CHAPTER 1. INTRODUCTION

A milkfat surplus has existed for the majority of the past decade. Two major factors, composition and functionality, contributed to the underutilization of milkfat (Rizvi, 1991). Increased consumer health consciousness has led to a decrease in the consumption of whole fat dairy products. Milkfat contains approximately 65-70% saturated fatty acids and 0.25-0.4% cholesterol (Rizvi, 1991). The saturated fat and cholesterol constituents in milkfat are linked to various dietary diseases, most notable being heart disease and obesity.

In food processing applications, milkfat exhibits limited functionality. The limited functionality of milkfat can be attributed to the presence of triglyceride molecules which tend to melt over a temperature range of 30-41^{o}C. Physical properties such as melting point raise processing concerns for food manufacturers. For example, the wide melting range of milkfat yields butter with poor spreadability and bakery products lacking tenderness and flakiness (Rizvi, 1991). Unfortunately, the spreadable range of butter falls between 16-24^{o}C. Therefore, butter is not spreadable at refrigeration temperatures. Fractionation of milkfat, however, has been used to improve the functionality of modified milkfat.

In previous years, the United States government was solely responsible for excess milkfat supplies. During the past 3 years milkfat sales have increased, resulting in a substantial decrease in surplus. A severe decrease in the price of butter from $1.35 to $0.64 per pound from 1989 to 1994 caused a reduction in reserves (Jimenez-Flores, 1997). As of July 1996, however, the price of butter has increased. In fact, the price rose beyond the 1989 price of $1.52 due to increased demand. The low price of milkfat is one of the principle driving forces behind increased milkfat usage by both consumers and food processors (Honer, 1993). In many markets, butter prices are almost equivalent to those of higher priced margarine products. Milkfat is also in high demand because of its irreplaceable contributions to the flavor, body, and texture characteristics of foods such as ice cream and cream cheese. Statistics indicate that the increase in milkfat sales has accompanied a 25% reduction in margarine sales, simply because consumers desire the good dairy flavor which margarine lacks (Honer, 1993).

The increase in milkfat utilization can also be attributed to the fact that dairy producers have assumed responsibility for existing milkfat surplus (Ahlberg, 1998). The dairy industry has explored new technologies for improving the nutritional and functional profile of milkfat. Such technologies include fractionation or separation procedures (Versteeg, 1991). These particular methods enhance the properties of milkfat by either chemical alteration or physical manipulation (Shukla et al., 1994). In particular, milkfat fractionation has proved to be one of the most successful techniques. During the process, milkfat is separated into different melting range fractions which differ in chemical and physical properties (Shukula et al., 1994). These milkfat fractions offer applications not previously applied to milkfat.

In further attempts to alter the melting range characteristics of milkfat, the dairy industry should have an awareness of the properties of milkfat and components used for emulsification. Although not commonly encountered in scientific literature, reformulation of cream to improve the composition and functionality of milkfat is possible. Common milk-derived components are rich in surface active molecules which contribute to emulsion stability. Creams reformulated with modified butteroils should have emulsion characteristics very similar to regular processed cream products, but may exhibit improved functionality. Incorporating milkfat into fluid dairy systems should yield oil-in-water emulsions with chemical and physical characteristics like natural cream.

In this project, a 20% milkfat cream was formulated using skim, sweet buttermilk, and butter-derived aqueous phase components to re-emulsify low-melting and medium melting modified butteroils. The reformulated creams should possess similar chemical and physical characteristics of natural cream. The primary goal was to determine the effect of milkfat fractions with different melting ranges on the emulsion characteristics and physical properties of the reformulated creams. Implication of separation temperature in obtaining components as contributors of milkfat globule membrane, whey proteins, and casein proteins for stabilizing the milkfat was also made.

CHAPTER 2. REVIEW OF LITERATURE

Factors affecting milkfat melting range. Milkfat composition and melting characteristics. Bovine milk consists of approximately 3.5 to 5% total lipid, existing as emulsified globules. Approximately 98% of the total lipid content is triglyceride, followed by 0.5-1.0% phospholipids and 0.2-0.5% sterols (Jensen et al., 1991). Monoglycerides and diglycerides account for only trace amounts of total lipids, comprising approximately 0.5% of total lipid collectively (Campbell and Marshall, 1975). Since triglycerides are the predominant form of lipid occurring in milkfat, they have the greatest effect on functionality.

The functionality of milkfat is also associated with interactions between solid and liquid phases that exist based on milkfat composition (Kaylegian and Lindsay, 1995). Above 40^{o}C, milkfat exists in the liquid state. However, as temperature is depressed below -40^{o}C, milkfat is a compact solid. Between these two temperature extremes, milkfat is a mixture of solid fat and liquid oil (German and Dillard, 1998). As a result, milkfat is characterized as having a melting range as opposed to a definite melting point.

Three melting categories exist for milkfat triglycerides. Low-melting triglycerides melt below 10^{o}C whereas high-melting triglycerides melt above 20^{o}C (Kaylegian and Lindsay, 1995). In between the two extremes is a group of middle-melting range triglycerides (Kaylegian and Lindsay, 1995). Three principal factors affect the observed melting range of milkfat. These factors include fatty acid composition, distribution of acids in glycerides, and polymorphic state of fat crystals (Campbell and Marshall, 1975).

Fatty acid composition. In considering natural fats, milkfat has the broadest range of fatty acids. Milkfat is very complex because of the diversity of fatty acids, ranging from 2-24 carbons in chain length, that make up the triglycerides. Overall, 400 different fatty acids have been identified in milkfat (German and Dillard, 1998). Realistically, however, the figure is 20 major fatty acids since a majority of the fatty acids occur in trace amounts (Kuksis, 1972). The fatty acids of milkfat are complex in structure, often falling into various structural categories such as monobranched, multibranched, dienes, trienes, hydroxy, cyclic, and others (Jimenez-Flores, 1997). In comparison to other sources of fat, milkfat contains a higher percentage of low molecular weight and saturated fatty acids. Since low molecular weight fatty acids exist in the liquid state at lower temperatures, they tend to lower the melting range of milkfat (Campbell and Marshall, 1975). Saturated fatty acids, which constitute approximately 65-70% of the fatty acid composition, however, elevate the melting range of milkfat.

The long chain saturated fatty acids have the greatest effect on melting range. Palmitic (C16), stearic (C18), and myristic (C14) are the major saturated fatty acids that melt well above 20^{o}C (Campbell and Marshall, 1975). The most abundant saturated fatty acid of milkfat is palmitic acid (C16). Palmitic acid accounts for 20-25% of the fatty acids in milkfat (German and Dillard, 1998). Falling in the 10-20^{o}C range, caprylic acid (C8) is

the only saturated fatty acid affecting the middle-melting point triglyceride group. Low molecular weight saturated fatty acids such as butyric acid (C4) and caproic acid (C6) and unsaturated fatty acids like palmitoleic acid (C16:1) and linolenic (C18:2) acid melt below 10^{o}C (Campbell and Marshall, 1975). Contributing 30-38% of total fatty acids, oleic acid (C18:1) is the most abundant unsaturated fatty acid (German and Dillard, 1998).

The contrasting melting points of long-chain saturated fatty acids and unsaturated fatty acids affect the melting range of milkfat. Long chain saturated fatty acids have an increasing effect whereas unsaturated fatty acids have a decreasing effect on the melting range of milkfat. Unsaturated fatty acids yield softer butters, particularly during the spring and summer due to incorporation of increasing amounts of unsaturated fatty acids in the diet of milking cows (Campbell and Marshall, 1975). Although large amounts of polyunsaturated fatty acids are incorporated in the diets of dairy cattle, polyunsaturated fatty acids only comprise 4% of the fatty acid profile of milkfat (Jensen and Newberg, 1995). The unsaturated fatty acids can be cis or trans isomers (Fox and McSweeney, 1998). Trans unsaturation has an elevating effect on melting point, somewhat higher than that of cis unsaturation (Goff and Hill, 1993). Odd-numbered and branched chains decrease the melting range, yielding softer butters because they do not form crystal structures (Goff and Hill, 1993).

Distribution of fatty acids. The abundance of 400 fatty acids occurring in milkfat also give rise to a potential of over 6 million triglycerides (Jimenz-Flores, 1997). Since it has been identified that approximately 20 fatty acids predominate, a possible figure of 8,000 different major triglycerides exist (Jensen and Clark, 1988). Due to the asymmetry of milkfat triglycerides, the amount can be reduced even further (Jensen and Clark, 1988). The distribution of fatty acids on the three positions of glycerol molecules also has an impact on the melting range of milkfat. The complexity and various configurations possible for triglycerides provide milkfat with a plastic yet spreadable form. Fatty acid distribution and esterification on the glycerol backbone is a nonrandom process (German and Dillard, 1998). The nonrandom distribution and esterification process of fatty acid attachment to glycerol can be attributed to metabolic, dietary, and biosynthetic factors (German and Dillard, 1998). The glycerol backbone of triglycerides consists of three distinct positions for fatty acid attachment. The upper position is referred to as the *sn*1 position, followed by the middle *sn*2 and bottom *sn*3 positions (German and Dillard, 1998). In general, the *sn*1 position is typically the site for a majority of longer carbon length fatty acids whereas the *sn*3 position is the preferred site for shorter carbon length and unsaturated fatty acids. Most C12-C16 are esterified to the *sn*2 position and C4 and C6 are associated with the *sn*3 position (Fox and McSweeney, 1998). For example, 97% of butyric acid (C4) is located on the *sn*3 position of milkfat triglycerides. The most predominant triglyceride molecules in milkfat are 1,2 dipalmitoyl-3-butyrolyl-sn-glycerol and 1-palmitoyl-2-myristoyl-3-butyroyl (German and Dillard, 1998). In each molecule, butyric acid is bound to the *sn*3 position whereas the longer carbon length palimitic and myristic acid are attached to the *sn*1 and *sn*2 positions, respectively.

Polymorphic state of fat crystals. In polymorphism, the carbon chains of milkfat fatty acids freely rotate perpendicularly on the glycerol portion of the triglyceride molecule (Campbell and Marshall, 1975). The rotation process gives rise to various configurations for one triglyceride molecule. As a result, triglyceride molecules tend to pack together in different forms upon crystallization. The resulting fat crystals have different melting points. The polymorphic forms of milkfat crystals are γ, α, β, and β' (Kaylegian and Lindsay, 1995). Of the forms, the γ form is the least stable whereas β is more stable. The γ form is considered to be a subform of the α form (Mulder and Walstra, 1974). The γ form arises when the α form is cooled very quickly, but the change is reversible (Mulder and Walstra, 1974). The α form has a rather loose spatial arrangement resulting in a low melting point. In contrast, the β form is tightly packed and has a high melting point (Kaylegian and Lindsay, 1995).

Upon rapid cooling, α crystals form first, followed by formation of the other forms (Mulder and Walstra, 1974). The initial formation of α crystals can be attributed to simple structure. Other forms follow as crystallization progresses. During storage or handling, milkfat is susceptible to partial melting. As a result, recrystallization occurs causing an increase in hardness, coarseness, and oiling-off at the surface.

Milkfat modification. Overview of fractionation. In the past, the United States government was responsible for existing milkfat surplus. Recently, however, the responsibility of milkfat excess was shifted from the U.S. government to dairy producers. As a result, the dairy industry has explored new applications for improving the nutritional profile and increasing the functionality of milkfat (Ahlberg, 1998). Research indicates that the cholesterol content of milkfat can be substantially lowered by various methods, most notably vacuum steam distillation (Versteeg, 1991), short path molecular distillation (Boudreau and Arul, 1993), and supercritical fluid extraction (Rizvi, 1991). Various methods of milkfat fractionation have proven to successfully increase the functionality of milkfat.

The functionality of milkfat can be improved by fractionation technology, thus increasing the potential for utilization of milkfat. In recent years, the dairy industry has utilized milkfat fractionation as an alternative to other technologies to improve the functional characteristics of milkfat under various processing parameters (Kaylegian and Lindsay, 1995). The technology provides two or more fractions which can be blended for specific product applications. Four major methods of milkfat fractionation are used by the dairy industry. These methods include dry fractionation (crystallization from melted milkfat), crystallization from solvent solution, supercritical carbon dioxide extraction, and short-path distillation (Kaylegian and Lindsay, 1995).

Fractionation has an effect on various physical properties of milkfat including crystallization behavior and polymorphism, melting range, and solid fat content (Kaylegian and Lindsay, 1995). Typically, crystals from very high melting and high melting fractions contained mixtures of stable β and β' crystals. Crystals from intermediate and low melting milkfat fractions consist of β' crystals (Kaylegian and Lindsay, 1995). The fractions obtained by fractionation are characterized as very high-

melting (>45°C), high melting (35-45°C), intermediate melting (25-35°C), low melting (10-25°C), and very low melting (< 10°C) (Kaylegian and Lindsay, 1995). As expected, very high and high melting fractions of fractionated milkfat demonstrate higher solid fat content behavior. The lowest solid fat content is associated with very low melting fractions. Thus, fractionation increases the range of milkfat products for use in food applications. From a chemical standpoint, fractions with higher amounts of saturated C4-C10 and unsaturated C16-C18 fatty acids and C24-C34 contributed to lower melting point fractions. Lowering the concentrations of these fatty acids, however, contributed to a higher melting point.

Dry fractionation. Dry fractionation is the most common method of milkfat fractionation (Kaylegian, 1995). The simplicity and independence from chemicals or additives during processing are key elements for widespread use of dry fractionation in altering milkfat to improve functionality. Dry fractionation has been successfully commercialized (Kaylegian and Lindsay, 1995).

