PROCESSING PARAMETER EFFECTS ON PHYSIOCHEMICAL PROPERTIES OF NATURAL AND REFORMULATED CREAMS

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ABSTRACT

Skim, sweet buttermilk, and butter-derived aqueous phase components were used to re-emulsify low-melt butteroil into creams with a desired 20% milkfat. The implications of pasteurization process, homogenization sequence, and formulation on the physicochemical properties of reformulated and natural creams were investigated.

Creams homogenized prior to pasteurization had significantly ($p<0.05$) greater amounts of milkfat surface material per gram of cream and per gram of lipid compared to creams homogenized after pasteurization. Significantly ($p<0.05$) higher percentages of available phospholipid also were associated with the milkfat surface material of creams homogenized prior to pasteurization. Phosphodiesterase, a marker enzyme within the native milkfat globule membrane, was in significantly ($p<0.05$) higher activity relative to protein on lipid globule surface when cream was homogenized prior to pasteurization. Creams that underwent pasteurization prior to homogenization had significantly ($p<0.05$) higher protein load associated with the milkfat surface material.

Natural cream homogenized prior to pasteurization had significantly ($p<0.05$) greater milkfat surface material per gram lipid than natural cream homogenized after pasteurization and buttermilk / aqueous phase (AP) reformulated cream homogenized after pasteurization. In contrast, natural cream homogenized after pasteurization and BM / AP reformulated cream homogenized after pasteurization had significantly ($p<0.05$) greater amounts of protein per 10 mg of milkfat surface material than all other formulation / homogenization sequence combinations. In addition, natural cream homogenized prior to pasteurization, and skim milk (SM) reformulated cream pasteurized prior to or after homogenization had significantly ($p<0.05$) higher percentages of available phospholipid associated with the milkfat surface material than natural cream homogenized following pasteurization.

Pasteurization temperature had a significant ($p<0.05$) effect on apparent viscosity of natural and reformulated creams. All UHT pasteurized natural and reformulated creams had significantly ($p<0.05$) greater apparent viscosities at all shear rates monitored than HTST pasteurized natural and reformulated creams. At a failing curve shear rate of 692 s^{-1} UHT pasteurized cream reformulated with BM / AP had significantly ($p<0.05$) greater viscosity than UHT pasteurized natural cream and cream reformulated with skim component.

HTST pasteurized natural cream had significantly ($p<0.05$) greater viscosities than HTST pasteurized cream reformulated with skim component. Moreover, at a shear rate of 2769 s^{-1} and a rising shear rate of 1384 s^{-1} HTST pasteurized natural cream had significantly

($p<0.05$) greater viscosity than HTST pasteurized cream reformulated with buttermilk and aqueous phase.

Creams formulated with skim component showed significant differences in creaming stability after 7 days of storage. On the other hand, natural cream and cream reformulated with buttermilk and aqueous phase showed significant differences in creaming stability after 9 days of storage. HTST pasteurized creams had greater creaming stability than UHT pasteurized creams. All creams feathered in a pH range of 5.09 to 5.31. Homogenization prior to UHT pasteurization resulted in creams rated "out of specification" because of poor sensory quality on day 1. Other processing sequences resulted in creams within sensory specifications.

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DEDICATION

I dedicate this work to my family: Mrs. Vicki Lee, Mr. Jay Bolling, Ms. Laura Bolling, Mr. Ed Digilio, Mr. Don Lee, and Mrs. Dot Bolling. Along with the dogs of the family: Merlin, Eli, Maggie, and Max. Without all of their support this work would definitely not have been possible. I would also like to dedicate this work to music. Without the sounds of my favorite bands I would have never completed this work. Most of all, I wish to dedicate this research to my late Granddaddy George, whom passed away on New Year's eve of 1999. He was an inspiration to me all of my life, and continues to influence my life. He taught me so much about overcoming adversity, and I will always remember him.

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CHAPTER I
B. INTRODUCTION

Modified milkfat, differing from natural milkfat in nutritional and functional attributes, requires emulsification for incorporation in many food systems. Emulsification can be accomplished by combining altered milkfat with surface active agents into reformulated dairy products such as creams. Milk-derived components such as skim milk, sweet buttermilk, butter-derived aqueous phase, whey proteins, casein dispersions, and purified milkfat globule membrane suspensions have proven to successfully emulsify butteroil (Smith and Dairiki, 1975; Oortwijn et al., 1977; Oortwijn et al., 1979; Kanno, 1989; Kanno et al., 1991, Rosenberg and Lee, 1993; Oehlmann et al., 1994; Tomas et al., 1994; Elling et al., 1996; Scott, 1999). Skim milk is an abundant source of whey and casein proteins while sweet buttermilk and butter-derived aqueous phase also are abundant in phospholipids from milkfat globule membrane fractions. Ideally, emulsification should produce a dairy product having physical attributes similar to natural dairy products. It is necessary to understand the effects of processing on the chemical structure of milk if this goal is to be accomplished.

Prior to processing, interactions between milk proteins (casein and whey) and milkfat are limited. Milkfat is contained within a milkfat globule membrane; whereas casein proteins, primarily in micelle form, and whey proteins are predominantly in the serum phase of milk. Processing such as heating and homogenization can alter this relationship.

When milk is heated, changes occur in the conformation of serum proteins and micelle proteins. In addition, changes may occur in the structure of the milkfat globule membrane. These effects can cause significant changes in the chemical structure of milk (Dalgleish and Sharma, 1993). Houlihan et al. (1992) found milkfat globule membrane to be involved in heat-induced interactions with beta-lactoglobulin and kappa-casein. Levels of incorporation of these components into the membrane were dependent on the extent of heat treatment. Kim and Jimenez-Flores (1995) hypothesized that milk serum proteins may deposit on the milkfat globule membrane (MFGM) with the displacement of polypeptides from the membrane material.

When milk is homogenized, new structures are formed. Size reduction of the milkfat globules causes a corresponding increase in surface area of the milkfat globule. The increased surface area of the milkfat cannot be covered by native milkfat globule membrane. Thus other milk constituents, mainly proteins, must fill the gaps in the milkfat membrane created by homogenization.

Dalgleish and Sharma (1993) proposed explanations for interaction of denatured proteins in homogenized, heated milk. The most likely explanation was interaction of denatured beta-lactoglobulin with kappa-casein complexed in micelle form on the surface of milkfat globule membrane. Because casein molecules adsorbed at the milkfat globule membrane interface have a greater amount of kappa-casein exposed, it is likely that denatured serum proteins form complexes with the membrane bound casein, mainly kappa-casein (Dalgleish and Sharma, 1993).

Following processing, many possibilities exist for constituents of the resulting membrane surrounding the milkfat globule. The method of processing dictates the resulting membrane constituents. For example, if homogenization precedes heat treatment, then denatured whey proteins will bind to casein micelles and casein protein (mainly kappa-casein) oriented at the membrane of the milkfat globule. However, if homogenization occurs after heat treatment, then serum proteins, having been denatured prior to homogenization, will already be complexed with casein micelles and will not be able to undergo any further reactions (Dalgleish and Sharma, 1993).

Elling et al. (1996) found the surface material of creams reformulated with skim and buttermilk resembled the components used to emulsify them. Protein was the major emulsifier in both reformulated creams. However, cream reformulated with buttermilk contained significantly greater amounts of phospholipid, indicating the presence of greater amounts of native milkfat globule membrane. Natural and reformulated creams homogenized at higher homogenization pressures had more surface material at the membrane interface than corresponding creams homogenized at lower pressures.

Scott (1999) investigated the effect of separation temperature on components used in reformulated creams and on the resulting milkfat surface material. No significant differences in chemical composition of milkfat surface material were attributed to separation temperature.

In summary, pasteurization and homogenization both affect the composition of the surface material on the milkfat globule. Different homogenization pressures affect the amount of protein that associates with the resulting surface membrane materials while differing pasteurization temperatures affect the type and amount of proteins that associate with the homogenized surface membrane material as well as the structure of the fat globule.

An effective reformulated cream would parallel homogenized natural cream in physical properties (appearance, composition, membrane material) (Elling and Duncan, 1996). The goal of this study was to examine the effect of high temperature short time (HTST) pasteurization, ultra high temperature (UHT) pasteurization, and homogenization sequences on the chemical and physical properties of natural cream and creams made up of low-melt fractionated butteroil and emulsified with skim component, sweet buttermilk component, and butter-derived aqueous phase. Low-melt fractionated butteroil was chosen because it contains lesser amounts of saturated fat when compared with medium-melt fractionated butteroil.

CHAPTER II
LITERATURE REVIEW

A. EMULSIFICATION OF MILKFAT INTO AQUEOUS SYSTEMS

When secreted, the milkfat globule is completely engulfed by a bilayer membrane that is believed to originate from the plasma membrane (Deeth, 1997; Mather and Keenan, 1975). Acting as a barrier at the interface between the fat globule and the serum phase of milk, the milkfat globule membrane (MFGM) is responsible for the oil-in-water emulsion typical of milk systems (Jensen et al., 1991; Mather and Keenan 1975). The structure of the MFGM is asymmetric with a hydrophobic inner compartment adjacent to the fat and a hydrophilic outer region oriented toward the serum phase (McPherson and Kitchen, 1983). Amphiphilic molecules (phospholipids and proteins) are located across the membrane. In addition to emulsifying the triglyceride globules in milk, the MFGM is also responsible for controlling the rate of chemical and physical reactions the binding of enzymes, release of products of lipolysis, creaming, clumping, and churning (Jensen et al., 1991).

The Milkfat Globule Membrane

The MFGM, which is 10 nm in diameter, is made up of proteins, phospholipids, glycoproteins, cholesterol, neutral glycerides, and enzymes (McPherson and Kitchen, 1983). It is estimated that proteins and lipids account for approximately 90% of the dry weight of the MFGM (Keenan and Dylewski, 1995). The relative proportions of these two constituents, however, is not clear due to different methods used to release and recover the membrane materials (Keenan and Dylewski, 1995). Keenan et al. (1988) estimated that protein makes up anywhere from 25% to 60% of the MFGM. Relative to the amount of protein, Keenan et al. (1988) estimated the total lipid concentration ranges from 0.5 mg/mg protein to 1.1 mg/mg protein. Kanno (1980) and McPherson and Kitchen (1983) found that 55% of the MFGM is made up of protein while 44% consists of lipid. Of the total lipid content, Keenan et al. (1988) found anywhere from 0.13 mg/mg protein to 0.34 mg/mg protein was phospholipid and 0.25 mg/mg protein to 0.88 mg/mg protein was made up of neutral lipid. Bracco et al. (1972) found that of the lipids found in the MFGM, 61.7% were triglycerides while 22.1% were phospholipids.

MFGM Proteins. The MFGM proteins constitute only a small portion of the milk lipid globules' total mass, yet they are fundamental for the stability of the fat globules (McPherson and Kitchen, 1983; Keenan and Dylewski, 1995). Because of their amphiphilic nature and origin, MFGM proteins are expected to be good emulsifying agents; however, their role in the milk lipid globule is not clearly understood. Using SDS-PAGE isolation techniques, Keenan et al. (1982) elucidated a number of proteins that have a role in the MFGM. These proteins may exist solely or in complexes (Keenan and Dylewski, 1995). It is unclear if these proteins are fixated at the outer or inner faces of the primary membrane, span the membrane, serve as an outer coating of the fat droplets, or are sandwiched between the droplet and the membrane (Keenan and Dylewski, 1995).

Butyrophilin makes up approximately 40% of the mass of the MFGM proteins (Jack and Mather, 1990). Jack and Mather (1990) concluded that butyrophilin is a protein that spans the membrane. However, the mechanism for interaction of butyrophilin with lipid droplets has not been elucidated.

Another major protein constituent of the MFGM is xanthine oxidase. It migrates with an approximated M_r of about 155 kDa and accounts for approximately 20% of the membrane proteins (Keenan and Dylewski, 1995). It appears that this protein is located between the lipid core and membrane of the milk fat globule (Keenan and Dylewski, 1995). Most xanthine oxidase proteins are released in soluble form upon disruption of the milk fat globule, while others remain. Keenan et al. (1982) hypothesized that the remaining xanthine oxidase is covalently bound to fatty acids.

PAS-I has a M_r ranging from 170 to 200 kDa and is a major glycoprotein of bovine MFGM (Snow et al., 1977; Patton and Patton, 1990). This protein has been isolated and its amino acid composition determined, revealing that this protein consists of 50% w/w carbohydrate. The function of this protein is not understood.

PAS-IV is considered a major MFGM glycoprotein. It has a M_r of about 76 kDa and is considered an integral membrane protein (Keenan and Dylewski, 1995). Five percent of this glycoprotein is carbohydrate.

Another MFGM protein is PAS-V (Mather and Keenan, 1975). It has a M_r of approximately 44 to 48 kDa. Because the MFGM contains a high amount of this protein in its reduced form, it is speculated that PAS-V serves as a peripheral protein constituent of the milkfat globule (Keenan and Dylewski, 1995). Basch et al. (1976) found this glycoprotein contains 14% w/w carbohydrate.

Keenan and Dylewski (1995) believe that the MFGM proteins interact with each other to form supermolecular complexes. However, the role of the MFGM proteins has not been fully elucidated.

Phospholipids of the MFGM. Phospholipids account for less than 1% total lipid content of milkfat, yet they play a crucial role in maintaining the structural integrity of the milkfat globule membrane (Bitman and Wood, 1990). McPherson and Kitchen (1983) estimated that 50% of the phospholipids present in milk reside in the milk fat globule membrane. Phospholipids have both lipophilic and hydrophilic properties contributing significantly to their excellent emulsification properties (Deeth, 1997). The hydrophobic tails of phospholipids react with the non-polar regions of milkfat while the polar tails of phospholipids extend in the aqueous medium. The estimated composition of the phospholipids is: sphingomyelin (25%), phosphatidyl choline (35%), phosphatidyl ethanolamine (30%), phosphatidyl inositol (5%), and phosphatidyl serine (3%) (Christie, 1995; Keenan and Dylewski, 1995).

The MFGM is asymmetric with inner and outer sides differing greatly in composition (Deeth, 1997). Phosphatidyl choline and sphingomyelin, which contain a choline group,

are positioned on the outer portion of the milkfat globule membrane (Deeth, 1997). Phosphatidyl inositol, phosphatidyl ethanolamine, and phosphatidyl serine are positioned on the inner portion of the milkfat globule membrane.

Bitman and Wood (1990) estimated that phospholipids consist of 25 to 30 types of fatty acids, a glycerol backbone, and a phosphorylated alcohol group. Sphingomyelin also had 25 to 30 types of fatty acids but has a sphingosine base with an attached choline group.

Because phospholipids contain long-chain polyunsaturated fatty acids, they are susceptible to autooxidation processes (Jensen et al., 1991). Deeth (1997) estimated that phosphatidyl ethanolamine and phosphatidyl choline have 40% to 60% unsaturated fatty acids. A third of these unsaturated fatty acids associated with phosphatidyl ethanolamine and phosphatidyl choline are polyunsaturated (Christie, 1995).

Composition of Milk Proteins

The main proteins of skim milk are the caseins. The caseins constitute 80% of the total protein in milk, while the whey proteins make up the majority of the other proteins (Leman and Kinsella, 1989).

Casein Proteins. Approximately 95% of the casein proteins in milk are associated in a micelle form (Fox and Mulvihill, 1982). There are four major casein proteins (alpha S1, alpha S2, beta, and kappa casein). The relative proportion of these are 45%, 12%, 34%, and 10% (Leman and Kinsella, 1989). Casein proteins contain high levels of proline (Leman and Kinsella, 1989). These high levels of proline limit the extent to which alpha helix or beta sheet secondary structures can exist due to stearic hindrance. Consequently, the casein proteins have very little secondary or tertiary structure, making them very flexible (Leman and Kinsella, 1989). In general, the caseins are considered to be hydrophobic. However, their polar groups are clustered, resulting in amphiphilic properties (Leman and Kinsella, 1989). Because of the amphiphilic properties of casein proteins, they are considered to be very surface active, which is beneficial in forming emulsions.

The alpha S2 caseins contain 8.5% proline evenly distributed throughout the molecule (Kinsella, 1984). As a result, alpha helix formation is rare. Negative charges in the form of ten to thirteen phosphoserine residues per molecule are concentrated near the N-terminus and positive charges are concentrated near the C-terminus. Some tertiary protein structure is present, however, in alpha S2 casein proteins because they contain 2 cysteine residues (Leman and Kinsella, 1989). Alpha S1 casein has two hydrophobic regions, containing all the proline residues, separated by a polar region, which contains all but one of eight phosphate groups. Compared to beta casein, the alpha S caseins have a higher charge and lower hydrophobicity, which causes them to be less susceptible to aggregation in the presence of calcium (Kinsella, 1984).

Beta casein is very hydrophobic; however, at its N terminal region (residues 1 to 50) contains hydrophilic groups (Kinsella, 1984). Five phosphorylated serine groups are contained in an approximately 170 residue hydrophobic segment that also contains 16%

proline residues, which prevent formation of alpha helix (Kinsella, 1984). These properties make beta casein highly amphiphilic and a good component in emulsions because it can orient hydrophilic groups towards the aqueous phase of milk and hydrophobic groups towards the lipid rich core of the milkfat globule due to the flexibility of the molecule.

Kappa caseins possess a hydrophilic carboxyterminal consisting of oligosaccharides (N-acetyneumonic acid, galactose, N-acetylglutamine) attached to threonine residues via glycosidic linkage (Kinsella, 1984). The presence of a charged oligosaccharide moiety and only one phosphoserine group distinguishes kappa-casein from alpha and beta casein. On the other hand, the N-terminal, which is made up of around 100 residues, is hydrophobic with 2 disulfide bonds (Kinsella, 1984). These two properties make kappa-casein amphiphilic and therefore very surface active. Treatment of kappa-casein with rennet cleaves the protein at the Phe 105-Met 106 bond. This eliminates the stabilizing properties of kappa-casein, leaving a hydrophobic portion known as para-kappa-casein and a hydrophilic portion known as caseinmacropeptide.

The casein proteins all share similar characteristics such as: 1) similar sizes (around 20,000 daltons); 2) hydrophilic regions as well as hydrophobic regions creating an amphiphilic nature; 3) random coil nature due to the presence of proline residues. These properties contribute to relatively high heat stability. Dickinson and Stainsby (1982) found that a 3% solution of casein protein at pH 7 can be heated up to a temperature of 140^{o}C for sixty minutes without major aggregation.

The amphiphilic nature of caseins and their phosphorylation facilitate interactions with each other and with calcium phosphate to form highly hydrated spherical complexes known as micelles (Varnum and Sutherland, 1994). The average diameter of these micelles range from 30 to 300 nm, and the micelles consist of 92% protein composed of alpha-S1, alpha-S2, beta-casein, and kappa-casein in an average ratio of 3:1:3:1 (Varnum and Sutherland, 1994). Inorganic constituents, mostly calcium phosphate, make up the remaining 8% of the micelle.

Each micelle is made up of an aggregate of almost spherical sub-micelles, which consist of more limited aggregates of casein molecules (Varnum and Sutherland, 1994). Each casein micelle is made up of 10 to 100 casein submicelles. In casein submicelles alpha-S and beta casein are linked by covalent or electrostatic interaction with colloidal calcium phosphate. Submicelles rich in kappa-casein occupy a surface position. Submicelles with lesser amounts of kappa-casein are located within the interior. Kappa-casein is found on or very close to the surface of the casein micelle. The hydrophobic portion of kappa-casein is bound to the core of the micelle, while the hydrophilic macropeptide forms a layer of highly hydrated hairs responsible for the steric stabilization of casein micelles (Varnum and Sutherland, 1994). These hairs project into the aqueous phase of milk.

