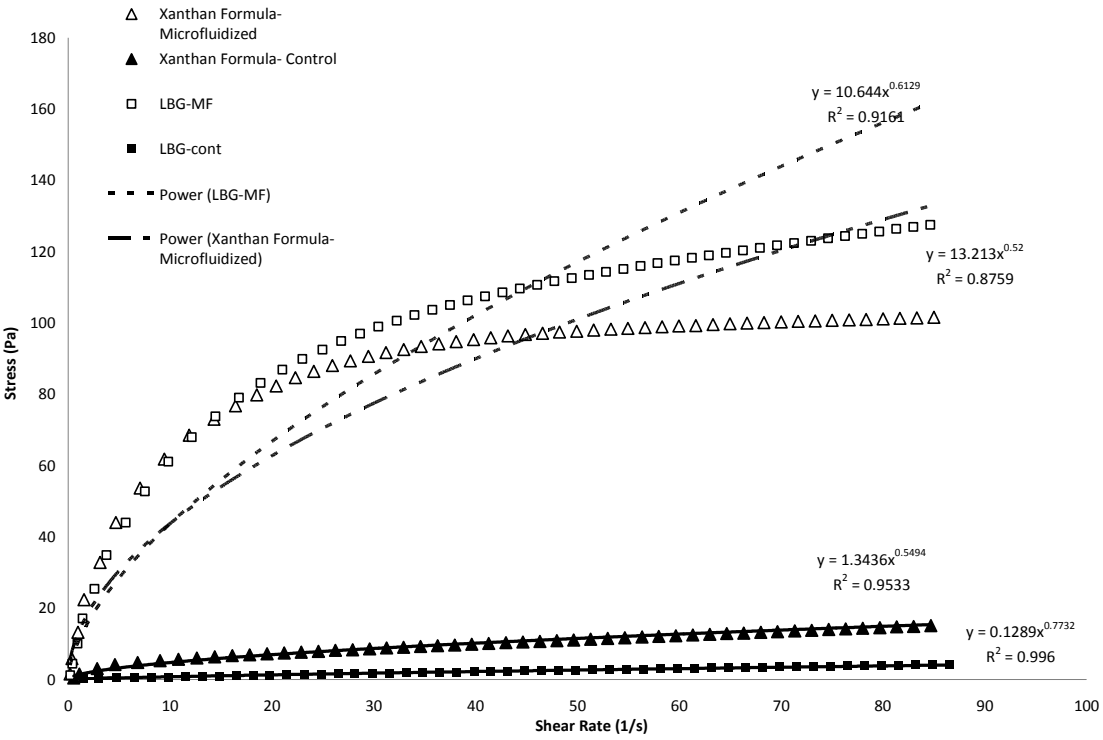


Figure 5.2: Stress vs. Shear-Rate

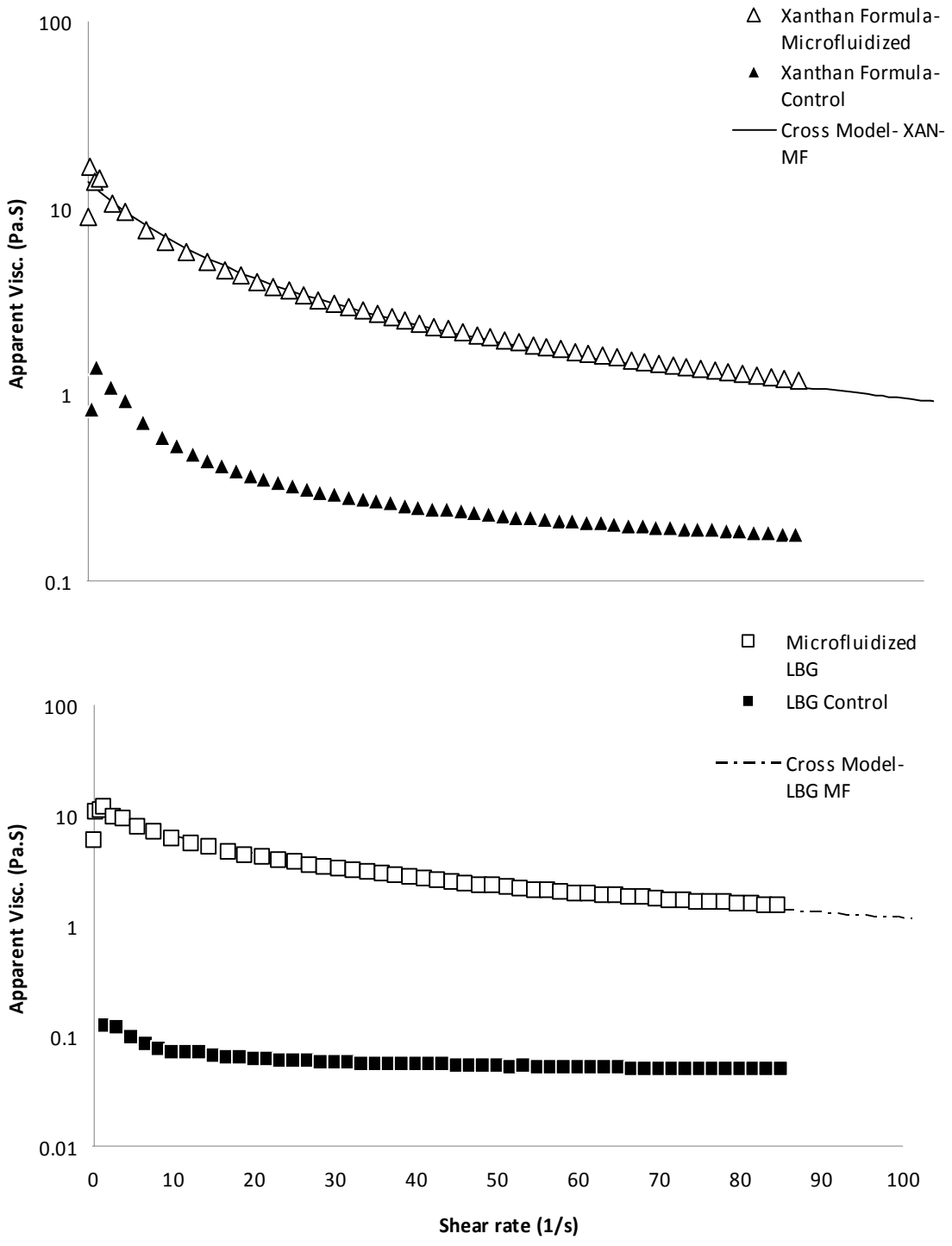


Figure 5.3: Apparent Viscosity of Ice Cream Mixes