The dry fractionation process involves four distinct stages: melting milkfat at 60°C, cooling (0.005-2°C/min.) and agitation (0-120 rpm) to form crystal nuclei, crystal growth, and separation between 18 and 28°C into high melting stearin (solid) and low melting olein (liquid) fractions (Kaylegian, 1995; German and Dillard, 1998). Single stage processing yields only one stearin and olein fraction, but multistage processes involve refractionation of these two fractions to produce three or more fractions. Fractions obtained from refractionation tend to exhibit a wider range of melting points than their parent fractions (Kaylegian, 1995). Single stage fractionation occurs at 17-34°C whereas a wider range, typically 4.5-34°C, is necessary for multistage fractionation (Kaylegian and Lindsay, 1995).

The fractionation equipment of the Tirtiaux dry fractionation process is made up of a control panel, a chiller for cooling, a stainless steel jacketed tank with an agitator for crystallization, and a vacuum or pressure filtration system for separation (Ahlberg, 1998). This particular system was used to obtain the fractions for this research project. Separation is governed by the melting points of triglycerides and usually occurs under vacuum on a Florentine filter, a membrane filter, or centrifugation system (Deffense, 1993). The obtained melting ranges for the stearin and olein fractions typically range from 40-46°C and 18-28°C, respectively (Deffense, 1993). Other processes used in the dry fractionation of milkfat include the De Smet process, the Alfa-Laval process, and vacuum filtration (Kaylegian and Lindsay, 1995). Disadvantages of the dry fractionation process, however, are that less pure fractions of milkfat are obtained and a limited temperature range for fractionation is used (Kaylegian and Lindsay, 1995).

Crystallization from solvent solution. Crystallization from solvent solution has similar operating procedures as dry fractionation (Kaylegian, 1995). Unlike dry fractionation, however, a chemical solvent is necessary for separation. Melted milkfat is treated in the

solvent prior to crystallization, usually 1 part milkfat to 4-10 parts solvent (Kaylegian and Lindsay, 1995). Acetone, ethanol, pentane, and hexane are solvents which optimize milkfat solubility in this particular process (Kaylegian and Lindsay, 1995). During crystallization, the milkfat precipitates from solution and is separated by vacuum filtration. In comparison to dry fractionation, more pure fractions are obtained and low temperatures can be utilized (Kaylegian and Lindsay, 1995). Disadvantages include the potential for solvent toxicity and off-flavor development (Kaylegian and Lindsay, 1995).

Several factors contribute to the results of fractionation processes based on crystallization (i.e. dry fractionation and crystallization from solvent solution). These factors include chemical composition of the milkfat, use of seeding, degree of supercooling, rate of cooling and agitation, separation temperature, amount of time used for crystallization, and separation method employed (Kaylegian, 1995). These factors can have an impact on the amount of crystals formed, crystal nuclei formation, crystal growth, and the retention of liquid milkfat in the stearin fraction (Kaylegian, 1995).

Supercritical fluid extraction. A third method of fractionation of milkfat is supercritical fluid extraction. Also used in the extraction of cholesterol, supercritical fluid extraction involves fractionation of milkfat on the basis of solubility in the supercritical fluid at different temperatures and pressures (Kaylegian, 1995). The supercritical fluid is passed through a vessel holding the milkfat and the milkfat dissolves in the supercritical fluid. The fractions obtained appear in order of increasing melting point (Kaylegian, 1995). A residual milkfat extract usually remains which has the highest melting point and can be utilized in food applications. Although other solvents are available for supercritical extraction , CO_2 is most commonly used and encountered in the literature (Arul et al., 1987; Kankare et al., 1989; Bhaskar et al., 1993; Shukula et al., 1994). The advantages associated with this particular method of fractionation are the nontoxic nature and high availability of CO_2, production of more distinct fractions, and simple removal from milkfat (Kaylegian and Lindsay, 1995; Jimenez-Flores, 1997). Concerns, however, have been raised about the expensive energy and capital investments required for supercritical fluid extraction (Boudreau and Arul, 1993).

Short path distillation. In short-path distillation, milkfat triglycerides are separated in a gas-free environment, generally a vacuum, according to molecular weight, melting temperature, volatility, and intermolecular interaction (Boudreau and Arul, 1993; Kaylegian and Lindsay, 1995). Short-path distillation has very limited applications in milkfat fractionation due to high energy requirements and potential loss of flavor due to high processing temperatures (Boudreau and Arul, 1993).

Applications of milkfat fractions. The various fractions obtained by fractionation have proved to increase milkfat functionality in a variety of food products. Additionally, functionality can be optimized to fit a product's characteristics by blending fractions.

In confectionery items, particularly chocolate, high melting fraction with a melting range of approximately 40 to 42^{o}C prevents softening and fat bloom at higher temperatures

(Rajah, 1994). High melting point fractions falling in this same melting point range are used in the processing of puff pastries. During rolling and folding of dough, unaltered milkfat readily melts into the dough, resulting in poor layering and variations in pastry lift (Versteeg et al., 1994). High melting fractions with a melting point of 36-38^{o}C are used to achieve desired flakiness and layering in Danish pastries (Rajah, 1994).

The low melting fractions also have various applications. Unlike their high melting fraction counterparts, low melting fractions can be used in the manufacturing of dressing and mayonnaise spreads (Rajah, 1994). Low melt fractions are also used in canned sauces and soups having emulsion characteristics (Rajah, 1994). Low melt fractions have also prevented fat bloom in shortbread and cookies. Fat bloom is the condition resulting from undesirable crystallization of milkfat triglycerides during cold storage (Eyres, 1989). Most notably, however, low melting fractions are used in the processing of cold-spreadable butters. In a recent investigation, spreadable butters manufactured from low melting milkfat fractions exhibited spreadability at refrigeration temperature (Kaylegian and Lindsay, 1992).

Milkfat fractions are often blended to optimize flavor and functional characteristics of foods. For example, fractions are combined to enhance the creaming properties of baked cakes (Versteeg et al., 1994). Traditionally, cake margarines and shortenings have been used in the preparation of cakes. Cake maragarines and shortenings were used largely because of fat melting characteristics and emulsifying agents. The use of blended melting point fractions enhance the creaming ability and flavor of the cake.

Oxidative stability and sensory characteristics of fractionated milkfat. Fractionation of milkfat yields fractions which not only differ in melting point, but also in texture and flavor characteristics (Lakshminarayana and Rama Murthy, 1985). Low melting fractions have a higher degree of unsaturated fatty acids than medium and high melting fractions, contributing to a lower melting point and softer texture. The greater extent of unsaturated fatty acids occurring in lower melting fractions, however, raises oxidation issues.

Bhat and Rama Murthy (1983) compared the rates of autoxidation of low and high melting fractions obtained from cow and buffalo milkfat. Comparisons were also made to whole cow and buffalo milkfat. Cream, obtained from a random pool of cows and buffalo, was churned into butter. Butter was melted at approximately 100^{o}C and subsequently filtered at 37^{o}C. The melted butter samples from buffalo and cow milk were stored overnight at 32^{o}C and 28^{o}C, respectively. Both samples were centrifuged at 1500 x g for approximately 15 min. The low melt fraction was decanted. Both low and high melting fractions from cow and buffalo milkfat were stored at 60^{o}C and analyzed for peroxide value.

The softening points for low melting fractions obtained from cow and buffalo milk were 23^{o}C and 25^{o}C, respectively (Bhat and Rama Murthy, 1983). High melting fractions from cow and buffalo milk were 41^{o}C and 43^{o}C, respectively. Peroxide development was highest in low melting fraction from buffalo milkfat followed by low melt fraction

from cow milkfat, buffalo whole milkfat, cow whole milkfat, high melt fraction of cow milkfat, and high melt fraction from buffalo milkfat. Low melting fractions oxidized more rapidly than whole milk and high melting fractions because of higher levels of unsaturated fatty acids. Also, samples obtained from buffalo milkfat had higher peroxide values due to its higher polyunsaturated fatty acid content. Buffalo milkfat commonly has fatty acids with 4 or 5 double bonds (Bhat and Rama Murthy, 1983).

Lakshminarayana and Rama Murthy (1985) analyzed oxidative stability of milkfat fractions by determining iodine values. Iodine values can be used as a means of determining oxidative stability because they reveal information regarding unsaturation. Cream from buffalo and cow milks was churned into butter. Subsequently, butter was clarified at 100^{o}C and filtered through filter paper. Milkfat was fractionated by a series of steps as described by Schaap et al. (1970) and Kankare (1974). Milkfat was melted at 70-80^{o}C and cooled to 31^{o}C in approximately 1 hour. Crystal formation was initiated by incubating the milkfat at 31^{o}C for 12 hr. Crystals were obtained by centrifugation at 1500 g for 2 min and decanting from the liquid portion. The liquid milkfat portion was obtained by cooling to 23^{o}C for approximately 12 hr. Crystals were obtained as previously described. The remaining liquid portion was cooled to 15^{o}C for 12 hr. A total of four fractions were obtained and designated as S_{31}, S_{23}, S_{15}, and L_{15}.

Mean melting points for whole milkfat, S_{31}, S_{23}, S_{15}, and L_{15} obtained from fractionated buffalo milkfat were 35.8, 37.5, 31.2, 19.0, and 14.5^{o}C, respectively. Similar values were obtained for milkfat from cows. Mean melting points were 34.2, 36.5, 30.5, 19.0, and 14.0 for whole milkfat, S_{31}, S_{23}, S_{15}, and L_{15}, respectively. Iodine values were measured to indicate a decrease in unsaturation of whole and fractionated milkfat samples. In most instances, as melting point decreased, iodine value increased. Reported mean iodine values for whole milkfat, S_{31}, S_{23}, S_{15}, and L_{15} from buffalo milkfat were 31.1, 28.8, 30.2, 34.8, 35.60, respectively. Whole milkfat, S_{31}, S_{23}, S_{15}, and L_{15} from cow milkfat had mean iodine values of 32.2, 30.1, 31.2, 33.4, and 35.2, respectively (Lakshminarayana and Rama Murthy, 1985).

Khalifa and Mansour (1988) determined the chemical and organoleptic characteristics of natural and fractionated butteroils over a 6 month storage period. Raw milk was separated into skim and cream. Subsequently, cream was pasteurized, cooled overnight, and churned into butter. Butter was melted at 70^{o}C to yield butteroil. Butteroil obtained from melting was placed in a separating funnel and incubated at 30^{o}C for approximately 24 hr. Crystallized fat, from the funnel, was retrieved by straining through muslin cloth. Overall, 4 fractions were obtained. The four fractions were characterized as solid at 35 (S-30), 25 (S-25), and 20^{o}C (S-20) and a liquid fraction at 20^{o}C (L-20). All fractions were refrigerated at 5^{o}C until analyzed for chemical or organoleptic properties.

Iodine and TBA values were analyzed on days 0, 30, 90, 135, and 180 of the 6 month storage period. Differences existed in iodine value among the fractionated butteroils. On day 0 of analysis L-20 had the highest iodine value of 38.92, followed by 36.12 for S-20, 35.07 for natural butteroil, 34.43 for S-25, and 32.35 for S-30 (Khalifa and Mansour, 1988). Very little difference existed in iodine values for each butteroil sample for the 6

month storage period. On day 0 all butteroils had no detectable TBA values. However, TBA values were detected after 30 days of refrigerated storage for all samples except S-30. By the end of the 6 month storage period, TBA values were 0.790, 0.395, 0.305, 0.210, and 0.140 for L-20, S-20, natural butteroil, S-25, and S-30, respectively (Khalifa and Mansour, 1988).

Organoleptic quality of butteroils was evaluated by ten panelists after 3 and 6 months of refrigerated storage. Texture and flavor characteristics were assessed by a 10 point rating scale. Flavor scores decreased over the storage period. Fresh butteroil sample scores ranged from 6.9 to 7.8, the former being L-20 and latter being natural butteroil (Khalifa and Mansour, 1988). Scores for S-20, S-25, and S-30 were 7.6, 7.4, and 7.3, respectively. Final flavor scores for L-20, S-20, S-25, S-30, and natural butteroil were 5.9, 6.8, 6.7, 6.6, and 6.9, respectively (Khalifa and Mansour, 1988). Texture scores were 2.4, 8.2, 8.3, 6.9, and 8.6 for fresh L-20, S-20, S-25, S-30, and natural butteroil, respectively. At the end of the storage period, texture scores were 2.1, 7.8, 8.0, 6.5, and 8.0 for L-20, S-20, S-25, S-30, and natural butteroil, respectively (Khalifa and Mansour, 1988).

Challenges of emulsifying fractionated butteroils. Incorporation of butteroils into stable oil-in-water emulsion systems can be difficult. Although milkfat fractions provide an increased functionality and contribute sensory attributes unique to milkfat, quality problems often result upon incorporation into a food system. Oehlmann et al. (1994) used skim milk, buttermilk, and butter-derived aqueous phase to emulsify a cholesterol reduced butteroil. Emulsification was achieved by either homogenization or ultrasound dispersal. Electron microscopy revealed that all formulations were characteristic of water-in-oil type emulsion, not typical oil-in-water emulsions.

A common quality defect related to the use of milkfat fractions as well as other forms of chemically modified milkfat is the presence of free oil due to separation. Oil is typically released from the food product because of the inability of milkfat to be completely emulsified into the product. Very little research exists on oil separation when fractionated milkfat is applied to a food system. However, Schroder and Baer (1990) used cholesterol reduced milkfat to improve the organoleptic properties of skim milk. Natural milkfat was added to skim milk as a control. The final fat contents of both control and cholesterol reduced milkfat milks were 1, 2, and 3.25%. Separation was determined by a homogenization efficiency test. Slight separation occurred in all three control samples. No separation was apparent in 1% cholesterol reduced milkfat milk. Definite separation, however, occurred in 2% and 3.25% fat samples when cholesterol reduced milkfat was added to skim milk.