Whey Proteins. Whey proteins are made up of four major proteins: beta lactoglobulin, alpha lactalbumin, bovine serum albumin, and immunoglobins. Whey proteins contain less proline than the casein proteins thus whey proteins possess greater amounts of alpha

helix and beta sheet secondary structure than casein proteins. The major whey proteins are beta lactoglobulin (54% of whey proteins) and alpha lactalbumin (21% of whey proteins) (Leman and Kinsella, 1989).

At ambient conditions beta lactoglobulin consists of 2 subunits linked through disulfide bridges to form a dimer (Leman and Kinsella, 1989). In addition, each monomer has a free thiol group. Because of the lesser amounts of proline, beta lactoglobulin has 11% of its structure existing as alpha helix. Because of its greater secondary, tertiary, and quaternary structures, beta lactoglobulin is more susceptible to thermal denaturation than the casein proteins. At temperatures above 65 ^{o}C, beta lactoglobulin undergoes conformational changes that expose its free thiol group (Kinsella, 1984). This conformational change caused by thermal denaturation helps optimize the interaction of beta lactoglobulin at the milkfat interface, resulting in a lower free energy (Dalgleish, 1996).

The role of alpha-lactalbumin in food systems has not been established. It is a globular protein with four intramolecular disulfide bonds but no free sulfhydral groups (Leman and Kinsella, 1989). These four disulfide bonds make this whey protein relatively heat stable.

B. EFFECTS OF PROCESSING ON NATIVE MFGM

Milk exists in a two-phase emulsion consisting of an oil phase enveloped in a water phase. When dealing with emulsions, it should be understood that a "stable" emulsion is where the unavoidable process of separation has been slowed to an extent that is not observable through a product's shelf-life (Friberg et al., 1990). Prior to processing, virtually no direct interactions between milk proteins (casein and whey) and milkfat occur. Milkfat is contained within a milkfat globule membrane; casein proteins are in micelle form; and whey proteins are in the serum phase of milk. Processing such as heating and homogenization can alter this by causing changes in the emulsion characteristics of milk.

Effect of Homogenization on the MFGM of Unprocessed Milk

Unhomogenized milk will exhibit a separation of cream and skim when stored for a period of time. Consequently, milk is homogenized to produce an emulsion with greater stability. Prior to homogenization, fat globules in whole milk have average diameters ranging from 2 to 6 um. Following homogenization, fat globules are reduced to less than 1 um in diameter and a corresponding 4 to 10 times increase in surface area is noted (McPherson et al., 1984, Walstra, 1975). Factors affecting fat globule distribution include homogenization pressure, valve number and type, and flow rate (Brunner, 1974; Mulder and Walstra, 1974). The treatment alters the original MFGM because there is insufficient native MFGM to cover the increased fat globule surface area induced by homogenization (Kanno et al., 1991). Casein proteins and, to a lesser extent, whey proteins fill the holes left by the lack of sufficient native MFGM to cover the fat globule surface. The adsorption process reduces the interfacial tension between water and oil to give a state of free energy lower than in the absence of the proteins (Tornberg et al., 1982).

This lower state of free energy occurs via two mechanisms. The first mechanism stabilization is caused by particles possessing charged surfaces (Walstra, 1987; Dickinson and Stainsby, 1982). The second is steric stabilization (Napper, 1983). An explanation of the mechanisms of charged protein stabilization is the formation of a charged double layer around the milkfat globule upon adsorption to the milkfat globule membrane. This charged double layer prevents other approaching milkfat globules from interacting with each other due to the resulting energy barrier being greater than thermal energy (Fisher and Parker, 1988). In addition, upon adsorption to the milkfat globule membrane, casein proteins may alter their conformation, resulting in extended structures that project a considerable distance out from the milkfat globule membrane. These extended hydrated layers form the basis for steric stabilization either through loss of configurational entropy of the flexible protein chain (Hesselink et al., 1971; Meier, 1967) or from water being placed between milkfat globules by osmotic effects (Hesselink, 1969). In summary, emulsifying activity is based on the strength of the adsorbed protein layer and its desirable properties of high charge or steric hindrance to prevent close association of milkfat droplets (Dalgleish, 1996).

Darling and Butcher (1978) pre-warmed (60^{o}C) and homogenized milk at 20 MN/m and examined the proteins of the MFGM. Casein and undenatured whey proteins were adsorbed to the fat-serum interface during homogenization. No preferential adsorption of either protein was observed. However, casein proteins were the predominant group of proteins adsorbed at the fat-serum interface. After washing, whey proteins were more easily removed, indicating that casein components had a closer association at the milkfat interface than whey proteins (Darling and Butcher, 1978.

Keenan et al. (1983b) compared the milkfat globule membrane of unprocessed milk and milkfat surface material (MFSM) of homogenized (1st stage = 3,515 mg/m^2; 2nd stage = 14,062 mg/m^2) milk. MFSM of homogenized milk contained more protein (8.0% vs. 3.4% recovery) but less total lipid (75.8% vs. 97.7% recovery) and phospholipid (54.1% vs. 67.4%) than the MFGM of unprocessed milk. The increased protein load found on the MFSM was attributed to the association of casein micelles at the fat-serum interface (Keenan et al., 1983b). The decreased amount of lipid and phospholipid found on the homogenized MFSM was attributed to the displacement of native MFGM material caused by homogenization. Keenan et al. (1983b) concluded homogenization caused some loss of membrane material from the lipid globules but the majority of the native membrane material remained on the lipid globule surfaces. The native membrane material, however, was altered in that native membrane material was spread over a larger surface area (Keenan et al., 1983b).

In summary, the homogenization of unprocessed milk causes milk proteins (casein and whey) to associate at the MFGM. Closer association of casein proteins, mainly in micelle form, is observed with less closer association of whey proteins. In addition, the majority of native milkfat globule membrane remains associated at the MFSM. However, the native milkfat globule membrane structure is altered as it covers a greater amount of milkfat surface area.

Effect of Heat Treatment on MFGM of Unprocessed Milk

Heat treatment of milk, as occurs in pasteurization, creates complexes between casein and whey proteins and native MFGM components (Dalgeish and Banks, 1991; Houlihan et al., 1992; Kim and Jimenez-Flores, 1995; Sharma and Dalgeish, 1993). Most commercial pasteurization systems operate at continuous high temperature short time (HTST) parameters. Milk is heated at 72^{o}C for 15 to 18 seconds. Other companies utilize ultra-high temperature (UHT) pasteurization systems, which involve heating milk at a higher temperature (138^{o}C) and shorter time (2 sec.) than HTST systems. Casein and whey proteins react differently with the native MFGM components depending on the temperature and duration of the thermal process. Heat treatment causes changes in the confirmation of casein and whey proteins. In addition, changes in the structure of the milkfat globule membrane may occur. These effects can cause significant changes in the chemical structure of milk, which determine product stability and resulting consumer acceptability (Dalgleish and Sharma, 1993, McPherson and Kitchen, 1983). In summary, it is necessary to understand the implications of thermal processing on the interactions of natural and processed milk globules with milk proteins to facilitate innovation of new dairy products.

Heating causes the whey proteins of milk (alpha-lactalbumin and beta-lactoglobulin) to denature. Alpha-lactalbumin does not exhibit heat induced interactions with other milk components unless severely heated. On the other hand, when beta-lactoglobulin is heated, a sulfhydryl group is exposed. This sulfhydryl group allows beta-lactoglobulin to interact with other denatured beta-lactoglobulin, with surface kappa-casein of casein micelles, and with milkfat globules (Dalgleish and Banks, 1991a; Mulvhill and Kinsella, 1987; Dalgleish, 1990b; Houlihan et al., 1992; Smits and VanBrouwershaven, 1980). Interactions between denatured beta-lactoglobulin molecules is minimal in milk systems because interaction between beta-lactoglobulin and casein micelles is more efficient (Dalgleish and Sharma, 1993). Despite casein micelles having 30 times the surface area of milkfat globules, beta-lactoglobulin preferentially binds to milkfat globule membrane upon heating (Dalgleish and Sharma, 1993). Consequently, the formation of a complex between beta-lactoglobulin and milkfat globule membrane is more efficient. Increased protein interaction with milkfat globule membrane is observed with increased heating time (Dalgleish and Banks, 1991b; Houlihan et al., 1992). Beta-lactoglobulin is the main protein that associates with the milkfat globule membrane upon heating. There are two possible explanations for this association. First, upon heating the milkfat globule membrane could rupture and beta-lactoglobulin bind directly to the milkfat. Second, the denatured beta-lactoglobulin could bind to milkfat globule membrane constituents. Because the second reaction is thermodynamically favored, it is the most likely explanation of interactions created between serum proteins and milkfat globules in milk systems (Phipps and Temple, 1982).

Heat treatment of milk causes proteins of the milkfat globule membrane to denature and expose cysteinyl residues. Consequently, sulfhydryl groups of denatured beta-lactoglobulin interact with exposed cysteinyl residues of the milkfat globule membrane

proteins, creating a secondary membrane layer around the milkfat globule (Dalgleish and Sharma, 1993).

Houlihan et al. (1992) studied the effects of heat on the MFGM and skim milk components. At 80°C more protein associated with the altered membrane of the fat globule as time increased. Protein concentration increased from 0.40 g/100 g fat to 0.48 g/100 g fat to 0.54 g/100 g milkfat at corresponding times of 2.5 min, 10 min, and 20 min, respectively (Houlihan et al., 1992). In addition, at all heating times examined, beta-lactoglobulin was the major protein adsorbed to the MFGM. MFGM is involved in heat-induced interactions with beta-lactoglobulin and, to a lesser extent, kappa-casein, and levels of incorporation of these components into the membrane are dependent on the extent of heat treatment (Houlihan et al., 1992). Houlihan et al. (1992) concluded that heating alone did not affect the major compositional changes in the milkfat globule membrane, rather it was the presence of skim milk proteins during heating that caused the observed changes.

Kim and Jimenez-Flores (1995) heated whole milk at 87°C for 60 min and examined the resulting protein complexes at the milkfat globule interface. Heat-induced disulfide linkages in the form of dimer and trimer complexes of beta-lactoglobulin or as a complex of beta-lactoglobulin and kappa-casein or alpha-lactalbumin were observed (Kim and Jimenez-Flores, 1995). In addition, other heat induced interactions between serum proteins and MFGM proteins were found to exist. The precise nature of these reactions was not elucidated. Kim and Jimenez-Flores (1995) hypothesized that milk serum proteins may deposit on the MFGM with the displacement of polypeptides from the membrane material.

Effect of Processing on the MFGM of Homogenized / Heat Treated Milk

Following processing, many possibilities exist for constituents of the resulting membrane surrounding the milkfat globule. The method of processing dictates the resulting membrane constituents. For example, if heat treatment occurs prior to homogenization, then serum proteins are denatured and interact with both the kappa-casein micelle and the native milkfat globule membrane (Dalgleish and Banks, 1991b). The following homogenization process causes the micellar complex of casein and serum proteins to adsorb on the newly formed milkfat interface. In contrast, when milk is homogenized prior to heat treatment, casein proteins, either as semi-intact micelles or as micellar fragments, cover the newly formed milkfat surface, and no whey proteins are present on the milkfat surface (Walstra and Oortwijn, 1982; Sharma and Dalgleish, 1993). Subsequent heating of this milk causes serum proteins and the adsorbed casein to complex on the increased milkfat surface area.

Effects of Homogenization Prior to Heat Treatment on MFGM Material. Previous discussion of the effects of processing of milk has described the effects of heating unprocessed milk. However, when milk is homogenized, new structures are formed. Size reduction of the milkfat globules causes a corresponding increase in surface area of the milkfat globule. The increased surface area of the milkfat globules cannot be covered by native milkfat globule membrane. Thus other milk constituents, mainly proteins, must

fill the gaps in the milkfat membrane created by homogenization. Little is known regarding the changes in the homogenized milkfat globule membrane upon heating.

Five possible explanations of interaction of denatured proteins in homogenized, heated milk have been proposed (Dalgleish and Sharma, 1993). These include (i) interaction with other denatured molecules of beta-lactoglobulin, (ii) interaction with kappa-casein on the surfaces of casein micelles in suspension, (iii) interaction with kappa-casein complexed in micelle form on the surface of milkfat globule membrane, (iv) interaction with residual membrane materials, (v) direct adsorption to the milkfat globule membrane (Dalgleish and Sharma, 1993).

Because casein molecules adsorbed at the milkfat globule membrane interface have a greater amount of kappa-casein exposed, it is likely that denatured serum proteins form complexes with the membrane bound casein, mainly kappa-casein. Dalgleish and Sharma (1993), using 6 M urea, broke all non-covalently bound protein complexes adsorbed on the fat globule surfaces, leaving only the proteins in close contact with the fat surface. Following treatment with 6 M urea, it was found that only beta-lactoglobulin and kappa-casein remained at the milkfat globule surface. Because kappa-casein is not the most surface active casein protein, it may be possible that the casein micelle rearranges upon heating to orient kappa-casein toward the milkfat globule interface and allow the binding of beta-lactoglobulin (Dalgleish and Sharma, 1993).

Darling and Butcher (1978) examined the effect of homogenization (single stage, 20 MN/m) followed by pasteurization (85^{o}C for 10 minutes) on the resulting membrane material surrounding the milkfat globule of 32% milkfat cream. During homogenization, caseins and undenatured whey proteins were adsorbed to the fat-serum interface. Following homogenization, the whey proteins were easily removed by washing while the caseins were difficult to remove. After subsequent pasteurization, however, there was a significant increase in both the adsorption of beta-lactoglobulin and in their resistance to desorption. Darling and Butcher (1978) concluded interfacial membrane between fat droplets and the serum phase consists of a protein composite material containing casein micelles, casein micellar sub-units, native milkfat membrane, and non-micellar protein. In addition, Darling and Butcher (1978) concluded that whey proteins are only strongly bound to the interface if the cream is heated after homogenization.

Effect of Pasteurization Prior to Homogenization on MFGM Material. Cano-Ruiz and Richter (1997) looked at the effect of homogenization pressure on the milkfat globule membrane proteins. Prior to homogenization, milk was heat treated at 65^{o}C for 30 minutes or 85^{o}C for 20 minutes. Following heat treatment, milk was homogenized at 30 MPa, 60 MPa, or 90 MPa. Protein load in processed milk increased with increasing homogenization pressure and decreased with increasing heat treatment. Casein proteins made up about 70% of the processed milk membrane material. Cano-Ruiz and Richter (1997) found that some of the casein proteins associated with native membrane proteins and whey proteins through disulfide linkages. Greater amounts of beta-lactoglobulin (8.22% vs. 5.10%) and alpha-lactalbumin (5.48% vs. 4.13%) were associated with the fat globule membrane of milk receiving the greater heat treatment. The amount of native

milkfat globule membrane associated at the milkfat globule interface made up approximately 10% of the membrane material for all processed milk treatments. Cano-Ruiz and Richter (1997) attributed the decrease in protein load of the higher heat treated milk to direct absorption of denatured beta-lactoglobulin and alpha-lactalbumin to the milkfat globule membrane; thus limiting the surface area available for casein micelles to be adsorbed and therefore decreasing the total protein load (Cano-Ruiz and Richter, 1997).

McPherson et al. (1984) isolated the milkfat globule membrane material from milk commercially HTST pasteurized or ultra-heat pasteurized and subsequently homogenized. Isolated membrane material from milk HTST pasteurized followed by homogenization contained mostly alpha-S casein proteins and lesser amounts of beta-casein; in addition, little or no kappa-casein was detected (McPherson et al., 1984). Using phospholipid content as an indicator of natural MFGM material present in the processed milkfat globule membrane, it was found that some of the natural MFGM material was lost in processing. The native MFGM material was not selectively removed because the processed MFGM component polypeptides produced a similar electrophoresis pattern to that of unprocessed MFGM (McPherson et al., 1984). In addition, it was found that a significant amount of para-kappa casein was present in the processed MFGM material. This could be a result of kappa-casein being adsorbed directly onto the processed milkfat globule membrane and proteolytic enzymes causing kappa-casein to change conformations to para-kappa casein, or para-kappa casein could be present in the raw milk before processing and subsequently preferentially adsorbed because of its increased hydrophobicity compared to kappa-casein (Farrell and Thompson, 1974). Milk, ultra heat treated followed by homogenization, had casein proteins and, to a lesser extent beta-lactoglobulin, associated at the membrane interface (McPherson et al., 1984). In addition, lesser levels of phospholipid were associated at the processed membrane interface of ultra-heat treated milk when compared with HTST milk. This indicates lesser amounts of native MFGM material present in ultra-heat treated milk when compared with HTST milk (McPherson et al., 1984).

Sharma and Dalgleish (1994) compared the MLGM of whole milk pasteurized (75, 80, 85, 90^{o}C for 2 to 120 min) and homogenized either prior to or following pasteurization. Results indicated that the amounts of serum proteins (mainly beta-lactoglobulin) present in the MLGM were smaller (0.48 mg/m^2) in milk heated prior to homogenization than in milk that had been homogenized prior to heating (0.64 mg/m^2) (Sharma and Dalgleish, 1993; Sharma and Dalgleish, 1994). Using photon correlation spectroscopy, the adsorbed layer of protein on milk heated prior to homogenization (20 nm) was smaller than milk homogenized prior to pasteurization (34 nm) (Sharma and Dalgleish, 1993; Sharma and Dalgleish, 1994).

In summary, pasteurization and homogenization both affect the composition of the MFGM. Different homogenization pressures affect the amount of protein that associates with the resulting surface membrane materials, while differing pasteurization temperatures affect the type and amount of proteins that associate with the homogenized surface membrane material as well as the shape of the fat globule.

REFORMULATED CREAMS: COMPONENTS AND PROCESSING EFFECTS

Dairy Components used for Reformulation of Butteroil

The envelopment of butteroil into a stable emulsion involves reconstitution with MFGM or other milk-derived components. Skim milk, sweet buttermilk, and butter derived aqueous phase all have components beneficial in emulsifying butteroil into a stable dairy emulsion.

Description of How Components are Obtained. Skim milk contains surface active proteins (casein and whey) that can help emulsify butteroil into a stable emulsion (Singh et al., 1993; Tomas et al., 1994; Oortwijn and Walstra, 1979; Elling et al., 1996, Elling and Duncan, 1996, Scott, 1999). The skim milk proteins adsorb to the MFGM, filling vacancies created by the reconstitution process (Mulder and Walstra, 1974). Skim milk is obtained by separating whole milk into cream (30-35% milkfat) and skim component (0.01-0.05% milkfat).

Sweet buttermilk is obtained from cream by the process of churning. It contains MFGM fragments released into sweet buttermilk during churning. MFGM is an efficient natural surface-active substance for emulsion formation. Successful butteroil in water emulsions have been made using the MFGM derived from buttermilk (Kanno, 1989; Kanno et al., 1991; Elling et al., 1996; Elling and Duncan, 1996; Corredig and Dalgleish, 1996; Scott, 1999). Sweet buttermilk frequently has not been used in milk products in the past. However, due to modern hygienic practices, quality standards of sweet buttermilk in the form of buttermilk powder have been improved. Sweet buttermilk powder has become affordable because the value of milk fat has recently decreased. In addition, Newstead (1999) manufactured milks produced with sweet buttermilk with improved flavor profiles compared to recombined milks produced with skim milk powder. The affordability, improved flavor profile, and emulsion characteristics of recombined milk products produced with sweet buttermilk make this component a feasible option for reformulation.

