# CHAPTER I

# INTRODUCTION

Coronary heart disease is the leading cause of death in America (American Heart Association, 2000b) and most industrialized countries (Gurr, 1995). In 1997, cardiovascular diseases were responsible for 41.2 % of all deaths in the US. The most common cardiovascular diseases in America are high blood pressure, coronary heart disease, stroke and rheumatic fever. Coronary heart disease is the largest killer of American women (American Heart Association, 2000c).

Thickening of the coronary arteries, known as atherosclerosis, causes a form of coronary heart disease. The thickening of arteries occurs by deposits of fatty substances, low-density lipoprotein cholesterol (LDL), calcium and other substances in the arteries. Elevated levels of cholesterol and triglycerides in blood are major causes of atherosclerosis. Cholesterol, present in all animal products (meat, dairy products, eggs), is used in the human body for production of hormones, membranes and tissues. There are two main forms of plasma cholesterol: LDL (low-density lipoprotein), which carries cholesterol to the peripheral tissues, and HDL (high density lipoprotein), which carries cholesterol away from the peripheral tissues back to the liver (American Heart Association, 2000a). High levels of cholesterol in the blood are associated with enhanced risk of heart attack. Cholesterol is produced by the human body and the amount in blood and tissues can be increased by consuming high cholesterol foods. Foods rich in saturated fatty acids also have been implicated in raising serum cholesterol concentrations (Grundy, 1997).

The American Heart Association recommends an intake of saturated fats at 7-10 % of total daily calories. They recommend the consumption of polyunsaturated fats (vegetable oils and low trans fatty acid margarines) instead of saturated fats like butter or lard, which are classified as atherogenic foods (foods that cause Atherosclerosis) (American Heart Association, 2000d).

Consumption of milk and some dairy products (butter, ice cream and whole milk cheeses) have been related to coronary heart disease due to the content of saturated fatty acids and cholesterol. Dairy products contribute approximately 25-29% of the dietary saturated fat intake in western countries and 40% in countries such as Australia (Noakes et al., 1996).

Per capita consumption of whole milk, butter and ice cream has declined between 1970 and 1996 (Nayga et al., 1999). For instance, the per capita consumption (milk equivalent pounds per person) of butter has decreased from 117.2 (1970) to 93.74 (1996). Ice cream also decreased from 75.17 (1970) to 69.42 (1996). This reduction might be due to the publicity campaign that relates dairy products to heart diseases as well as development and increased consumption of low fat dairy products (Nayga et al., 1999; Kaylegian and Lindsay, 1995) and margarines (Keogh, 1995). In contrast, a 75% increase has been seen in the consumption of foods containing high levels of polyunsaturated fatty acids (PUFA), possibly due to the capacity of PUFA for lowering cholesterol levels as compared to saturated fatty acids (Newton, 1996). Milkfat is composed of 98% triglycerides, from which 66% are saturated fatty acids, 30% monounsaturated fatty acids and 4% polyunsaturated fatty acids (Lin et al., 1996a).

Modification of the fatty acid profile in milkfat to decrease the saturated fatty acid content, by increasing the polyunsaturated fatty acid content, has been one of the major research focuses in the dairy industry. Different techniques such as dietary manipulation of the cows, cholesterol removal from milk, fractionation of milkfat, and cream tempering (Kaylegian and Lindsay, 1995) are used not only to modify the fatty acid content, but to modify physical and chemical properties of the milkfat or other type of fat or oil and consequently, the final product.

Chemical and physical properties of milkfat that are modified through dietary manipulation of the cow could increase consumer acceptability of high unsaturated products. For instance, cold butter is known to have poor spreadability when refrigerated (Kaylegian and Lindsay, 1995). It has been shown that butter texture (spreadability) changes to a softer texture due to a higher content of unsaturated fatty acids (Baer et al., 2001; Stegeman et al., 1992). Other changes that can be observed in milkfat when the fatty acid profile is modified to a more unsaturated type include color change, flavor, increased oxidation rate, texture, and crystallization, among others (Kaylegian and Lindsay, 1995).

The objectives of this research were to study oxidation, texture and sensory perception of butter and ice cream made with milkfat with an enhanced content of linoleic acid or milkfat with an enhanced content of oleic acid. Chemical and physical characteristics were measured on butter and ice cream. Sensory characteristics were also measured in ice cream.

# CHAPTER II

# REVIEW OF LITERATURE

Milkfat, when secreted from the mammary gland, exists as globules. These globules have an inner composition of 98% triglycerides surrounded by a lipid membrane composed of phospholipids, mono and diglycerides, cholesterol, other sterols, carotenoids, vitamin A and other components (Kaylegian and Lindsay, 1995; Keenan and Dylewski, 1995). Triglycerides are composed of fatty acids, distributed along a glyceral backbone in different positions. Fatty acid composition and positioning influence milkfat functionality. The fatty acid composition of milkfat depends primarily on genetic, environmental and dietary factors. There are two main types of fatty acids: saturated fatty acids, which account for approximately 66% of total fatty acids in milk, and unsaturated fatty acids, which represent 30% of the total (Kaylegian and Lindsay, 1995).

## Saturated Fatty Acids

Saturated fatty acids have single bonds along the carbon chain; they can be divided into groups based on fatty acid chain length. These groups are often classified as: short (C4-C10), medium (C12- C16) and long ( 18) chain fatty acids. Short -chain fatty acids represent approximately 10.6- 12.8 wt% of total fatty acids in milk fat. Medium-chain fatty acids represent 42.4 - 44.0 wt% of total fatty acids (Molkentin, 1999). Palmitic (C16:0) and myristic acid (C14:0) account for the majority of the saturated fatty acids in milkfat. These two medium-chain fatty acids and lauric acid (C12:0) have been associated with increased total cholesterol and LDL-cholesterol in plasma (Grundy, 1997). Stearic acid (C18:0), a long-chain saturated fatty acid (9.2-12.6 wt%) in milk fat appears not to have a major influence in increasing LDL-cholesterol (Molkentin, 1999).

## Polyunsaturated Fatty Acids (PUFAs)

Unsaturated fatty acids are classified depending on the number and position of double bonds present in the carbon chain. In standard IUPAC terminology, the position of the double bond is identified by the number of the carbon, counting from the caroboxyl end of the carbon chain. For example, linoleic acid is an 18 carbon fatty acid with two double bonds at positions

9 and 12. An “omega” shorthand notation is often used which counts from the methyl end. Hence linoleic acid is 18:2 w6 (deMan, 1999; Fennema, 1996).

In milk products, the most common polyunsaturated fatty acid is linoleic acid (C18: 2 w-6) which accounts for only 1.5-2.0 wt% of the total fatty acids (Molkentin, 1999). In contrast, major dietary sources of linoleic acid are corn (57.0%), soybean (53.2%) and safflower oils (77.7%) (Chow, 2000). Linoleic acid is an essential fatty acid in the diet because it is a precursor of arachidonic acid (20:4 w-6) (Newton, 1996). Research has proven that linoleic acid has a cholesterol lowering capacity when compared to saturated fatty acids and to a less extent, oleic acid (Grundy, 1997).

α-Linolenic acid (C18: 3 w-3) is another PUFA present in milk fat. α-Linolenic acid has the same cholesterol lowering effect as linoleic acid but represents only 0.5-0.7-wt% of the total fatty acids in milk (Molkentin, 1999). This PUFA is found in high concentrations in linseed oil (52.7%) and canola oil (9.2%) (Chow, 2000). α-Linolenic acid has been shown to have cardioprotective effects, antithrombotic effect and anti-inflammatory effects (Connor, 1999).

Both linoleic and α-linolenic acid are precursors of essential fatty acids (20 and 22-carbon w-6 and 20-22 carbon w-3 long-chain fatty acids respectively), which also produce eicosanoids and leukotrienes. Eicaosanoids and leukotrienes are related to cardiovascular, renal and immune functions. Therefore, the consumption of linoleic and α-linolenic acids is essential for health maintenance (Newton, 1996).

On the other hand, recent studies (Grundy, 1997) have demonstrated that linoleic acid, although it has some beneficial effect on lowering cholesterol, might have a relationship in producing malignant tumors (Molkentin, 1999), arthritis, inflammation and immune functions, when consumed in high proportions. The minimum requirement for linoleic acid, in order to avoid deficiency is 1.5% of the dietary calories but a high consumption of linoleic acid (10-15 g per day) has been observed in the United States and possibly implicated in incidence of other diseases such as cancer and diabetes mellitus (Berry, 1997).

Linoleic acid also has shown to cause a small decrease in HDL-cholesterol and to have a higher tendency to produce oxidation of membrane phospholipids, as well as LDL oxidation. These cytotoxic compounds could cause tumor growth, cancer and accelerated aging (Grundy, 1997; Molkentin, 1999). N-3 fatty acids, as opposed to n-6 fatty acids, show protective properties against tumor production (Molkentin, 1999).