Emulsification of milkfat into aqueous systems. Origin of milkfat globules. During milk synthesis, the native milkfat is enveloped in the milkfat globule membrane. Milk lipids are formed from small drops of triglyceride on or in the surfaces of the rough endoplasmic reticulum membranes (Mather and Keenan, 1998). The triglycerides collect in the form of microlipid droplets (0.5 μm or less in diameter) in the cytoplasm. It is believed that microlipid droplets, precursors of lipid globules, originate in the endoplasmic reticulum membrane. The microlipid droplets are coated with a layer of

protein and polar lipid. Subsequently, microlipid droplets are capable of fusing with others to give rise to bigger cytoplasmic lipid droplets (1-5 μm in diameter). Differences in densities exist between microlipid droplets and cytoplasmic lipid droplets; however, surface coat material on both kinds of lipid droplets is identical in protein and lipid composition (Mather and Keenan, 1998).

The droplets are eventually carried to the apical cytoplasm where they are secreted from the cell (Mather and Keenan, 1998). Two mechanisms are used to explain lipid secretion. The first mechanism, an apical mechanism, involves the envelopment of lipid droplets with apical plasma membrane. A second mechanism, the secretory-vessicle mechanism, has been proposed suggesting that fat droplets are placed around secretory vessicles in the cytoplasm (Mather and Keenan, 1998). In this mechanism, the fat droplets are released by exocytosis from intracytoplasmic vacuoles.

The milkfat globule membrane. The milkfat globule membrane is primarily responsible for maintaining the emulsion stability of milkfat globules. The milkfat globule membrane is also involved in limiting several chemical and physical reactions such as enzyme binding, lipolysis, globule fusion, and destabilization of the emulsion by creaming, clumping, churning, and heating (Jensen et al., 1991).

The membrane, approximately 10 nm in diameter, is composed of proteins, phospholipids, glycoproteins, triglycerides, cholesterol, and enzymes (McPherson and Kitchen, 1983). Research indicates that proteins and lipids comprise over 90% of the membrane's dry weight (Patton and Keenan, 1975). The approximate gross composition of the milkfat globule membrane is 41% protein, 27% phospholipid, 3% cerebrosides, 2% cholesterol, 14% neutral glycerides, and 13% water (Mulder and Walstra, 1974).

The native milkfat globule membrane typically consists of proteins which are not associated with the skim phase (Fox and McSweeney, 1998). Most of the proteins of the milkfat globule membrane are glycoproteins, consisting of approximately 2.8-4.15% hexose, 2.5-4.2% hexosamine, and 1.3-1.8% sialic acid (Fox and McSweeney, 1998). The principal proteins are xanthine oxidase, butyrophilin, and glycoprotein B (Fox and McSweeney, 1998). Butyrophilin makes the greatest contribution to the milkfat globule membrane. This particular protein has a hydrophobic character, thus being strongly attracted to milk lipids (Fox and McSweeney, 1998).

Enzymes also make a contribution to the milkfat globule membrane. The enzymes associated with the milkfat globule membrane arise from cytoplasm and secretory cell membrane (Fox and McSweeney, 1998). They come in contact with the milkfat globule membrane via globule excretion from cells. Xanthine oxidase is a dimer consisting of two identical subunits (Walstra and Jenness, 1984). The subunits each have a flavin-adenine dinucleotide, 1 molybdenum atom, 4 iron atoms, and four sulfur atoms. As noted by its enzymatic name, xanthine oxidase is responsible for oxidation catalysis of numerous compounds, including xanthine, aldehydes, purines, pyrimidines and pterins (Keenan et al., 1988). Its activity is increased by storage at refrigeration temperature, heating at 70^{o}C for approximately 5 min, or homogenization (Walstra and Jenness, 1984).

Phosphodiesterase I serves as a marker enzyme for the milkfat globule membrane and catalyzes the hydrolysis of phosphate diesters (Jenness and Patton, 1976).

The ratio of phospholipids to neutral lipids is usually 2:1 (Fox and McSweeney, 1998). The neutral lipid content of the milkfat globule membrane is approximately 83-88% triglycerides, 5-14% diglycerides, and 1-5% free fatty acids (Fox and McSweeney, 1998). The amount of diglycerides associated with the milkfat globule membrane is somewhat higher than that observed in whole milkfat because of their surface-active characteristics (Fox and McSweeney, 1998). The structure of the milkfat globule membrane is asymmetric in nature with a hydrophobic inner compartment adjacent to the fat and a hydrophilic outer region extending to the aqueous phase (McPherson and Kitchen, 1983).

The milkfat globule membrane contains 5-25% of the Cu and 30-60% of the Fe found in milk (Fox and McSweeney, 1998). Trace levels of Co, Ca, Na, Mg, K, Mn, Mo, and Zn have also been found in the milkfat globule membrane.

Neutral lipids, mainly triglycerides, make the greatest contribution to the lipid content of the native milkfat globule membrane. The relationship of lipids to the milkfat globule membrane has been investigated. Bracco et al. (1972) characterized the types of lipid associated with the milkfat globule membrane in unhomogenized bovine milk. Milkfat globule membrane material obtained by ultracentrifugation revealed that the milkfat globule membrane of bovine milk is rich in high-melting triglycerides, phospholipids, sterols, alkanes, and acyclic terpenes (Bracco et al., 1972). Triglycerides constituted approximately 61.7% of the total lipid of the milkfat globule membrane (Bracco et al., 1972). Wooding and Kemp (1975) isolated lipids of the milkfat globule membrane by three successive chloroform:methanol extractions. Approximately 90% of the triglycerides and majority of phospholipids associated with the milkfat globule membrane were successfully removed. Wooding and Kemp (1975) concluded that since the extraction did not have an effect on the structure or width, the dense material of the milkfat globule membrane is derived from proteins as opposed to lipids.

Jensen and Nielsen (1996) compared the fatty acid content of the milkfat globule membrane to total fatty acid content. Fatty acids of the milkfat globule membrane contribute 18-27g/kg to the total fatty acid content of milk (Jensen and Nielsen, 1996). A considerable amount of the fatty acids are long-chain, polyunsaturated fatty acids (Jensen and Nielsen, 1996). A total of 9-17 g/kg of the polyunsaturated fatty acids occurring in milk were associated with the milkfat globule membrane (Jensen and Nielsen, 1996).

Over fifty percent of the total phospholipid content of milk is in the milkfat globule membrane (McPherson and Kitchen, 1983). The polar head groups of phospholipids play a significant role in stabilizing the fat globule in milk (Shimizu et al., 1980). Like triglycerides in the membrane, phospholipids generally contain high levels of long-chain fatty acids, particularly palmitic acid and oleic acid (McPherson and Kitchen, 1983). Short-chain fatty acids are usually present in low concentrations. The phospholipids include high concentrations of sphingomyelin, phosphatitidylcholine, and phosphatidylethanolamine (Patton and Keenan, 1975). Less abundant are inositol and

serine phosphatides as well as choline and ethanolamine plasmogens (Patton and Keenan, 1975).

The protein occurring in the native milkfat globule membrane accounts for only 1% of the total milk protein content. However, the small percentage plays a vital role in maintaining the integrity of the milkfat globule membrane. The proteins of the milkfat globule membrane are associated with the lipids by hydrophobic interaction (Shimizu et al., 1976). The proteins of the milkfat globule membrane contain high levels of glutamic acid, aspartic acid, leucine, serine, and threonine (Anderson and Cawston, 1975). Mather and Keenan (1975) used SDS-polyacrylamide gel electrophoresis to characterize proteins of the milkfat globule membrane. At least 21 polypeptides were observed in the molecular weight range of 15,000-240,000 Daltons (Mather and Keenan, 1975). Mather and Keenan (1975) also observed the presence of glycoproteins associated with the milkfat globule membrane.

Processing steps are essential in preserving the safety and shelf-life of milk and other dairy products. Common processes which improve milk quality include homogenization and pasteurization. Processing, however, causes structural changes in the milkfat globule membrane. For example, homogenization decreases the milkfat globule size, yielding an increase in surface area. Since the original components of the milkfat globule membrane are insufficient to cover the increase in fat globule surface area, casein and whey proteins are adsorbed to the surface of lipid globules. In addition, physical properties such as creaming stability and size and shape are affected (Elling and Duncan, 1996). Homogenization of high fat creams typically increases the incidence of feathering and clustering (Elling and Duncan, 1996). The changes accompanied by homogenization and heating will be discussed in much more detail in future sections.

Recombination of butteroil into dairy emulsions. Milkfat fractionation and cholesterol removal processes, applied to improve functionality or nutritional characteristics of milkfat, are conducted on anhydrous milkfat (butteroil). Incorporation of modified butteroil into an oil-in-water fluid emulsion requires the envelopment of lipid globules by the milkfat globule membrane or proteins provided by the aqueous portion of milk or cream or by added components. Butteroil can be emulsified into dairy emulsions using skim component, purified milkfat globule membrane extracts, whey proteins, sweet buttermilk, casein protein dispersions, butter-derived aqueous phase, and emulsifying agents (Oortwijn et al., 1977; Oortwijn and Walstra, 1979, 1979; Oortwijn and Walstra, 1982; Kanno et al., 1991; Singh et al., 1993; Rosenberg and Lee, 1993; Oehlmann et al., 1994; Tomas et al., 1994; Elling et al., 1996). The resulting chemical and physical properties are very much dependent on the components used to emulsify the butteroil.

Skim component is commonly used to emulsify butteroil due to its rich casein and whey protein content. Sweet buttermilk, obtained when butter is churned from cream, is abundant in proteins, phospholipids, and cholesterol as some of the membrane components are removed from the surface layer of the milkfat during the churning

process (Mulder and Walstra, 1974). Since membrane material is found in buttermilk, reemulsification of butteroil is possible using this by-product of buttermaking.

Characteristics of surface active molecules. An emulsion is defined as "a heterogeneous system consisting of one or more phases dispersed in a continuous phase" (Kinsella and Whitehead, 1989). Emulsion stability is accomplished by amphiphatic surface active agents, such as phospholipids and proteins which have an attraction for both phases. The responsibility of emulsifying agents is to decrease the interfacial energy and maintain dispersal of the continuous phase (Kinsella and Whitehead, 1989). Proteins are associated at emulsion interfaces in a way in which hydrophilic and charged amino acids are anchored in the aqueous phase while hydrophobic regions react with the lipid phase. Research also indicates the proteins fold at oil-water interfaces to minimize exposure of hydrophobic regions (Phillips, 1981). As a result, the free energy of the system is lowered, increasing stability. Factors which affect the emulsion stability include size and charge of molecules comprising both the continuous and discontinuous phases, tension at the interface, properties of the adsorbed film, the ratio of weight between the continuous and discontinuous phase, ionic strength, pH, and viscosity of the continuous phase (Friberg, 1976; Graham and Phillips, 1976).

Phospholipids. Although phospholipids contribute less than 1% to total lipid content of milkfat, they play a vital role in maintaining the structural integrity of the milkfat globule membrane (Bitman and Wood, 1990). Approximately 50% of the phospholipids of milk are in the milkfat globule membrane (McPherson and Kitchen, 1983). Those phospholipids that are not associated with the milkfat globule membrane occur in skim milk as lipoprotein entities (Jensen and Clark, 1988). Emulsifying properties of phospholipids can be attributed to their amphiphilic nature. Due to their nature, they are not completely soluble in either aqueous media or fat. The polar tails of phospholipids are extended in the aqueous medium whereas hydrophobic tails react with nonpolar regions.

The major phospholipids associated with the milkfat globule membrane are sphingomyelin, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, and phosphatidyl ethanolamine, with phosphatidyl ethanolamine, sphingomyelin and phosphatidyl choline being most abundant (Jensen et al., 1991). The approximate percentages of milk phospholipids are 35% phosphatidyl choline, 30% phosphatidyl ethanolamine, 25% sphingomyelin, 5% phosphatidyl inositol, and 3% phosphatidyl serine (Christie, 1995; Keenan and Dylewski, 1995). Phospholipids with a choline group, phosphatidyl choline and sphingomyelin, are positioned on the outside of the milkfat globule membrane (Deeth, 1997). Glycolipids are also located on the outside of the membrane (Deeth, 1997). Neutral lipids, phosphatidyl ethanolamine, phosphatidyl serine, and phosphatidyl inositol are found on the inner portion of the milkfat globule membrane (Deeth, 1997).

Most of the phospholipids consist of fatty acids, a glycerol backbone, and a phosphophorylated alcohol group (Bitman and Wood, 1990). Sphingomyelin, like its phospholipid counterparts, also has a fatty acid, but has a sphingosine base with an

attached choline group. Lesser degrees of plasmogens, cerebrosides, and lyphophospholipids have been associated with the milkfat globule membrane (Jensen et al., 1991). Cerebrosides are unique in that they are based on sphingosine with a hexose group but demonstrate similar patterns as other phospholipids (Mulder and Walstra, 1974).

Phospholipids have been characterized as containing long-chain polyunsaturated fatty acids, thus, they are susceptible to autoxidation processes (Jensen et al., 1991). Phosphatidyl choline and phosphatidyl ethanolamine have approximately 40-60% unsaturated fatty acids (Deeth, 1997). At least a third of the unsaturated fatty acids associated with phosphatidyl choline and phosphatidyl ethanolamine are polyunsaturated (Christie, 1995). The phospholipids of the milkfat globule membrane typically have an abundance of palmitic and oleic acid (McPherson and Kitchen, 1983). Shorter chain fatty acids occurring in milkfat globule membrane phospholipids are less common (McPherson and Kitchen, 1983). Thus, there is concern about their stability during extended storage and processing. During heat processing, phospholipids are degraded to lyso-type phospholipids and free fatty acids. The degradation compounds produced result in flavor and quality defects.