When butter is melted, remaining MLGM on fat globule surface is released in the butter-derived aqueous phase. The aqueous phase of butter differs from buttermilk in that it has 2 to 3 times the lipid content of buttermilk (Deeney et al., 1985; Keenan and Patton, 1993; Elling et al., 1996; Scott, 1999).

Surface Active Agents Present in Components used for Emulsification of Butteroil. Buttermilk, skim milk, and butter-derived aqueous phase all contain surface active agents such as phospholipids and proteins for emulsifying butteroil into a stable dairy emulsion. Elling et al. (1996) measured the amount of surface active agents in skim milk, buttermilk, and aqueous phase components used in reformulation. Skim milk, buttermilk, and butter-derived aqueous phase all had comparable amounts of surface active protein available for emulsification (28.01 mg/g, 27.00 mg/g, and 31.75 mg/g) (Elling et al., 1996). However, amounts of surface active phospholipid differed significantly ($p<0.05$) between these components. Butter-derived aqueous phase had significantly greater amounts (4.95 mg/g) of phospholipid when compared with buttermilk (0.89 mg/g) and skim milk (0.12 mg/g) (Elling et al., 1996). In addition,

buttermilk component had significantly greater amounts of surface active phospholipid than skim milk component (Elling et al., 1996).

Effect of Separation Temperature of Cream on Surface Active Agents Utilized by Components for Emulsification. Scott (1999) examined the effect of separation temperature of whole milk on the amount of surface active protein and phospholipid of skim milk, buttermilk, and buttermilk-derived aqueous phase. Separation temperature (49^oC vs. 55^oC) did not significantly ($p>0.01$) affect the amount of surface active protein or phospholipid present in the components. However, formulation did affect the amount of protein available for emulsification in the creams. At a separation temperature of 55^oC, skim component (30.19 mg/g) and buttermilk component (28.93 mg/g) had significantly ($p<0.01$) greater amounts of protein than butter-derived aqueous phase (26.12 mg/g) (Scott, 1999). In addition, at a separation temperature of 55^oC, butter-derived aqueous phase (4.634 mg /g) had greater amounts of surface active phospholipid than sweet buttermilk component (1.004 mg/g) and skim milk component (0.156 mg/g) (Scott, 1999). Sweet buttermilk component had significantly ($p<0.01$) greater amounts of surface active phospholipid than skim milk component (Scott, 1999).

Effect of Processing on Reformulated Cream

Resulting surface material of reformulated creams differs from the native MFGM. Having examined the effect of processing on natural milk systems, it is necessary to compare the effects of processing on reformulated cream. Examples of processes that affect the emulsifying components of reformulated creams are cooling, heating, and homogenization. Consequently, the way the MFGM reacts to these treatments will determine the stability of the product and product acceptability (McPherson and Kitchen, 1983).

Reformulated creams have components that react differently to pasteurization and homogenization treatments. An effective reformulated cream should parallel homogenized natural cream in physical properties (appearance, composition, membrane material) (Elling and Duncan, 1996).

Effects of Processing on Reformulated Cream Composition. Oehlmann et al. (1994) emulsified cholesterol-reduced and natural butteroils (35%) with sweet buttermilk, aqueous phase derived from melted butter, skim phase, or a combination of these components. These components were mixed, warmed (40^oC), and dispersed either by homogenization or ultrasound. Usage of cholesterol reduced butteroil had an insignificant effect on the emulsion stability, churn time, and protein composition of the cream (Oehlmann et al., 1994).

Emulsions formed were characterized as water-in-oil types as opposed to the typical oil-in-water emulsions occurring in natural creams (Oehlmann et al., 1994). Protein composition of reformulated emulsions was very similar to protein composition of starting components (Oehlmann et al., 1994). Regardless of what emulsifying component was used, the amount of protein associated with emulsified butteroil was comparable to the amount of protein before emulsification. For example, stable emulsions were

produced with approximately 50 mg protein/g butteroil, approximately 50.5 +/- 4.9 mg protein/g butteroil reassociated upon reformulation (Oehlmann et al., 1994). In addition, phospholipid content among the components and emulsions were also similar (Oehlmann et al., 1994).

Elling et al. (1996) prepared three reformulated creams consisting of 1) 20% cholesterol-stripped butteroil and 80% skim component, 2) 20% cholesterol-stripped butteroil and 80% sweet buttermilk, 3) 20% cholesterol-stripped butteroil, 70% sweet buttermilk, 10% butter-derived aqueous phase and compared them to a 20% natural cream. Natural and reformulated creams were batch pasteurized (30 min, 65.5^{o}C) and homogenized at a total pressure of 10.2/3.4 MPa (1st stage = 10.2 MPa, 2nd stage = 3.4 MPa) or at a total pressure of 13.6/3.4 MPa (1st stage = 13.6 MPa, 2nd stage = 3.4 MPa). Resulting emulsions were of the oil-in-water type. Elling et al. (1996) found reformulated creams consisting of sweet buttermilk or sweet buttermilk and butter-derived aqueous phase exhibited protein concentration and phospholipid concentration most similar to that of natural homogenized cream. Formulation did not have a significant effect ($p>0.05$) on the total fat and total protein contents of the creams. The natural cream had a protein concentration of approximately 26.5 mg protein/g cream while all reformulated creams had a range of 24.8 to 27.9 mg protein/g cream (Elling et al., 1996). The main protein fraction associated with lipid globules of natural and reformulated creams was casein. Formulation had a significant effect for phospholipid content of the creams. Similar phospholipid contents (0.60 mg phospholipid/g cream) were obtained for the control creams and creams having only buttermilk as an emulsifying component (Elling et al., 1996). The formulation consisting of sweet buttermilk and butter-derived aqueous phase had significantly ($p<0.05$) higher amounts of phospholipids (approximately 1.0 mg phospholipid/g cream) (Elling et al., 1996). On the other hand, the formulation consisting of 80% skim and 20% butteroil had significantly ($p<0.05$) lower amounts of phospholipid (0.15 mg phospholipid/g cream) than other formulations (Elling et al., 1996).

Scott (1999) examined the effect of formulation, separation temperature, and butteroil composition on the chemical characteristics of eight reformulated creams prepared from medium or low melt butteroil and emulsifying components (skim milk, buttermilk, aqueous phase) obtained at two separation temperatures (49^{o}C or 55^{o}C). Natural and reformulated creams were processed in the same manner as Elling et al. (1996). Separation temperature and butteroil fraction had no significant effect on chemical composition. However, formulation did have a significant effect protein content (Scott, 1999). Creams reformulated with skim component had greater amounts of protein than natural cream (40.25 mg protein/g cream vs. 28.83 mg protein/g cream) (Scott, 1999). In addition, significant ($p<0.01$) differences existed for phospholipid content of the creams. Creams reformulated with buttermilk (0.527 mg phospholipid/g cream) and natural creams (0.489 mg phospholipid/g cream) had greater amounts of phospholipid than creams reformulated with skim component (0.065 mg phospholipid/g cream) (Scott, 1999).

Effect of Processing on Reformulated Cream Milkfat Surface Material. Following processes such as heating and homogenization, the resulting milkfat surface material dictates the emulsifying properties of the particular reformulated cream. It is important to understand the effects of processing on the MFGM of reformulated cream if innovative dairy products are to be realized.

Kanno (1989) examined the effects of homogenization speed, emulsification time, temperature of emulsification, milkfat concentration, milkfat globule concentration, pH, and temperature on emulsifying activity and stability of milkfat emulsified with milkfat globule membrane. Milkfat globule membrane was obtained from cream centrifuged at 186,000 x g at 4^{o}C for 40 min and from melting butter granules obtained from churning at 45^{o}C. Emulsions were prepared by mixing 1% MFGM with 25% milkfat and homogenizing the mixture for 60 sec at 19,000 rpm. A positive correlation existed between homogenization speed marks (5, 7, 9, and 11) and emulsifying activity and emulsion stability (Kanno, 1989). Extended emulsification time (0.5 to 5 min), however, caused a decrease in both emulsion stability and emulsifying activity (Kanno, 1989). The greatest emulsion stability was achieved at 1 min emulsifying time. The greatest emulsifying activity and stability were obtained at a homogenization temperature of 45^{o}C. Emulsifying activity and stability decreased with increasing milkfat concentration (5, 15, 25, 35, 45%) in the presence of 1% MFGM due to insufficient coverage of lipid droplets by MFGM. A corresponding increase in emulsifying activity and stability was noted with increasing MFGM concentration (20, 40, 60, and 80 mg/g fat) (Kanno, 1989). Optimum emulsion activity and stability was obtained at pH 4. The effect of heat treatment was investigated by processing emulsions at 4, 10, 15, 25, 35, 45, and 55^{o}C for 30 min. Stable emulsions were obtained at temperatures below 25^{o}C. However, at temperatures above 25^{o}C, emulsion stability decreased with increasing temperature (Kanno, 1989).

Kanno et al. (1991) examined the physicochemical properties of milkfat emulsified with MFGM. Creams were prepared using the method of Kanno (1989). As MFGM concentration increased, greater amounts of protein was absorbed on the globules. The percentage of protein actually adsorbed onto the fat globules, however, decreased as increasing concentrations of MFGM were used for emulsification. Using 20, 40, 60, and 80 mg/g MFGM, 51.5, 43.6, 36.2, and 32.7 percent protein was adsorbed (Kanno et al., 1991). Using 40 mg MFGM/g fat, at pH values of 4, 5, 6, 7, and 8, the percentage of adsorbed protein was 79.6, 74.2, 50.2, 37.2, and 40.1%, respectively (Kanno et al., 1991). The higher adsorbance of proteins at pH 4 was attributed to the greater degree of exposure of hydrophobic regions of proteins below the isoelectric point. Due to its amphipathic nature, Kanno et al. (1991) concluded that MFGM was an efficient emulsifying agent for foods and other materials.

Oortwijn et al. (1979) determined the quantity of proteins adsorbed on milkfat droplets in recombined cream consisting of milkfat emulsified with skim component, whey, and casein dispersions. Components were mixed and warmed to 40^{o}C followed by homogenization at 10 MPa. Oortwijn et al. (1979) found protein load (mg/m^2) for milkfat in casein, skim component, and whey were 20, 10, and 2.5 mg/m^2, respectively.

Oortwijn et al. (1979) concluded that casein proteins were more efficiently adsorbed to fat globules.

Oortwijn and Walstra (1982) produced recombined creams consisting of milkfat and whey and/or skim component and determined their corresponding emulsion stability. Emulsion stability was analyzed using the properties of coalescence and clustering. The quantity of available protein was the principal factor affecting the properties under study (Oortwijn and Walstra, 1982). Emulsions were prepared in the same manner as described by Oortwijn et al. (1979). Creams were heated to 45^{o}C and homogenized at a pressure of 5 MPa. Cluster formation was noted when protein per ml fat was less than 0.15 g (Oortwijn and Walstra, 1982). Stability to coalescence was analyzed over a storage period of seven days. Creams emulsified with whey containing less than 10% milkfat changed minimally during the storage. However, when protein load was decreased below 0.5 mg/m^2, coalescence occurred (Oortwijn and Walstra, 1982). After heat treatment of emulsions at 80^{o}C, coalescence of fat globules was noted. Stability to coalescence at heat treatment of 120^{o}C was noted as protein load increased from 7 to 35 mg protein/ml fat (Oortwijn and Walstra, 1982). Oortwijn and Walstra (1982) concluded that coalescence, resulting from heat treatment of whey based emulsions, was dependent on the amount of available protein.

Tomas et al. (1994) analyzed the effects of the ratio of fat to protein on the mean droplet diameter, surface of interface, amount of protein adsorbed, and protein surface coverage. Oil-in-water emulsions were obtained from milkfat and skim component, varying in fat (4-30 g/100g) and protein (0.4-3.2 g/100g). Anhydrous milkfat and skim component were emulsified using a microfluidizer operating at 5000 psi at 52^{o}C. The final ratio of milkfat to protein varied between 1 and 13. Proteins from the skim phase were mainly responsible for maintaining emulsion stability. Preferential adsorbance of casein proteins over whey proteins was observed, though some whey proteins were adsorbed at the milkfat/serum interface (Tomas et al., 1994). The results revealed that as the fat to protein mass ratio increased, the droplet size increased. This was most apparent when the fat to protein mass ratio was greater than 6 (Tomas et al., 1994). In addition, as the fat to protein ratio increased, the interfacial surface area increased due to an increase in fat droplet quantity (Tomas et al., 1994). This resulted in a corresponding increase in the fraction of protein adsorbed. Maximum protein adsorbance was achieved at 85% (Tomas et al., 1994). At fat to protein ratios below 11 mg/m^2 , maximum protein surface coverage was observed. A slight decrease in protein surface coverage was observed at protein concentrations greater than 11 mg/m^2 (Tomas et al., 1994).

In order to determine the degree of reassociation of native components, Elling et al. (1996) analyzed the chemical composition of isolated milkfat globule surface material in control and reformulated creams. Formulation had a significant effect ($p<0.05$) on the relative amount of milkfat globule surface material, protein, and phospholipid content in the surface material reassociated with lipid. The reformulated cream consisting of 80% skim component and 20% butteroil had significantly more isolated milkfat globule surface material than all other creams (Elling et al., 1996). The amount of milkfat globule surface material retrieved from the cream composed of 80% skim component and

20% butteroil and homogenized at 13.6/3.4 Mpa was 112.6 mg/g lipid (Elling et al., 1996).

Formulations consisting of sweet buttermilk only or buttermilk (70%) and butter-derived aqueous phase (10%) had similar amounts of milkfat globule membrane associated with the lipid globules when compared to that of the control cream (Elling et al., 1996). For example, at a homogenization pressure of 13.6/3.4 MPa, the membrane material for the control was 75.4 +/- 3.38 mg surface material / g lipid in cream, 70.7 +/- 9.4 mg surface material/g lipid in cream for creams composed of 80% buttermilk and 20% milkfat, and 76.9 +/- 3.9 mg surface material / g lipid in cream for creams composed of 70% buttermilk, 20% butteroil, and 10% butter-derived aqueous phase (Elling et al., 1996).

Elling et al. (1996) found that protein associating at the lipid surface was consistent among formulations with the exception of the 80% skim component and 20% butteroil. This formulation homogenized at 13.6/3.4 MPa was significantly higher ($p<0.05$) in the amount of protein occurring in the milkfat surface material / g of lipid in cream when compared to buttermilk formulations with and without butter-derived aqueous phase and homogenized at 13.6/3.4 Mpa (Elling et al., 1996). Creams formulated with skim component and homogenized at 10.2/3.4 MPa had significantly higher ($p<0.05$) amounts of protein associated with lipid globule surfaces than creams formulated with sweet buttermilk and butter-derived aqueous phase homogenized at 10.2/3.4 MPa. Regardless of homogenization pressure, the reported values for protein content of the milkfat surface material associated with creams processed from skim component were 46-52mg protein/g lipid in cream (Elling et al., 1996). At 10.2/3.4 MPa and 13.6/3.4 MPa, the protein content of the milkfat surface material of the control cream was 45.1 +/- 2.66 mg protein/ g lipid in cream and 48.0 +/- 2.90 mg protein/ g lipid in cream, respectively (Elling et al., 1996). Lower values were obtained for creams reformulated with buttermilk and buttermilk and butter-derived aqueous phase. At 10.2/3.4 MPa, reformulated cream processed from 20% butteroil, 70% buttermilk, and 10% butter-derived aqueous phase had 38.4 +/- 1.75 mg protein/g lipid in cream and 20% butteroil and 80% buttermilk had 41.3 +/- 2.24 mg protein/g lipid in cream (Elling et al., 1996). At the higher homogenization pressure of 13.6/3.4 MPa, cream consisting of only buttermilk as a component and buttermilk and butter-derived aqueous phase had 42.8 +/- 3.31mg protein/g lipid in cream and 45.4 +/- 2.57 mg protein/g lipid in cream, respectively (Elling et al., 1996).

The amount of native milkfat globule membrane fragments reassociated with lipid was estimated using phospholipid content. Compared to all formulations consisting of buttermilk, the formulation comprised of skim component had significantly lower ($p<0.05$) amounts of phospholipid in the milkfat surface material (Elling et al., 1996). At 10.2/3.4 MPa, the reformulated cream consisting of sweet buttermilk component and cream consisting of sweet buttermilk component and butter-derived aqueous phase exhibited milkfat globule surface material with phospholipid compositions of 0.55 +/- 0.003 mg phospholipids/g lipid in cream and 0.54 +/- 0.001 mg phospholipids/g lipid in cream, respectively (Elling et al., 1996). However, at the same homogenization pressure, the skim component formulation had only 0.001 +/- 0.24 mg phospholipid/g lipid in

cream while at 13.6/3.4 MPa phospholipid content was 0.33 +/- 0.02 mg phospholipid/g lipid in cream (Elling et al., 1996). Slightly higher values of phospholipid content were observed when creams were homogenized at 13.6/3.4 MPa. For example, the control, sweet buttermilk, and sweet buttermilk combined with butter-derived aqueous phase emulsions had 0.56+/- 0.06, 0.58 +/- 0.04, and 0.67 +/- 0.05 mg phospholipid/g lipid, respectively, in cream (Elling et al., 1996).

Scott (1999) determined the composition of milkfat surface material (SM) (SM/lipid in cream, protein in SM/lipid in cream, % total protein associated with lipid globules, phospholipid SM/lipid in cream, % phospholipid associated with lipid globules) isolated from lipid globules of natural and reformulated creams processed from components obtained at two different separation temperatures (49^{o}C and 55^{o}C) and butteroils having different melting range characteristics (low-melt and medium melt fractions). No significant ($p>0.01$) differences in chemical composition of milkfat surface material were attributed to separation temperature or melting range characteristics. However, significant ($p<0.01$) differences were noted in chemical composition of milkfat surface material of creams, depending on their formulation (Scott, 1999).

A greater percentage of protein was found in the milkfat surface material of natural creams when compared to creams reformulated with skim component (33.5% vs. 23.5%). In addition, a greater amount of phospholipid was found in the milkfat surface material of creams reformulated with buttermilk and aqueous phase (1.00 mg phospholipid in surface material/g lipid) or natural cream (1.05 mg phospholipid in surface material/g lipid) when compared with creams reformulated with skim component (0.048 mg phospholipid in surface material/g lipid) (Scott, 1999).

Corredig and Dalgleish (1998) investigated the changes occurring in the MFGM when cream was heated. Soybean oil was emulsified with either sweet buttermilk or MFGM isolates and heated either by HTST pasteurization or batch heating at controlled temperatures ranging from 60-85^{o}C. Protein solubility, protein adsorption, and emulsifying properties of the MFGM isolates were determined as a function of temperature and compared with an unpasteurized control cream. Emulsions prepared with 2% (w/v) buttermilk isolates from unpasteurized cream had similar diameter distributions of emulsions (0.1um to 1um) when compared with 2%(w/v) buttermilk isolates from creams heated at 76, 78, 85^{o}C (Corredig and Dalgleish, 1998). It was determined that casein proteins were the primary proteins adsorbed at the oil-water interface. Consequently, casein proteins determined the behavior of the emulsions.