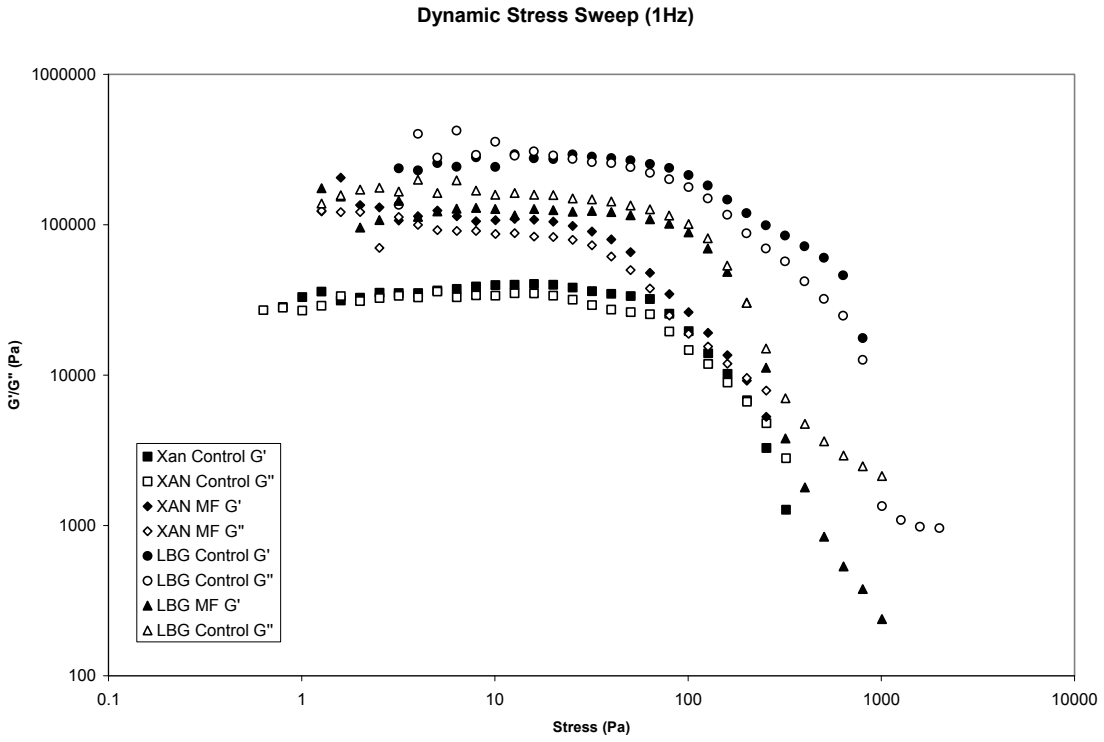


Figure 5.4: Dynamic Stress Sweeps of Solid Ice Cream

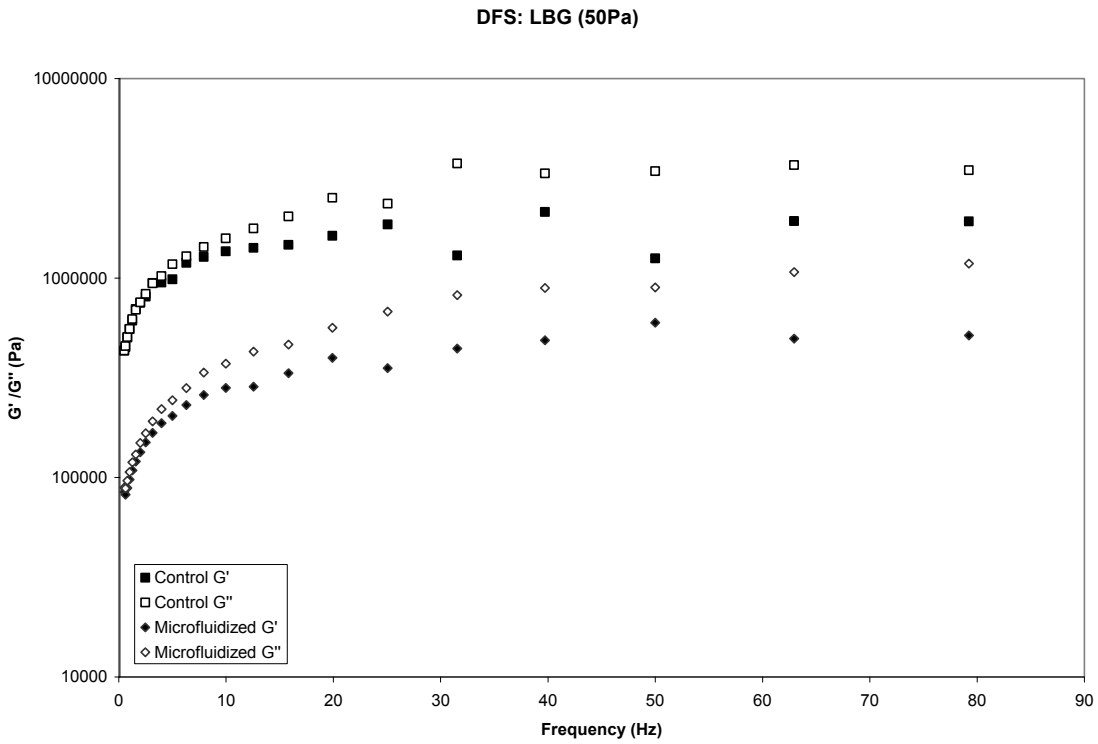


Figure 5.5: Dynamic Frequency Sweeps of Ice Cream Made with Locust Bean Gum