Nakanishi and Kaya (1970) determined the changes that phospholipids undergo during extensive heating. Approximately 200 ml of raw milk was heated at 63 and 93^{o}C for 10, 20, and 30 min. A solution consisting of 60 mg of phospholipids and 200 ml of water which was heated at 93^{o}C for 10, 20, and 30 min served as the control. Lipids were extracted using a 2:1 mixture of chloroform-methanol. Phospholipids were subsequently separated by silicic acid column chromatography and identified by thin-layer chromatography. Total milk phospholipid decreased to 96% while lyso-type phospholipids increased to 2.7% of total phospholipid after heat treatment at 63^{o}C for 30 min (Nakanishi and Kaya, 1970). After heating milk at 93^{o}C for 30 min, phospholipids decreased to 85.8% whereas lyso-type phospholipids increased to 4.2% of total phospholipids (Nakanishi and Kaya, 1970). Phospholipids in the control which was heated for 30 min at 93^{o}C decreased only to 96.6% while lyso-type phospholipids increased to 2.1% (Nakanishi and Kaya, 1970). Higher percentages of individual milk phospholipids were associated with the 63^{o}C heat treatment for 30 min when compared to the 93^{o}C heat treatment for 30 min (Nakanishi and Kaya, 1970). Phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, and spihingomyelin were 34.0, 29.2, 30.1, 30.1, and 30.1% respectively in samples heated at 63^{o}C for 30 minutes (Nakanishi and Kaya, 1970). When heated at 93^{o}C for 30 minutes, however, phosphatidyl ethanolamine, phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, and sphingomyelin were 27.9%, 25.9%, 27.8%, 27.8%, and 27.8%, respectively (Nakanishi and Kaya, 1970).

Smith and Dairiki (1975) analyzed the creaming stability of oil-in water emulsions made from 25% milkfat and 0-2.0% of various emulsifying agents. Emulsion stability was determined by measuring the percent of fat in the upper phase and issuing a stability index (Smith and Dairiki, 1975). The most stable emulsions had lower percent upper

phase separation, thus higher stability index values. Emulsion stability was evaluated after 24 hours of storage at 23^{o}C. Using phospholipids as an emulsifier, the milkfat emulsion was stable to creaming. Additionally, increasing the phospholipid concentration enhanced the creaming stability of the emulsion. Phospholipids from soybeans produced slightly more stable emulsions than phospholipids in milkfat. When using 0.25, 0.5, 1.0, and 2.0% phospholipid in total fat, milk phospholipids had 19, 12, 7, and 4% fat separated in the upper phase (Smith and Dairiki, 1975). Percent values obtained from 0.25, 0.5, and 1.0% milk phospholipid were averaged for comparison to other sources of phospholipids. Of the phospholipid classes used, some individual phospholipids provided more stable emulsions and these are the minor phospholipids in milk membranes (Smith and Dairiki, 1975). For 0.25, 0.5, and 1.0% phospholipid used, sphingomyelin, phosphatidyl choline, phosphatidyl ethanolamine did not stabilize the emulsions well and on average had 39.7, 34.7, 22.3% fat in the upper phase, respectively (Smith and Dairiki, 1975). Milk phospholipids, phosphatidyl inositol, and phosphatidyl serine on average had 12.7, 5.3, and 2.0% fat separated in the upper phase (Smith and Dairiki, 1975).

Proteins. Milk proteins fall into two general classes, caseins and whey proteins. Casein and whey proteins are most commonly associated with the aqueous phase of milk. When the original milkfat globule membrane is disrupted during processing, however, these particular proteins readily adsorb to fat globules. Casein proteins are flexible and unstructured while whey proteins are characterized as having well-defined three-dimensional structures (Dalgleish, 1996). Caseins comprise approximately 80% of total milk proteins whereas whey proteins contribute the remaining 20% (Wong et al., 1996). Caseins can further be divided into α_{s1}-casein, α_{s2}-casein, β–casein and κ-casein which occur as 45, 12, 34, and 10% of total casein, respectively (Leman and Kinsella, 1989). The whey proteins are α-lactalbumin, β-lactoglobulin, bovine serum albumin, and immunoglobulins (Leman and Kinsella, 1989). The structures and amino acid compositions of the different milk proteins must be known to understand functionality and physiochemical reactivity in food products.

Caseins have several unique structural features which provide unique physiochemical functions. Casein proteins are abundant in phosphoseryl residues that assist with binding calcium ions and stabilizing micelle structures (Leman and Kinsella, 1989). Also, caseins have high levels of the amino acid proline, limiting α-helix and β-sheet structures. The presence of proline in the structure of caseins distinguishes these proteins from whey proteins, resulting in open and flexible structures (Leman and Kinsella, 1989). A total of 17, 10, 35, and 20 proline residues in a mole of α_{s1}, α_{s2}, β and κ-casein, respectively, have been identified (Fox and McSweeney, 1998). Tertiary structures are also absent, resulting in increased resistance to heat denaturation. Stability to heat denaturation is related to the very minor amount of structure unfolded under heat conditions. Casein proteins possess phosphate groups which are esterified to serine groups, thus increasing the amphiphatic nature of these particular proteins (Leman and Kinsella, 1989). At 20^{o}C at pH 4.6, caseins can be precipitated from raw milk (Wong et al., 1996).

Around 95% of casein is associated in micelles (Fox and McSweeney, 1998). Other components of casein micelles include calcium, phosphate, citrate, and trapped milk serum (Fox and McSweeney, 1998). The driving forces behind casein micelle formation occur by a combination of hydrophobic interactions, hydrogen bonding, electrostatic interactions, and calcium cross bridging (Leman and Kinsella, 1989). It has been proposed that casein micelles are constructed of submicelles (Walstra and Jenness, 1984). The submicelles are characterized as having closely packed hydrophobic cores with a loosely constructed hydrophilic outer regions (Walstra and Jenness, 1984). Casein micelles are spherical in shape and have a diverse range of 50-500 nm diameter (Fox and McSweeney, 1998). κ-casein is located on the outside of the casein micelle, playing a vital role in stabilization of the more sensitive α_{s1} and α_{s2} caseins (Walstra and Jenness, 1984).

Much is known about the structure of α_{s1}-casein. Currently five genetic variants are known for α_{s1}-casein. These variants are referred to as A, B, C, D, and E (Eigel et al., 1984). The B genetic variation of this particular protein is comprised of 199 amino acid residues while the A variant lacks 13 residues which occur in the N-terminus between residues 14 and 26 (Wong et al., 1996). The C genetic variant has a glycine substitution for glutamic acid at residue 192 whereas threonine-P replaces alanine at residue 53 (Eigel et al., 1984). In the E variant glycine replaces glutamic acid at residue 192 and lysine replaces glutamine at residue 59 (Eigel et al., 1984). Acidic amino acids predominate over basic ones in the structure of α_{s1}-casein, yielding a net negative charge of 22 at pH 6.5 (Wong et al., 1996). Since the remaining amino acids are apolar, the rest of the protein is uncharged (Leman and Kinsella, 1989). Tertiary structure is almost nonexistent due to the presence of 8.5% proline in the polypeptide chain (Wong et al., 1996). Between residues 45 and 89, seven of its eight phosphoseryl groups occur in a hydrophilic region (Wong et al., 1996). In the same region, three hydrophobic regions occur at sites 1 to 44, 90 to 113, and 132 to 199 (Wong et al., 1996).

The amino acid sequence of α_{s2}-CN A-11P consists of 207 residues (Wong et al., 1996). Four genetic variants, called A, B, C, and D, have been identified for this protein. Positively charged side chains occur, especially in the C-terminus (Wong et al., 1996). Negative charges are more concentrated at the N-terminus of the protein molecule. The α_{s1} and α_{s2}-caseins tend to be more sensitive to calcium precipitation than the β and κ casein (Kinsella, 1984).

β-casein is negatively charged and has a total of five phosphate groups. Four of its five phosphate groups are situated at the N-terminus, falling between residues 13 and 21 (Leman and Kinsella, 1989). The C-terminus portion of β-casein is characterized as being very hydrophobic (Wong et al., 1996). The contrasting hydrophilic and hydrophobic characteristics of the N-terminus and C-terminus improves the surface activity of β-casein in foods. β-casein has about 16% proline with very little α-helix between residues 97 to 150 (Leman and Kinsella, 1989). The β-CN A1-5P polypeptide chain has 209 residues (Wong et al., 1996).

Only one phosphoseryl residue occurs in the κ-casein polypeptide, thus it has the lowest phosphate content (Kinsella, 1984). The C-terminus of κ-casein is very hydrophilic due the attachment of oligosaccharide residues such as N-acetylneuraminic acid, galactose, and N-acteylgalactosamine (Kinsella, 1984). The C-terminus is also hydrophilic because of the abundance of the amino acids aspartic and glutamic acids (Wong et al., 1996). The carbohydrates are not distributed evenly throughout the chain (Wong et al., 1996). An o-glycosidic link attaches these oligosaccharides to the amino acid threonine (Kinsella, 1984). The N-terminus is very hydrophobic. κ-casein is the only casein protein which has cysteine (Wong et al., 1996). It has two disulfide bonds and some secondary structure (Kinsella, 1984). κ-casein, the casein protein which is most resistant to binding calcium ions, associates with a_{s1}-casein to stabilize it against precipitation by calcium (Brunner, 1981). κ-casein is susceptible to cleavage at the Phe105-Met106 bond by rennin (Eigel et al., 1984). The by-products of the cleavage are hydrophobic para-k-casein and hydrophilic macropeptide (Wong et al., 1996). K-CN B-1P has 169 amino acid residues (Wong et al., 1996). Two genetic variants, A and B, have been designated for k-casein (Eigel, et al., 1984). Functionality of κ-casein can be attributed to the fact that it is a glycoprotein with some apolar character.

Whey proteins are the group of proteins which are still soluble in milk serum or whey after casein has been precipitated at 20^{o}C at pH 4.6 (Eigel et al., 1984). β-lactoglobulin and α-lactalbumin constitute 54% and 21% of total whey proteins (Leman and Kinsella, 1989). The remaining 25% is comprised of bovine serum albumin, the immunoglobulins, and various polypeptides. Whey proteins are characterized as having compact globular structures, resulting in structural restriction and stabilization (Kinsella and Whitehead, 1989). Whey proteins have several structural features which distinguish them from caseins. Whey proteins are unique due to the presence of discrete disulfide bonds (Kinsella, 1984). In comparison to the caseins, whey proteins are more susceptible to denaturation by heat, less sensitive to precipitation by calcium, and engage in thiol-disulfide exchanges (McKenzie, 1971; Fox and Mulvihill, 1982; Swaisgood, 1982).

β-lactoglobulin is the most abundant whey protein. It consists of 162 amino acid residues and 7 genetic variants (Eigel et al., 1984). β-lactoglobulin is a dimer made up of two similar subunits (Leman and Kinsella, 1989). β-lactoglobulin has dimer characteristics because of electrostatic interactions between Asp130 of one monomer with Glu134 of the other monomer (Kinsella and Whitehead, 1989). Each monomer has a free thiol group at Cys121 and β-lactoglobulin has two disulfide linkages, one between Cys66 and Cys160 and another between Cys106 and Cys119 (Leman and Kinsella, 1989). As temperatures increase beyond 40^{o}C, β-lactoglobulin separates into its monomer constituents (Leman and Kinsella, 1989). During heat processing, β-lacotglobulin has the potential to engage in disulfide exchange with thiol containing proteins (Leman and Kinsella, 1989). Since β-lactoglobulin is the most characterized milk protein, its secondary structure is known. β-lactoglobulin consists of approximately 15% α-helix, 51% β-sheet, 17% reverse turn, and 17% aperiodic structure (Kinsella and Whitehead, 1989). A unique structural feature of β-lactoglobulin is that it comprised of eight strands of antiparallel β sheets (Wong et al., 1996). These antiparallel β sheets wrap to form a β barrel (Wong et al., 1996). The β

barrel is lined with hydrophilic amino acids, but its interior is highly hydrophobic (Wong et al., 1996).

α-lactalbumin has three genetic variants and the B variant has a total of 123 amino acids (Eigel et al., 1984). The polypeptide chain is abundant in various amino acids, including lysine, leucine, threonine, tryptophan, and cysteine (Kinsella and Whitehead, 1989). Four disulfide linkages have been identified occurring between Cys6 and Cys120, Cys28 and Cys111, Cys61 and Cys77, and Cys 73 and Cys91 (Wong et al., 1996). Furthermore, α-lactalbumin is divided into two lobes, forming a deep cleft (Wong et al., 1996). One side of the cleft consists of four helices whereas the other side is composed of two β strands (Wong et al., 1996). α-lactalbumin has unique binding sites for calcium ions as well as other ions such as zinc, cadmium, copper, manganese, and aluminum (Wong et al., 1996). The ability of α-lactalbumin to bind ions protects the protein from denaturation by heat (Kinsella and Whitehead, 1989). As a result, α-lactalbumin is the most heat resistant of the whey proteins. The heat stability of α-lactalbumin is dependent on the presence of bound calcium, thus decreasing if the ion is removed (Leman and Kinsella, 1989).

Bovine serum albumin is a very large globular protein with approximately 580 amino acid residues (Kinsella and Whitehead, 1989). Seventeen disulfide bonds occur throughout the polypeptide chain, with one free thiol group at residue 34 (Kinsella and Whitehead, 1989). Bovine serum albumin is divided into a total of three domains, two large loops and one small loop (Eigel et al., 1984). The binding of bovine serum albumin to free fatty acids and other lipids inhibits thermal denaturation.

Immunoglobulin proteins in bovine milk can be divided into four categories. Those categories are IgG, IgA, IgM, and IgE (Kinsella and Whitehead, 1989). The immunoglobulins are monomer or polymer units of a basic unit which is comprised of four polypeptide chains (Kinsella and Whitehead, 1989). The polypeptide chains are covantlently linked by disulfide bridges (Kinsella and Whitehead, 1989). The immunoglobulin IgM plays a role in the cold agglutination of cold, raw milk.