Emulsions prepared with MFGM alone had greater amounts of MFGM and lesser amounts of skim proteins associated at the membrane interface than emulsions prepared with buttermilk isolate. Compared to unpasteurized creams emulsified with MFGM, MFGM isolates from creams heated by HTST pasteurization temperatures between 76 and 85^{o}C exhibited far worse emulsifying properties (Corredig and Dalgleish, 1998). All emulsions prepared from MFGM and heat treated had a bimodal distribution of fat globules with a large number of globules being greater than 10 um and a large number of fat globules being less than 1 um. On the other hand, the MFGM emulsion that did not

undergo heat treatment produced fat globules between the range of 0.1 um and 1 um (Corredig and Dalgeish, 1998).

Corredig and Dalgleish (1998) found that all emulsions heat treated and formulated with either MFGM or buttermilk had beta lactoglobulin associated with the membrane of the fat globules. These results differ from previous studies on whole milk where both beta-lactoglobulin and alpha-lactalbumin where found to associate with the MFGM (Dalgleish and Banks, 1991; Corredig and Dalgleish, 1996). Thus the heat induced protein interactions in whole milk and cream may differ (Corredig and Dalgleish, 1998).

Corredig and Dalgleish (1998) investigated the emulsifying properties of MFGM isolates obtained from buttermilk heated at temperatures ranging from 60 to 85^{o}C and compared them to MFGM isolates obtained from unheated buttermilk. Emulsions comparable to emulsions prepared with MFGM obtained from unheated buttermilk were obtained with MFGM isolates obtained from buttermilk heated at 60 and 62^{o}C. However, MFGM isolates obtained from buttermilk heated at temperatures greater than 62^{o}C produced emulsions with greater fat globule diameters than emulsions prepared from MFGM isolates obtained from unheated buttermilk (Corredig and Dalgleish, 1998). A significant increase ($p<0.05$) in average fat globule diameter of emulsions prepared from MFGM isolates obtained from buttermilk heat treated at temperatures greater than 65^{o}C compared to emulsions prepared from MFGM isolates obtained from unheated buttermilk was observed (Corredig and Dalgleish, 1998).

Corredig and Dalgleish (1998) also found that the amount of beta-lactoglobulin increased significantly ($p<0.05$) at temperatures greater than 65^{o}C. This agreed with the findings of McPherson et al. (1984) and Kim and Jimenez-Flores (1995). Corredig and Dalgleish (1998) hypothesized that beta-lactoglobulin interactions with MFGM might occur through non-covalent interactions simultaneously or sequentially with disulfide interchange. Disulfide interchange may occur between xanthine oxidase, which upon heating at temperatures above 65^{o}C releases its iron compounds exposing cysteine residues (Berglund et al., 1996) for disulfide interaction with cysteine containing proteins (kappa-casein, beta-lactoglobulin) (Corredig and Dalgleish, 1998).

INFLUENCE OF PROCESSING PARAMETERS ON EMULSION STABILITY

Cream is defined as the "liquid milk product high in fat separated from milk, which may have been adjusted by adding thereto: Milk, concentrated milk, dry whole milk, skim milk, concentrated skim milk, or nonfat dry milk" (Code of Federal Regulations, 1999). The Code of Federal Regulations (1999) divides cream products into 4 different categories based on fat content: half and half (10.5-18% fat), light cream (18-30% fat), light whipping cream (30-36% fat), and heavy cream (>36% fat). Cream has a natural sensory quality that gives it a full bodied texture. It is important that a reformulated cream mimic these properties in order for it to be accepted by the public. The resulting physical characteristics of reformulated creams are dependent on the stability of the emulsion which is decreased by phenomena such as flocculation and coalescence. Several factors, including viscosity, creaming stability, and feathering, have been investigated.

Following homogenization, if there is not sufficient amount of surfactant to cover the oil-water interface of the fat globule, then instability during emulsion formation will occur (Dalgleish, 1997). Adsorbing proteins will cover the greatest amount of fat globule surface area as possible. However, if their are gaps left in the interfacial layer, then oil droplets will coalesce, decreasing the total surface area until their is enough surfactant for sufficient coverage (Fang and Dalgleish, 1993). Coalescence normally occurs during or immediately after homogenization, but may continue for some time in a viscous product such as cream (Dalgleish, 1997).

Another possibility resulting from the lack of sufficient protein coverage caused by homogenization is bridging flocculation. Bridging flocculation results in fat droplets being bridged by casein micelles. A second homogenization at a lower pressure prevents this type of flocculation. However, in emulsions where a single protein molecule serves as a linking agent, the linking bridges do not break in this way and more surfactant is needed (Dalgleish, 1997).

Processing of cream must produce emulsions with proper resistance to flocculation. The net charge of the proteins adsorbed to the milkfat interface will affect emulsion stability to flocculation. A stable emulsion will have a charge that is high enough to prevent the close approach of fat globules (Dalgleish, 1997)

Viscosity

Viscosity is the property of liquid that describes the magnitude of the resistance due to shear forces with in the liquid. Viscosity is measured by dividing shear rate by shear stress. Viscosity is measured in units of poise. Common methods for measuring viscosity include rotational viscometers and capillary tube methods.

At temperatures greater than 40^{o}C fluid milk exhibits Newtonian flow because varying shear rates and resulting measurements of shear stress produce a linear relationship when plotted against each other. However, cooled raw milk and cream exhibit non-Newtonian fluid flow because the shear rate affects the velocity (Walstra and Jenness, 1984). At temperatures below 40 ^{o}C, immunoglobins form bridges between fat globules; as a result, extra work is needed to break these bridges (Prentice, 1992). This is reflected in an increase in viscosity. At temperatures above 40 ^{o}C the bridges disappear and the viscosity of the cream exhibits Newtonian flow.

Viscosity changes over time of interfacial layers of protein coated emulsions have been observed. Consequently, the deduction can be made that the nature of the interfacial layer changes after the time of its formation (Dickinson et al., 1990). Emulsions prepared with beta-casein have shown little change in interfacial viscosity over time (Dickinson et al., 1988). However, emulsions prepared with globular proteins (whey) had significant increases in interfacial viscosity over time (Dickinson et al., 1990). Examining the initial conformational structure of these proteins, it can be seen that beta-casein has little defined structure and seems to adopt an extended structure upon adsorption to an oil-water surface (Graham et al., 1984; Dalgleish, 1990a). In contrast, the globular whey

proteins have greater degrees of defined structure and denature upon adsorption to the oil-water interface (Phillips, 1977). These observations may account for the differences observed in interfacial viscosity over time.

Langley (1984) and Phipps (1982) conducted studies on viscosity changes that occurred in processed (homogenized/pasteurized) creams. Both developed equations with the following factors: mean globule diameter, % fat of cream, storage time, and initial viscosity of the cream. Langley (1984) and Phipps (1982) reported increases in viscosity of creams as storage time increased. Phipps (1982) used single stage homogenization ranging from 10.8 to 25.8 MPa and lesser percent fat creams while Langley (1984) used single stage homogenization (6.9 MPa and 13.8 MPa) on 20%, 40%, and 50% milkfat creams with different pasteurization processes (74^{o}C, 125^{o}C for 10-15 sec). Phipps (1982) attributed increased viscosity with time to progressive flocculation of fat globules and to the strengthening of the structure formed by the gradual accretion of casein micelles on the globule surfaces and their bridging. Langley (1984) found increased homogenization pressure, decreased heat-treatment temperature, and increased fat content of the creams all contributed to greater increases in viscosity with time. Both of these studies examined cream viscosity over a longer storage period. Langley (1984) used a 5 week storage period and did not use ultra high temperature pasteurization temperatures, while Phipps (1982). Phipps (1982) attributed increases in viscosity due to globules combining due to increased strengthening of the structure formed by conglomerating casein micelles on the surface of the globules.

Kanno et al. (1991) reconstituted butteroil and MFGM and looked at the effects of emulsifying time, MFGM concentration, and pH on viscosity. MFGM and butteroil were emulsified at 19,800 rpm for various lengths of time. At four min emulsification time, the highest viscosity was noted. After four min, however, a sharp drop in viscosity occurred due to the incorporation of air bubbles (Kanno et al., 1991). Viscosity was also monitored while varying the concentration of MFGM. Viscosity of the reconstituted milkfat globules remained constant at MFGM concentrations ranging from 20 to 40 mg of MFGM/g fat. However, a linear increase in viscosity was noted between 40 to 80 mg of MFGM/g fat. This could be attributed to an increase in proteins absorbed on the surface of the fat globules causing increased resistance to shear (Kanno et al., 1991).

Elling and Duncan (1996) determined the viscosities of reformulated and natural creams over a 13 day storage period. All control creams were less viscous than reformulated creams at all stages of the study. On days one through seven, creams reformulated with skim or sweet buttermilk and butter-derived aqueous phase and homogenized at 13.6/3.4 MPa were not statistically different from the control. However, by day 13 of the study, cream reformulated with sweet buttermilk and butter-derived aqueous phase was the lone formulation not statistically different from the control cream. In addition, the study revealed that creams homogenized at 10.2/3.4 MPa did not significantly differ in viscosity from creams homogenized at 13.6/3.4 MPa (Elling and Duncan, 1996).

Scott (1999) determined viscosities of reformulated and natural creams over a 13 day storage period. Creams reformulated with medium-melt butteroil had significantly

($p<0.01$) greater viscosity values than creams formulated with low-melt butteroil. This observation could be explained by the higher degree of unsaturated fatty acids in the low-melt butteroil which increase fluidity (Scott, 1999).

Creaming Stability

Besides the full bodied, viscous, mouth-feel of cream, consumers also look at the appearance of their cream. The most common defect in cream is the rising of fat globules and subsequent formation of a cream layer due to the fat globules being lighter than the plasma phase (Brunner, 1974). Stokes' law describes this phenomenon:
$V = (r^2*2*(d_1 - d_2)*g) / 9n$ where: V = rate of the rise, r = radius of the fat globules, d_1 = density of the plasma phase, d_2 = density of the fat phase, g = the gravitational constant, n = specific viscosity of the plasma phase. Factors that affect the cream layer and its rate of formation include: absolute size of the fat globules, the composition of the milk, the extent to which the milk has been agitated (homogenization pressure and time), and the temperature history of the milk (Brunner, 1974). Dalgleish (1997) found that bridging flocculation causes an emulsion to cream more rapidly.

Fink and Kessler (1985) examined the effect of heating on the creaming stability of 30% milkfat natural cream. Creams were heated (70^{o}C) and 2 stage homogenized (1st stage = 70 bar; 2nd stage = 7 bar) then pasteurized (85^{o}C to 145^{o}C at 0.8 to 90 sec). Fink and Kessler (1985) found heat induced aggregation of the casein spread out in submicelles over the surface of the fat globules resulted in the formation of fat globule aggregates. Consequently, the milkfat globule membrane loses its uniform structure and holes within the milkfat globule membrane begin to develop and more free fat was observed (Fink and Kessler, 1985). This was especially apparent at Pasteurization temperatures greater than 105^{o}C where destabilization of the milkfat globule membrane could be detected immediately after heating. Overall, Fink and Kessler (1985) concluded extractable free fat content of the cream increased with increasing holding times and increasing temperatures above 105^{o}C.

Elling and Duncan (1996) conducted studies to monitor the creaming stability of natural creams and creams reformulated with skim component, buttermilk component, or butter-derived aqueous phase component. Creams were stored (4^{o}C) for 2 weeks and monitored at various times for creaming stability. Creaming stability was measured by taking the % fat of the top layer and the % fat of the bottom layer over a designated storage time (Tornberg and Hermansson, 1977). Two factors were found to influence the creaming stability: homogenization pressure and length of storage time (Elling and Duncan, 1996). All creams homogenized at the higher pressure (13.6/3.4 MPa) showed better creaming stability than those homogenized at the lower homogenization pressure (10.2/3.4 MPa). By day 13, fat contents obtained from the top layer of reformulated creams ranged from 16% to 41.5%, depending on homogenization pressure. No significant difference was noticed between the creaming stability of the different reformulated creams (Elling and Duncan, 1996).

Using the methods of Elling and Duncan (1996), Scott (1999) examined creaming stability of 20% milkfat natural and reformulated creams. Processing variables in

reformulation explored in this experiment included temperature at which separation of cream and skim occurred (49°C vs. 55°C) and type of butteroil used for reformulation (low-melt butteroil vs. medium-melt butteroil). No significant differences in creaming stability were noted between natural creams and reformulated creams.

Feathering

Cream is often added to coffee. A common defect known as feathering occurs when cream coagulates when mixed with hot coffee. The presence of this feathering phenomena is influenced by 3 factors: type of coffee, treatments carried out on cream, and the formulation of the creams themselves (Geyer and Kessler, 1989). The type of coffee varies in acidity and temperature, both of which have marked effects on feathering. Treatments such as homogenization and pasteurization can have varied effects on feathering based on the parameters at which they were carried out. Various formulations of cream have components that react differently when placed in a high temperature, high acid environment, like that of coffee.

Anderson et al. (1977) examined the effect of a ten week storage period on feathering and composition of UHT treated 18% milkfat cream. Substituting coffee solutions with acetate buffers with pH values ranging from 4.7 to 5.6, Anderson et al. (1977) found that creams feathered in the pH range of 4.70 to 5.20. In addition, as storage period increased, the incidence of feathering increased. This could be attributed to an increase in the amount of casein and calcium surrounding the fat globules over the ten week storage period (Anderson et al., 1977).

Elling and Duncan (1996) examined 3 different reformulated creams 20% butteroil/80% skim milk (w/w), 20% butteroil/80% buttermilk (w/w), 20% butteroil/70% buttermilk/ 10% aqueous phase for feathering in coffee at different pH. Formulation, homogenization pressure, and length of storage did not have a marked effect on feathering. It was found that all creams feathered in a pH range of 4.86 to 5.09 (Elling and Duncan, 1996).

Scott (1999) examined the extent of feathering of reformulated and natural creams. Formulation and separation temperature had no significant effect on the degree of feathering of the cream. Creams typically feathered in the pH range of 4.86 to 5.09 (Scott, 1999).

Previous studies have shown that processing (heating, cooling, separation temperature) affect natural and reformulated creams. However, the interactions of these processes and their effect on the emulsion stability of natural and reformulated creams has not been thoroughly investigated. The goal of this study was to examine the effects of homogenization and pasteurization sequence on the physicochemical emulsion characteristics of 20% milkfat natural and reformulated creams.

CHAPTER III
PROCESSING PARAMETER EFFECTS ON PHYSICOCHEMICAL PROPERTIES OF 20% MILKFAT NATURAL AND REFORMULATED CREAMS

A. ABSTRACT

Skim, sweet buttermilk, and butter-derived aqueous phase components were used to re-emulsify low-melt butteroil into desired 20% milkfat creams. The implications of pasteurization process, homogenization sequence, and formulation on the physicochemical properties of reformulated and natural creams were investigated.

Homogenization sequence had a significant ($p<0.05$) effect on the chemical properties of the creams. Creams homogenized prior to pasteurization had greater amounts of milkfat surface material at the milkfat interface than creams homogenized following pasteurization, which contained greater amounts of protein and lesser amounts of phospholipid at the milkfat interface. Natural cream and buttermilk/aqueous phase reformulated cream homogenized after pasteurization had significantly ($p<0.05$) lesser amounts of milkfat surface material with greater amounts of protein associated at the milkfat interface when compared with other formulation homogenization sequence combinations. Creams reformulated with skim component had significantly ($p<0.05$) greater percentages of phospholipid adsorbed at the milkfat interface.

Ultra high temperature (UHT) pasteurized natural and reformulated creams had significantly ($p<0.05$) greater viscosity at all shear rates investigated when compared with high temperature short time (HTST) pasteurized creams. In addition, HTST pasteurized natural cream was significantly ($p<0.05$) more viscous than HTST pasteurized reformulated creams at most shear rates investigated.

Creams reformulated with buttermilk showed creaming stability most comparable to natural cream. HTST pasteurized creams had greater creaming stability than UHT pasteurized creams. All creams feathered in a pH range of 5.09 to 5.31.

Homogenization prior to UHT pasteurization resulted in creams rated "out of specification" because of poor sensory quality on day 1. Other processing sequences resulted in creams within sensory specifications.

B. INTRODUCTION

Modified milkfat, differing from natural milkfat in nutritional and functional attributes, requires emulsification in many food systems. Emulsification can be accomplished by combining altered milkfat with surface active agents into reformulated dairy products such as creams. Milk-derived components such as skim milk, sweet buttermilk, butter-derived aqueous phase, whey proteins, casein dispersions, and purified milkfat globule membrane suspensions have proven to successfully emulsify butteroil (Smith and Dairiki, 1975; Oortwijn et al., 1977; Oortwijn et al., 1979; Kanno, 1989; Kanno et al., 1991, Rosenberg and Lee, 1993; Oehlmann et al., 1994; Tomas et al., 1994; Elling et al., 1996; Scott, 1999). Ideally, emulsification should produce a dairy product having chemical attributes similar to natural dairy products. It is necessary to understand the effects of processing on the chemical structure of milk if this goal is to be accomplished.

Prior to processing, interactions between milk proteins (casein and whey) and milkfat are limited. Milkfat is contained within a milkfat globule membrane; casein proteins, primarily in micelle form, and whey proteins predominantly in the serum phase of milk. Processing such as heating and homogenization can alter this relationship.

When milk is heated, changes occur in the conformation of serum proteins and micelle proteins. In addition, changes may occur in the structure of the milkfat globule membrane. These effects can cause significant changes in the chemical structure of milk (Dalgleish and Sharma, 1993). Houlihan et al. (1992) found milkfat globule membrane to be involved in heat-induced interactions with beta-lactoglobulin and kappa-casein. Levels of incorporation of these components into the membrane were dependent on the extent of heat treatment.

When milk is homogenized, new structures are formed. Size reduction of the milkfat globules to less than 1 uM or less causes a corresponding 4 to 10 times increase in surface area of the milkfat globule (Brunner, 1974; Walstra, 1975). The increased surface area of the milkfat globules cannot be covered by native milkfat globule membrane. Thus other milk constituents, mainly proteins, must fill the gaps in the milkfat membrane created by homogenization.

In summary, pasteurization and homogenization both affect the composition of the surface material at the serum-milkfat interface. Different homogenization pressures affect the amount of protein that associates with the resulting surface membrane materials while differing pasteurization temperatures affect the type and amount of proteins that associate with the homogenized surface membrane material as well as the structure of the fat globule.

An effective reformulated cream would parallel homogenized natural cream in physical properties (appearance, composition, membrane material) (Elling and Duncan, 1996). The goal of this study was to examine the effect of high temperature short time (HTST) pasteurization, ultra high temperature (UHT) pasteurization, and homogenization sequences on the chemical and physical properties of natural cream and creams made up

of low-melt fractionated butteroil and emulsified with skim component, sweet buttermilk component, and butter-derived aqueous phase.