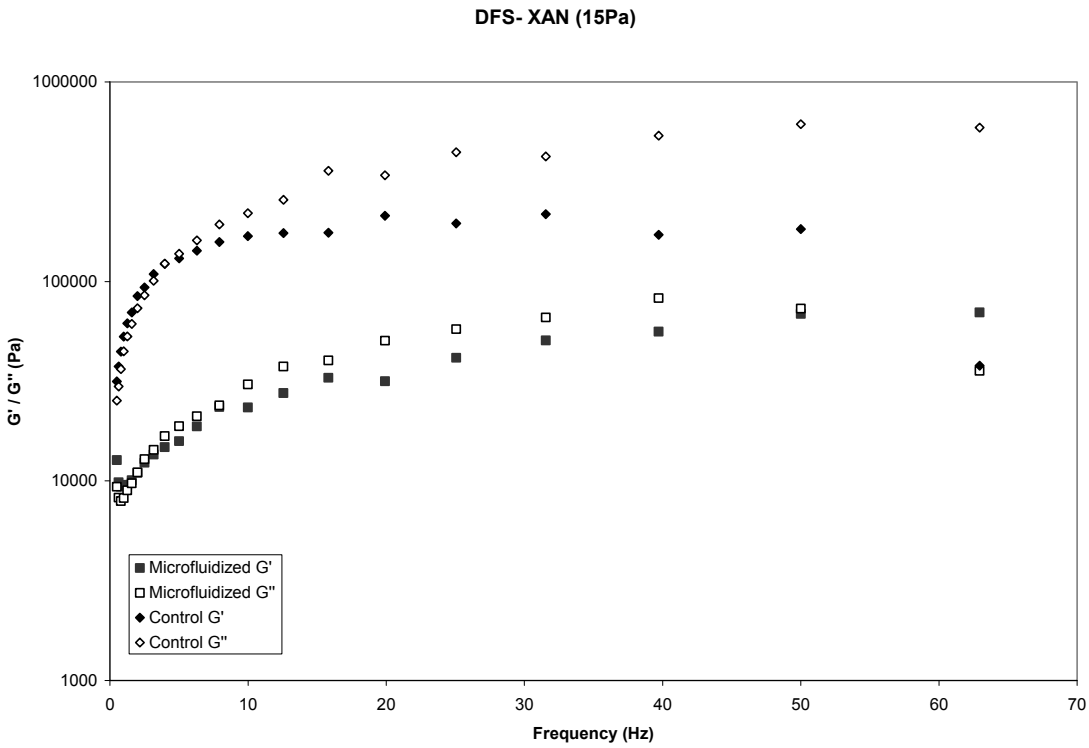


Figure 5.6: Dynamic Frequency Sweeps of Ice Cream Made with Xanthan Gum

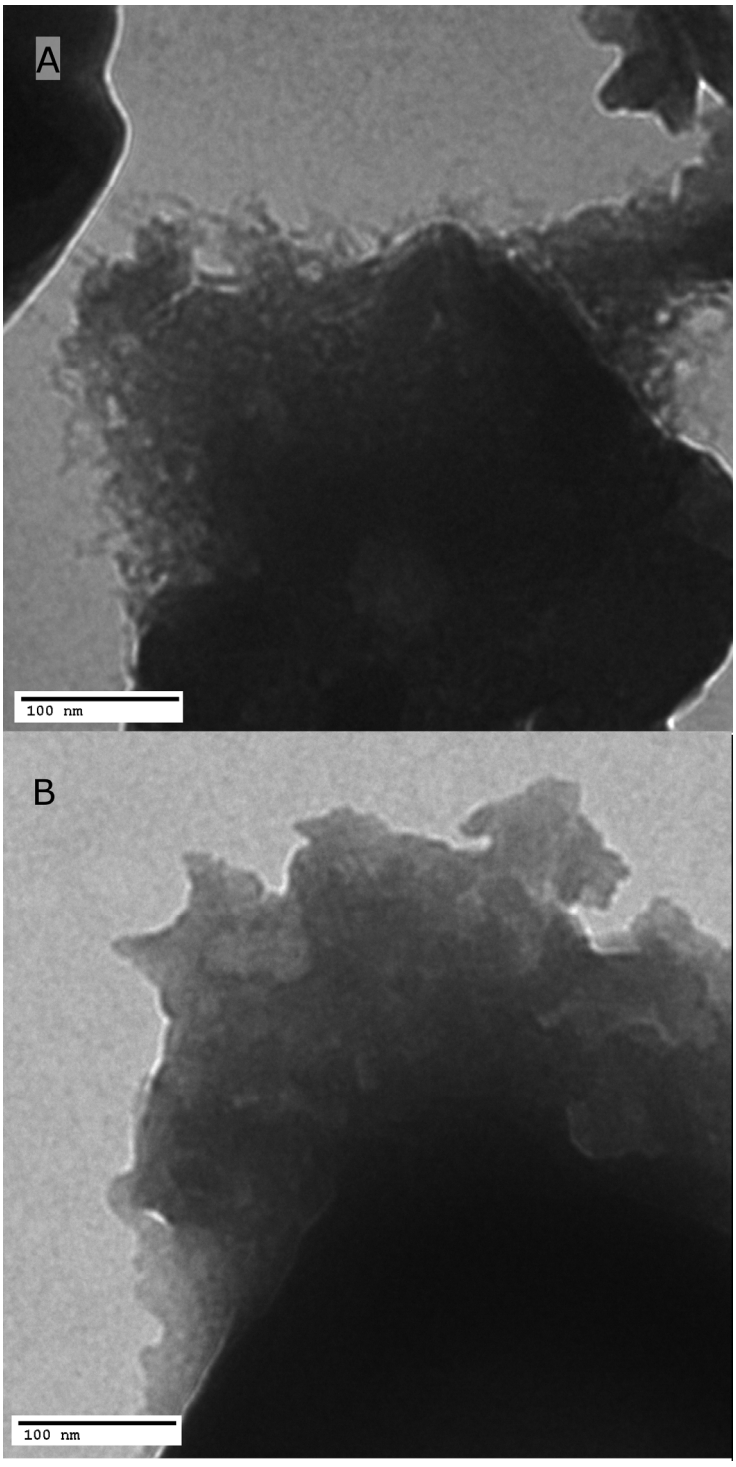


Figure 5.7: TEM Micrograph of