In contrast to the health concerns of high linoleic acid consumption, oleic acid (18:1 n-9) has been shown to have some beneficial properties such as lowering LDL- cholesterol, though at a lower rate when compared to linoleic acid concentrations.

**Oleic Acid (Monounsaturated Fatty Acids) (MUFA)**

Oleic acid is a monounsaturated fatty acid, present in milk fat at 20.1-20.8 wt% of the total fatty acids (Molkentin, 1999). Oleic acid has been shown to be more stable to oxidation as compared to linoleic acid (Noakes et a., 1996) and does not affect HDL-cholesterol levels significantly, as compared to linoleic acid (Aigster et al., 2000;Molkentin, 1999).

Recent research on the beneficial ratios between monounsaturated fatty acids and polyunsaturated fatty acids established that no more than 30% of the total energy consumptions should come from fats (Grundy, 1997) and that less than 10% should be from saturated fats (O'Byrne et al., 1998). The American Heart Association recommends, therefore, an intake of about 15-16% of oleic acid as the fat source (Grundy, 1997). This would lead to a more monounsaturated type of diet rather than a polyunsaturated one.

**Trans Fatty Acids**

Trans fatty acids are produced by partial hydrogenation of polyunsaturated fatty acids by rumen bacteria (Molkentin, 1999). The most common trans fatty acid in milk products is trans-octadecenoic acid (trans-C18: 1) which has been related to increased LDL-cholesterol levels and decreased HDL-cholesterol levels in plasma.

On the other hand, another trans fatty acid, known as the isomer C18:2 9 cis 11trans (rumenic acid) is a conjugated linoleic acid (CLA) (Bessa et al., 2000) from the group of conjugated dienes. The term "CLA" refers to the group of conjugated dienoic fatty acids, but C18:2 9 cis 11trans (rumenic acid) is considered to be the active constituent of this group of isomers (Adlof, 1999). This isomer is the principal CLA isomer in dairy fat (Pariza, 1999) and has been shown to have beneficial health properties such as anticarcinogenic, antiatherogenic, inmunomodulating, growth-promoting, and antidiabetic properties (Parodi, 1999). The isomer C18:2 10 trans 12 cis, CLA, has shown to have not only anticarcinogenic properties, but

influence in lipid metabolism and changes in body compostion (Pariza,1999). Ruminant animals produce CLA as an intermediate in the biohydrogenation of dietary linoleic acid (Figure I-1) by an enzyme from the bacteria *Butyrivibrio fibrisolvens,* which is present in rumen (Parodi, 1996). CLA is found primarily in milk fat but its percentage depends on the biohydrogenation intermediates. These intermediates depend, at the same time, on the source of fatty acids from pasture and nitrogen content in the cow diet. CLA average content in milk varies between 0.3 and 0.6% of total fatty acids (Dhiman et al., 2000). It is also found in similar amounts in meat (lamb, beef) and fish (Fritsche and Steinhart, 1998). Several studies (Baer et al., 2001; Lawless et al., 1998) suggest that the daily human intake of CLA that may prevent cancer should be 3 g. Baer et al. (2001) suggests even lower concentrations (1.33 g/day) after taking into consideration transformation of transvaccenic acid (18:1  11 trans)  to CLA in human body.. Ritzenthaler et al. (2001) estimated that the rumenic acid (CLA C18:2  9 cis  11trans ) human intake that could deliver the same anticarcinogenic properties as in animal studies (Ip et al., 1999) is around 620 and 441 mg/d for men and women, respectively (Ritzenthaler et al., 2001). According to some current studies concerning CLA intake conducted by Herbein.J  (personal comunication) in the Dairy Science Department of Virginia Tech, the CLA intake based on a 2500 Kcal human diet should be around 625 mg/day to have similar anticarcinogenic properties shown in animal models by Ip et al. (1999). Further studies are being done to determine a specific concentration that would impact human health Ritzenthaler et al. (2001) also estimates that the common rumenic acid daily intake in U.S. men is around 150 mg/day.

Recent studies have shown that the presence of transvaccenic acid (18:1  11 trans (TVA)), which is the second product from biohydrogenation of linoleic acid (Figure 2-1) increases the content of CLA available in the mouse body, once TVA has been ingested (Baer et al., 2001; Bessa et al., 2000). TVA is converted to C18:2  9 cis  11trans, CLA, by action of the  9 - desaturase enzyme (Ip et al., 1999). Further investigation should be done to determine if transvaccenic acid could help synthesize CLA in the human body (Bessa et al., 2000).

**Modification of Fatty Acids in MilkFat**

Milk fat is not only a source of essential fatty acids and vitamins, but it also delivers flavor and rheological properties to dairy products (Fox and McSweeney, 1998). The fatty acid composition of milk fat is one of the most important chemical characteristics and can influence milk functionality (Kaylegian and Lindsay, 1995).

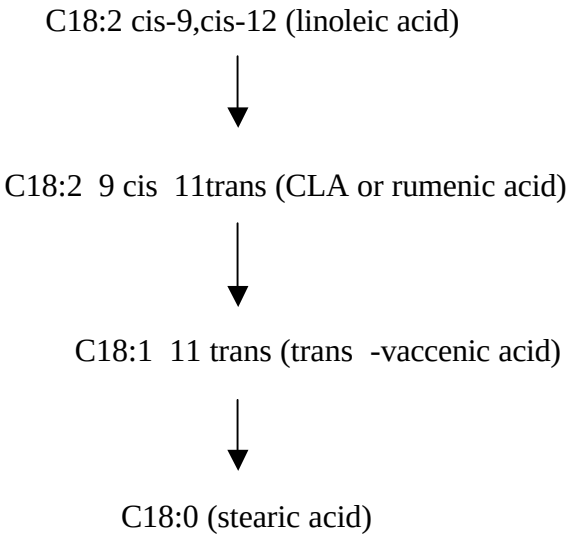


Figure 2- 1. Linoleic acid biohydrogenation (Bessa et al., 2000)

Fatty acid composition and physical properties of the milkfat can be varied by stage of lactation of the cow, seasonal variation, breed, (Kaylegian and Lindsay, 1995) and feeding regimen of the cow (fiber content, proportion of concentrate to forage, degradability of starch and the rumen inertness and digestibility of the fat supplement) (Ashes et al., 1997).

***Influence of Bovine Diet Manipulation***. Bovine dietary manipulation has been studied in recent decades (Ashes et al., 1997) to obtain milk with a healthier fatty acid composition and similar physicochemical and sensory properties of traditional milk. Fatty acids in milkfat can be originated in two ways:

- Mammary gland synthesis: the fatty acids produced are known as de novo fatty acids. These are the short and medium-chain fatty acids (C4- C14 and some C16 fatty acids) (Lin et al., 1996).
- Fatty acids added through the diet: long chain fatty acids are transferred to the mammary gland from the food. Around 90% of the long chain fatty acids are produced this way (Lin et al., 1996).

In order to modify the profile of milkfat to contain less saturated and more unsaturated fatty acids, fats and oils are added to the cow's diet (Hawke and Taylor, 1995). The nutritional supplement has to fulfill protein and energy demand also (Ashes et al., 1997). When oils are added directly to the diet, it is expected that medium-chain fatty acids will be decreased while long chain fatty acids, specifically C18:0 and C18: 1, will increase in milk. Polyunsaturated fatty acids can increase but moderately, compared to C18:0. The increase levels of specific fatty acids in milk will depend on the type of supplement that has been added. If a specific fatty acid is added to the diet, then its content will increase in milk (Hawke and Taylor, 1995).

Different types of fat and oils, as supplements, have been developed and studied in order to determine the effect on diet and final fatty acid profile. Some of the different supplements include plant oils, fish oils, free fatty acids, whole oilseeds, calcium soaps of fatty acids and protected fat (Hawke and Taylor, 1995). Other preharvest techniques that could change fatty acid profiles in milkfat include abomasal infusions, manipulation of the rumen fermentation (Ashes et al., 1997; Chouinard, 1999), and genetic manipulation of the dairy cow. Postharvest techniques include milkfat fractionation, cholesterol removal of milk, cream tempering, hydrogenation and interesterification (in other type of oils) (Kaylegian and Lindsay, 1995).