C. MATERIALS AND METHODS

Separation of Cream and Skim

On day 0 of processing, raw milk obtained from the Virginia Tech dairy farm was separated (55°C) into 30-35% milkfat cream and skim milk using a pilot plant separator (Elecrem, model 1G, 6400 rpm, Bonanza Industries, Inc., Calgary, Alberta). Skim milk from separation was subsequently used for reformulation of creams. Modified Babcock procedure (Marshall, 1993) was used to determine the percent milkfat of the separated cream. If not within range, creams were standardized to 30-33%. Cream was preheated to 80°C and ultra high temperature (UHT) pasteurized at 148°C for 2 sec in a tubular heat exchanger (Microthermics UHT/high temperature short time (HTST) (Lab 25-HV, Microthermics, Inc., Raleigh, NC) to insure proper pathogen elimination for sensory studies. After cooling (21.5°C), cream either was stored (13°C) for 14 h for subsequent churning or stored (4°C) for further processing. The skim portion to be used for reformulation was not heat treated and was stored at 4°C.

Preparation of Sweet Buttermilk and Butter-Derived Aqueous Phase Components

On day 1 of processing, sweet buttermilk was obtained using the methods of Elling et al. (1996). Tempered cream (13°C) was mechanically churned (Gem Dandy Standard Electric Churn, Bonanza Industries, Inc., Calgary, Alberta) to produce sweet buttermilk and butter. Sweet buttermilk was separated from butter granules by pouring through cheesecloth and pressing excess buttermilk from butter granules with a stainless steel spoon. The resulting sweet buttermilk was stored at 4°C until further processing and subsequent usage as a component for reformulation of creams.

Butter-derived aqueous phase was received from a commercial processor (Grasslands Dairy Products, Inc., Greenwood, WI) on day 1 of processing for use as a reformulation component. Butter-derived aqueous phase was obtained from a commercial processor due to the high quantity needed for this experiment.

Characterization of Low-Melt Fractionated Butteroil

Low-melt fractionated butteroil was obtained from anhydrous milkfat utilizing the Tirtiaux fractionation procedure at the Wisconsin Center for Dairy Research (University of Wisconsin, Madison). Low-melt fractionated butteroil had a dropping point between 10-25°C. At a temperature between 25°C and 30°C, percent solid fat was zero. The low-melt fractionated butteroil had medium-yellow coloration and butter-like flavors.

Cream Reformulation

A natural 20% milkfat cream formulated from skim and cream component was used as a control formulation. Low-melt butteroil was melted (45-50°C) and combined with components into two experimental formulations (Table 3-1). Each formulation underwent each of the following processing sequences:

1) homogenization followed by HTST (77°C for 15 sec) pasteurization;
2) HTST pasteurization followed by homogenization;
3) homogenization followed by UHT (148°C for 2 sec) pasteurization;
4) UHT pasteurization followed by homogenization.

Table 3-1. Description of processing sequence and pasteurization process of 20% natural and reformulated creams.

Reformulated Cream Description	Homogenization[1] Sequence	Type of Pasteurization[2]	Components %	Low-Melt Butteroil %
20% Natural Cream	before	HTST	Control	Control
20% Natural Cream	after	HTST	Control	Control
20% Natural Cream	before	UHT	Control	Control
20% Natural Cream	after	UHT	Control	Control
80% Skim + 20% Butteroil	before	HTST	80% Skim	20% Butteroil
80% Skim + 20% Butteroil	after	HTST	80% Skim	20% Butteroil
80% Skim + 20% Butteroil	before	UHT	80% Skim	20% Butteroil
80% Skim + 20% Butteroil	after	UHT	80% Skim	20% Butteroil
70% Buttermilk + 10% Butter Derived Aqueous Phase + 20% Butteroil	before	HTST	70% Buttermilk 10% Aqueous Phase	20% Butteroil
70% Buttermilk + 10% Butter Derived aqueous Phase + 20% Butteroil	after	HTST	70% Buttermilk 10% Aqueous Phase	20% Butteroil
70% Buttermilk + 10% Butter Derived Aqueous Phase + 20% Butteroil	before	UHT	70% Buttermilk 10% Aqueous Phase	20% Butteroil
70% Buttermilk + 10% Butter Derived Aqueous phase + 20% Butteroil	after	UHT	70% Buttermilk 10% Aqueous Phase	20% Butteroil

[1] 1st stage = 13.6 MPa, 2nd stage = 3.4 MPa; "before" or "after" pasteurization
[2] HTST (high temperature short time) = 77°C, 15 sec; UHT (ultra high temperature) = 148°C, 2 sec

HTST pasteurization and UHT pasteurization were accomplished using a tubular laboratory pasteurization system (Microthermics UHT/HTST Lab 25-HV, Microthermics, Inc., Raleigh, NC). Homogenization at a total pressure of 13.6/3.4 MPa (1st stage = 13.6 MPa, 2nd stage = 3.4MPa) was accomplished using a laboratory homogenizer (APV Gaulin, Inc., Model 15MR, Everett, MA).

Three treatments were HTST pasteurized (77^{o}C, 15 sec) prior to homogenization. Sanitizer was then run through the homogenizer prior to homogenization, limiting post pasteurization contamination. Formulations were warmed (27.7^{o}C) and stirred using a hand mixer prior to pasteurization. Outlet temperature valve of the pasteurizer was 55^{o}C. Creams then were homogenized and stored at 4^{o}C.

Three HTST treatments were homogenized prior to pasteurization. Formulations were warmed (55^{o}C) and stirred with a hand mixer to ensure uniformity, homogenized, cooled (27.7^{o}C), and pasteurized. Homogenized and pasteurized creams were then stored (4^{o}C).

After all treatments receiving HTST pasteurization were completed, the pasteurizer was reconfigured for UHT pasteurization parameters (148^{o}C, 2 sec) and the homogenizer cleaned and sanitized. All treatments receiving UHT pasteurization parameters then were processed as previously described.

Fat, Protein, and Phospholipid Determination of Creams and Membrane Material

Protein content was analyzed by a dye binding assay (DC Bio Rad assay, BioRad Laboratories, Hercules, CA). The modified Babcock procedure (Marshall, 1993) was used to measure fat content of reformulated and control creams. Total lipid contents of reformulated and control creams were determined using the methods of Folch et al. (1957). Determination of phospholipid content required lipid extraction using the methods of Folch et al. (1957). Using a silicic acid column, phospholipids in the extracts were separated (Rouser et al., 1966). A quantitative analysis of the amount of phosphorous in the phospholipid extract was made using a spectrophotometric method (Rouser et al., 1966). The value obtained from the analysis was multiplied by a factor of 25 to convert phosphorous content to phospholipid content (Anderson et al., 1977).

Analysis of Milkfat Surface Material

The amount of phospholipid and protein adsorbed on the surface of the milkfat globule was determined as described by Elling et al. (1996). Creams were centrifuged (60 min., 2^{o}C, 175,000 x g) in a Beckman L2-65B Ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA). Each cream was separated into two components, a lipid rich cream plug and a skim phase. The cream plug which contained lipid associated membrane material, including lipid complexed with protein, was collected. Two cycles of slow freezing and thawing of the cream plug followed by centrifugation (60 min., 2^{o}C, 175,000 x g) in a Beckman L2-65B Ultracentrifuge (Beckman Instruments Inc., Palo Alto, CA) allowed release of components of the milkfat surface material from the milkfat. A pellet, containing milkfat associated surface material, was obtained and lyophilized in a freeze drier (Freezemobile 12 SL, Virtis Co., Inc., Gardiner, NY) with drying chamber (10-MR-SM Vacuum Stoppering and Manifold Drying Chamber, Virtis Co., Inc., Gardiner, NY).

Pellets were ground, using a mortar and pestle, into powder. Lipid extraction of the pellet then was carried out using the methods of Bligh and Dyer (1959). The amount of protein on the milkfat surface was determined using a dye binding assay (DC Bio Rad Protein Assay,Bio Rad Laboratories, Hercules, CA). Phospholipids were obtained by lipid extraction (Bligh and Dyer, 1959) and phosphorous content was determined by a spectrophotometric method (Rouser et al., 1966). This value then was multiplied by a factor of 25 to convert to phospholipid content (Anderson et al., 1977). Phosphodiesterase I activity was determined as described by Brown et al. (1976).

Creaming Stability

The emulsion stability of all creams was analyzed over a two week period (Elling and Duncan, 1996). Cream was placed in 100 ml graduated cylinders, capped, and stored at 4^{o}C on day 0 of storage. Initial fat content on day 0 of storage then was determined using a modified Babcock procedure (Marshall, 1993). The top and bottom layers of each cream were evaluated for fat content using a modified Babcock procedure (Marshall, 1993) on days 1, 3, 5, 7, 9, 11, and 13 of storage. The top layer was first collected using a 10 ml pipette. The bottom layer then was collected using a 10 ml glass pipette. Measurements were made in duplicate. The following equation was used to determine the changes occurring in fat percentage of each layer compared to the initial fat content:
Changes in fat percentage = ((Fat content of top or bottom layer) / (initial fat content)*100)-100)

Viscosity

On day 0, each treatment was placed in a 16x150 mm glass tubes and stored (4^{o}C). Viscosity measurements were made on days 1, 7, and 13 using a Haake Rotovisco RV-12 viscometer equipped with a Haake NV spindle cup (Haake-Buchler Instruments, Paramus, NJ) (Elling and Duncan, 1996; Scott, 1999). Measurements were made at 7^{o}C maintained by a HaakeA92 cooling unit. Shear stress measurements were taken at the following shear rates (s^{-1}): 173->346->692->1385->2770->1385->692->346->173. Viscosity values were then obtained by dividing shear stress by the shear rate.

Feathering Stability

Feathering assays were carried out at 4^{o}C and 85^{o}C at various pH values (pH = 4.70-5.60) in sodium acetate buffer to examine cream feathering. The feathering test was carried out on days 1, 7, and 13 of storage (4^{o}C). On day 0 of storage, each treatment was poured into 16x150 mm glass tubes, capped, and stored (4^{o}C). The assay was performed using the methods of Anderson et al. (1977). Measurements were made in duplicate. The test mimics feathering conditions found in coffee. Scores from 5 to -5 were used to assess the degree of feathering in each sample. The lowest value at which feathering failed to occur was used as the feathering score. Highly stable creams typically had scores of 5 to 3 (pH 4.70 to 4.81) whereas stable creams were given feathering scores ranging from 2 to 1 (pH 4.86 to 4.92) (Atherton and Newlander, 1977). Moderately stable creams had feathering scores of 0 (5.00) and slightly unstable creams had feathering scores of -1 to -2 (5.09 to 5.20). Unstable creams having feathering scores of -3 to -5 (5.31 to 5.60) were considered unmarketable to consumers (Atherton and Newlander, 1977).

Microbiological Analysis

Standard plate count, modified psychrotrophic bacterial count, and coliform bacteria count methods of enumeration were conducted on Day 0, 6, and 12 of storage. These tests were carried out to make certain creams were properly pasteurized and maintained satisfactory microbial quality. Aerobic Count and Coliform Count Petrifilm (3M, St. Paul, Minnesota) were used for plating samples. Dairy blanks were made using solutions of $MgPO_4$, KCl, and deionized water (Marshall, 1993). Dilutions of 10^{-1}, 10^{-2}, and 10^{-3} were employed for microbial analysis. All counts were carried out in duplicate. Petrifilm was incubated at 21°C for 25 h for the modified psychrotrophic bacteria count and at 32°C for 48 h for the standard plate count and coliform plate count methods of enumeration.

Sensory Evaluation of Cream Quality

Creams were evaluated for sensory characteristics on days 1, 7, and 13 of storage. Ten panelists from the Department of Food Science and Technology, familiar with the sensory properties of milk or cream, used the In/Out Method to evaluate the creams (Munoz et al., 1992). This method was used to determine if creams met quality specifications. Panelists were familiarized with the characteristics of an "In" specification cream. A cream "In" specification was described as having the perceived mouthfeel thickness of a 20% milkfat cream along with being free of objectionable off-flavors. When creams began to display moderate levels of off flavors, they were deemed "Out" of specification. Creams were deemed acceptable in quality if 60% of total responses were "In" specification.

Approximately 20 ml of each cream were poured into 1 oz portion size plastic soufflé cup on the day the sensory test was to be conducted and stored at 4°C. Samples were identified with three digit codes and randomized for presentation to panelists. Panelists were given a total of 12 samples (one sample/treatment) at a time. Panelists independently evaluated cream samples in the sensory laboratory of the Department of Food Science and Technology, Virginia Tech under incandescent lighting.

Statistical Analysis

Three repetitions of this study were carried out. A split plot was used with subsampling. The whole plot factor was the cream formulation. The split plot factor was a 2 * 2 factorial with factors of homogenization sequence and pasteurization type. For comparisons on effects, a procedure mixed version was used. A multivariate split plot (repeated measures with a split plot structure between subjects) was also used in this study.

D. RESULTS AND DISCUSSION

Elling et al. (1996) and Scott (1999) processed reformulated creams using sweet buttermilk and butter-derived aqueous phase emulsified modified milkfat with emulsion characteristics similar to natural cream. Reformulated creams using skim component and modified butteroil were different in composition and viscosity compared to natural creams (Elling et al., 1996; Elling and Duncan, 1996). Both studies used low temperature long time (65.5^{o}C, 30 min) to thermally process the creams. Emulsions were determined to be of the oil-in-water type upon examination by transmission electron microscopy.

In this study, HTST and UHT thermal processes in sequence with homogenization of natural and reformulated creams were investigated. Chemical and physical measurements were made on natural and reformulated creams to explain the emulsion stability of the creams. All emulsions in this study were determined to be of oil-in-water type upon examination by light microscope. Creams had fat contents ranging from 15.94% to 18.88% +/- 0.22% milkfat. These values were lower than the target fat content of 20% for the creams. This could be attributed to the loss of milkfat during isolation of the milkfat. Natural creams had lesser amounts of protein (mg/g cream) available for emulsification while skim reformulated creams and buttermilk/aqueous phase creams had similar amounts of protein (mg/g cream) available for emulsification (Appendix B, Table B-1). Natural cream and buttermilk/aqueous phase creams had greater amounts of phospholipid (mg/g) available for emulsification than skim reformulated creams (Appendix B, Table B-1).

Changes in Milkfat Surface Material

Homogenization sequence had an effect on the resulting milkfat surface material. Homogenization reduces mean milk fat globule diameter to 1 um or less and increases surface area of milkfat globules from 4 to 10 times (Brunner, 1974; Walstra, 1975). The increased surface area of the milkfat globules cannot be covered by native milkfat globule membrane. Thus other milk constituents, mainly proteins, must fill the gaps on the milkfat globule surface created by homogenization. Creams pasteurized prior to homogenization and creams pasteurized after homogenization have different milkfat surface material. For example, if heat treatment occurs prior to homogenization, then serum proteins are denatured and interact with both the kappa-casein micelle and the native milkfat globule membrane (Dalgleish and Banks, 1991b). The following homogenization process causes the micellar complex of casein and serum proteins to adsorb on the newly formed milkfat interface. In contrast, when milk is homogenized prior to heat treatment, casein proteins, either as semi-intact micelles or as micellar fragments, cover the newly formed milkfat surface, and no whey proteins are present on the milkfat surface (Walstra and Oortwijn, 1982; Sharma and Dalgleish, 1993). Subsequent heating of this milk causes serum proteins and the adsorbed casein to complex on the increased milkfat surface area.

Creams homogenized prior to pasteurization had significantly ($p<0.05$) greater amounts of milkfat surface material per gram of cream and per gram of lipid compared to creams

homogenized after pasteurization (Table 3-2). In addition, significantly ($p<0.05$) greater amounts of protein per gram cream were associated with creams homogenized prior to pasteurization. Significantly ($p<0.05$) higher percentages of available phospholipid also were associated with the milkfat surface material of creams homogenized prior to pasteurization (Table 3-2). Phosphodiesterase, a marker enzyme within the native milkfat globule membrane, displayed significantly ($p<0.05$) greater activity when cream was homogenized prior to pasteurization (Table 3-2).

Natural and reformulated creams homogenized prior to pasteurization probably already had casein proteins and, to a lesser extent, whey proteins, associated at the milkfat interface. In other words, the milkfat surface material was probably fixed. Upon HTST or UHT pasteurization, additional casein and whey proteins associated with the milkfat membrane material either through bonds between casein proteins, bonds between whey protein and native milkfat globule membrane through disulfide linkage, or bonds between whey protein and casein protein through disulfide linkage (Sharma and Dalgleish, 1993). This could account for increased amount of milkfat surface material per gram cream and per gram lipid associated with creams homogenized prior to pasteurization.

Natural and reformulated creams homogenized prior to pasteurization had significantly ($p<0.05$) greater amounts of protein per gram cream than natural and reformulated creams homogenized after pasteurization (Table 3-2). The method of isolation of the proteins could account for this observation. The amount of protein in the natural and reformulated creams, regardless of homogenization sequence, should be the same because there was the same initial amount of protein in the creams.

Natural and reformulated creams homogenized prior to pasteurization had greater amounts of native milkfat globule membrane associated in the milkfat surface material. This was demonstrated by phosphodiesterase, a marker enzyme within the milkfat globule membrane, displaying significantly ($p<0.05$) greater activity in creams homogenized prior to pasteurization, and the percent phospholipid adsorbed being significantly ($p<0.05$) higher in creams homogenized prior to pasteurization. Natural and reformulated creams homogenized prior to pasteurization most likely had undenatured native milkfat globule membrane material available for emulsification prior to pasteurization. The undenatured native milkfat globule membrane fragments, having more efficient emulsifying properties, oriented themselves at the milkfat globule interface to a greater extent when compared with denatured milkfat globule membrane (Corredig and Dalgleish, 1997; Corredig and Dalgleish, 1998). As a result, lesser surface area is available for casein and whey proteins at the milkfat interface and more native milkfat globule membrane is present at the milkfat interface of creams homogenized prior to pasteurization.

In contrast, creams that underwent pasteurization prior to homogenization had significantly ($p<0.05$) higher protein load associated with the milkfat surface material. Pasteurization denatures milkfat globule membrane proteins. Consequently, the denatured milkfat globule membrane constituents are not as efficient emulsifying agents as undenatured proteins of the milkfat globule membrane (Corredig and Dalgleish, 1997;

Table 3-2. Chemical composition of natural and reformulated cream homogenized[1] prior to (Before) and following (After) pasteurization.

Variable	Homogenization Sequence		Standard Error
	Before	After	
gm MFSM[2] / gm cream	0.028[a]	0.020	0.002
gm MFSM / gm lipid	0.146[a]	0.111	0.009
protein (mg / g cream)	42.54[a]	40.35	0.88
protein (mg / 10 mg MFSM)	3.329	4.759[a]	0.212
% phospholipid adsorbed	63.22[a]	47.51	5.18
uM phosphodiesterase / mg protein / minute	48.19[a]	33.69	4.67

[2] Milk Fat Surface Material (MFSM)

[a] Mean is significantly greater than corresponding mean within row ($p<0.05$)

[1] Two-stage homogenization (1st stage = 13.4 MPa; 2nd stage = 3.4 MPa)

Corredig and Dalgleish, 1998). Milkfat globule membrane fragments probably were lost to the aqueous phase of milk and were not available for emulsification. Consequently, more serum proteins (mainly caseins) were needed to cover the surface area created by homogenization of creams pasteurized prior to homogenization. Complexes between whey, casein, and native milkfat globule membranes occurred prior to homogenization. These proteins, having been denatured prior to homogenization, probably could no longer undergo further reactions. The previously mentioned complexes most likely took up a greater amount of surface area on the resulting milkfat surface. In addition, there was probably a lesser extent of secondary layer formation around the processed milkfat globule. These two observations may account for the decrease in milkfat surface material found in natural and reformulated creams homogenized following pasteurization in comparison to natural and reformulated creams homogenized prior to pasteurization. In addition, since proteins are the main source of emulsifiers for all creams, regardless of formulation, a greater amount of proteins per gram of milkfat surface material could be associated with creams pasteurized prior to homogenization.