The complex structural features of casein and whey proteins have several implications for their functionality in food products. In comparison to other sources of food proteins, casein proteins tend to have superior surface activity properties. Surface active proteins are those proteins which are capable of adsorbing at the interface or surface of an emulsion (Kinsella and Whitehead, 1989). Upon adsorption at the interface, surface active proteins spread and rearrange themselves to attain the lowest free energy (Kinsella and Whitehead, 1989). As a result of its surface activity characteristics, casein proteins are commonly used in foods in which foaming and emulsion stability are important (Fox and McSweeney, 1998). The surface activity of casein proteins can be attributed to its hydrophilic and hydrophobic regions, which are capable of stabilizing the water and lipid phases of an emulsion. Casein proteins have an amphiphatic nature due to the presence of phosphoseryl residues, permitting interaction with the aqueous phase of an emulsion. The hydrophobic and hydrophilic regions of caseins do not occur in an ordered pattern. Therefore, good surface activity is also a result of the highly disordered structure of

caseins. The structure of caseins is further characterized as high in proline content (Dickinson, 1989). The proline residues are responsible for interruption of α-helix and β-sheet formation, allowing a somewhat disorganized structure which enables successful emulsification (Dickinson, 1989). Other structural properties which enable caseins to display good surface activity are low molecular weight, high surface hydrophobicity, and chain flexibility (Dickinson, 1989; Fox and McSweeney, 1998).
Caseins also have an impact on the viscosity of foods. Due to their open, flexible structures and ability to bind water, caseins tend to have an increasing effect on viscosity (Fox and McSweeney, 1998). Casein proteins tend to be less sensitive to heat processes than whey proteins and fail to undergo true denaturation processes due to their open structures. At refrigeration temperatures, casein micelle aggregation is suppressed. However, when milk is heated to temperatures exceeding 40^{o}C initial changes are observed in casein micelle structure. These particular changes occur at a very slow rate during the initial heating process. The somewhat spherical shape of micellar casein is lost and irreversible aggregation occurs (Prentice, 1992). The result is an increase in the viscosity of the product. The heat induced chemical changes occurring in casein micelles include dephosphorylation, some cross-linking reactions, and partial hydrolysis (Walstra and Jenness, 1984).

Casein functionality is affected by pH changes which are dependent on temperature (Fox and McSweeney, 1998). Decreasing the pH has a destabilizing effect on micellar casein. Casein precipitation potentially occurs over a wide pH range such as 3.0-5.5 at 70^{o}C or higher. Precipitation will not occur at temperatures below 5-8^{o}C (Fox and McSweeney, 1998). Pasteurization temperatures alone have a slight effect on the buffering capacity and pH of milk. The pH is depressed and buffering capacity is altered due to the removal of carbon dioxide and precipitation of calcium phosphate accompanied by discharge of hydrogen ions (Pyne, 1962). The initial signs of casein destabilization are dissolution of colloidal calcium phosphate and lower micelle voluminosity (Walstra and Jenness, 1984). Reduction to pH 5.5 or lower results in casein aggregation, thus micelles tend to enlarge. At approximately pH 4.8 nearly all phosphate has been dissolved in the aqueous phase. When pH reaches 4.6, the isoelectric point of casein has been achieved and the micelles have disintegrated. At this point casein precipitates out of colloidal suspension (Walstra and Jenness, 1984).

The structure of whey proteins influences various functional characteristics, most importantly functionality during heat processing. Whey proteins readily denature at temperatures above 65^{o}C (Kinsella, 1984). Denaturation occurs when globular proteins unfold from a compact structure to a rather loose, unorganized structure. The denaturation process is also characterized by aggregation after unfolding and eventually precipitation. Since whey proteins exist in compact three-dimensional structures with well-defined secondary and tertiary structure, they are very susceptible to heat denaturation. Another factor which impacts denaturation of whey by heat treatment is amino acid composition. During heat treatment whey proteins, particularly β-lactoglobulin and bovine serum albumin, participate in protein aggregation via thiol/disulfide interchanges when these particular groups become exposed (de Wit, 1981).

Several intrinsic factors should be considered when determining the extent of whey denaturation by heat. These factors include pH, lactose, and salts (de Wit, 1981). At pH 4.6 whey proteins are most susceptible to denaturation by heat (Kinsella, 1984). Resistance to heat denaturation is typically greatest at pH 2.5-3.5 due to good solubility at this pH range. Milk sugars, principally lactose, tend to protect whey proteins from thermal denaturation by loss of solubility (de Wit, 1981). The same effect has been observed with salts such as NaCl.

During heat treatment, complex interactions occur between whey proteins as well as whey proteins and casein micelles. Heat induced interactions occur between the principal whey proteins, β-lactoglobulin and α-lactalbumin (Kinsella, 1984). The two major proteins of whey associate via sulphydryl/disulphide interaction. Of the denaturable whey proteins, β-lactoglobulin is the most sensitive to heat whereas α-lactalbumin is the most heat resistant (Kinsella, 1984). Through interaction with β-lactoglobulin, heat sensitivity to denaturation increases for a-lactalbumin (Hunziker and Tarassuk, 1965). For α-lactalbumin interaction with κ-casein to occur, β-lactoglobulin must be present (Kinsella, 1984). Heat induced association of denatured β-lactoglobulin with κ-casein and α_{s1}-casein occurs when milk is heated at 70^{o}C (Walstra and Jenness, 1984). The key element behind the interactions between β-lactoglobulin and κ-casein is the presence of a thiol group in the β-lactoglobulin which participates in disulfide bonding (Kinsella, 1984). Factors affecting the complex between β-lactoglobulin and κ-casein are pH and ionic strength. The interaction is enhanced as ionic strength increases and decreases as pH is raised from 6.8 to 7.3 (Kinsella, 1984). This particular protein interaction has implications on the structure of casein micelles, resulting in problems with rennetting during processing (Kinsella, 1984; Walstra and Jenness, 1984).

Chemical characteristics of components used for emulsification. Purified milkfat globule membrane extract. The milkfat globule membrane, rich in phospholipids and proteins, acts as a natural emulsifier. The emulsifying ability of the milkfat globule membrane can be attributed to its surface-active components, proteins and phospholipids. Kanno (1989) analyzed the effects of homogenization speed, emulsification time, temperature of emulsification, milkfat concentration (5-45%), milkfat globule membrane concentration (0.5-2%), pH, and temperature on emulsifying activity and emulsion stability of emulsions based on milkfat globule membrane and milkfat. For standard treatments, cream was separated from raw milk, chilled, and churned. Milkfat globule membrane was obtained by centrifugation at 186,000 x g at 4^{o}C for 40 min. Milkfat globule membrane was treated in a 0.01 M sodium phosphate buffer at pH 7.0 prior to use. Milkfat globule membrane was also obtained from melting butter granules at 45^{o}C obtained from churning. Emulsions were prepared by mixing 1% milkfat globule membrane with 25% milkfat. Emulsions were heated to 45^{o}C and homogenized 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). However, prolonged emulsification time (0.5-5 min) caused a decrease in both emulsifying activity and emulsion stability (Kanno, 1989). Optimum emulsion stability was observed at approximately 1 min. of emulsification. In considering effect of

emulsifying temperature (30-65°C), both maximal emulsifying activity and emulsion stability were achieved at 45°C. As milkfat concentration increased (5, 15, 25, 35, and 45%), emulsifying activity and emulsion stability decreased in the presence of 1% milkfat globule membrane. Decreases in both emulsifying activity and emulsion stability resulting in increased milkfat concentration can be attributed to insufficient coverage of lipid droplets by milkfat globule membrane. As milkfat globule membrane concentration (20, 40, 60, and 80 mg/g fat) increased, emulsifying activity and emulsion stability increased (Kanno, 1989). When analyzing the effect of pH (pH 4-9), emulsifying activity and emulsion stability were highest at pH 4 with drastic reduction at pH 5.0 (Kanno, 1989). To determine the effect of temperature on emulsion properties, samples were heated at 4, 10, 15, 25, 35, 45, and 55°C for 30 min. Stable emulsions were obtained at temperatures below 25°C, however, as temperatures increased stability decreased (Kanno, 1989).

Kanno et al. (1991) prepared emulsions consisting of 25% milkfat, 1% milkfat globule membrane, and phosphate buffer. Cream from raw milk was separated and churned into buttermilk. Butter serum was obtained from melted butter granules. The buttermilk and butter serum were combined and centrifuged at 100,000 x g for 60 min at 4°C for recovery of the milkfat globule membrane. Upon collection, the milkfat globule membrane pellet was dispersed in a 10 mM phosphate buffer and stored at –80°C. Protein and lipid accounted for approximately 56% and 44% of total solids content of the milkfat globule membrane and phosphate suspension. Emulsification consisted of combining 7.5 ml 10 mM sodium phosphate buffer + 1% milkfat globule membrane and 25% milkfat. The milkfat preparations were heated to 45°C and homogenized at 19,800 rpm for 60 sec. The effects of varying milkfat globule membrane concentration (20, 40, 60, and 80 mg/g fat) and pH (4, 5, 6, and 7) were analyzed. Kanno et al. (1991) determined that the amount of proteins absorbed on the surface emulsions fortified with milkfat globule membrane extract was dependent on the pH, concentration of milkfat globule membrane, and amount of milkfat. Under standard conditions (40 mg milkfat globule membrane/g fat), at pH values 4, 5, 6,7, and 8, the percentages of adsorbed protein were 79.6, 74.2, 50.2, 37.2, 39.3, and 40.1%, respectively (Kanno et al., 1991). At neutral pH, the amount of adsorbed protein increased as the concentration of the milkfat globule membrane increased (Kanno et al., 1991). The highest amount of protein absorbed, 16 mg/g fat, was displayed by the emulsion with the highest concentration of milkfat globule membrane (80 mg/g fat) (Kanno et al., 1991).

Skim component. The emulsification of anhydrous butteroil with skim component and proteins has been thoroughly investigated by Oortwijn, Walstra, and others (Oortwijn et al., 1977, Oortwijn and Walstra, 1979: Oortwijn and Walstra, 1982; Melsen and Walstra, 1989). Oortwijn et al. (1977) demonstrated emulsification of butteroil with skim component, whey, and casein dispersions. Milkfat used in the emulsion was obtained from churned cream. The butter was melted and filtered through cotton wool. Skim milk was obtained from fresh milk. The source of whey for emulsification was rennet whey obtained from low temperature pasteurized skim milk. Casein was obtained by centrifugation of fresh low temperature pasteurized skim milk at 35,000 g followed by washing with NaCl and $CaCl_2$ solutions. Emulsions were prepared at 40°C with a valve

homogenizer operating at less than 1 MPa. Electron microscopy revealed that casein and whey proteins interacted at the fat-lipid interface (Oortwijn et al., 1977).

Oortwijn and Walstra (1979) determined the quantity of proteins adsorbed on milkfat droplets in recombined cream consisting of milkfat in either skim component, whey, or casein protein dispersions. Milkfat was obtained from fresh cream whereas skim was obtained from fresh milk or reconstituted low heat treated milk powder. Whey was obtained from ultrafiltration and drying procedures and casein used in formulations was obtained from centrifugation of fresh milk and subsequent washing with milk ultrafiltrate. All creams were passed through a valve type homogenizer at 40^{o}C at 10 MPa. In comparison to other proteins of the aqueous phase and whey proteins, casein proteins were more adsorbed to fat globules (Oortwijn and Walstra, 1979). The protein load (mg/m^2) for milkfat in casein, skim component, and whey were 20, 10, and 2.5 mg/m^2 (Oortwijn and Walstra, 1979).

Oortwijn and Walstra (1982) determined the emulsion stability of recombined creams consisting of milkfat and whey and/or skim component. The properties analyzed for stability included clustering and coalescence. The principal factor affecting these particular properties was quantity of available protein (Oortwijn and Walstra, 1982). Emulsions were prepared by the same methods outlined in Oortwijn and Walstra (1979). When protein:fat ratio was varied, clusters formed at a constant homogenization pressure of 5 MPa at 45^{o}C and constant fat content of 5% (m/m) if protein per ml fat was less than 0.15 g (Oortwijn and Walstra, 1982). In whey based emulsions, cluster formation did not occur at fat contents below 20% (Oortwijn and Walstra, 1982). Stability to coalescence was analyzed over a 7 day storage period. Emulsions with whey containing less than 10% milkfat changed slightly during the storage period. Coalescence occurred, however, when protein load was decreased below 0.5 mg/m^2 (Oortwijn and Walstra, 1982). Coalescence resulting from heat treatment of whey based emulsions was also dependent on the amount of available protein (Oortwijn and Walstra, 1982). Coalescence was evident after heat treatment at 80^{o}C (Oortwijn and Walstra, 1982). As protein load increased from 7 to 35 mg protein/ml fat, stability to coalescence at 120^{o}C was enhanced (Oortwijn and Walstra, 1982).

Tomas et al. (1994) made oil-in-water emulsions from milkfat and skim component, varying in the fat (4-30 g/100g) and protein (3.2-0.4 g/100g) contents. The objective was to analyze the effects of ratio of fat to protein on the mean droplet diameter, surface of interface, amount of protein adsorbed, and surface coverage. The emulsions were processed by pre-heating and combining skim component and anhydrous milkfat. Dispersal was achieved by using a microfluidizer operating at 5000 psi at 52+2^{o}C. Variations in fat to protein mass ratio were achieved by altering protein content from 0.4 to 3.2 g/100 g or by modifying fat content from 4 to 30 g/100g while protein content was constant at 2.3g/100g. The final ratio of milkfat to protein varied between 1 and 13. Proteins contributed from the skim phase of milk played a significant role in maintaining emulsion stability. Both casein and whey proteins were adsorbed, however, preferential adsorbance of casein over whey proteins occurred (Tomas et al., 1994). SDS-PAGE also indicated that proteins which were not adsorbed were predominantly whey proteins

(Tomas et al., 1994). The results indicated that as the fat to protein mass ratio increased, the droplet size increased, especially with fat to protein mass ratio greater than 6 (Tomas et al., 1994). Varying the fat content also affected the interfacial surface area. As fat content increased in the fat to protein ratio, the interfacial surface area increased due to an increase in fat droplet quantity (Tomas et al., 1994). As the fat to protein mass ratios increased, the fraction of protein adsorbed increased (Tomas et al., 1994). Maximum adsorbance achieved was 85% (Tomas et al., 1994). Protein surface coverage was highest in fat to protein mass ratios below 4 (11 mg/m^2), but slightly decreased beyond a fat to protein mass ratio of 4 (Tomas et al., 1994).