Recent studies have focused on increasing the CLA level in milkfat due to its anticarcinogenic effect (Baer et al., 2001; Chouinard, 1999; Dhiman et al, 2000; Lawless et al., 1998). Recently the relationship of transvaccencic acid (trans-11 C18:1) with CLA synthesis and its increase in milkfat has been studied through feeding techniques (Baer et al., 2001). Other studies are determining which fat supplement protection method is more efficient in reducing biohydrogenation in the rumen and further trans fatty acid production (Bayourthe et al., 2000; Bessa et al., 2000; DeLuca and Jenkins, 2000; Dhiman et al., 1999 a; Dhiman et al., 2000; Jenkins, 1998; Lawless et al., 1998; Lin et al., 1996). One of the major objectives of these investigations is to know how to manipulate biohydrogenation in order to lower trans fatty acid production but increase CLA production (lower trans C18:1/CLA ratio) (Bessa et al., 2000). Different protection methods to avoid biohydrogenation have been developed and are currently under study. Extruded, exploded, crushed chemically treated oilseeds, calcium salts of long-chain fatty acids, formaldehyde-treated oils, and, most recently, the use butylamides such as oleamide (a butylamide that is resistant to biohydrogenation) have been utilized as fat supplements (Ashes et al., 1997; Bayourthe et al., 2000; DeLuca and Jenkins, 2000; Jenkins,

1998). Other factors that have to be taken into consideration, when choosing a supplement and determining the diet are ratio of forage to concentrate, lactation stage and season (Hawke and Taylor, 1995).

When the fatty acid composition is changed, chemical and physical properties of the mikfat are consequently changed. Milkfat functionality is the interaction between the physical and chemical properties of the fat. Diet modification, resulting in a subsequent change in fatty acid profile, and processing are some of the factors that influence milkfat functionality (Kaylegian and Lindsay, 1995).

**Chemical Properties Of Milk Fat**

***Fatty Acid Composition***. As mentioned earlier, the fatty acid composition of milk fat is very complex and influences several chemical and physical properties. The number of double bonds in fatty acids influences melting behavior and oxidative stability of the fat while distribution of the fatty acids in the triglyceride structure influences crystallization behavior and nutritional aspects (Kaylegian and Lindsay, 1995).

Around 400 fatty acids have been identified in milkfat but only 10 influence physical properties (C4, C6, C8, C10, C12, C14, C16, C18, C18:1(cis) and C18:1 (trans)) (Spreer, 1998). The position of the fatty acids in the triglyceride is important too, since this also influences physical properties. For instance, in milkfat C16:0 is commonly found in positions sn-1 and sn-2, while C18:1 is predominantly found in sn-1 and sn-3. When an increase in C18:1 or C18:0 (sn-1 position) is produced, a reduction in C16:0 is obtained and more C18:1 in position sn-3 is found (Hawke and Taylor, 1995). High molecular weight triglycerides are those that have three long-chain fatty acids on the glycerol backbone, while low molecular weight triglycerides have 1 short-chain and 2 long-chain fatty acids (Hawke and Taylor, 1995). The amount of high or low molecular triglycerides and the presence of unsaturated fatty acids will affect the melting point of the fat.

Chemical reactions affected by the fatty acid profile are lipid oxidation and subsequent off-flavor production, as well as the physical properties of milkfat, crystallization behavior and melting behavior, among others (Hawke and Taylor, 1995; Kaylegian and Lindsay, 1995).

***Lipid Oxidation.*** Lipid oxidation is a chemical reaction which involves the reaction of oxygen with free radicals, originated from unsaturated fatty acids, to yield off flavors and odors in lipids and fatty foods (deMan, 1999). This reaction is a critical quality control point in the

production and preservation of several food products due to its relationship with food deterioration and spoilage. Different types of lipid oxidation include photoxidation, thermal oxidation and enzymatic oxidation (hydrolytic rancidity in dairy products) (deMan, 1999) but the autoxidation of unsaturated fatty acids is the most common mechanism (Labuza, 1975).

Oxidation of lipids is influenced by the presence of oxygen, light, metals (copper, iron), salts of fatty acids, enzymes, temperature, pH, water activity, fatty acid composition, microbiological population, homogenization and fermentation (O'Connor and O'Brien, 1995).

The main steps of autooxidation are: initiation, propagation and termination (Figure 2-2). Initiation is the production of a free radical (R*) that occurs by extracting a hydrogen from the methylene group adjacent to the double bond in an unsaturated fatty acid. This reaction can be initiated by factors such as active oxygen species (produced by light exposure), metals, irradiation and enzymes (O'Connor and O'Brien, 1995; deMan, 1999). Propagation follows initiation and involves a cyclic reaction in which hydroperoxides are formed. The free radical, produced in the initiation step, reacts with oxygen to produce a peroxi-free radical ($RO*_2$). This free radical extracts another hydrogen from the unsaturated molecule (O'Connor and O'Brien, 1995) and produces a hydroperoxide (ROOH) and another free radical to start the cycle again. Hydroperoxides are the primary oxidation products (deMan, 1999) of this reaction. They are not related to direct flavor transmission to the product but are very unstable and are the precursors of secondary and tertiary products such as aldehydes, alkanols, hydrocarbons (alkanes, alkenes, etc) that are responsible for different off-flavors and odors. The production of peroxides mediates the oxidation reaction but peroxide stability will determine the development of oxidative rancidity (Fox and McSweeney, 1998). Oxidative rancidity is referred, under these terms, as the group of off-flavors which are caused by the autoxidation of unsaturated fatty acids and that are detectable in advanced stages of autoxidation (Hoffman, 1962). The autoxidation cycle can be terminated by the formation of stable non-radical components by the reaction of the free radicals with themselves. An interruption of the free radical chain takes place (Badings, 1970; deMan, 1999).

The initial stages of autooxidation can be measured by the peroxide value which detects the amount of peroxides produced. Peroxides increase slowly at initial stages but, at the end of the induction period, the concentration of peroxides increases rapidly and then declines to almost zero (Labuza, 1975) (Figure 2-3) as they are decomposed to secondary products.

Secondary products can be determined organoleptically or by other analytical methods such as anisidine value, Kreis tests, Thiobarbituric acid test, and others (deMan, 1999). According to some authors (Rossell 1989; Hoffmann, 1962), fats should have a peroxide value of less than 1 meq/Kg to be considered fresh. At this level no off-flavor can be perceived in

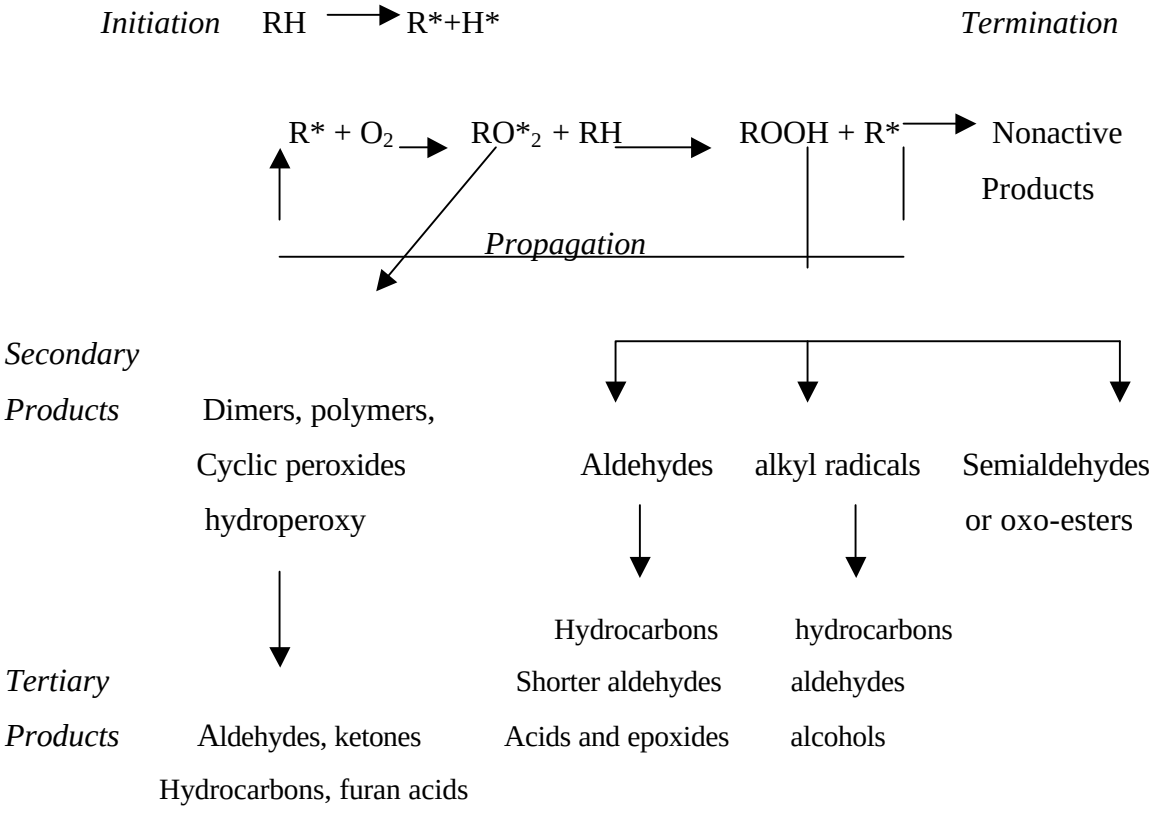


Figure 2-2. Autooxidation scheme (deMan, 1999; Fennema, 1996)

milkfat. In oils and fats, after refining and storage, a peroxide value of 10 meq/Kg is accepted before off-flavors can be developed (Rossell, 1989).