Differences in milkfat surface material as a function of processing may be further explained by considering the formulation. Statistically, there was a two-way interaction ($p<0.05$) between homogenization sequence and cream formulation.

Natural cream homogenized prior to pasteurization had significantly ($p<0.05$) greater milkfat surface material per gram lipid than natural cream homogenized after pasteurization and low-melt butteroil (LMBO), buttermilk (BM), aqueous phase (AP) reformulated cream homogenized after pasteurization (Table 3-3). In contrast, natural cream homogenized after pasteurization and LMBO, BM, AP reformulated cream homogenized after pasteurization had significantly ($p<0.05$) greater amounts of protein per 10 mg of milkfat surface material than all other formulation / homogenization sequence combinations (Table 3-3). In addition, natural cream homogenized prior to pasteurization, and LMBO, skim milk (SM) reformulated cream pasteurized prior to or after homogenization had significantly ($p<0.05$) higher percentages of available phospholipid associated with the milkfat surface material than natural cream homogenized following pasteurization (Table 3-3).

Emulsifying properties of native milkfat globule membrane are greatly decreased with increased heat treatment (Corredig and Dalgleish, 1998). It followed that natural creams and LMBO, BM, AP reformulated creams homogenized after pasteurization, which had higher amounts of native milkfat globule membrane available for emulsification, had lesser amounts of milkfat surface material when compared to the corresponding formulations homogenized prior to pasteurization. Denatured native milkfat globule membrane fragments do not adsorb well as undenatured milkfat globule membrane fragments (Corredig and Dalgleish, 1998). Consequently, casein and whey proteins did not form complexes with the denatured milkfat globule proteins and a lesser amount of milkfat surface material was found per gram of lipid.

Table 3-3. Formulation interaction with homogenization sequence effect on the chemical composition of natural and reformulated creams.

Formulation[1]	Homogenization Sequence[2]	gm MFSM[3] / gm Lipid	mg Protein / 10mg MFSM	% Phospholipid Adsorbed
20% milkfat natural cream	Before	0.178 [b]	3.90 [a]	64.47 [b]
20% milkfat natural cream	After	0.099 [a]	5.87 [b]	27.23 [a]
20% LMBO, 80% SM	Before	0.120 [a, b]	2.48 [a]	71.99 [b]
20% LMBO, 80% SM	After	0.139 [a, b]	3.01 [a]	76.18 [b]
20% LMBO, 70% BM, 10% AP	Before	0.140 [a, b]	3.58 [a]	53.20 [a, b]
20% LMBO, 70% BM, 10% AP	After	0.095 [a]	5.40 [b]	39.13 [a, b]
Standard Error		0.016	0.37	8.98

[1] LMBO = low melt fractionated butteroil; SM = Skim Milk; BM = Sweet Buttermilk; AP = Butter-derived Aqueous Phase

[2] Before: Homogenized prior to pasteurization; After: Pasteurized prior to homogenization

[3] MFSM = Milk Fat Surface Material

[a, b, c, d] different letters within a column are significantly different ($p<0.05$) based on means from three repetitions

The fact that skim milk formulated creams had similar amounts of milkfat surface material, regardless of homogenization sequence, supports this argument (Table 3-3). Because skim milk mainly uses protein as an emulsifying component, there is minimal emulsification properties contributed by native milkfat globule membrane. Thus, regardless of homogenization sequence, upon pasteurization, more proteins probably associated with proteins already adsorbed in the milkfat surface material and similar amounts of milkfat surface material were noted for the skim formulated creams.

Creams reformulated with buttermilk/aqueous phase had the greatest amount of milkfat globule membrane present for potential incorporation into the resulting milkfat globule membrane (Elling et al., 1996; Scott, 1999). Consequently, lesser percentages of phospholipid adsorbed were associated with creams reformulated with buttermilk/aqueous phase. Because creams reformulated with skim milk mainly rely on casein and whey proteins as emulsifying agents, the percent phospholipid adsorbed is relatively high because there was little native milkfat globule membrane present initially (Table 3-3).

Homogenization sequence affected the amount and type of milkfat surface material present in the natural and reformulated creams yet had minimal effects on the physical properties of the creams. Pasteurization temperature played a greater role in affecting the physical properties of the creams investigated in this study.

Processing Effects on Physical Properties of Natural and Reformulated Creams

Viscosity. Apparent viscosity of reformulated and natural creams was monitored over a two week storage ($4^{o}C$) period to determine effects of pasteurization temperature and homogenization sequence. Thirteen days of storage ($4^{o}C$) did not have a significant effect ($p>0.05$) on viscosity of creams. Consequently, viscosity values from day one of storage are reported. In contrast, Langley (1984) and Phipps (1982) reported increases in viscosity of creams as storage time increased. Phipps (1982) used single stage homogenization ranging from 10.8 to 25.8 MPa and lesser percent fat creams while Langley (1984) used single stage homogenization (6.9 MPa and 13.8 MPa) on 20%, 40%, and 50% milkfat creams with different pasteurization processes ($74^{o}C$, $125^{o}C$ for 10-15 sec). Phipps (1982) attributed increased viscosity with time to progressive flocculation of fat globules and to the strengthening of the structure formed by the gradual association of casein micelles on the globule surfaces and their bridging. Langley (1984) found increased homogenization pressure, decreased heat-treatment temperature, and increased fat content of the creams all contributed to greater increases in viscosity with time. Both of these studies examined cream viscosity over a longer storage period. Different processing conditions and percent milkfat creams could account for these observed differences.

Creams in this study displayed non-Newtonian behavior at shear rates ranging between 692 s^{-1} to 2496 s^{-1}. Hysteresis curves were generated by taking viscosity values at increasing shear rates then noting viscosity values at the same shear rate but in decreasing order (Appendix D, Table D-2, Figure D-1). Non-Newtonian fluid flow is characterized

by a decrease in apparent viscosity due to an increase in shear rate (Sherbon, 1988). This was observed in the failing curve portion of a hysteresis curve. In addition, non-Newtonian fluid flow was observed when an increase in viscosity was noted as shear rate was lowered. Comparisons of failing curves and rising curves showed higher apparent viscosity values associated with failing curves. This observation can be attributed to break down of colloidal aggregate particles as increasing shear was applied during the first stage of viscosity measurements (Fox and McSweeney, 1998).

Pasteurization temperature had a significant ($p<0.05$) effect on apparent viscosity of natural and reformulated creams. All UHT pasteurized natural and reformulated creams had significantly ($p<0.05$) greater apparent viscosities at all shear rates monitored than HTST pasteurized natural and reformulated creams (Table 3-4). Corredig and Dalgleish (1996) found UHT pasteurized cream to have greater amounts of beta-lactoglobulin (2.66 mg/g fat) in the milkfat surface material when compared to HTST pasteurized cream (0.584 mg/g fat). Casein has little defined structure and can adapt to an extended structure at the membrane interface thus creating emulsion stability through steric stabilization. On the other hand, globular whey proteins have greater degrees of defined structure and denature upon adsorption to the oil-water interface (Graham et al., 1984; Dalgleish, 1990b; Phillips, 1977). Because of the defined structure of beta-lactoglobulin, it could be possible that a greater resistance to shear is observed in UHT pasteurized cream due to the presence of homogenization clusters. Homogenization clusters often occur in high fat products such as cream and may result from a lack of steric stabilization caused by the inability of whey proteins to adapt extended structures and the resulting close association of the milkfat globules. These conditions may lead to a greater degree of sharing of membrane material between the milkfat globules.

Protein load and composition on the milkfat globule surface varies based on the composition of the components used for formulation (Elling et al. 1996; Scott, 1999). Viscosity was further explained by the effects of pasteurization process on the different cream formulations. Statistically, interaction of pasteurization process with cream formulation had a significant ($p<0.05$) effect on the apparent viscosity of natural and reformulated creams.

At a failing curve shear rate of 692 s^{-1} UHT pasteurized cream reformulated with buttermilk / aqueous phase had significantly ($p<0.05$) greater viscosity than UHT pasteurized natural cream and cream reformulated with skim component (Table 3-5, Figure 3-1). This could be accounted for by the position of native milkfat globule membrane fragments at the lipid interface of the milkfat globules. UHT pasteurized creams reformulated with buttermilk / aqueous phase probably had a greater amount of

Table 3-4. Apparent viscosity (mPa*s) of both natural and reformulated creams at various shear rates for two pasteurization processes.

	Pasteurization Type		
Shear Rate (s^{-1})	HTST[1]	UHT[2]	Standard Error
173.12	19.09	37.41[a]	1.94
346.24	28.06	55.46[a]	1.87
692.48	42.17	71.95[a]	1.77
1384.98	39.58	64.95[a]	1.41
2769.92	34.35	47.57[a]	0.72
1384.92	36.85	57.63[a]	0.99
692.48	38.26	62.16[a]	1.27
346.24	23.06	42.77[a]	1.10
173.12	14.77	26.78[a]	1.16

[a]Viscosity is significantly greater than the corresponding value taken at the same shear rate but processed at a different pasteurization temperature ($p<0.05$); based on means of three repetitions

[1]HTST: high temperature short time; 77°C at 15 sec.

[2]UHT: ultra high temperature; 148°C at 2 sec.

Table 3-5. Viscosity (mPa*s) of natural and reformulated creams formulated at two pasteurization temperatures at five shear rates.

		Failing Curve				Rising Curve				
Formulation[1]	Pasteurization[2]	Rate of 173.1 s^{-1}	Rate of 346.2 s^{-1}	Rate of 692.4 s^{-1}	Rate of 1384.8 s^{-1}	Rate of 2769.6 s^{-1}	Rate of 1384.8 s^{-1}	Rate of 692.4 s^{-1}	Rate of 346.2 s^{-1}	Rate of 173.1 s^{-1}
20% Milkfat Natural Cream	HTST	23.82 [a, b]	34.83 [a]	50.76 [b]	47.70 [b]	39.20 [b]	43.51 [b]	44.85 [b, c]	29.45 [b]	18.65 [b]
20% Milkfat Natural Cream	UHT	32.76 [b]	51.64 [b, c]	69.40 [c]	64.09 [c]	45.67 [c]	56.75 [c]	60.24 [c, d]	37.66 [b, c]	23.08 [b, c]
20% LMBO, 80% SM	HTST	18.42 [a. b]	25.92 [a]	35.23 [a]	34.01 [a]	31.18 [a]	32.50 [a]	32.97 [a]	20.29 [a]	14.05 [a]
20% LMBO, 80% SM	UHT	40.07 [c]	49.59 [b]	65.34 [c]	62.39 [c]	47.21 [c]	56.05 [c]	59.93 [c, d]	39.89 [c]	28.19 [c]
20% LMBO, 70% BM, 10% AP	HTST	15.02 [a]	23.43 [a]	40.53 [a, b]	37.02 [a, b]	32.68 [a]	34.59 [a]	36.95 [b]	19.41 [a]	11.60 [a]
20% LMBO, 70% BM, 10% AP	UHT	39.41 [c]	65.16 [c]	81.11 [d]	68.37 [c]	49.83 [c]	60.10 [c]	67.36 [d]	50.77 [d]	29.07 [c]
Standard Error (s^{-1})		3.65	3.25	3.07	2.45	1.24	1.71	2.20	1.90	2.00

[1] LMBO = low melt fractionated butteroil; SM = Skim Milk; BM = Sweet Buttermilk; AP = Butter-derived Aqueous Phase

[2] HTST: high temperature short time; 77°C at 15 sec.
UHT: ultra high temperature; 148°C at 2 sec.

[a, b, c, d] different letters within a column are significantly different ($p<0.05$); Means based on three repetitions.

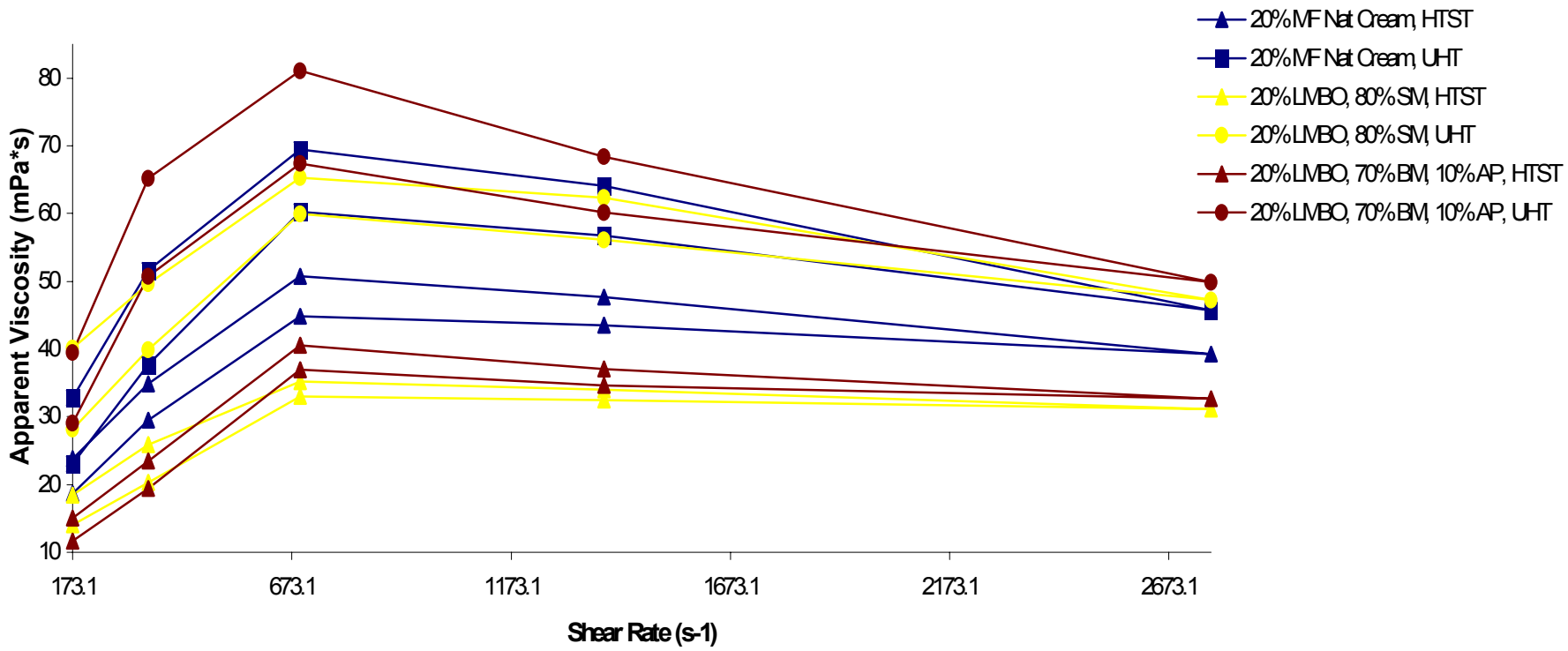

Figure 3-1. Hysteresis curve at 7°C of apparent viscosity of natural creams and creams reformulated with low-melt butteroil (LMBO), skim milk (SM), buttermilk (BM), and aqueous phase (AP) and pasteurized high temperature short time (HTST: 77°C at 15 sec) or ultra high temperature (UHT: 148°C at 2 sec).

native milkfat globule membrane fragments with the potential for interaction with beta-lactoglobulin when compared with natural cream and cream reformulated with skim component. Two reasons could explain this observation. Cream reformulated with skim component has lesser amounts of phospholipids and milkfat globule membrane proteins associated at the lipid interface when compared to natural cream, and creams reformulated with buttermilk and butter-derived aqueous phase (Elling et al., 1996; Scott, 1999). In addition, the reformulation process could orient native milkfat globule membrane in positions more susceptible to interaction with beta-lactoglobulin. Beta-lactoglobulin has preferential adsorption to milkfat globule membrane proteins, despite the fact casein proteins make up the majority of milkfat surface material (Dalgleish and Sharma, 1993). As a result, beta-lactoglobulin probably adsorbed to a greater extent on the milkfat surface membrane of creams reformulated with buttermilk / aqueous phase when compared with natural cream and cream reformulated with skim component. Because beta-lactoglobulin has a greater degree of defined structure, this protein cannot adopt an extended structure upon adsorption to the milkfat surface material (Phillips, 1977). Consequently, there is little emulsion stability brought upon by steric hindrance and homogenization clusters form. The presence of greater amounts of homogenization clusters in UHT pasteurized creams formulated with buttermilk and aqueous phase could cause higher apparent viscosity. The greater amount of homogenization clusters was probably caused by the increased sharing of milkfat surface material between milk fat globules.

All UHT processed creams at a shear rate of 692 s^{-1} of the failing curves had higher viscosities than HTST processed creams. However, at a shear rate of 692 s^{-1} of the rising curve, UHT pasteurized natural cream and UHT pasteurized cream reformulated from skim component had similar viscosity to HTST pasteurized natural cream (Table 3-5). This could be due to the degree of shear at that point causing the majority of homogenization clusters to be broken. As a result, similar viscosities were noted between the creams because homogenization clusters caused by the incorporation of beta-lactoglobulin at the milkfat membrane surface were no longer contributing to increased resistance to shear. Creams reformulated with buttermilk and aqueous phase, however, most likely had significantly ($p<0.05$) higher viscosities than HTST processed creams due to the presence of greater amounts of homogenization clusters caused by a greater amount of MFGM fragments oriented at the milkfat globule interface.

At all shear rates where creams displayed non-Newtonian flow, HTST pasteurized natural cream had significantly ($p<0.05$) greater viscosities than HTST pasteurized cream reformulated with skim component (Table 3-5, Figure 3-1). Moreover, at a shear rate of 2769 s^{-1} and a rising shear rate of 1384 s^{-1} HTST pasteurized natural cream had significantly ($p<0.05$) greater viscosity than cream reformulated with buttermilk and aqueous phase (Table 3-5, Figure 3-1). These findings disagree with those of Elling and Duncan (1996) who found batch pasteurized (30 min, 65.5^{o}C) 20% milkfat creams reformulated with skim component or buttermilk / aqueous phase to have greater apparent viscosity when compared with 20% milkfat natural cream. However, these results do agree with the findings of Scott (1999) who found higher apparent viscosity values to be associated with natural creams, followed by creams reformulated with skim

milk and creams reformulated with buttermilk and butter-derived aqueous phase. Scott (1999) used a low-melt fractionated butteroil similar to the one utilized in this study. Elling and Duncan (1996) however, used a cholesterol reduced butteroil with different melting properties from the low-melt fractionated butteroil utilized in this study. This coupled with the differing processing parameters of the experiments could explain the differences found in apparent viscosity of the creams.

In summary, interaction of pasteurization process and cream formulation on viscosity could be attributed to two factors. UHT pasteurization temperatures cause beta-lactoglobulin to associate with milkfat surface material. This association mainly occurs through disulfide linkage of beta-lactoglobulin with the proteins (mainly xanthine oxidase) of the native milkfat globule membrane because beta-lactoglobulin possesses greater degrees of defined structure and cannot adapt to extended structures like casein proteins (Phillips, 1977; Graham et al., 1984; Dalgleish, 1990b). As a result, there is a increased viscosity resulting from the inability of beta-lactoglobulin to provide proper steric hindrance between milkfat globules. The second factor attributing to the differences in apparent viscosity of the creams caused by the interaction of pasteurization process and cream formulation is the butteroil used in formulating the creams. Low-melt fractionated butteroil contains higher degrees of unsaturation when compared to natural butteroil. This higher degree of unsaturation contributes to lesser apparent viscosity values of the HTST pasteurized reformulated creams in comparison to the UHT pasteurized natural cream.