Sweet buttermilk and butter-derived aqueous phase. Investigations also support the use of sweet buttermilk and butter-derived aqueous phase components as emulsifying agents. Since buttermilk is abundant in surface active agents such as proteins and phospholipids, it can be utilized in cream reformulation applications. In comparison to skim component obtained from milk, buttermilk has more phospholipid content. Oehlmann et al. (1994) and Elling et al. (1996) incorporated butteroils into creams consisting of skim milk, buttermilk, and an aqueous phase derived from butter.

Oehlmann et al. (1994) incorporated cholesterol-reduced and natural butteroils into emulsion systems consisting of sweet buttermilk, aqueous phase of melted butter, skim, or combinations of these particular components. Fresh milk was separated into cream (35% lipid) and skim phases. The cream was either unwashed or washed twice by suspending it in water at a temperature of approximately 35^{o}C. The cream was churned, yielding butter and buttermilk, which were separated. The milkfat globule membrane was obtained by centrifugation of the buttermilk phase. The final step consisted of melting the butter at 40^{o}C to separate the butteroil from the aqueous phase. Recombination was accomplished by mixing the butteroil with buttermilk, aqueous phase of melted butter, skim, or combinations of these particular components. Dispersal of butteroil was accomplished by either homogenization or ultrasound dispersal. Use of a cholesterol-reduced butteroil had an insignificant effect on the emulsion stability, churn time, and protein composition of the cream (Oehlmann et al., 1994).

Protein load at the fat-lipid interface was higher when buttermilk and the aqueous phase of butter came from washed cream (Oehlmann et al., 1994). Oehlmann et al. (1994) discovered that protein composition of reformulated emulsions was very similar to protein composition of starting components. Regardless whether the emulsifying component was milkfat globule membrane suspension, sweet buttermilk, or sweet buttermilk and butter-derived aqueous phase, the amount of protein associated with the emulsified butteroil was comparable to the amount of protein before emulsification (Oehlmann et al., 1994). In all formulations, stable emulsions were produced with approximately 50 mg protein/g butteroil (Oehlmann et al., 1994). Approximately 50.5 $\pm$ 4.9 mg protein/g butteroil reassociated upon reformulation (Oehlmann et al., 1994). Phospholipid content among the components and emulsions were also similar (Oehlmann et al., 1994). The degree of reassociation of protein, total lipid, and phospholipid classes were dependent upon if cream was washed or unwashed (Oehlmann et al., 1994). When

using buttermilk and butter-derived aqueous phase as emulsifying components, less protein, total lipid, and phospholipid reassociated when unwashed cream was the starting material (Oehlmann et al., 1994). The percentages for protein, total lipid, and phospholipid associated with unwashed cream were 60, 71, and 45%, respectively (Oehlmann et al., 1994). Percentages obtained for washed cream and cholesterol-reduced butteroil were more comparable. Protein, total lipid, and phospholipid content for emulsions consisting of buttermilk plus butter-derived aqueous phase from washed cream were 74, 82, and 71%, respectively (Oehlmann et al., 1994). The percent values for cholesterol-reduced butteroil were 71, 75, and 69%, respectively (Oehlmann et al., 1994). Upon inspection with electron microscopy, however, the emulsions formed in the experiment were characterized as water-in-oil types as opposed to the typical oil-in-water emulsions occurring in natural creams (Oehlmann et al., 1994).

Elling et al. (1996) prepared three reformulated creams which consisted of 1) 20% cholesterol-stripped butteroil and 80% skim component, 2) 20% cholesterol-stripped butteroil and 80% sweet buttermilk, and 3) 20% cholesterol-stripped butteroil, 70% sweet buttermilk, and 10% butter-derived aqueous phase. The components were obtained similarly to the methods described by Oehlmann et al. (1994). Buttermilk was obtained from churning butter. The aqueous phase was obtained from separating melted butter from the aqueous component of butter. Total protein (mg/g), phospholipid (mg/g), and fat (%) were determined for skim, sweet buttermilk, and butter-derived aqueous phase components. Butter-derived aqueous phase, buttermilk, and skim components were found to have 31.75 ± 0.89, 27.00 ± 0.84, and 28.01 ± 0.68 mg protein/g, respectively (Elling et al., 1996). For total phospholipid content, 4.95 ± 0.51, 0.89 ± 0.07, and 0.12 ± 0.00 mg phospholipid/g were associated with the butter-derived aqueous phase, buttermilk, and skim milk components, respectively (Elling et al., 1996). Approximately $1.50 \pm 0.36\%$, $1.13 \pm 0.15\%$, and $0.02 \pm 0.00\%$ total fat were found in the butter-derived aqueous phase, buttermilk, and skim components, respectively (Elling et al., 1996). Natural homogenized cream (20% milkfat) served as a control.

Elling et al. (1996) determined that reformulated creams consisting of sweet buttermilk or sweet buttermilk and butter-derived aqueous phase exhibited protein and phospholipid composition most similar to 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 whereas all reformulated creams had a range of 24.8 to 27.9 mg protein/g (Elling et al., 1996). SDS-PAGE results indicated that casein was the predominant protein type associated with the lipid globules of control and reformulated creams (Elling et al., 1996). Formulation, however, had a significant effect on the phospholipid content of the creams. Similar phospholipid contents were obtained for the control and creams having only buttermilk as an emulsifying component, averaging at 0.60 mg phospholipid/g for all replications (Elling et al., 1996). The formulation consisting of sweet buttermilk and butter-derived aqueous phase had significantly higher ($p \leq 0.05$) amounts of phospholipids (approximately 1.0 mg phospholipid/g) (Elling et al., 1996). The formulation consisting of 80% skim and 20% butteroil, however, was significantly lower ($p \leq 0.05$) in phospholipid content (0.15 mg phospholipid/g) than the other formulations. Unlike the

formulations processed by Oehlmann et al. (1994), each formulation was an oil-in-water emulsion.

Milkfat globule surface material in processed creams. Elling et al. (1996) analyzed the chemical composition of isolated milkfat globule surface material in control and reformulated creams in order to determine the degree of reassociation of native components. Significant effects ($p \leq 0.05$) were obtained for the relative amount of milkfat globule surface material and protein, and phospholipid in the surface material reassociated with the lipid due to formulation (Elling et al., 1996). Of the formulations, the reformulated cream consisting of 80% skim component + 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 recovered from the cream consisting of 80% skim + 20% butteroil formulation was 112.6 mg/g lipid in cream for homogenization pressure of 13.6/3.4 Mpa (Elling et al., 1996).

The formulations consisting of sweet buttermilk only or buttermilk (70%) and aqueous phase derived from butter (10%) were comparable to control creams in the amount of milkfat globule membrane material associated with the lipid globules (Elling et al., 1996). At 10.2/3.4 and 13.6/3.4 MPa homogenization, the membrane material for the control was 72.0 $\pm$ 3.98 mg surface material/g lipid in cream and 75.4 $\pm$ 3.38 mg surface material/g lipid in cream, respectively (Elling et al., 1996). The amount of obtained milkfat globule surface material from the formulations consisting of buttermilk were quite similar regardless of homogenization pressure (Elling et al., 1996). At a homogenization pressure of 10.2/3.4 MPa, the reformulated cream comprised of 20% butteroil and 80% buttermilk had a value of 67.4$\pm$4.82 mg surface material/g lipid in cream whereas the creams formulated from 20% butteroil, 70% buttermilk, and 10% aqueous phase a value of 61.1$\pm$4.83 mg surface material/g lipid in cream (Elling et al., 1996). At 13.6/3.4 MPa, 70.7$\pm$9.4 mg surface material/g lipid in cream was associated with creams formulated from 20% butteroil and 80% buttermilk processed at 13.6/3.4 MPa (Elling et al., 1996). At the same homogenization pressure, 76.9 mg $\pm$ 3.9 surface material/g lipid in cream occurred in creams having 70% buttermilk, 20% butteroil, 10% butter-derived aqueous phase emulsion (Elling et al., 1996).

Protein associating at the lipid surface was fairly consistent among formulations (Elling et al., 1996). The 80% skim component + 20% butteroil formulation homogenized at 13.6/3.4 MPa, however, was significantly ($p \leq 0.05$) higher in 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 except for cream formulated with sweet buttermilk and 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 ($p \leq 0.05$) 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. The values reported for protein content of the milkfat surface material associated with creams processed from skim component were approximately 46-52 mg protein/g lipid in cream at 10.2/3.4 MPa and 13.6/3.4 MPa homogenization pressures (Elling et al., 1996). At 10.2/3.4 MPa and

13.6/3.4 MPa, the protein contents of the milkfat surface material were 45.1±2.66 mg protein/g lipid and 48.0±2.90 mg protein/g lipid, respectively, (Elling et al., 1996). Lower values were obtained for creams manufactured from buttermilk and buttermilk and butter-derived aqueous phase formulations. At 10.2/3.4 MPa, reformulated cream processed from 20% butteroil, 70% buttermilk, and 10% aqueous phase had 38.4 ± 1.75 mg protein/g lipid and 20% butteroil+80% buttermilk had 41.3 ± 2.24 mg protein/g lipid in cream (Elling et al., 1996). At 13.6/3.4 MPa, cream consisting of only buttermilk as a component and sweet buttermilk and butter-derived aqueous phase had 42.8 ± 3.31 mg protein/g lipid in cream and 45.4 ± 2.57 mg protein/g lipid in cream, respectively (Elling et al., 1996).

Phospholipids served as an indication of the amount of native milkfat globule membrane fragments reassociated with the lipid. The formulation comprised of skim component had significantly lower ($p \leq 0.05$) amounts of phospholipid in the milkfat surface material than the control and all formulations consisting of buttermilk (Elling et al., 1996). The buttermilk formulated creams had more phospholipid contributing to the surface material. At 10.2/3.4 MPa, the reformulated cream consisting of sweet buttermilk component and cream consisting of sweet buttermilk and aqueous phase gave milkfat globule surface material with 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). At the same homogenization pressure, however, the skim component formulation only had 0.24 ± 0.001 mg phospholipids/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). In comparison to 13.6/3.4 MPa homogenization pressure, slightly higher values were obtained at the lower initial homogenization pressure (Elling et al., 1996). 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 in cream (Elling et al., 1996).

Electron microscopy of milkfat emulsions. Electron microscopy has proved to be a beneficial tool in the analysis of milkfat emulsions. Optical microscopy has limited ability in analyzing the structures of casein micelles and fat globules because of shallow focus depth and limited resolution (Kalab, 1993). Electron microscopy, however, extends resolution so that researchers are able to examine molecular aggregates as well as their spatial arrangement in more detail (Kalab, 1993).

Oortwijn et al. (1977) utilized electron microscopy to view the structure of recombined fat globules. Electron microscopy revealed that serum proteins and caseins were active in forming newly adsorbed milkfat surface material . Serum proteins were found to participate by forming a thin layer around the fat globules. Caseins, however, were readily incorporated into the membrane (Oortwijn et al., 1977).

Rosenberg and Lee (1993) analyzed the microstructure of emulsions consisting of milkfat (30-50%) and 5% (w/w) whey protein isolate. Scanning electron microscopy, cryo-scanning electron microscopy, and transmission electron microscopy were used to analyze microstructures. The whey protein isolate was mixed with deionized water at 25^{o}C. Emulsification occurred in two steps. Prior to homogenization, each emulsion was

heated to 50^{o}C and maintained at this temperature during the homogenization process. The first step consisted of mixing the milkfat and whey solution, and subsequently homogenizing at 13,500 rpm for 30 sec. Next, each emulsion was homogenized in 4 to 12 successive steps at either 50 or 80 MPa. Electron microscopy proved that whey proteins were good emulsifying agents. Whey protein films surrounding milkfat globules were visible. Fat globule aggregation into clusters occurred at the higher homogenization pressure (80 MPa) and/or when emulsions were passed through the homogenizer 12 successive times. These particular conditions are known to favor aggregation or clustering. Aggregation of fat globules can be attributed to insufficient protein coverage due to increased surface area.

Oehlmann et al. (1994) analyzed electron micrographs of various formulated cream. Electron micrographs revealed that creams reformulated with cholesterol-stripped butteroil were water-in-oil emulsions under the conditions of the study (Oehlmann et al., 1994). Elling et al. (1996), however, determined that reformulated creams with cholesterol-stripped butteroil were oil-in-water emulsions like those occurring in control creams. The emulsions were characterized as lipid globules surrounded by milkfat globule membrane components in the serum phase (Elling et al., 1996). Caseins were identified in the surface layer around the milkfat globules. Globule diameter, degree of clustering, and shape differed with formulation and processing (Elling et al., 1996). Globule diameter was greatest for the formulations consisting of skim component, followed by creams consisting of sweet buttermilk and butter-derived aqueous phase, and control creams (Elling and Duncan, 1996). Electron microscopy also revealed that unpasteurized creams had more spherical lipid globules and pasteurized creams had lipid globules which were more misshapen (Elling and Duncan, 1996). Clustering, regardless of homogenization pressure, was more associated with pasteurized cream (Elling and Duncan, 1996).

Influence of processing parameters. Dairy destabilization. Like other food emulsions, a dairy emulsion does not exist in thermodynamic equilibrium because it is not at its lowest energy state (Friberg et al., 1990). Energy is stored at the water-oil interface (Friberg et al., 1990). The forces which impact emulsion stability are electrostatic repulsion from the creation of an electrical double layer around a charged particle, attraction forces (i.e. van der Waals forces, steric forces from adsorbed components (i.e. proteins), hydrophobic interactions, and applied external fields (Friberg et al., 1990). Activation energy must be overcome, however, for emulsion instability to occur (Walstra, 1983). Factors which have the greatest impact on emulsion stability are temperature, ratio of liquid to solid fat, fat globule size, fat content, plasma composition, membrane characteristics, and presence of air (Mulder and Walstra, 1974).