The threshold peroxide value for off-flavor is 20-50 meq/Kg (Hoffman, 1962). According to the International Dairy Federation the standard peroxide value for milkfat is 0.2 meq oxygen/Kg fat (Kaylegian and Lindsay, 1995).

Research has shown that a high content of unsaturated fatty acids in milk fat increases the risk of oxidation and production of off-flavors (Ashes et al., 1997; Focant et al., 1998; Im and Marshall, 1998; Lin et al., 1996). For instance, milk with a higher content of polyunsaturated fatty acids can develop oxidized flavors within 24 hours of refrigerated storage (McDonald and Scott, 1977).

Off-flavors in butterfat can be carried to second products, like ice cream, and affect consumer acceptance (El-Rahman et al., 1997 b). However high monounsaturated fatty acid concentrations in milk, when compared to milk with high polyunsaturated fatty acid concentrations, did not exhibit oxidation problems (Lin et al., 1996a; Lin et al., 1996b).

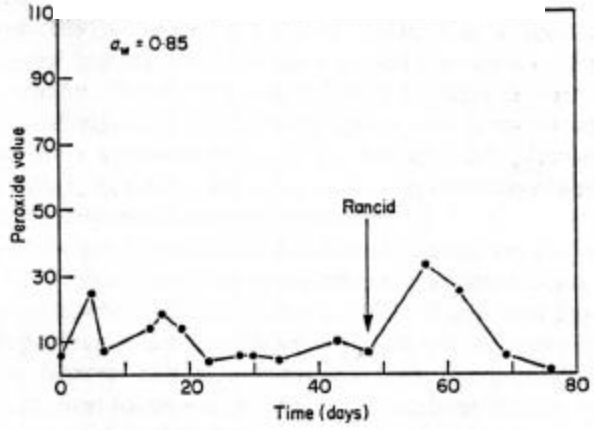


Figure 2-3. Variation of peroxide values with time in a intermediate moisture food at 35 C. The point of rancidity determined organoleptically (Labuza, 1975)

Antioxidants are used in a variety of food products to prevent oxidation however the addition of synthetic antioxidants in milk is not allowed (O'Connor and O'Brien, 1995).

Research on the use of natural antioxidants, primarily vitamin E, in milk has been conducted, as well as the use of spices, like rosemary (O'Connor and O'Brien, 1995).

***Flavor.*** Flavor is a sensory perception that is influenced by certain fatty acids that are precursors of flavor compounds. Depending on fatty acid stability, off-flavors or desired flavors can be obtained (Jenness and Patton, 1976; Kaylegian and Lindsay, 1995). The primary flavor compounds in milk are lactones, methyl ketones, low molecular weight, and branched chain fatty acids and aldehydes (Kaylegian and Lindsay, 1995).

**Physical Properties of Milkfat**

Physical properties of milkfat are influenced by melting and crystallization properties (Kaylegian and Lindsay, 1995).

***Crystallization Behavior***. Fatty acid composition and triglyceride structure determine the crystallization behavior of milkfat. Other factors such as temperature, cooling and agitation rates influence crystallization (Kaylegian and Lindsay, 1995). The process involves two steps:

1. Nucleus formation: the number and size of the nuclei influences the growth of the crystal which would determine the melting properties.
2. Crystal growth: crystal size and morphology vary according to rapid or slow cooling. Reduced cooling rates and high temperatures produce more stable crystals (β'), less firmness and less solid fat content. The sizes of the crystals depend on processing conditions too (Kaylegian and Lindsay, 1995).

Crystallization behavior will be modified by the type of triglycerides present, which is influenced by the fatty acid profile. Three types of crystals have been identified in milkfat: á, â and â'. â' is the most stable crystal form in milkfat and is characterized by having a higher melting point (Kaylegian and Lindsay, 1995). Milkfat crystals can have different sizes depending on cooling rates and processing conditions. When cooling is slow crystals range between 2 and 40ìm. With rapid cooling crystals are around 1-2 ìm (Kaylegian and Lindsay, 1995) and a higher incorporation of high melting triglycerides takes place, promoting more solid fat content and higher firmness. Crystallization affects consistency of high fat dairy products like butter and ice cream. Crystallization behavior of milkfat can be modified by fractionation methods like crystallization and fraction separation (Walstra el al., 1995).

***Melting Behavior of MilkFat.*** Melting point of fatty acids increases with saturation and chain length (Walstra et al., 1995). The melting behavior of fat is understood as the rate at which solid milkfat changes to liquid milkfat (Kaylegian and Lindsay, 1995). It also depends on the fatty acid profile and distribution in the triglycerides. Melting point, thermal profile and solid fat content profiles are analyses that describe the melting behavior of milkfat. Melting point is the measurement of the temperature at which milkfat becomes clear and free of crystals (Kaylegian and Lindsay, 1995). The melting point of fat is influenced by the fatty acid profile. The higher number of double bonds present in the fatty acid chain, the lower the melting point. Cis isomers have lower melting points than trans isomers (Walstra, 1995). Position of the double bonds in the chain also influences melting point.

Thermal profile explains the melting behavior of milkfat. It includes all melting types of glycerides: low-melting (melt below 10°C); middle melting (melt between 10- 20 C) and h igh melting glycerides (melt above 20 C) (Kaylegian and Lindsay, 1995; Lin et al., 1996b).

Solid fat content is the measurement of the percentage of solid fat at a specific temperature (Kaylegian and Lindsay, 1995). Solid fat content is one of the factors that will determine the fat solid phase functionality, others factors are type of crystals, and melting point of solid crystals. It has been reported that by increasing the content of short-chain saturated fatty acids (C4-10), long chain unsaturated fatty acids (C16:1-18:1) and decreasing the content of saturated fatty acids (C16:0-18:0) in milkfat, the melting point and solid fat content profile decrease due to an increase in low melting species (Kaylegian and Lindsay, 1995).

When milkfat was modified to a more unsaturated fat (35% of cis C18:1 and 8% C18:2) a softer milkfat was obtained, influencing properties such as melting point and processes such as churning (Ashes et al., 1997). When milkfat is modified to a higher percentage of unsaturated triglycerides both high and low molecular weight, melting point of the fat decreases and produces a softer butter at any temperature (Ashes et al., 1997).

**Textural Characteristics of Milkfat**

Textural properties of milkfat are influenced by solid fat content, liquid phase and melting point. Rheological properties such as viscosity and texture properties like firmness and hardness describe the texture of some products (Walstra, 1995; Kaylegian and Lindsay, 1995).

Firmness of milkfat is determined by the ratio of solid to liquid fat; this is influenced by the fatty acid composition, distribution and processing treatments (Walstra, 1995). Firmness is expressed by the melting behavior and textural properties (Kaylegian and Lindsay, 1995).

Viscosity is a rheological property. It is a function of the liquid phase of the milkfat but it also depends on the solid fat content. It is known as the internal friction of a liquid and is a function of shear stress and rate (Kaylegian and Lindsay, 1995). Viscosity is also influenced by product composition, pH, temperature, thermal profile of fat and processing operations (Walstra, 1995).

**Butter**

Butter is a water in oil emulsion, with 80% fat. It is considered a product that leads to atherosclerosis when consumed regularly (American Heart Association, 2000 d). Due to its high-saturated fatty acid content, butter consumption has declined and has been substituted by margarine. Other problems that affect butter popularity include poor spreadability at low temperatures (refrigeration temperature) and firmness (Prentice, 1992; Kaylegian and Lindsay, 1995). Research has been done to improve butter spreadability with methods like cream tempering, modifying churning conditions and butter tempering, interesterification and milkfat fractionation (Kaylegian and Lindsay, 1995). Spreadability and firmness of butter depends on the solid fat and liquid fat content. Solid and liquid fat content depend on the fatty acid profile and the fatty acid distribution in the triglycerides. Studies have shown that butter with higher content of unsaturated fatty acids is softer (Baer et al., 2001; Kaylegian and Lindsay, 1995; Lin et al., 1996b; Stegeman et al., 1992).