Creaming Stability. A decrease in creaming stability occurs when a cream plug forms at the surface of a milkfat emulsion. Following homogenization, milkfat globules should be reduced to the size of 2 um or less. A homogenization efficiency test is a method used to test for the degree of milkfat globule size reduction occurring in a homogenization process. Milk is properly homogenized if after 48 hours of storage at 4.4^{o}C no visible cream separation occurs within the milk. Cream viscosity and formation of cream plugs in some skim milk reformulated creams that were homogenized prior to pasteurization and cream reformulated with buttermilk/aqueous phase hindered the evaluation and statistical comparison of the changes in fat content of the top layer of the emulsion. This observation suggests inefficient homogenization efficiency. Under further examination by light microscope, creams displaying early cream plug formation had many small milkfat globules (<0.3 um) surrounding larger milkfat globules (2-4 um) Milkfat progressively rises to the top of a milkfat emulsion as storage time increases. Consequently, bottom layer creaming stability values were used.

Changes in creaming stability of the bottom layer of the natural and reformulated creams were minimal throughout the first week of storage. After seven days of storage, however, significant ($p<0.05$) differences in creaming stability were noted for both natural and reformulated creams. Scott (1999) found 20% milkfat natural creams and creams made-up of 20% low-melt fractionated or medium-melt fractionated butteroils and emulsified with either skim or buttermilk/aqueous phase showed significant differences in creaming stability after one week of storage.

As length of storage time increased, the milkfat surface material of the natural and reformulated creams was most likely altered. This probably was due to proteins changing conformations over a period of time (Fink and Kessler, 1985). Thus the proteins became less efficient emulsifiers. Deterioration of this milkfat surface material may have eventually caused coalescence among milkfat globules due to the rupturing of the milkfat surface material. Coalescence probably continued until the surface area of the milkfat globule was reduced to the point where there was enough milkfat surface material for efficient coverage. This is known as flocculation clustering where milkfat globules share milkfat surface material.

After nine days of storage, natural cream, regardless of processing conditions, showed significant ($p<0.05$) differences in creaming stability of the bottom layer (Figure 3-2). LMBO, SM reformulated cream showed significant ($p<0.05$) differences in bottom layer creaming stability after seven days of storage (Figure 3-2).
After 9 days of storage, LMBO, BM, AP reformulated creams showed significant ($p<0.05$) differences in creaming stability of the bottom layer (Figure 3-2).
Consequently, regardless of processing conditions, LMBO, BM, AP reformulated cream had comparable bottom layer creaming stability to that of natural cream. On the other hand, LMBO, SM reformulated cream had less stability to creaming when compared with natural creams.

These differences could be attributed to LMBO, BM, AP reformulated creams having greater amounts of native milkfat globule membrane components such as phospholipids which have greater resistance to coalescence. Low-melt butteroil, SM reformulated creams had very little native milkfat globule membrane components and relied mainly on casein and whey proteins as emulsifying material (Elling et al., 1996; Scott, 1999). Casein, and to a lesser extent, whey proteins did not resist coalescence as well as the milkfat surface material constituents of LMBO, BM, AP reformulated cream.

Statistically, interaction of pasteurization temperature with length of storage on creaming stability had a significant ($p<0.05$) effect on the creaming stability of the bottom layer. HTST pasteurized natural and reformulated creams had greater creaming stability than UHT pasteurized natural and reformulated creams (Figure 3-3). UHT pasteurized natural and reformulated creams became significantly less stable after 7 days of storage. On the other hand, HTST pasteurized natural and reformulated creams became significantly less stable after 9 days of storage. Emulsion stability through steric stabilization was probably not as efficient with UHT pasteurized creams because whey proteins found at the milkfat surface were unable to prevent close association and eventual coalescence of the milkfat globules. These results agree with those of Fink and Kessler (1985) who found the free fat content of cream to rise over time with increasing processing temperatures ($>105^{o}C$).

Feathering. Feathering is a defect occurring in cream upon addition to coffee. This is used to document stability of cream to active conditions with and without heat to document if reformulated creams could be utilized in coffee and fermented products or as

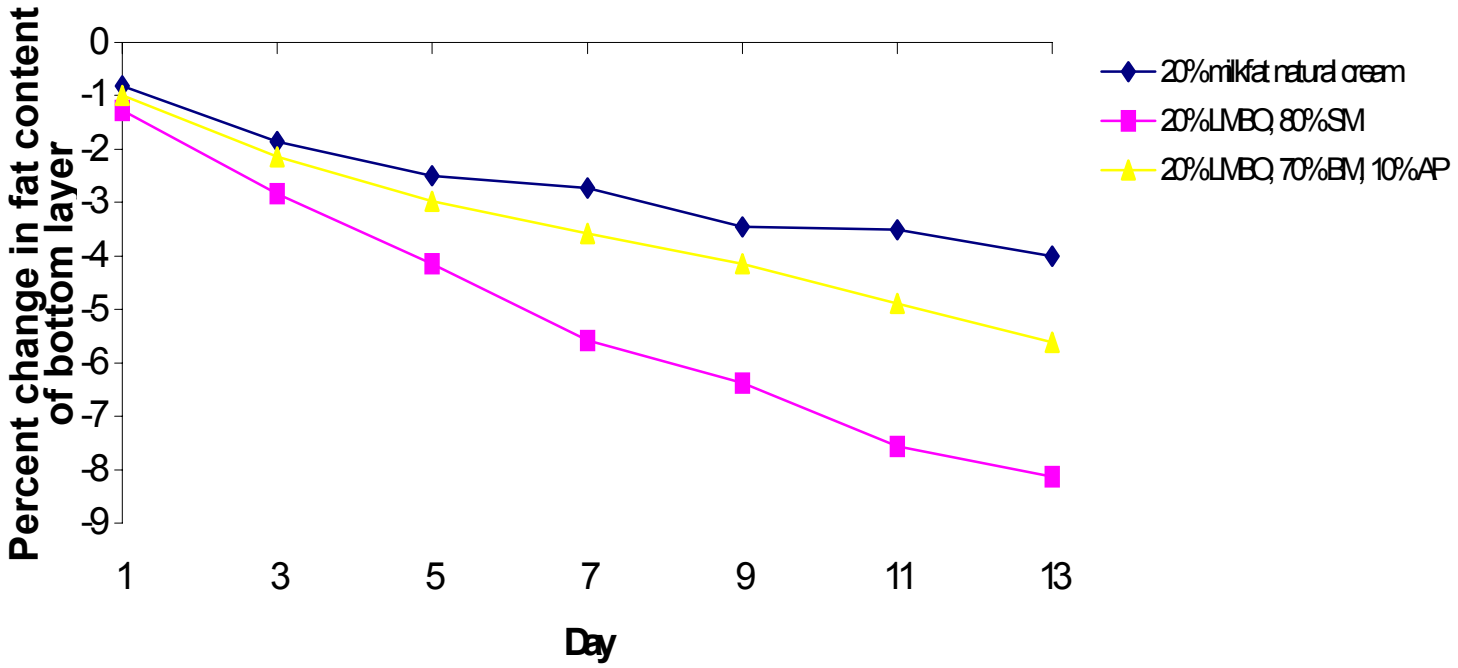

Figure 3-2. Creaming Stability (percent change in the fat content of the bottom layer) of natural creams and creams formulated with low-melt butteroil and either skim milk (SM), sweet buttermilk (BM), or butter-derived aqueous phase (AP) regardless of pasteurization process or homogenization sequence over a two week storage period at 4°C.

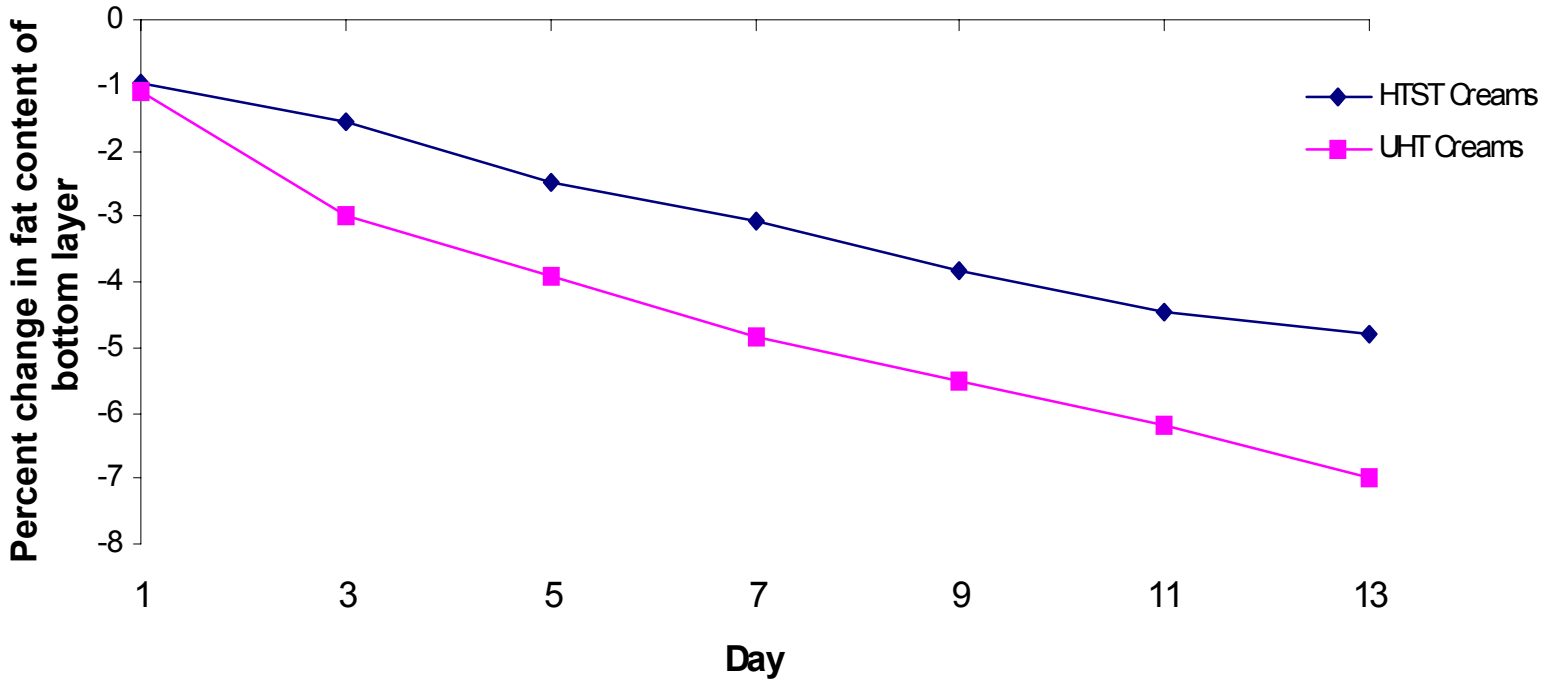

Figure 3-3. Creaming Stability (percent change in the fat content of the bottom layer) of natural and reformulated creams either HTST or UHT pasteurized over a two week storage period at 4°C.

an ingredient in food systems. Feathering is characterized by the formation of undesirable particulate matter caused by destabilized proteins and milk fat globules (Fox and McSweeney, 1998). Proteins and fat globules are destabilized in coffee by the hot and acidic environment. During the processing of natural and reformulated creams, multiple heat process steps (i.e. pasteurization of components and creams) contributed to sensitivity to denaturation of the creams when added to hot (85^{o}C) and cold (4^{o}C) buffer solutions. Creams were examined for feathering at cold temperatures to mimic fermentation conditions. Homogenization sequence nor pasteurization type did not significantly ($p>0.05$) affect the stability of natural and reformulated creams to feathering at either temperature investigated (Appendix D, Table D-4). These results agree with those of Geyer and Kessler (1989) who found UHT treated and HTST pasteurized 12% milkfat creams had similar degrees of feathering. Consequently, feathering scores of 0 to -2 were issued for most creams. These scores translate to creams being moderately stable or slightly unstable to feathering at a pH range of 5.00 to 5.20. Scott (1999) and Elling and Duncan (1996) found that natural and reformulated 20% milkfat creams feathered in a pH range of 4.70-5.09. Differences in feathering values between studies probably occurred because of differences in the processing of the creams. In this study, components used for pasteurization were initially UHT pasteurized thus exposing proteins to a greater degree of thermal denaturation when compared with Elling and Duncan (1996) and Scott (1999). The greater degree of thermal denaturation caused the natural and reformulated creams in this study to be more susceptible to feathering at higher pH values.

Sensory Analysis

Little research has been aimed at evaluating the sensory properties of cream. Less research has been done on the sensory evaluation of reformulated cream. A high quality cream has a clean, slightly sweet, and slightly cooked flavor (Jensen and Poulsen, 1992). The body and texture of cream should be smooth, free of fat plugs and lumps. The presence of off-flavors and texture defects may be indicative of various factors such as microbial growth or enzymatic activity, processing, storage conditions, or low emulsion stability.

A large number of the natural and reformulated creams were observed to have a "cooked" flavor to them. However, depending on the panelists, not all creams were deemed "Out" of specification if a "cooked" flavor was noted. This observation could be due to some panelists having more experience in sensory evaluating cream than. As time of storage increased, panelists marked an increasing number of creams "Out" of specification (Appendix D, Table D-6, D-7, D-8). Increases in the number of "Out" of specification scores were primary attributed to textural defects. The majority of the creams demonstrated separation around day 7 of storage. Consequently, day 1 sensory evaluation results of natural and reformulated cream will be discussed in relation to off-flavors.

All processed cream treatments were considered "In" at a 60% acceptance rate except for natural cream and LMBO, BM, AP reformulated cream homogenized prior to UHT pasteurization, and LMBO, SM reformulated creams UHT pasteurized either prior to or

after homogenization (Appendix D-6). Scott (1999) found 20% LMBO, 80% skim component reformulated HTST pasteurized cream to be "Out" of specification by a panel of 12 trained panelists. Panelists noted oxidized, flat, and cooked off-flavors associated with LMBO, SM reformulated creams. This was attributed to LMBO being more susceptible to oxidation and skim milk having little notes capable of masking the oxidized flavor.

Microbiological Analysis

Cream serves as an excellent environment for microbial growth. Cream has nutrients, pH, and water activity conducive to microbial growth and proliferation. Spoilage bacteria cause deteriorating reactions, resulting in off-flavors. Pasteurization and proper refrigeration are used to control microbial growth and proliferation.

Aerobic, modified psychrotrophic bacteria count, and coliform bacteria count methods of enumeration were conducted on days 1, 7, and 13 of storage. Microbiological analysis was carried out to insure creams were pasteurized properly and creams were microbiologically safe for consumption by sensory panelists. Due to efficient pasteurization and proper refrigeration, bacterial counts for all creams were low (<100 cfu/ml) (Appendix D, Table D-5). Greater counts were associated with the aerobic plate count because psychrotrophic bacteria are more susceptible to heat treatment than mesophilic bacteria. In addition, as storage time increased aerobic plate counts for each respective microbiological analysis increased in HTST pasteurized creams (Appendix D, D-5).

E. CONCLUSIONS

The research was designed to examine the effects of high temperature short time (HTST) and ultra high temperature (UHT) and homogenization sequence on the physicochemical properties of natural and reformulated creams manufactured with a low-melt fractionated butteroil and milk-derived components. All creams were of the oil-in-water emulsion type. Pasteurization temperature and homogenization sequence both affected the physicochemical properties of the creams investigated in this study.

Viscosity of a fluid cream product can be greatly influenced by thermal process and chemical composition of the formulating components. For example, in this study UHT pasteurized natural and reformulated creams were significantly more viscous than HTST natural and reformulated creams. This may be valuable in developing a reduced fat milk or cream with viscosity similar to a higher fat product.

Homogenization sequence affected the type and amounts of surface active agents at the milkfat interface. For example, creams homogenized following pasteurization had greater amounts of protein and lesser amounts of phospholipid oriented at the milkfat surface. These chemical differences brought about by processing could be used to produce other dairy products such as ice cream, cheese, or whipped cream with unique physicochemical properties not currently utilized.

Reformulated cream utilizing buttermilk and aqueous phase milk components for reformulation best mimicked the physicochemical properties of natural cream. The ratio of these in reformulation could be further studied to find ideal sensory and emulsion stability qualities of this formulation.

More research is needed to identify correlation between position of surface active agents (proteins and phospholipids) at milkfat and their corresponding conformations at milkfat interfaces and how these relate to emulsion stability of reformulated creams.

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APPENDIX A

CHEMICAL METHODOLOGY

Folch lipid extraction, modified babcock procedure, biorad protein assays on cream and membrane material, phospholipid assays on cream and membrane material, isolation of membrane material, and phosphodiesterase activity of membrane material were all accomplished using the methods of Scott (1999).

PHYSICAL METHODOLOGY

Creaming Stability, feathering, viscosity, and sensory methods were accomplished using the protocol set forth by Scott (1999).

APPENDIX B

Table B-1. Chemical composition[1] (% lipid, protein, phospholipid) of natural and reformulated creams homogenized before or after pasteurization treatment (HTST, UHT).

Formulation	Homogenization Sequence	Pasteurization Treatment	Fat (%)	Protein (mg/g)	Phospholipid (mg/g)
20% lmbo + 80% Skim	before	HTST	17.05	47.20	0.071
20% lmbo + 80% Skim	after	HTST	17.67	43.23	0.067
20% lmbo + 80% Skim	before	UHT	17.13	47.36	0.046
20% lmbo + 80% Skim	after	UHT	17.16	47.85	0.056
20% lmbo + 70% BM +10% AP	before	HTST	18.88	46.36	0.668
20% lmbo + 70% BM +10% AP	after	HTST	17.64	43.59	0.755
20% lmbo + 70% BM +10% AP	before	UHT	18.42	49.31	0.739
20% lmbo + 70% BM +10% AP	after	UHT	18.12	45.86	0.745
Natural Cream	before	HTST	17.68	32.19	0.522
Natural Cream	after	HTST	17.25	30.38	0.491
Natural Cream	before	UHT	17.15	32.80	0.517
Natural Cream	after	UHT	15.94	31.21	0.545
Standard Error			0.216	0.790	0.027

[1]Values are means and standard errors for 3 replications

Table B-2. Composition[1] (protein content) of milkfat globule membrane (MFGM) isolated from lipid globules of natural and reformulated creams homogenized before or after pasteurization treatment (HTST, UHT).