Over an extended period of storage, dairy emulsions will exhibit instability by separating into two distinct layers. Less dense in nature, lipid globules rise to the top of the emulsion whereas the skim phase remains at the bottom. The phenomena of milkfat rising to the top of the emulsion due to differences in density is known as creaming. Creaming can be predicted by Stoke's law, $v = g(\rho_s-\rho_f)d_2/18\eta s$. In the equation, "v" represents the rising speed of a fat globule of given diameter "d" which is influenced by

gravity "g," fat and skim phase, densities "ρ,"and viscosity "η" of the serum phase (Walstra, 1983).

Other indications of creaming instability are flocculation and coalescence. Flocculation results when milkfat globules join together to form a collective unit. The three kinds of flocculation are floccules, clumps, and clusters (Mulder and Walstra, 1974). Floccules are fat globules that flocculate but maintain their identity. Floccules are held together by rather weak forces, thus, making redispersal simple. Clumps result when part solid/part liquid fat globules aggregate if they come in contact with each other. When clumps form, they prove to be hard to redisperse. Clusters form when fat globules share parts of their interfacial layers. Strong bonds are formed, making redispersal difficult (Mulder and Walstra, 1974).

Coalescence is the merging of two emulsion droplets, ultimately disrupting the film of the continuous phase between two drops that are in close association with each other (Walstra, 1983). Coalescence is affected by agitation, temperature, fat content, creaming, freezing, surface layer of fat globules, and globule size. Agitation may result in partial coalescence, however disruption of globules, particularly larger ones, tend to occur. Partial coalescence is more likely to occur during agitation mechanisms when milkfat is part solid (15-30^{o}C). In creams with very high fat contents, coalescence is a result of agitation. Agitation mechanisms promote pressing of fat globules in high fat creams. In the packing of fat globules in cream layers, changes in crystallization patterns can potentially cause partial coalescence of fat globules (Walstra, 1983). As a result, a cream plug is formed. Freezing initiates partial coalescence with the growth of ice crystals which ultimately damage fat crystals. The surface layer of fat globules impacts coalescence when the membrane components are degraded by enzymes. Larger globules coalesce more readily than smaller ones.

Several observations have been made from the coalescence of fat droplets. Coalescence may result in quick creaming in dairy products, increased viscosity of cream, and transport of components of the milkfat globule membrane to the plasma phase due to a reduction in surface area of fat globules (Walstra, 1983). To increase stability to creaming, flocculation, and coalescence, heating and homogenization are necessary (Elling, 1995; Darling, 1982; Keenan et al., 1988; Mulder and Walstra, 1974).

Pasteurization. Heat treatment to cream and other dairy products is necessary to kill pathogenic microorganisms and increase the shelf-life. Heat treatment brings about changes in the structure of the milkfat globule surface material and stability of milkfat globules in reformulated creams (Elling, 1995).

Heat treatment causes changes in the proteins and phospholipids of the milkfat globule membrane (van Boekel and Walstra, 1995). Above 70^{o}C the major whey proteins, α-lactalbumin and β-lactoglobulin, tend to denature. In its denatured state, α-lactalbumin fails to engage in any heat induced reactions with other milk constituents until after drastic heating (Doi et al., 1983). β-lactoglobulin, unlike α-lactalbumin, is highly reactive upon heat denaturation (McKenzie et al., 1971). High reactivity is due to the

presence of an unpaired, reactive sulfydryl group which is activated by the protein's denaturation (van Boekel and Walstra, 1995). When the sulfydryl group is activated, β-lactoglobulin can react with k-casein of micellar casein and milkfat globules (Dalgleish and Banks, 1991; Houilihan et al., 1992). At temperatures above 70^{o}C, serum proteins such as κ-casein and β-lactoglobulin become incorporated into the milkfat globule membrane (van Boekel and Walstra, 1995; Dalgeish and Banks, 1991; Houlihan et al., 1992). Also during heating, phospholipids migrate from lipid globules to the aqueous phase (Radema, 1956; Koops and Tarassuk, 1959, Houlihan et al., 1992; van Boekel and Walstra, 1995). Heat may also cause phospholipids to hydrolyze (Nakanishi and Kaya, 1970).

Chemical changes induced by heat can have positive as well as negative implications on the physical stability of milkfat globules (van Boekel and Walstra, 1995). Since heating prevents cold agglutination of fat globules, creaming will be depressed. Cold agglutination occurs in cold raw milk. The phenomenon is a result of the immunoglobulin IgM adsorbing to fat globules, causing them to flocculate and rise to the top (Walstra, 1983). If a cream layer forms, however, it will be more compact. Partial coalescence is a potential as fat becomes more crystalline in structure (van Boekel and Walstra, 1995).

McPherson et al. (1984) analyzed the protein and phospholipid content of isolated milkfat globule membrane extract in homogenized and ultra-heat treated milk. Caseins and whey were major components of the milkfat globule surface material. Use of a SDS-PAGE on 15% acrylamide gel demonstrated that serum proteins, mainly caseins and whey proteins, were the main components of milkfat globule fractions obtained by centrifugation. Proteins and phospholipids associated with the native milkfat globule membrane were found in small amounts. The caseins, mainly α and β-casein, were incorporated in the low density protein material. Lower levels of κ-casein were present. Whey proteins were associated with high density material. Phospholipids in ultra-heat treated milkfat globule surface materials were much lower in comparison to lipid surface materials associated with other milks (McPherson et al., 1984).

Houlihan et al. (1992) found that heating raw milk at 80^{o}C for a time range of 2.5-20 min resulted in an increase in proteins and a loss of triglycerides. The increase in protein material can be attributed to the incorporation of skim milk proteins into the milkfat globule surface (Houlihan et al., 1992). The predominant serum protein associated with the milkfat globule interface was β-lactoglobulin. β-lactoglobulin predominated after 2.5 min, increasing as heating time progressed. Lower levels of κ-casein and α-lactalbumin were present. Like β-lactoglobulin, κ-casein increased in concentration at the lipid surface with a progression in heating time (Houlihan et al., 1992).

Kim and Jimenez-Flores (1995) observed the interactions between the proteins of the milkfat globule membrane and skim milk at 72^{o} and 87^{o}C. The temperature variables 72 and 87^{o}C were chosen in relation to pre-warming and pasteurization conditions. Whole milk samples were heated for 2.5, 5, 10, 20, 30, and 60 min. SDS-PAGE analysis was

used to separate the proteins. Approximate molecular masses were 150, 67, 62.5, 51, and 49 kDa for bands 1 thorough 5, respectively (Kim and Jimenez-Flores, 1995). At 72^{o}C, β-lactoglobulin and other serum proteins slightly reacted with the proteins bound at the milkfat globule interface. However, at 87^{o}C, serum proteins were very reactive at the milkfat globule surfaces (Kim and Jimenez-Flores, 1995).

Homogenization. Prevention of a cream plug is the main priority of homogenizing cream and other dairy products. Other reasons for homogenizing dairy creams include improving flavor and mouthfeel, increasing whitening power, and sometimes increasing viscosity (Kessler, 1981). In unhomogenized full cream milk, fat globules range from 2 to 10 μm in diameter. The homogenization process reduces fat globule size to 1 μm or less in diameter (McPherson et al., 1984). In most instances, milk is forced through homogenization valves, narrow slits, at 2500 psi (Keenan et al., 1988). The size distribution of the fat globules is determined by homogenization pressure, valve number and type, and flow rate (Brunner, 1974; Mulder and Walstra, 1974; Kurzhals, 1973; Walstra, 1975).

Dispersal of fat globules can be attributed to changes occurring in the milkfat globule membrane due to homogenization. The original milkfat globule membrane is partially disrupted by homogenization. Thus, the concentration of the original milkfat globule membrane fails to completely cover the increased surface area of the fat globules caused by homogenization (Cano-Ruiz and Richter, 1997). However, proteins from the skim phase of milk become adsorbed on the lipid surface so that interfacial tension decreases (Kessler, 1981). Thus, proteins from the milk serum, particularly casein proteins, become adsorbed onto globule surfaces (Keenan et al., 1988). As whey and casein proteins stabilize small fat globules, the density increases and retards the rising rate (Keenan et al., 1988).

Fat globule clustering in homogenized dairy products can be attributed to either high homogenization pressure or fat content (Kessler, 1981). Fat globule clustering in homogenized products results when at least two fat globules are bonded by a casein micelle, which is a simultaneously a component of each fat globules' surface layer (Walstra, 1983). If homogenization pressure and fat content are too high, the surface area of the homogenized fat globules becomes too large for sufficient coverage by surface active agents (Kessler, 1981). When homogenization pressure is too high, the fat globules leave the homogenizer insufficiently covered by protein (Walstra, 1983). Fat globules are not completely covered with protein because of the limited passage time (Walstra, 1983). Homogenization can be either a single-stage or two-stage process. In single-stage homogenization, viscosity of the product is greatly enhanced due to the formation of fat clusters (Kessler, 1981). Increases in viscosity is controlled by two-stage homogenization since fat globule clusters are broken apart during the second stage (Kessler, 1981).

Keenan et al. (1983) compared lipid globules from homogenized milk to globules from unprocessed milk. Lipid globule and skim milk phases were separated for comparison. Lipid globules from homogenized milk possessed more protein and less total lipid,

phospholipid, and cholesterol than globules from unprocessed milk. Total lipid and phospholipid recovery were equal to 70 and 57% in floating globules from homogenized milk, respectively (Keenan et al., 1983). In unprocessed milk, recovery of total lipids and phospholipids was 95-98% and 60-65% in lipid globules, respectively. Phospholipid distribution was similar when skim and lipid globule phases were compared in both homogenized and unprocessed raw milk. More casein micelles, at the expense of serum proteins, associated with the milkfat globule membrane of homogenized milk. Electron microscopy revealed that several casein micelles were in close relation with lipid globule surfaces (Keenan et al., 1983). Although homogenization resulted in loss of original membrane components, a large fraction of it remained intact with the surfaces of lipid globules (Keenan et al., 1983).

McPherson et al. (1984) determined the protein composition of milkfat globule surface material from homogenized milk. The gross composition of milkfat globule membrane material of homogenized milk was consistent with that observed in raw milk. However, homogenization had a decreasing effect on native milkfat globule membrane proteins due to disruption of the original membrane. Higher concentrations of casein and whey proteins from the serum phase were located at the milkfat globule membrane (McPherson et al., 1984). The major serum protein associated with the newly adsorbed surface material was β-lactoglobulin. Also, homogenization increased the neutral lipid content of the milkfat globule membrane (McPherson et al., 1984).

Cano-Ruiz and Richter (1997) analyzed the effect of high pressure homogenization on milkfat globule membrane proteins in 1.5 and 3.0% fat milk subjected to heat treatments of 65^{o}C for 30 min or 85^{o}C for 20 min. A positive correlation existed between protein load per surface area and homogenization pressure (Cano-Ruiz and Richter, 1997). At 30, 60, and 90 MPa, protein load values were 6.12, 9.79, and 11.88 mg/m^2, respectively. Protein load per surface area, however, decreased with heat treatment. Protein load values for 65 and 85^{o}C heat treatments were 9.69 and 8.84 mg/m^2, respectively. Fat concentration had little impact on protein load. For 1.5% milk, protein load was 9.24 mg/m^2 whereas 3.0% fat samples had a protein load value of 9.28mg/m^2. Proteins adsorbed as milkfat globule surface material were characterized as native membrane proteins, caseins, α-lactalbumin, and β-lactoglobulin (Cano-Ruiz and Richter, 1997). The majority of the proteins associated with the newly adsorbed surface material were caseins, which accounted for 70% of adsorbed proteins (Cano-Ruiz and Richter, 1997). In samples heated at 65^{o}C for 20 min, the ratios of adsorbed α-lactalbumin and β-lactoglobulin in comparison to caseins were lower than milk heated to 85^{o}C for 30 min (Cano-Ruiz and Richter, 1997). This finding is in contrast to β-lactoglobulin reported as the predominant protein occurring in homogenized milk by McPherson et al. (1984). At 65^{o}C, β-lactoglobulin and α-lactalbumin constituted 5.10 and 4.13% of proteins associated with the newly formed surface material (Cano-Ruiz and Richter, 1997). β-lactoglobulin and α-lactalbumin were 8.22 and 5.48% of milkfat globule surface proteins at 85^{o}C (Cano-Ruiz and Richter, 1997).

Definition and Physical Characteristics of Cream. Definition of cream. 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, 1998). The Code of Federal Regulations (1998) 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 ($\geq$ 36% fat).

The resultant physical characteristics of reformulated and natural creams are dependent on the stability of the emulsion. Several physical factors, including creaming stability, viscosity, and feathering have been investigated.

Creaming stability. A decrease in creaming stability is caused by alterations at fat globule surfaces (Yamauchi et al., 1982). In most instances, decreased creaming stability is noted by the formation of a thick cream plug on the surface of the milk product. Creaming stability is typically analyzed by measuring the depth of the cream plug and determining the percent of fat collected in the cream layer and the percent fat remaining in the bottom aqueous phase (Tornberg and Hermansson, 1977).

Yamauchi et al. (1982) analyzed the loss of creaming stability in ultra-heat-treated milk over a 12 month storage period. Samples were stirred and centrifuged at 2000 rotations per min and subsequently assessed a stability rating. The stability rating was a ratio of the fat content of sample aspirated from bottom of the sample to the initial fat content. After 1 month of storage, a cream plug on the surface of the UHT milk was visible (Yamauchi et al., 1982). As time progressed, the stability rating decreased, indicating that the milkfat globule membrane disintegrates under extended storage conditions and fat globule clustering was promoted (Yamauchi et al., 1982). Proteins were analyzed by extraction with 0.1M NaCl and 5M urea solutions and characterized as 1). Proteins loosely attached to the milkfat globule membrane by weak interactions; 2). proteins having fairly strong interactions with fat globules; and 3). proteins tightly adsorbed on the surfaces of milkfat globules (Yamauchi et al., 1982). During the 12 month storage period, concentration of loosely-bound proteins decreased. Up to the first 3 months of storage, concentration of tightly adsorbed proteins increased (Yamauchi et al., 1982). SDS-PAGE revealed that proteins adsorbed on the lipid surface were composed mostly of whey and casein proteins (Yamauchi et al., 1982).