LBG Formulation: A-Control, B-Microfluidized

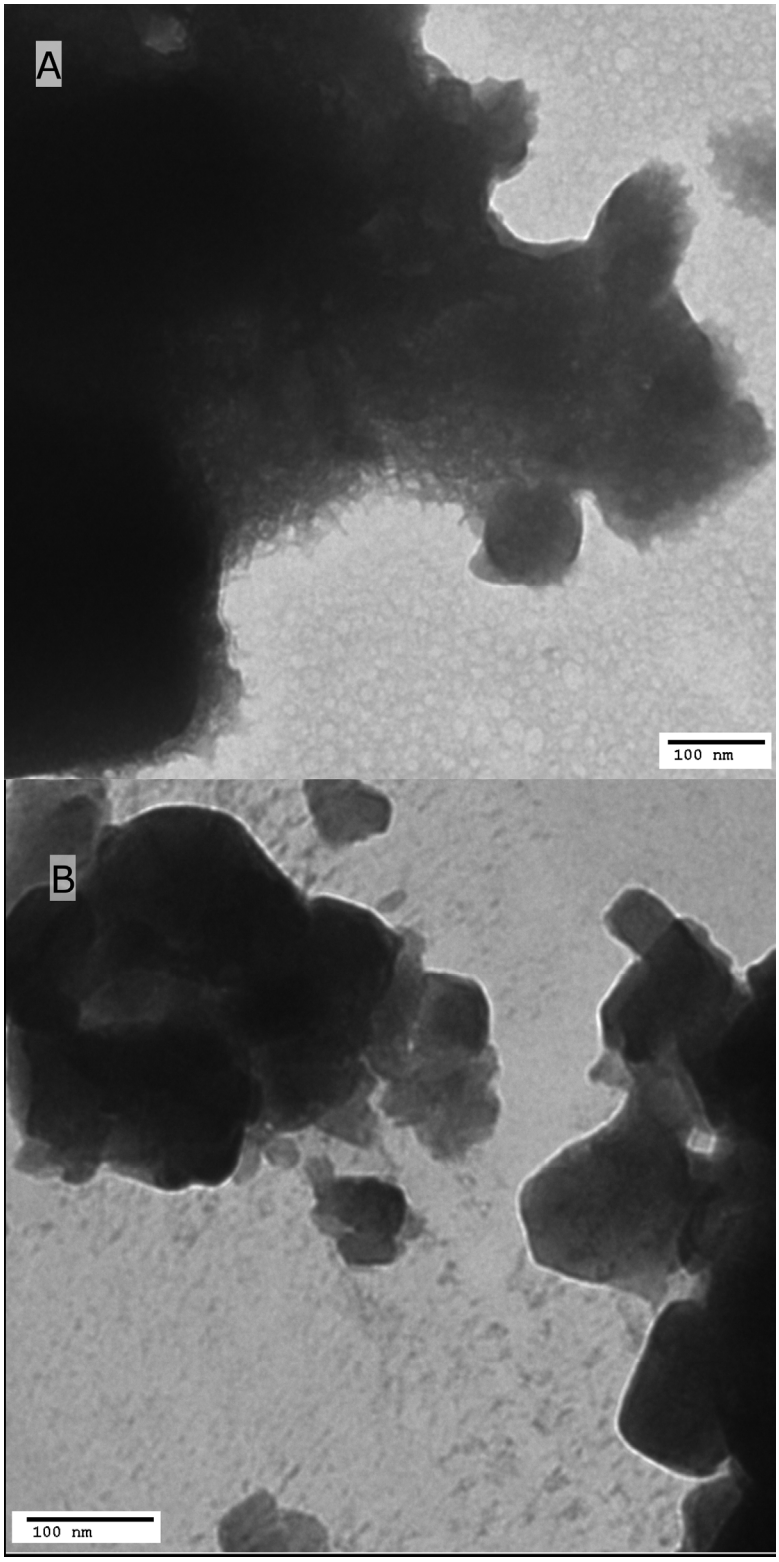


Figure 5.8: TEM Micrograph of Xanthan Formulation: A-Control, B-Microfluidized

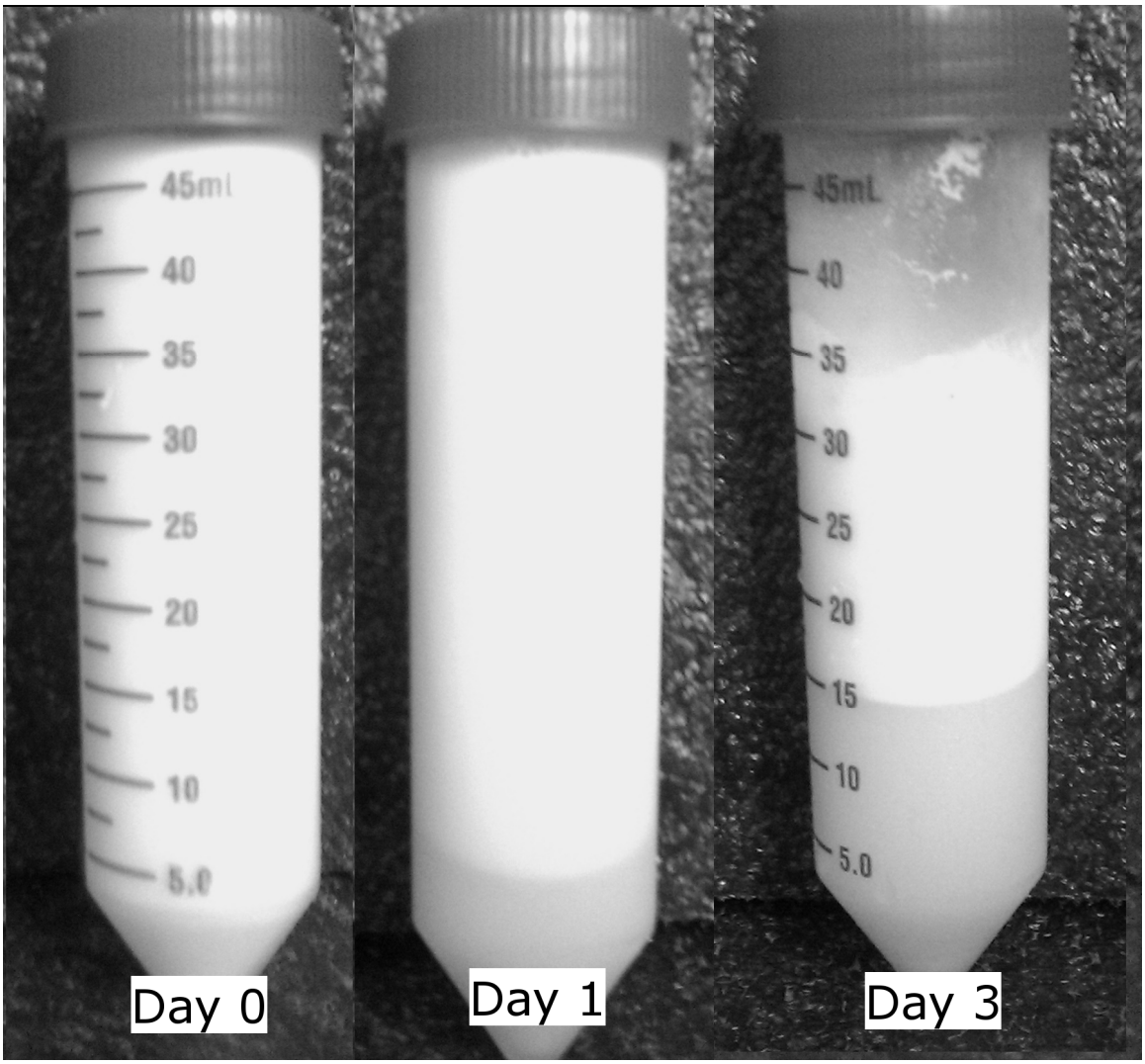


Figure 5.9: Separation of Ice cream mix made with LBG. *Tubes marked Day 0 and day 1 are the same sample, while the Day 3 tube was another aliquot of a different volume.*