Formulation	Homogenization Sequence	Pasteurization Treatment	MFGM/ lipid in cream (mg/g)	total protein/ MFGM (mg/g)	% total protein associated with lipid globules
20% lmbo + 80% Skim	before	HTST	123.11	24.69	11.68
20% lmbo + 80% Skim	after	HTST	139.70	30.30	14.39
20% lmbo + 80% Skim	before	UHT	116.42	24.93	10.70
20% lmbo + 80% Skim	after	UHT	137.93	29.90	11.88
20% lmbo + 70% BM +10% AP	before	HTST	117.49	40.08	20.53
20% lmbo + 70% BM +10% AP	after	HTST	113.22	48.92	23.55
20% lmbo + 70% BM +10% AP	before	UHT	162.24	31.28	22.12
20% lmbo + 70% BM +10% AP	after	UHT	76.53	59.07	17.26
Natural Cream	before	HTST	156.70	43.12	38.86
Natural Cream	after	HTST	105.14	56.67	36.24
Natural Cream	before	UHT	200.13	34.79	39.00
Natural Cream	after	UHT	91.89	60.64	33.32
Standard Error			10.83	1.60	2.11

[1]Values are means and standard errors for 3 replications

Table B-3. Composition[1] (phospholipid content) of milkfat surface material (SM) isolated from lipid globules of natural and reformulated creams homogenized before or after pasteurization treatment (HTST, UHT).

Formulation	Homogenization Sequence	Pasteurization Treatment	Phospho-lipid/ lipid in cream (mg/g)	% total phospho-lipid in lipid phase
20% lmbo + 80% Skim	before	HTST	0.071	0.045
20% lmbo + 80% Skim	after	HTST	0.067	0.026
20% lmbo + 80% Skim	before	UHT	0.046	0.032
20% lmbo + 80% Skim	after	UHT	0.056	0.045
20% lmbo + 70% BM +10% AP	before	HTST	0.668	0.359
20% lmbo + 70% BM +10% AP	after	HTST	0.755	0.414
20% lmbo + 70% BM +10% AP	before	UHT	0.739	0.384
20% lmbo + 70% BM +10% AP	after	UHT	0.745	0.394
Natural Cream	before	HTST	0.522	0.308
Natural Cream	after	HTST	0.491	0.309
Natural Cream	before	UHT	0.517	0.323
Natural Cream	after	UHT	0.545	0.345
Standard Error			0.027	0.015

[1]Values are means and standard errors for 3 replications

Table B-4. Composition[1] (phospholipid content) of milkfat globule membrane (MFGM) isolated from lipid globules of natural and reformulated creams homogenized before or after pasteurization treatment (HTST, UHT).

Formulation	Homogenization Sequence	Pasteurization Treatment	Phospho-lipid MFGM/ total MFGM (mg/g)	% total phospho-lipid associated with lipid globules
20% lmbo + 80% Skim	before	HTST	3.13	79.56
20% lmbo + 80% Skim	after	HTST	2.70	74.33
20% lmbo + 80% Skim	before	UHT	2.26	64.42
20% lmbo + 80% Skim	after	UHT	2.97	78.04
20% lmbo + 70% BM +10% AP	before	HTST	12.19	45.01
20% lmbo + 70% BM +10% AP	after	HTST	13.00	38.63
20% lmbo + 70% BM +10% AP	before	UHT	12.79	61.39
20% lmbo + 70% BM +10% AP	after	UHT	19.39	39.63
Natural Cream	before	HTST	9.67	54.84
Natural Cream	after	HTST	7.55	31.00
Natural Cream	before	UHT	10.34	74.10
Natural Cream	after	UHT	7.51	23.45
Standard Error			0.101	4.71

[1]Values are means and standard errors for 3 replications

Table B-5. Phosphodiesterase activity of surface material (SM) of natural and reformulated creams homogenized before or after pasteurization treatment (HTST, UHT).

Formulation	Homogenization Sequence	Pasteurization Treatment	uM Phospho-diesterase I/ mg protein in cream/ minute	uM Phospho-uM Phospho-diesterase/ 10 mg SM
20% lmbo + 80% Skim	before	HTST	64.015	11.974
20% lmbo + 80% Skim	after	HTST	58.382	11.707
20% lmbo + 80% Skim	before	UHT	55.824	11.727
20% lmbo + 80% Skim	after	UHT	54.153	11.512
20% lmbo + 70% BM +10% AP	before	HTST	46.177	8.087
20% lmbo + 70% BM +10% AP	after	HTST	28.652	5.912
20% lmbo + 70% BM +10% AP	before	UHT	75.775	9.060
20% lmbo + 70% BM +10% AP	after	UHT	39.775	7.962
Natural Cream	before	HTST	22.088	4.642
Natural Cream	after	HTST	10.808	3.678
Natural Cream	before	UHT	25.239	4.162
Natural Cream	after	UHT	10.397	3.866
Standard Error			4.455	0.393

[1]Values are means and standard errors for 3 replications

APPENDIX C

Table C-1. Incidence of feathering (pH of buffer at which feathering occured) of 20% milkfat natural cream, 20% low-melt fractionated butteroil (LMBO), 80% skim milk (SM) reformulated cream, and 20% LMBO, 70% buttermilk (BM), 10% aqueuous phase (AP) reformulated cream homogenized prior to (Before) either high-temperature short time (HTST) or ultra-high temperature (UHT) pasteurization or homogenized following (after) either HTST or UHT pasteurization.

Formulation	Homogenization Sequence	Pasteurization Type	Day	Feathering Value	pH
20% milkfat natural cream	before	HTST	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% milkfat natural cream	after	HTST	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% milkfat natural cream	before	UHT	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% milkfat natural cream	after	UHT	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% LMBO, 80% SM	before	HTST	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% LMBO, 80% SM	after	HTST	1	-1	5.09
			2	-3	5.31
			3	-2	5.20
20% LMBO, 80% SM	before	UHT	1	-1	5.09
			2	-3	5.31
			3	-2	5.20
20% LMBO, 80% SM	after	UHT	1	-1	5.09
			2	-3	5.31
			3	-2	5.20
20% LMBO, 70% BM, 10% AP	before	HTST	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% LMBO, 70% BM, 10% AP	after	HTST	1	-1	5.09
			2	-2	5.20
			3	-2	5.20
20% LMBO, 70% BM, 10% AP	before	UHT	1	-1	5.09
			2	-3	5.31
			3	-3	5.31
20% LMBO, 70% BM, 10% AP	after	UHT	1	-2	5.20
			2	-2	5.20
			3	-2	5.20

Table C-2. Number of "In" and "Out" of specification responses for natural and reformulated creams within 1 day of storage at 4.0°C. Responses are included for 20% milkfat natural cream, 20% low-melt fractionated butteroil (LMBO) and 80% skim milk (SM) reformulated cream, and 20% LMBO, 70% buttermilk (BM), 10% aqueous phase (AP) reformulated cream either pasteurized prior to (Before) either high-temperature short time (HTST) pasteurization or ultra high temperature (UHT) pasteurization or following (After) HTST or UHT pasteurization.

Formulation	Homogenization Sequence	Pasteurization Type	# of "Out" Responses	# of "In" Responses	In/Out Specification
20% Natural Cream	Before	HTST	10	20	In
20% Natural Cream	After	HTST	12	18	In
20% Natural Cream	Before	UHT	13	17	Out
20% Natural Cream	After	UHT	9	21	In
20% LMBO, 70% BM, 10% AP	Before	HTST	7	23	In
20% LMBO, 70% BM, 10% AP	After	HTST	11	19	In
20% LMBO, 70% BM, 10% AP	Before	UHT	14	16	Out
20% LMBO, 70% BM, 10% AP	After	UHT	10	20	In
20% LMBO, 80% SM	Before	HTST	7	23	In
20% LMBO, 80% SM	After	HTST	11	19	In
20% LMBO, 80% SM	Before	UHT	14	16	Out
20% LMBO, 80% SM	After	UHT	21	9	Out

Table C-3. Number of "In" and "Out" of specification responses for natural and reformulated creams after 7 days of storage at 4.0°C. Responses are included for 20% milkfat natural cream, 20% low-melt fractionated butteroil (LMBO) and 80% skim milk (SM) reformulated cream, and 20% LMBO, 70% buttermilk (BM), 10% aqueous phase (AP) reformulated cream either pasteurized prior to (Before) either high-temperature short time (HTST) pasteurization or ultra high temperature (UHT) pasteurization or following (After) HTST or UHT pasteurization.

Formulation	Homogenization Sequence	Pasteurization Type	# of "Out" Responses	# of "In" Responses	In/Out Specification
20% Natural Cream	Before	HTST	14	16	Out
20% Natural Cream	After	HTST	15	15	Out
20% Natural Cream	Before	UHT	18	12	Out
20% Natural Cream	After	UHT	9	21	In
20% LMBO, 70% BM, 10% AP	Before	HTST	8	22	In
20% LMBO, 70% BM, 10% AP	After	HTST	16	14	Out
20% LMBO, 70% BM, 10% AP	Before	UHT	16	14	Out
20% LMBO, 70% BM, 10% AP	After	UHT	16	14	Out
20% LMBO, 80% SM	Before	HTST	15	15	Out
20% LMBO, 80% SM	After	HTST	16	14	Out
20% LMBO, 80% SM	Before	UHT	21	9	Out
20% LMBO, 80% SM	After	UHT	15	15	Out

Table C-4. Number of "In" and "Out" of specification responses for natural and reformulated creams after 13 days of storage at 4.0°C. Responses are included for 20% milkfat natural cream, 20% low-melt fractionated butteroil (LMBO) and 80% skim milk (SM) reformulated cream, and 20% LMBO, 70% buttermilk (BM), 10% aqueous phase (AP) reformulated cream either pasteurized prior to (Before) either high-temperature short time (HTST) pasteurization or ultra high temperature (UHT) pasteurization or following (After) HTST or UHT pasteurization.

Formulation	Homogenization Sequence	Pasteurization Type	# of "Out" Responses	# of "In" Responses	In/Out Specification
20% Natural Cream	Before	HTST	17	13	Out
20% Natural Cream	After	HTST	15	15	Out
20% Natural Cream	Before	UHT	24	6	Out
20% Natural Cream	After	UHT	20	10	Out
20% LMBO, 70% BM, 10% AP	Before	HTST	21	9	Out
20% LMBO, 70% BM, 10% AP	After	HTST	22	8	Out
20% LMBO, 70% BM, 10% AP	Before	UHT	---[1]	---[1]	Out
20% LMBO, 70% BM, 10% AP	After	UHT	---[1]	---[1]	Out
20% LMBO, 80% SM	Before	HTST	24	6	Out
20% LMBO, 80% SM	After	HTST	16	14	Out
20% LMBO, 80% SM	Before	UHT	---[1]	---[1]	Out
20% LMBO, 80% SM	After	UHT	21	9	Out

[1]Indicates cream could not be sampled due to textural defects

Table C-5. Aerobic, modified psychrotrophic, and coliform plate counts[1] (cfu/ml) of 20% milkfat (MF) natural cream, 20% skim milk (SM), 80% low-melt fractionated butteroil (LMBO) reformulated cream, 20% LMBO, 70% buttermilk (BM), 10% aqueous phase (AP) reformulated cream either homogenized prior (Before) or following (After) either HTST or UHT pasteurization. Counts were taken on days 1, 7, 13 of storage at 4.0°C.

Formulation	Homogenization Sequence	Pasteurization Type	Method of Enumeration Aerobic Count (cfu/ml)	coliform (cfu/ml)	mPBC (cfu/ml)
20% MF natural	Before	HTST			
Day 1			<25	<25	<25
Day 7			67	<25	<25
Day 13			133	<25	<25
20% MF natural	After	HTST			
Day 1			<25	<25	<25
Day 7			82	<25	<25
Day 13			203	<25	<25
20% MF natural	Before	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day 13			<25	<25	<25
20% MF natural	After	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day 13			<25	<25	<25
20% LMBO, 80% SM	Before	HTST			
Day 1			<25	<25	<25
Day 7			56	<25	<25
Day 13			109	<25	<25
20% LMBO, 80% SM	After	HTST			
Day 1			<25	<25	<25
Day 7			82	<25	<25
Day 13			189	<25	<25
20% LMBO, 80% SM	Before	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day 13			<25	<25	<25
20% LMBO, 80% SM	After	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day 13			<25	<25	<25
20% LMBO, 70% BM, 10% AP	Before	HTST			
Day 1			<25	<25	<25
Day 7			168	<25	<25
Day 13			244	<25	<25
20% LMBO, 70% BM, 10% AP	After	HTST			
Day 1			<25	<25	<25
Day7			54	<25	<25
Day 13			211	<25	<25
20% LMBO, 70% BM, 10% AP	Before	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day13			<25	<25	<25
20% LMBO, 70% BM, 10% AP	After	UHT			
Day 1			<25	<25	<25
Day 7			<25	<25	<25
Day 13			<25	<25	<25

APPENDIX D

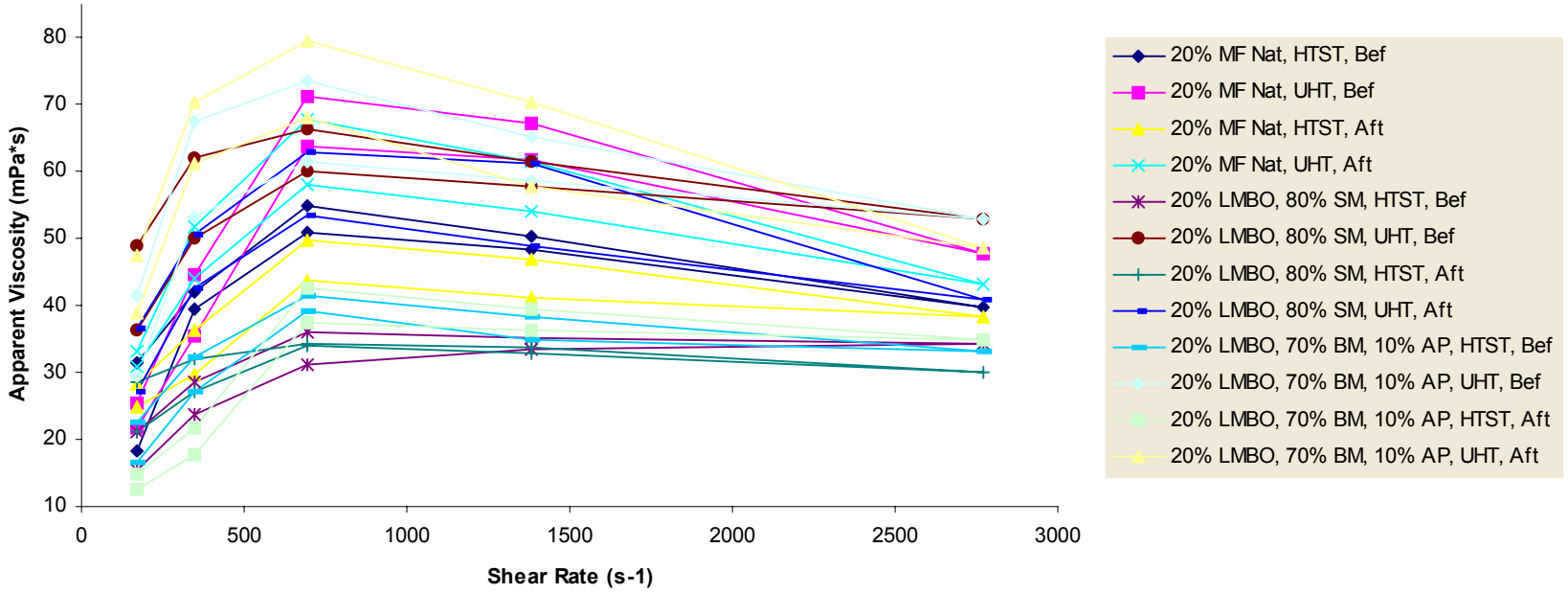
Table D-1. Changes in percent fat content of the top layer of creams.

Treatment	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
20% Natural Cream (HTST, Before)	-0.08	0	0.50	0	2.00	2.17	2.75
20% Natural Cream (HTST, After)	-0.08	0	0.25	0.33	2.75	2.83	3.17
20% Natural Cream, (UHT, Before)	0.17	1.33	1.25	1.42	3.17	3.75	6.42
20% Natural Cream, (UHT, After)	0.17	0.75	1.42	1.50	2.33	4.25	3.42
80% SM, 20% LMBO, (HTST, Before	0.50	0.92	1.17	2.08	3.50	4.25	6.67
80% SM, 20% LMBO, (HTST, After)	1.00	1.75	2.42	2.08	2.75	3.50	3.67
80% SM, 20% LMBO, (UHT, Before)	0.33	2.00	2.75	9.17	9.83	10.67	10.92
80% SM, 20% LMBO, (UHT, After)	0.33	0.75	1.00	2.75	3.42	4.50	6.67
70% BM, 10% AP, 20% LMBO, (HTST, Before)	0.67	0.92	2.75	3.17	3.42	4.67	6.58
70% BM, 10% AP, 20% LMBO, (HTST, After)	2.17	2.50	2.83	3.33	3.50	4.33	6.78
70% BM, 10% AP, 20% LMBO, (UHT, Before)	1.00	1.58	2.08	4.08	4.58	6.08	8.50
70% BM, 10% AP, 20% LMBO, (UHT, After)	1.25	1.42	1.58	3.17	4.08	4.92	5.08
Standard Error	0.06	0.09	0.18	0.47	0.89	0.65	1.2

Table D-2. Apparent viscosity values for all treatments in study

	173.12	346.24	692.48	1384.96	2769.92	1384.96	692.48	346.24	173.12
20% MF Nat, HTST, Bef	31.4	42.2	54.9	50.4	39.7	48.4	50.9	39.4	18.3
20% MF Nat, UHT, Bef	25.5	44.5	71.3	67.2	47.8	61.7	63.9	35.4	21.6
20% MF Nat, HTST, Aft	28.3	36.4	49.8	47	38.4	41.3	43.9	29.8	24.8
20% MF Nat, UHT, Aft	33.2	51.9	67.7	61.4	43.2	54.1	58.1	44.2	31
20% LMBO, 80% SM, HTST, Bef	21.2	28.5	36	35.1	34.3	33.5	31.1	23.7	15.4
20% LMBO, 80% SM, UHT, Bef	48.8	62	66.4	61.5	52.8	57.7	60.2	50.1	36.3
20% LMBO, 80% SM, HTST, Aft	28.6	32.1	34.4	33.8	30	32.8	34	27.2	21.1
20% LMBO, 80% SM, UHT, Aft	36.7	50.7	63.1	61.1	40.9	49	53.5	42.7	27.2
20% LMBO, 70% BM, 10% AP, HTST, Bef	22.6	32.3	41.6	38.2	33.3	35	39.3	27.1	16.5
20% LMBO, 70% BM, 10% AP, UHT, Bef	41.6	67.4	73.6	65.2	52.9	58.7	61.5	53.2	29.5
20% LMBO, 70% BM, 10% AP, HTST, Aft	14.9	21.6	42.6	39.5	35	36.3	37.4	17.7	12.7
20% LMBO, 70% BM, 10% AP, UHT, Aft	47.4	70.3	79.6	70.5	48.7	57.7	68.1	61.3	38.9

Figure D-1. Hysteresis curves for all treatments examined in this study.



VITAE

James C. Bolling

James C. Bolling was born to Mr. and Mrs. Jay Bolling on July 1, 1976. Mr. Bolling graduated from Cave Spring High School in 1994, and entered Virginia Polytechnic Institute the fall semester of that year. He obtained his Bachelor of Science degree in Food Science and Technology in May of 1998. His interest include product development and sensory aspects of product development and he specializes in dairy chemistry. Mr. Bolling entered the graduate program in Food Science and Technology in the fall of 1998 under the direction of Dr. Susan Duncan. She received her Master of Science degree in Food Science and Technology in February 2001.