Melsen and Walstra (1989) created stable emulsions by recombining skim milk and anhydrous milkfat, with variations in globule size distribution. Emulsions were also treated with phospholipids to analyze effects on stability. Both cream and skim milk were obtained by centrifugation of fresh milk at 40^{o}C. Commercial samples of egg and soy lecithin were used to determine the effects of phospholipids. Cream was recombined by mixing milkfat and skim milk at 50^{o}C in an inlet vessel of a laboratory homogenizing system. Recombined cream was more stable to coalescence and clumping than natural cream. Partial coalescence was observed in recombined fat globules only when the average globule diameter exceeded 6 μm. Also, phospholipids associated with fat globules did not interfere with proteins interacting with the fat globule surface (Melsen

and Walstra, 1989). Phospholipids also did not influence the coalescence stability of emulsions. An abundance of phospholipids, however, led to a decrease in stability (Melsen and Walstra, 1989).

Elling and Duncan (1996) determined the creaming stability of 20% milkfat reformulated creams by measuring the fat contents of the top and bottom layers of the creams at two day intervals for two weeks. All formulated creams homogenized at 13.6/3.4 MPa exhibited a significant decrease in creaming stability compared to natural creams. Further, a greater loss in creaming stability was observed for all formulations homogenized at 10.2/3.4 MPa. A decrease in creaming stability was noted by an increase in fat content in the top layer and decrease in fat content of the bottom layer of formulated creams, which was evident by day 7 of the analysis (Elling and Duncan, 1996).

Viscosity. According to definition, viscosity is resistance to flow due to internal friction between molecules of a particular substance as they shear each other (Atherton and Newlander, 1977). Viscosity can also be defined by the equation, $\eta = F/(dv/dx)$ in which η is the coefficient of viscosity, F is the force in dynes cm^{-2} required to maintain a unit velocity gradient between two parallel planes separated by unit distance, and dv/dx is the velocity gradient in sec^{-1} perpendicular to the planes (Sherbon, 1988). Newtonian fluids have viscosity coefficients which are dependent only on temperature and pressure, but independent of shear rate (Sherbon, 1988). Cream is considered a Newtonian fluid only when shear rate is moderate, milkfat content is lower than 40%, and temperatures exceed $40^{o}C$ (Fox and McSweeney, 1998). At temperatures above $40^{o}C$ milkfat is liquid, thus cold agglutination does not occur.

Non-Newtonian characteristics of cream are manifested when the fat content of cream is increased and/or temperature is lowered below $40^{o}C$ (Fox and McSweeney, 1998). Non-Newtonian fluids are those fluids in which their apparent viscosity is inversely related to shear rate. This phenomenon is referred to as shear thinning or thixotropic behavior. Non-Newtonian fluids also display hysteresis in which the coefficient of viscosity is dependent on increases and decreases in shear rate (Sherbon, 1988). Colloidal dispersions such as cream consist of aggregate particles that have a large effective volume because of irregularities in shape (Fox and McSweeney, 1998). As shear rate increases, increased shear stress is applied to aggregates (Fox and McSweeney, 1998). The result is dispersal of smaller and more round forms of the aggregates. This particular process decreases the interstitial space between fat globules, resulting in both a lower total volume fraction of the fat phase and apparent viscosity (Fox and McSweeney, 1998). When shear rate is high, however, turbulence is possible, resulting in a rise in viscosity (Walstra and Jenness, 1984).
Fat content, temperature, storage, and processing conditions are factors which are most critical in influencing the viscosity of cream (Elling and Duncan, 1996; Phipps, 1982; Langley, 1984; Prentice, 1992). There is a direct relationship between the fat content of cream and its apparent viscosity. As the fat content of cream increases, the apparent viscosity increases (Prentice, 1972). Increasing the fat content of cream results in a decrease in the distance between fat globules. Reduction in space between fat globules

promotes an increase in the hindering effect among fat globules (Prentice, 1972). Partial coalescence of fat globules in high fat creams can cause an increase in apparent viscosity since milk serum is entrapped in aggregates and irregular shapes form (Fox and McSweeney, 1998).

Reducing temperature promotes non-Newtonian behavior of cream. Low storage temperatures enhance cold agglutination of fat globules, thus increasing the apparent viscosity of cream (Fox and McSweeney, 1998). As temperature decreases, the forces between fat globules become stronger, causing higher apparent viscosity values (Walstra and Jenness, 1984).

Heat treatment also has an impact on the viscosity of cream and other dairy products. Overall, increasing heat treatment has a decreasing effect on viscosity (Prentice, 1992). Above approximately 65-70^{o}C, denaturation of β-lactoglobulin becomes irreversible by a series of 3 types of aggregation steps (Fox and McSweeney, 1998). In type I aggregation intermolecular disulphide bonds are formed. Type II aggregation is accompanied by a series of non-specific interactions, mostly hydrophobic and electrostatic bonding. The final stage, Type III aggregation, occurs when sulphydryl groups become blocked. Above 75^{o}C, disulphide-linked interactions are possible between β-lactoglobulin and κ-casein. In the literal sense, casein proteins do not undergo denaturation reactions like their globular, whey protein counterparts. When temperature increases above ambient temperature, the hydration shell of casein micelles tend to decrease. Also, the voluminosity of casein micelles falls from approximately 3 at 20^{o}C to 2 at 35^{o}C (Prentice, 1992). If temperature continues to increase, casein micelles may even disintegrate. Severe heat treatment, however, results in dephosphoylation, aggregation, and formation of disulphide complexes with other proteins (Fox and McSweeney, 1998). When cream is separated above 40^{o}C, it demonstrates more Newtonian behavior. Immunoglobulins, which are responsible for cold agglutination, are lost in the skim milk (Fox and McSweeney, 1998). As a result, less cold agglutination occurs. Most of the changes associated with heat treatment occur at pasteurization temperature. In considering pasteurization, low-temperature-long-time pasteurization has a greater influence on viscosity than high-temperature-short time pasteurization due to its longer holding time (Prentice, 1992).

Homogenization increases the viscosity of cream. As fat globules are passed through the homogenizer, they diminish in size. The milkfat globule membrane surrounding the fat globules is disrupted, causing caseins to adsorb on the fat globule surfaces. The casein subunits are highly hydrated and have diameters that are approximately 10 nm (Prentice, 1992). Therefore, the casein adsorption to the fat globules has an increasing effect on the viscosity of cream (Prentice, 1992). Homogenization, particularly at high pressures, results in the formation of homogenization clusters. Homogenization clusters are controlled by the use of two-stage homogenization processing (Kessler, 1981).

Langley (1984) determined the effects of shear rate, fat content, homogenization pressure, and heat treatment on the viscosity of processed cream stored at 5^{o}C. All processed creams displayed shear thinning, a decrease in apparent viscosity as a result of

increasing shear rate. Increasing fat content (20, 40, and 50%) and homogenization pressure (0, 1000, and 2000 psi) caused the viscosity to rise (Langley, 1984). Cream heated to 125^{o}C had a lower viscosity than cream heated to only 74^{o}C. Also, as storage time progressed, viscosity of all creams steadily increased (Langley, 1984).

Kanno et al. (1991) analyzed the effects of emulsifying time (0-6 min.), milkfat globule membrane concentration (20, 40, 60, and 80 mg/g fat), and pH (4-7) on the viscosity of reconstituted milkfat emulsions. Increasing the emulsifying time had an increasing effect on the viscosity of reconstituted milkfat emulsions. Increase in viscosity was apparent at an emulsification time of 4 min and higher. A similar pattern was observed with milkfat globule membrane concentration. As the milkfat globule membrane concentration increased, the viscosity increased. The increase in viscosity observed with increased milkfat globule membrane concentration can be attributed to the increase of protein adsorption on the membrane (Kanno et al., 1991). Viscosity was highest at pH 5 and 4, decreasing at pH values 6 and 7 (Kanno et al., 1991).

Elling and Duncan (1996) determined the viscosities of reformulated creams during a 13 day storage period. In comparison to formulated creams, control creams were less viscous at all stages of the study. On days of one and seven, formulations consisting of skim component or sweet buttermilk and butter-derived aqueous phase processed at 13.6/3.4 MPa were statistically comparable to the control. By day 13 of 4^{o}C storage, cream consisting of sweet buttermilk and butter-derived aqueous phase was the only formulation that was not statistically different from the control cream. The formation of homogenization clusters in formulated creams was suspected as a reason for the differences in viscosities between control and reformulated creams. Creams homogenized at 10.2/3.4 MPa were not significantly different in viscosity from those homogenized at 13.6/3.4 MPa (Elling and Duncan, 1996).

Feathering. Feathering is a common quality defect which occurs in coffee. Feathering is the tendency of particulate precipitate to form upon the addition of cream to coffee (Towler, 1982). Homogenized creams are susceptible to feathering because newly adsorbed protein comprising the new membrane destabilizes and flocculate together with fat globules in hot, acidic coffee (Geyer and Kessler, 1989a). The proteins which associate with fat globules as a result of homogenization are whole casein micelles, submicelles, and some whey proteins (Geyer and Kessler, 1989a). Fewer whey proteins associate with the fat globules and their attachment is less strong in comparison to casein's attachment (Geyer and Kessler, 1989b). Unhomogenized creams are less susceptible to feathering because the protein material is very small, causing feathering which is undetectable by the human eye (Geyer and Kessler, 1989b).

Coffee has an impact on cream susceptibility to feathering. The acid content of coffee is the primary contributing factor to cream feathering (Geyer and Kessler, 1989b). The acidity of coffee is affected by the type of coffee, processing procedure, and quality of water used during used to prepare the coffee (Geyer and Kessler, 1989b). The high temperature range of 60-80^{o}C also increases the incidence of cream feathering in coffee

(Geyer and Kessler, 1989b). Therefore, the combination of acid pH and high temperature cause cream feathering to occur.

Excess calcium salts, high homogenization pressures with low temperatures, single stage homogenization, and high ratio of fat to serum solids are also linked to feathering in coffee. Resistance to feathering can be improved by use of two-stage homogenization, addition of calcium sequestering agents, and use of additives such as sodium phosphate or sodium citrate (Towler, 1982).

Anderson et al. (1977) determined the effect of a 10 week storage period on feathering and composition of UHT treated 18% milkfat cream. Acetate buffering systems with pH values ranging from 4.7 to 5.6 were used to substitute for coffee solutions. When creams were stored for the same amount of time, feathering pH range was 4.70-5.20 (Anderson et al., 1977). As storage period progressed, the incidence of feathering increased. Also, the amount of casein and calcium surrounding the fat globules increased over the 10 week storage period causing the incidence of feathering to increase (Anderson et al., 1977).

Elling and Duncan (1996) observed the extent of feathering of reformulated cholesterol reduced creams in sodium acetate buffers at various pH values. Formulation, homogenization, and length of storage period had a very little effect on the extent of feathering in cream, suggesting the reformulated creams were stable to variations in pH (Elling and Duncan, 1996). Feathering typically occurred from pH 4.86-5.09 (Elling and Duncan, 1996).

Sensory quality. Very little research has been conducted on the sensory characteristics of cream. Cream is typically evaluated on flavor and body and texture characteristics (Prentice, 1992). The ideal cream should possess a pleasing, clean, slightly sweet, and slightly cooked flavor (Jensen and Poulsen, 1992). The potential for flavor defects is high since cream has such a bland taste (Jensen and Poulsen, 1992).

The body of cream should be smooth even though the fat content is quite high (Jensen and Poulsen,1992; Prentice, 1992). Good texture, a sign of emulsion stability, is indicated by homogeneous liquid-like characteristics free from graininess, grittiness, and lumps (Jensen and Poulsen,1992; Prentice, 1992). The factors which affect emulsion stability are size distribution of fat globules, seasonal variations, processing conditions, and storage temperature (Jensen and Poulsen, 1992).

Fat lumps, cream plugs, and fat deposition are indicative of poor emulsion stability (Jensen and Poulsen, 1992). These particular quality defects are a result of damage to fat globule membranes by mechanical or thermal means during processing and storage (Jensen and Poulsen, 1992). When the milkfat globule membrane is damaged by mechanical processing, such as homogenization, fat leaks out and forms clusters with other fat globules (Jensen and Poulsen, 1992). Storage temperature also has an influence on emulsion stability. High temperatures, typically between 10 and 13^{o}C, have two

detrimental effects on emulsion stability. Storing cream within this temperature range enhances fat deposition and increases firmness in cream layers (Eibel and Kessler, 1986).

Off-flavors in cream are similar to those which are common in milk. Typical off-flavors in cream are categorized as microbial, lipolyzed, oxidized, light-induced, and heated flavors (Jensen and Poulsen, 1992). Other potential off-flavors include feed, foreign, lacks freshness, and flat (Jensen and Poulsen, 1992).

Since cream contains a high fat percentage, lipolyzed and oxidized flavor development is of great concern. Lipolyzed flavors are caused by naturally occurring milk lipases or microbial lipases, which hydrolyze milkfat triglycerides (Jensen and Poulsen, 1992). As a result, short chain fatty acids such as butyric and caproic acid are released. The flavor is commonly referred to as rancid. Oxidation of unsaturated fatty acids in milkfat results in oxidized flavor. In lipid oxidation reactions, lipids react with oxygen to yield unstable hydroperoxides (Jensen and Poulsen, 1992). Subsequently, the hydroperoxides decompose to volatile carbonyl compounds. The off-flavor is often described as cardboardy or tallowy.

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