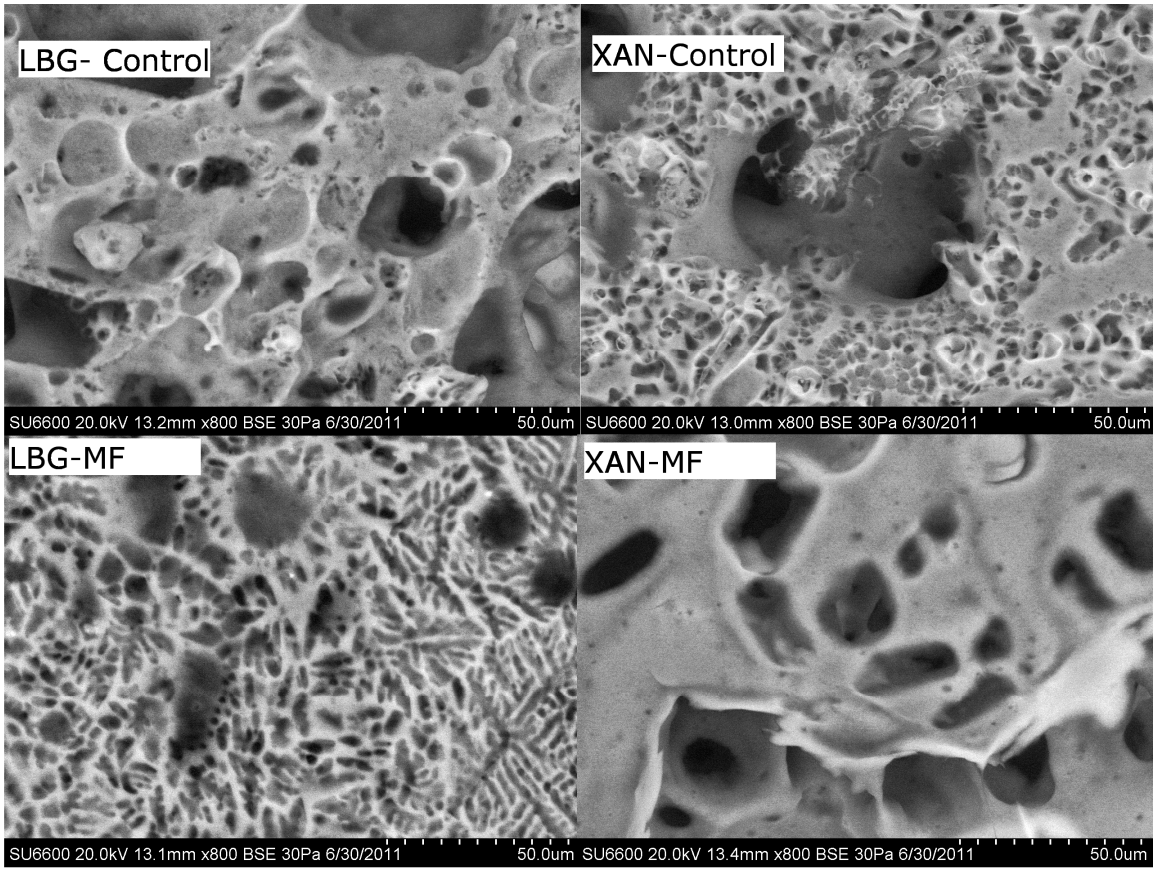


Figure 5.10 SEM (800X) of Frozen Ice Creams

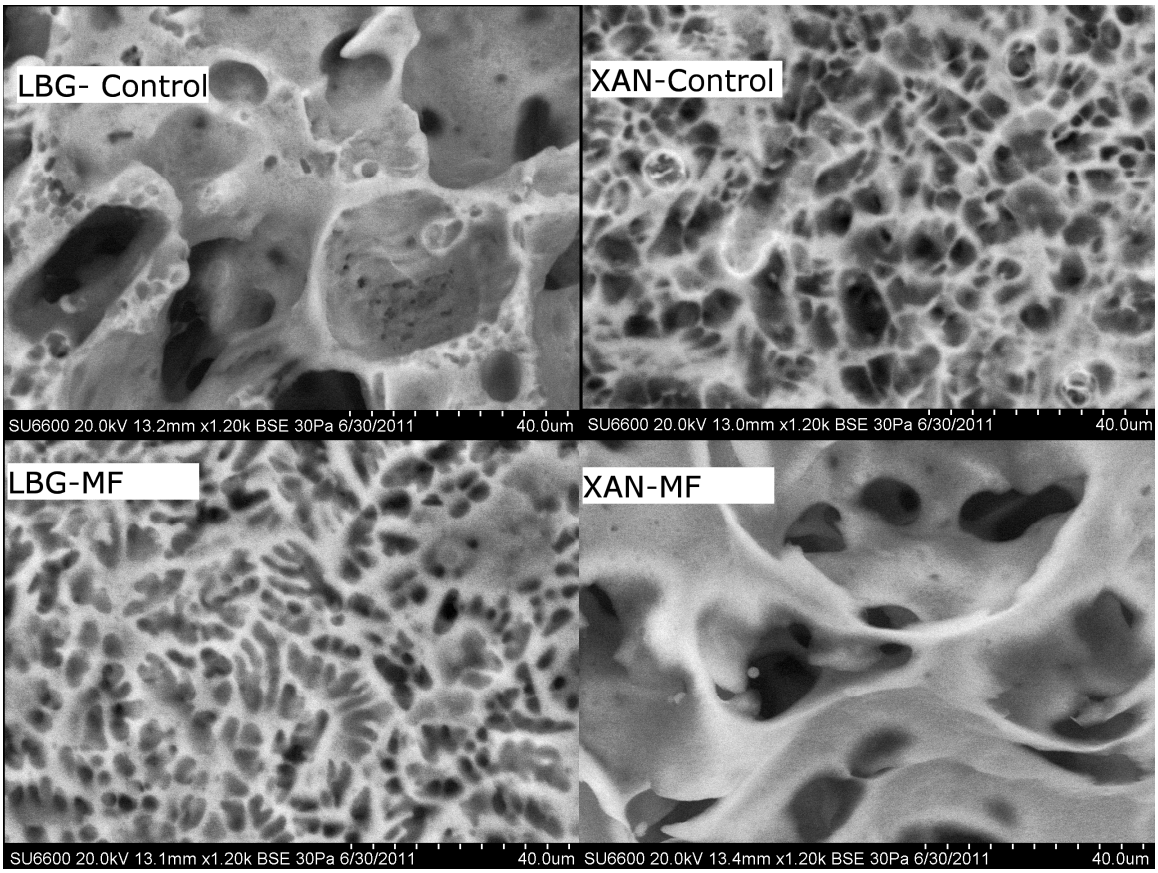


Figure 5.11 SEM (1200X) of Frozen Ice Creams

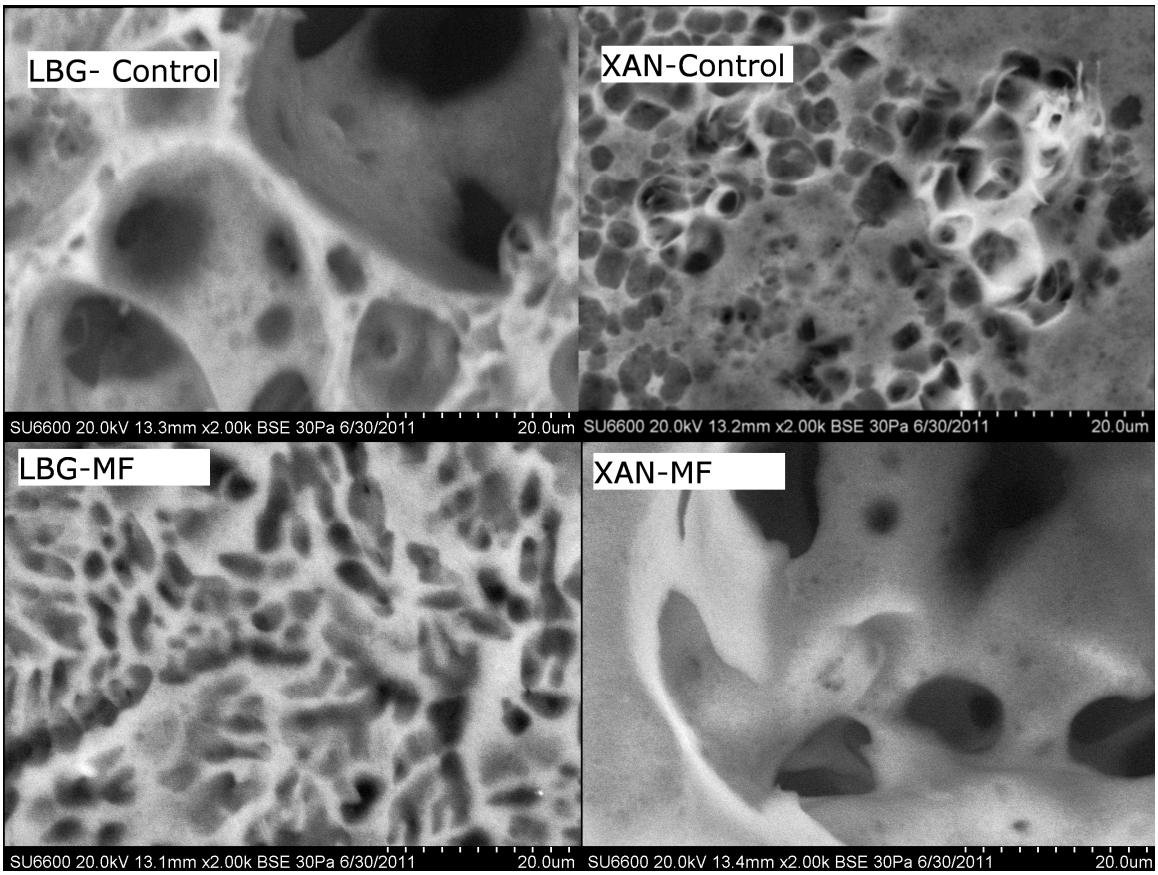


Figure 5.12 SEM (2000X) of Frozen Ice Creams

CHAPTER 6:

CONCLUSIONS

Continuous high pressure processing (CHPP), as it is typically thought of, is not truly a "non-thermal process." That much is apparent from the data in the third chapter: while CHPP can effect a microbial reduction of 8 log or more, this is primarily due to the temperature rise seen in the pressure release component. When that increase is controlled through cooling at the point of decompression, the microbial reduction levels are far more modest. These modest levels may be useful in the design of processes using hurdle technologies, but in and of themselves, they cannot replace pasteurization.