**Ice Cream**

Ice cream is one of the most consumed dairy products in the world, and the United States is the leading country in production and consumption of ice cream. Per capita consumption of ice cream in the U.S. is around 23.4 liters (Gorski, 1997). In Europe, ice cream consumption is high too; specifically in countries such as Sweden, Finland, Italy, Norway and Ireland show high ice cream per capita consumption (Hoyer, 1997). Around the world, ice cream production uses approximately 800,000 ton of fat per year. Ice cream is a dairy product prepared with ingredients such as milk fat, sweeteners, stabilizers, emulsifiers and flavorings. Milkfat plays an important

role in ice cream production, specifically in properties like flavor, texture, melting properties and crystal structure (Walstra and Jonkman, 1997). There are different types of fat that can be used to produce ice cream, but the best source of fat is fresh cream (Arbuckle, 1986). Low-fat ice cream and fat-free ice cream have been produced to satisfy weight control and other health issues of concern to consumers groups. Research on modified fat ice cream has also been done to improve textural properties of the ice cream (Elling et al., 1995; Abd El-Rahman et al, 1997 a; Abd El-Rahman et al, 1997 b), as well as production of ice cream with vegetable oils instead of cream to produce a nutritionally healthier, low- cholesterol product (Im and Marshall, 1998; Otero et al., 1999).

Ice cream is composed of four phases: ice, air, fat and the matrix (sugars, proteins and stabilizers) (Goff, 1999). The main steps in ice cream production are mix manufacture (mixing ingredients), pasteurization, homogenization, mix aging and freezing. After the fat is melted in the pasteurization step, the mix is homogenized, reducing fat globules size and increasing emulsification. The ice cream mix composition and stability will influence the final texture (Arbuckle, 1986) of the ice cream and the mix properties are also important to determine energy requirements to properly freeze the mix (Prentice, 1992). The ice cream mix is an oil in water emulsion, which suffers changes due to processing conditions like agitation, freezing, concentration and aeration (Arbuckle, 1986). The dispersion of the milkfat in ice cream mix is of great importance to determine texture and body characteristics of ice cream. The way that fat globules aggregate depends on the behavior of the fat globule surface and further relationships of solid and liquid fat ratios of the globules with temperature and processing conditions (freezing, thawing whipping) (Arbuckle, 1986). Physical properties of the oil in water phase in ice cream can be understood by viscosity measurements. Viscosity is an important property of ice cream mix and its affected by several factors such as composition and amount of fat and stabilizer, heat, salts, processing and handling of the mix, and temperature (Arbuckle, 1986). When viscosity of the ice cream mix increases, resistance of melting increases and smoothness of texture increases. The normal viscosity of ice cream mix varies from 50-300 cP (Arbuckle, 1986).

The emulsion is frozen, where ice crystallization takes place and produces the structural components of ice cream: the foam, a network of partially coalesced fat around the air bubbles, and ice crystals (Goff, 1999). Fat crystallization starts to take place during the mix aging process, where the high melting triglycerides crystallize first, followed by the low melting triglycerides

(Goff, 1999). This early process helps improve the overrun and stability of the foam in the structure. Ice cream's rheological properties are influenced by the ice cream mix properties but are more complex (Prentice, 1992).

Ice cream can have defects in flavor and texture due to several factors: type of fat, fat oxidation, freezing process, temperature differential, storage time and place and packaging. Flavor defects can be due to chemical changes like oxidation of fat and acidity (Arbuckle, 1986) or the use of old ingredients.

Research on ice cream produced with modified milkfat and its impact in flavor changes is limited but, according to Abd El- Rahman et al. (1997 b), oxidized flavors in butter fat are carried to the final product (ice cream). In this research (Abd El- Rahman et al., 1997 b) they substituted milkfat with anhydrous milkfat and low and high melting milkfat fractions. Oxidation flavors were found in the ice cream produced with the fractions and peroxide values were higher when compared to control ice cream.

Other defects in ice cream are those related to its texture. Terms like sandy (due to large lactose crystals), coarse or icy (large ice crystals), weak (lack of firmness) are used to describe defects in body and texture. Research on hardness and firmness of ice cream produced with fat fractions or with vegetable oils is also limited, but those completed have found no significant textural differences between treatments (El- Rahman et al., 1997 a; El-Rahman et al, 1997 b; Im and Marshall, 1998; Otero et al., 1999). On the other hand, a study by Im and Marshall (1998) showed that fatty acid composition of the ice cream fat and homogenization pressures affect firmness. This behavior was observed in ice cream with a higher content of unsaturated fatty acids. Less firmness in the final product was observed at a constant overrun and different homogenization pressures (Im and Marshall, 1998).

In ice cream, rheological properties are important for consumer acceptance (Abd El-Rahman et al., 1997 a). Ice cream with a ratio of 1:1:1 monounsaturated, polyunsaturated, saturated was compared to a control ice cream (Im and Marshall, 1998). Lower viscosity values were obtained for the vegetable oil ice cream due to differences in sizes and dispersion of the fat globules, when compared to the control. Temperature and concentration remained constant (Im and Marshal, 1998).

Melting point in ice cream is also measured to describe textural properties when the fatty acid profile has been modified. Abd El-Rahman et al, (1997 a) showed that ice cream produced

with high melting fat fractions melted slower than low melting fat fraction ice cream. Another factor that influences melting point is the content of fat. In ice cream studies (Campbell and Pelan, 1997) the rate of meltdown was reduced when milkfat was increased.

## REFERENCES


Abd El-Rahman., A.M, Madkor., S.AS., Ibrahim, F.S., and Kilara, A. 1997 a. Physical characteristics of frozen desserts made with cream, anhydrous milk fat, or milk fat fractions. J Dairy Sci. 80: 1926-1935.

Abd El-Rahman, A.M., Shalabi, S.I., Hollender, R., and Kilarara, A. 1997 b. Effect of milk fat fractions on the sensory evaluation of frozen desserts. J Dairy Sci. 80: 1936-1940.

Adlof, R.O. 1999. Preparation of unlabeled and isotope-labeled conjugated linoleic and related fatty acid isomers. Ch3 in Advances in Conjugated Linoleic Acid Research, Volume 1, Yurawecz, M.P., Mossoba, M.M., Kramer, J.K.G., Pariza,M.W., and Nelson, G.J (ed), p. 12-20.AOCS Press. Illinois.

Aigster, A., Sims, C., Staples, C., Schmidt, R., and O'Keefe, S.F. 2000. Comparison of Cheeses made from milk having normal and high oleic fatty acid compositions. J.Food Sci 65(5) 920-924.

American Heart Association."Cholesterol."*Heart and Stroke Guide". 2000 a.* <http://www.americanheart.org.com >(17 April, 2001).

American Heart Association."Cardiovascular Disease Statistics." *Heart and Stroke Guide.2000 b.* <http://www.americanheart.org.com >(17 April, 2001).

American Heart Association."Women Heart Disease and Stroke Statistics." *Heart and Stroke Guide.2000 c.* <http://www.americanheart.org.com >(17 April, 2001).

American Heart Association."Fat." *Heart and Stroke Guide.2000 d.* <http://www.americanheart.org.com >(17 April, 2001).

Ashes, J.R., Gulati, S.K., and Scott, T.W. 1997. Potential to alter the content and composition of milk fat through nutrition. J Dairy Sci. 80:2204-2212.

Arbuckle.W.S. 1986. Ice Cream, Fourth Edition.Van Nostrand Reinhold., New York.

Badings, H.T. 1970. Autoxidation processes of fatty acids.Ch2 and Ch3 in Cold Storage defects and their relation to the autoxidation of unsaturated fatty acids. Netherlands Milk and Dairy Journal. 24 (3/4):151-176.

Baer, R.J., Ryali, J., Schingoethe, D.J., Kasperson, K.M.,Donovan, D.C., Hippen, A.R., and Franklin, S.T. 2001. Composition and properties of milk and butter from cows fed fish oil. J. Dairy Sci. 84: 345-353.

Bayourthe, C., Enjalbert, F., and Moncoulon, R. 2000. Effects of different forms of canola oil fatty acid plus canola meal on milk composition and physical properties of butter. J. Dairy Sci. 83:690-696.

Berry, E.M. 1997. Dietary fatty acids in the management of diabetes mellitus. Am. J. Clin. Nutr. 66 (suppl):991S.

Bessa, R.J.B., Santos-Silva, J., Ribeiro, J.M.R., and Portugal, A.V. 2000. Reticulo-rumen edible products with linoleic acid conjugated isomers. Livestock Production Science. 63(2000): 201-211.

Campbell, I.J., and Pelan, B.M.C. 1997. The influence of emulsion stability on the properties of ice cream. In Ice Cream: Proceedings of the International Symposium. Bucheim, W(Ed.), p25-34. Internati Dairy Fede., Athens, Greece.

Chouinard, P.Y. 1999. Milk yield and composition during abomasal infusion of conjugated linoleic acids in dairy cows. J. Dairy Sci 82: 2737-2745.