In the realm of property modification, CHPP shows more promise. It can significantly increase the viscosity of Ice cream mix, which, in turn, changes the physical properties of the final product. These changes are quite profound, and depending on formulation can improve the properties and the acceptability of the final product. Locust bean gum seems to have better functional properties after microfluidization as evidenced by the viscosity changes to the mix and the structural changes in the frozen product. Mixes made with xanthan gum also experiences similar structural changes in the mix, but appear to behave differently from LBG when frozen. This apparently contradictory result is thought to be the result of too great of an increase in viscosity due to treatment.

Finally, while this work answered several important questions, there are still many more avenues of research left to be explored. While the non-thermal effects of CHPP are modest, there is no reason a hybrid process could not be designed, using other

technologies like supercritical fluids, ultrasound and mild temperature treatments. Further, a better understanding of the complex interactions between valve geometry, mechanisms of action, cell size and culture density could be discovered using modern computer modeling.

With regard to the physiochemical changes seen in ice cream mixes, the results seen here are promising and the field would benefit from further work. This work includes comparing the effects of various different gums stabilizers at differing levels on ice cream quality so that the potential to use the technology to reduce ingredient cost could be examined. Further, studies on how the process effects changes in the structure of various gums would offer insight onto the mechanisms of the changes seen in this work and others. By designing experiments with gum backbone structures, side chain distribution and size as the treatment variables, and looking at average molecular weight and one or more physical properties, a good statistical model could be developed, and the level of prediction it gives would give us much more insight as to how the structures are modified by the process.

APPENDIX A

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