Chow, K.C. 2000., Fatty acids in Foods and their Health Implications. Marcel Decker, Inc. New York.

Connor, W.E. 1999. á-linolenic in health and disease. Am. J. Clin. Nutr. 69:827-828.

DeLuca, D.D and Jenkins, T.C. 2000. Feeding oleamide to lactating jersey cows 2. Effects on nutrient digestibility, plasma fatty acids and hormones. J. Dairy Sci. 83:569-576.

DeMan,J.M. 1999. Lipids.Ch 2 in Principles of Food Chemistry, p 33-81. Aspen Publishers, Inc., Maryland.

Dhiman, T.R., Anand, G.R., Satter, L.D., and Pariza, M.W. 1999 a. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci 82:2146-2156.

Dhiman, T.R., Helmink, E.D., Mcmahon, D.J., Fife, R.L., and Pariza, M.W. 1999 b. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412-419.

Dhiman. T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K., and Tolosa, X. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 83:1016-1027.

Elling, J., Harris, J., and Duncan, S.E. 1995. Incorporation of modified butteroil into high-fat dairy products: ice cream manufactured with reduced-cholesterol reformulated cream. Dairy, Food and Environmental Sanitation 15(12); 738-744.

Fennema, O.R. 1996. Food Chemistry. Third Edition. Marcel Dekker, Inc., New York.

Focant, M., Mignolet, E., Marique, M., Clabots, F., Breyne, T., Dalemans, D., and Larondelle, Y. 1998. The effect of vitamin E supplementation of cow diets containing rapeseed and linseed on the prevention of milk fat oxidation. J Diary Sci. 81:1095-1101.

Fox, P.F and McSweeney, P.L.H.1998. Dairy Chemistry and Biochemistry. Blackie Academic and Professionall, New York.

Frithsche, J and Steinhart, H. 1998. Amounts of conjugated linoleic acid (CLA) in german foods and evaluation of daily intake. Lebensm Unters Forsch A. 206:77-82.

Goff, D.H. 1999. Ice Cream. Ch12 in Lipid Technologies and Application, Gunstone, F.D and Padley, F.B (ed), p:329-351. Marcel Dekker, Inc., New York.

Gorski, D. 1997. American market trends. Session V: Market trends in ice cream.proceedings of the international symposium, Buchheim, W (ed), p145-151. Internati. Dairy Fed., Athens, Greece.

Grundy, S.M. 1997.What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet. Am. J. Clin. Nutr. 66(suppl): 988S.

Gurr, M.I. 1995. Nutritional significance of lipids. Ch 9 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.349-402. Chapman & Hall., Great Britain.

Hawke, J.C. and Taylor, M.W. 1995. Influence of nutritional factors on the yield, composition and physical properties of milkfat. Ch. 2 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.37-88. Chapman & Hall., Great Britain.

Hoffman, G.1962. Vegetable oils. Ch.12 in Symposium of Foods: Lipids and their Oxidation, Schultz, H.W(Ed),p. 215-226. Avi Publishing Company, Inc.,Westport, Connecticut.

Hoyer,C. 1997. European market trends. Session V: Market trends in ice cream. Proceedings of the International Symposium, Buchheim, W (ed), p145-151. Internati. Dairy Fed., Athens, Greece.

Im, Ji-S. and Marshall, R. 1998. Effects of homogenization pressure on the physical, chemical and sensory properties of formulated frozen. Food Sci. Biotechnol. 7(2):90-94.

Ip,C., Banni, S., Angioni, E., Carta,G., McGinley, J., Thompson, H., Barbano, D., and Bauman D. 1999. Conjugated linoleic acid-enriched butter fat alters mammary gland morphogenesis and reduces cancer risk in rats. J. Nutr.129: 2135-2142.

Jenkins, T.C. 1998. Fatty acid composition of milk from holstein cows fed oleamide or canola oil. J. Dairy Sci. 81: 794-800.

Jenness, R. and Patton, S. 1976. Principles of Dairy Chemistry. R. E. Krieger Publishing Co., New York.

Kaylegian, K.E. and Lindsay, R.C.1995. Handbook of Milkfat Fractionation Technology and Application. AOCS Press., Champaign, IL.

Keenan, T.W. and Dylewski, D.P. 1995. Intracellular origin of milk lipid globules and the nature and structure of the milk lipid globule membrane. Ch. 3 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.131-178). Chapman & Hall, Great Britain.

Keogh, M.K.1995. Chemistry and technology of milk fat spreads. Ch. 6 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.213-245. Chapman & Hall, Great Britain.

Labuza, T.O. 1975. Oxidative changes in foods at low and intermediate moisture levels. In Food Science and Technology. A series of monographs "Water Relations of Foods", Duckworth, R.B (ed), p. 455-472. Academic Press., NewYork.

Lawless, F., Murphy, J.J., Harrington, D., Devery, R. and Stanton, C. 1998. Elevation of conjugated cis-9, trans-11-octadecadienoic acid in bovine milk because of dietary supplementation. J. Dairy Sci. 81:3259-3267.

Lin, M-P., Staples, C.R., Sims, C.A., and O'Keefe, S.F. 1996. Modification of fatty acids in milk by feeding calcium- protected high oleic sunflower oil. J. Food Sci. 61(1): 24-27.

McDonald, I.W. and Scott, T.W.1977. Food of ruminant origin with elevated content of polyunsaturated fatty acids. World Rev. Nutr. Diet. 26:144-207.

Molkentin, J. 1999. Bioactive lipids naturally occuring in bovine milk. Nahrung 43(3):185-189.

Nayga,.R.M. and Siebert, J.W. 1999. Analysis of at-home consumption of dairy products in the United States. Journal of Food Products Marketing. 5(3):65-77.

Newton, I.S. 1996. Long chain fatty acids in health and nutrition. J. Food Lipids. 3:233-249.

Noakes, M., Nestel, P.J., and Clifton, P.M. 1996. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am. J. Clin. Nutr. 63: 42-46.

O'Connor, T.P. and O'Brien, N.M. 1995. Lipid oxidation. Ch 8 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.309-347. Chapman & Hall., Great Britain.

Otero, M., Rodriguez,T., Camejo, J., Regalado,O.L., and Fung,V. 1999. Uso de grasa marina y aceite vegetal en helado. Alimentaria 299:61-63.

Pariza, M.W.1999. The biological activities of conjugated linoleic acid. Ch. 2 in Advances in Conjugated Linoleic Acid research, Volume 1, Yurawecz,M.P., Mossoba, M.M., Kramer, J.K.G., Pariza, M.W., and Nelson, G.J. (ed), p. 12-20.AOCS Press., Illinois.

Parodi, P.W. 1996. Milk Fat components: possible chemopreventive agents for cancer and other diseases. Austra. J. Dairy Technol. 51: 24-31.

Parodi, P.W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. J. Dairy Sci. 82:1339-1349.

Prentice, J.H, 1992. Dairy Rheology A Concise Guide. Hui, Y.H (ed). VCH Publishers, Inc., New York.

Ritzenthaler, K.L., McGuire, M.K., Falen, R., Shultz, T.D., Dasgupta, N., and McGuire, M.A. 2001. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J. Nutr. 131: 1548-1554.

Rossell, J.B.1989. Measurement of rancidity. Ch.2 in Rancidity in Foods, Allen, J.C and Hamilton, R.C (Ed.), p.23-30. Elsevier Science Publishers LTD., USA.

Spreer, E. 1998. Milk as a raw material in food. Ch. 2 in Milk and dairy product technology, Mixa,A. (ed), p. 2-26. Marcel Dekker, Inc., New York.

Stegeman, G.A., Baer, R.J., Shingoethe, D.J., and Casper, D.P. 1992. Composition and flavor of milk and butter from cows fed unsaturated dietary fat receiving bovine somatotropin. J. Dairy Sci. 75: 962-970.

Walstra, P., van Vlet, T., and Kloek, W. 1995. Crystallization and rheological properties of milkfat. Ch.5 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.179-211.Chapman & Hall., Great Britain.

Walstra. P. 1995. Physical chemistry of milkfat globules. Ch. 4 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.131-178). Chapman & Hall, Great Britain.

Walstra,P., and Jonkman, M. 1997. The Role of milkfat and protein in ice cream. Session I: Emulsion and foam stabilization in ice cream. Proceedings of the International Symposium, Buchheim, W (ed), p145-151. Internat. Dairy Fed., Athens, Greece.

# CHAPTER III

# OXIDATION AND TEXTURAL CHARACTERISTICS OF BUTTER AND ICE CREAM WITH MODIFIED FATTY ACID PROFILES

**S.Gonzalez[1], S.E. Duncan[1], S.S. Sumner[1]**

**S.F. O'Keefe[1] and J. Herbein[2].**

Virginia Tech, Blacksburg 24061

[1]Department of Food Science and Technology and

[2]Department of Dairy Science

ABSTRACT

Milk fat composition determines specific rheological, sensory and physicochemical properties of dairy products such as texture, melting point, flavor, color, oxidation rates, and viscosity. Previous studies have shown that milkfats containing higher levels of long chain polyunsaturated fatty acids have lower melting points and decreased solid fat contents which leads to softer-textured products. An increased risk of higher oxidation rates can be a disadvantage of high levels of polyunsaturated fatty acids.

Three different milkfat compositions were obtained through dietary manipulation of cows: high oleic content, high linoleic content and control milkfat. Ice cream and butter were processed from the treated and control milks. Butter and ice cream samples then were analyzed to measure differences in fatty acid profiles and firmness. High-oleic and high-linoleic milkfat had lower concentrations of saturated fatty acids, such as C 16:0. Conjugated linoleic acid content was increased in the high-linoleic milkfat. High-oleic milkfat resulted in a softer butter. Ice cream samples were analyzed to measure differences in viscosity, melting point, oxidation rate and sensory perception. Significant differences ($P<0.05$) were found in the fatty acid profiles of milkfat, ice cream mix viscosity, peroxide values of ice cream after 3 to 5 months of storage, butter color, and butter firmness. Sensory analyses included a scooping test at -18°C to detect differences in texture. A difference test was conducted to determine oxidation flavor differences between the three ice cream treatments at extended storage times. No significant differences were found in the scooping test or the oxidation flavor difference.

Manipulation of the cow's diet increased the total amount of unsaturated fatty acids. It also influenced firmness of butter and behavior of peroxide values during extended storage of high-linoleic ice cream.

**Key words**: ice cream, butter, oleic acid, linoleic acid, conjugated linoleic acid (CLA), firmness, oxidation rate, peroxides

# INTRODUCTION

Consumption of milk and some dairy products (butter, ice cream and whole milk cheeses) have been related to coronary heart disease due to the content of saturated fatty acids and cholesterol (Aigster et al., 2000; Noakes et al., 1996). Dairy products contribute to approximately 25-29% of the dietary saturated fat intake in western countries and 40% in countries such as Australia (Noakes et al., 1996). Palmitic (C16:0) and myristic acids (C14:0) account for the majority of the saturated fatty acids in milkfat. These two medium-chain fatty acids and lauric acid (12:0) have been associated with increased total cholesterol and LDL-cholesterol in plasma (Grundy, 1997). Stearic acid, a long-chain fatty acid (9.2 to 12.6 wt%) in milkfat appears not to have a major influence in increasing plasma LDL- cholesterol (Molkentin, 1999).

Modification of the fatty acid profile in milkfat to decrease the saturated fatty acid content, by increasing the polyunsaturated fatty acid content, has been one of the major research areas in the dairy industry. Different techniques such as dietary manipulation of the cows, cholesterol removal from milk, fractionation of milkfat, and cream tempering (Kaylegian and Lindsay, 1995) are used not only to modify the fatty acid content, but to modify physical and chemical properties of the milkfat or other type of fat or oil and consequently, the final product.

In order to modify the fatty acid profile of milkfat to lower saturated and increase unsaturated fatty acids, fats or oils are added to the cows diet (Hawke and Taylor, 1995). When oils are added to the diet, it is expected that medium-chain fatty acids will be reduced, while the long chain fatty acids will increase, especially C18:0 and C18:1 (Hawke and Taylor, 1995). Polyunsaturated fatty acids can increase moderately, when compared to C18:0. The increase in percentage of a specific fatty acid will depend on the type of supplement that has been added. If a specific fatty acid is added to the diet, then its content will increase in milk (Hawke and Taylor, 1995).

Chemical and physical properties of milkfat that are modified through dietary manipulation of the cow to increase unsaturated fatty acids could increase consumer acceptability of high fat dairy products. For instance, butter is known to have poor spreadability when refrigerated (Kaylegian and Lindsay, 1995). It has been shown that butter texture (spreadability) changes to a softer texture due to a higher content of unsaturated fatty acids (Baer et al., 2001; Stegeman et al., 1992) or monounsaturated (Lin et al., 1996b). Other changes that

can be observed in milkfat when the fatty acid profile is modified to a more unsaturated type include color change, flavor, increased oxidation rate, texture and crystallization, among others (Kaylegian and Lindsay, 1995).

Several studies (Ashes et al., 1997; Baer et al., 2001; DeLuca and Jenkins, 2000; Dhiman et al., 2000; Jenkins, 1998; Lawless et al., 1998; Lin et al., 1996a; Lin et al., 1996b; Noakes et al., 1996; Parodi,1999; Stegeman et al., 1992) have shown that supplementation of cows diet with different sources of unsaturated fatty acids modifies the fatty acid profile. Butter fat and milkfat have been analyzed to measure changes in some chemical and physical properties (Ashes et al., 1997, Stegeman et al., 1992; Baer et al., 2001) like fatty acid profile, cholesterol and firmness in butter. Limited research has been done in modified ice cream fat (Ashes et al., 1997; Im and Marshall, 1998) and influence in oxidative stability and storage as well as changes in texture parameters. Studies relating oxidation and high content of unsaturated fatty acids also are limited (Ashes et al., 1997; Baer et al., 2001; Focant et al., 1998; Kaya, 2000; Stegeman et al., 1992), however some of them have shown that a high content of unsaturated fatty acid increases oxidation rates (Baer et al., 2001; Focant et al., 1998).

The manufacture of a wide variety of dairy products with modified fatty acid composition is a trend in food and dairy science. Improvement of products to deliver increased health benefits without altering the desirable chemical and physical properties should increase consumer acceptance.

In order to understand the influence of modified fatty acids in chemical and physical properties of other dairy products besides milk, the main objective of this research was to study oxidation, texture and sensory perception of butter and ice cream made with milkfat with a high content of linoleic acid or milkfat with a high content of oleic acid.

## MATERIALS AND METHODS

### Modification of Bovine Diet to Alter Milkfat

Four Holstein cows were fed two diets (Table 3-1) to produce milk with different fatty acid composition (high oleic acid or high linoleic acid). The diets included safflower oil as the fat supplement, delivering increased amounts of oleic or linoleic acid. Both oils were provided by Columbus Food Co., North Albany, IL. The high oleic safflower oil contained 79% oleic acid, while the high linoleic safflower oil contained 76% linoleic acid. Based on the diet formulations, the cows received 555 mg of oleic acid (oleic diet) per gram of total fatty acids, while the cows on the linoleic diet consumed 573 mg of linoleic acid per gram of total fatty acids.

Oils were mixed with the concentrate (corn grain, soybean meal, vitamin mix, dicalcium phosphate and limestone) (Table 3-1) and stored in sealed barrels for a week maximum. This mixture was blended into the forages (corn silage, alfalfa silage and orchard grass hay) every day and fed to the cows selected for the study. Feeding was controlled to assure ingestion of the diet.

Table 3-1. % Total diet dry matter in bovine diets with different fatty acid concentrations

| Ingredient | % Total diet dry matter |
|---|---|
| Corn silage | 11.0 |
| Alfalfa silage | 28.1 |
| Orchard grass hay (chopped) | 13.4 |
| Corn grain (ground) | 31.6 |
| Soybean meal | 11.9 |
| Mineral / vitamin mix | 0.9 |
| Dicalcium phosphate | 0.1 |
| Limestone | 0.5 |
| Safflower oil (high oleic or high linoleic)[1] | 2.5 |
| **TOTAL** | **100.00** |

[1] High oleic safflower oil delivers 79% oleic acid; high linoleic safflower oil delivers 76% linoleic acid

Milk was collected individually from each cow receiving special diets as well as milk from the farm bulk tank, representing milk from cows fed a traditional diet without an oil supplement.

- **Control milk** obtained from untreated cows (from bulk tank);
- **High-oleic acid milk** obtained from the four cows when fed the diet supplemented with high oleic safflower oil;
- **High-linoleic acid milk** obtained from the four cows when fed the diet supplemented with high linoleic safflower oil.

Milk was collected twice a day in three periods (June, July and August). The first collection took place two weeks after the oils were added to the diets. Diets were rotated for the second and third collection period (Table 3- 2). Milk from cows fed like-diets (oleic vs linoleic) was commingled and each treatment milk was kept separate to retain compositional integrity. The control milk was obtained from the Virginia Tech Dairy Center bulk tank, during the first three days of each collection period.

**Table 3- 2. Oleic and Linoleic diet rotations assigned to each cow**

| Cow | Period 1 (June 26-June 20, 2000) | Period 2 (July 17- July 22$^{th}$, 2000) | Period 3 (July 31- August 4, 2000) |
|---|---|---|---|
| 1 | O* | L | O |
| 2 | O | L | L |
| 3 | L** | O | L |
| 4 | L | O | O |

* Oleic diet

** Linoleic diet

**Postharvest Milk Processing**

Raw milk from each treatment was preheated (55 C) and separated into 30 to 35% cream and skim milk using a pilot plant separator (Elecrem separator, model IG, 6400rpm, Bonanza Industries. Inc., Calgary. Alberta). Fat content was determined by the modified Babcock procedure (Marshall, 1993) on the creams. Peroxide values (AOAC, 1996) and percentage of

free fatty acids (Goff and Hill, 1993) also were determined on creams to evaluate the release of fatty acids and the oxidation process at this stage of the process. Skim milks of each treatment were pasteurized at 63ºC for 30 min and stored at 4ºC for subsequent use in the corresponding ice cream mix.

Creams were vat pasteurized at 68.8 ºC for 30 min and cooled to 13 C in an ice bath. They were tempered in the incubator overnight at 13 C before butter production. A portion of each cream was churned to produce butter samples. The remaining cream portions were used in formulation of 10 % fat ice cream.

**Butter and Butteroil Processing**

Butter samples were produced from the pasteurized creams of each treatment. Creams were churned using electric churns, until butter kernels were formed (13ºC; 30-45 min approximately). The buttermilk was separated from the butter kernels by draining through cheesecloth. Butter was pressed with a spatula to remove excess buttermilk. Butter samples were packaged in 5 oz. plastic cups and sterile plastic bags for further analyses. Butteroil was extracted by melting the butter at about 55ºC- 60ºC. Melted butter then was refrigerated at 3.3º C for 30 min to separate the butteroil from the aqueous phase (Scott, 1999). Butter was analyzed for color, firmness and fatty acid profile.

**Ice Cream Production**

Ice cream mixes were produced from cream and skim milks obtained from each pre-harvest production approach. Creams were mixed with the appropriate ingredients to formulate a 10 % fat ice cream mix (Table 3-3). Formulations were calculated by a computer program (DAYSY. Penn State Dairy Formulation System, Komputerwerk, Inc, 1988).

The different ice cream mixes were preheated at 54.4 C, homogenized and then pasteurized at 68.3 ºC for 30 min. Ice cream mixes were aged at -4 C for 24 h. Each ice cream mix was evaluated for composition (fat, protein, moisture, ash), fatty acid profile, peroxide value, free volatile fatty acids, freezing point and viscosity.

Ice cream mixes were frozen in a batch freezer (Emory Thompson Freezer 2HSC A, Emory Thompson Machine and Supply CO., New York) with 75 % overrun. Overrun is an important variable in hardness and other quality issues of ice cream so it was constantly measured (Arbuckle, 1986) during the process to assure consistency.

Table 3-3. 10% Fat ice cream formulation

| Ingredient | Percentage |
|---|---|
| Milkfat | 10.00 |
| Nonfat Dry Milk | 10.50 |
| Sweetener[1] | 16.66 |
| Stabilizer[2] | 0.20 |

[1]Sweetener= sucrose (60%), corn syrup (40%)

[2]Stabilizer= Kontrol 0.25( mono-diglycerides, cellulose gum, guar gum, polysorbate 80 carrageenan)

Ice cream samples were packaged in 4.6 L plastic containers and in 5 oz plastic cups and stored in the freezer at –20 C until sensory, textural and chemical analyses took place. Frozen ice cream samples were tested for microbial analyses, firmness, melting point, total acidity, peroxide value and sensory analysis such as ease of dipping and overall difference.

**Chemical Analyses**

***Compositional Analyses.*** Composition of ice cream was determined to verify that all treatments had similar characteristics except for variation in fatty acid composition. Fat was determined by the Pennsylvania test (Richardson, 1985). Protein was determined by a dye-binding method (Bio-rad, Hercules,CA) using a spectrophotometer (Spectronic 1001 Split Beam Spectrophotomer, Milton Roy Company, Rochester, NY). Moisture and solid fat content were measured using an infrared analyzer (Infrared Analyzer 115 Vac, Denver Instrument Company, Arvado, CO); ash was assessed by gravimetric analysis (AOAC 920.117; AOAC, 1997).

***Fatty Acid Profile.*** Fatty acid content was determined in ice cream mixes and butters to measure variation of oleic acid, linoleic acid, medium chain fatty acids and possible increases in CLA content. By knowing the content of these fatty acids a relationship between the physical and chemical properties of the product could be better understood. Lipids were extracted from ice cream mix (Bligh and Dyer, 1959) and analyzed by gas liquid chromatography. Fatty acid methyl esters were produced by the methylation procedure that includes sodium methoxide ($NaOCH_3$) and methanolic HCL (Kramer et al., 1997). The esters were separated and analyzed by gas liquid chromatography with a SP2560 100 m column (100m x 0.25 mm i.d., Supelco Park, PA). A Shimadzu QP5050A GC-MS was used to analyze the fatty acid methyl esters and,

for quantifying the esters, a total ion chromatogram was used. Helium was used as the carrier gas at 30 cm/sec.

***Oxidation Analyses.*** Unsaturated fatty acids, especially polyunsaturated fatty acids undergo oxidation due to different factors such as light, enzymes, oxygen and metals (copper and iron) (Fox and Mc Sweeney, 1998). Milk with a higher content of polyunsaturated fatty acids may have oxidized flavors within 24 hours of storage (McDonald and Scott, 1977). The oxidation of the product also may be increased during processing by factors such as temperature and processes such as agitation, homogenization and freezing.

Milkfat was extracted from milks, creams, ice cream mixes (fresh product) and stored frozen ice cream samples (AOAC 960.32; AOAC, 1997) to obtain the oil and analyze it for peroxide and free fatty acids. Both analyses were determined at different stages during the process:

- Raw milk within 24 hours of harvest;
- Cream after separation and pasteurization;
- Ice cream mix after homogenization and before pasteurization;
- Ice cream after freezing and packaging.

These analyses were performed within the first week after freezing and every 4 weeks after production of ice creams through 3-5 months of storage and again at 7-9 months of storage when sensory evaluation occurred.

Initial products of oxidation were measured with the determination of the peroxide value (AOAC CD 8-53). This method determined milliequivalents (meq) of peroxide/ kg of lipid (AOAC, 1997). Free fatty acids present in milk were assessed using the AOAC method Ca 5a-40 (AOAC, 1997). Free fatty acids can be expressed as total acidity, which reflects the potential for lipolyzed flavors resulting after reaching certain values of free fatty acids (Goff and Hill, 1993).

***Freezing Point.*** Freezing point may be used to determine the weight of solutes in solution (Goff and Hill, 1993). It is dependent on the concentration of the soluble constituents and varies with the composition of the ice mix (Arbuckle, 1986). Freezing point data can help to understand the relationship between the percent solids in the water and the amount of ice frozen related to texture properties such as firmness (Goff, and Hill, 1993). Freezing point was measured on the ice cream mixes with a thermistor cryoscope ( Advanced Milk Cryoscope

Model 4C, Advanced Instrument Inc., MA) on the Hortvett ( H) scale. A 1:4 dilution of the ice cream mix was used for this determination (Marshall, 1993). Samples were introduced in the cryoscope after calibration of the instrument. The freezing point was obtained in degrees Hortvet ( H) and changed to degrees Celsius with the following equation:

C = 0.9 ( H - 0.0024) (dilution factor)

***Color.*** Color was determined on butter and ice cream samples. A Minolta (CR-2000, Japan) colorimeter was used to determine a, b, and L values. This analysis was done after 3-5 months of storage on butter and ice cream cups.

**Texture And Physicochemical Analyses**

Fat content and emulsion characteristics have a great influence on the texture and melting properties (Walstra, 1995). Modification of fatty acids may alter the texture of the ice cream and produce less firm products (Im and Marshall, 1998). Milkfat melting point and spreadability in butter can be altered by unsaturated fat ( Lin et al., 1996b; Otero et al., 1999).

Butteroil samples were sent to Wisconsin Center for Dairy Research (Madison, University of Wisconsin) for determination of solid fat index. The dropping point was also measured. The dropping point is a modification of the softening point determination, in which fat is melted under control. The temperature at which the molten fat drops is recorded and interpreted as the dropping point (Kaylegian and Lindsay, 1995). Solid fat content of the fat was determined through nuclear magnetic resonance at 10ºC. The ratio of liquid to solid fat is an important factor to understand changes in properties such as spreadability and melting point. It has been seen in previous studies that higher concentration of unsaturated fatty acid influences the ratio of solid to liquid fat (Bayourthe et al., 2000).

Viscosity can be interpreted as the flow properties of the ice cream mix. It is the resistance of a liquid to flow and is influenced by concentration, temperature and dispersion of particles (Im et al., 1998) and processes such as pasteurization, homogenization and aging (Arbuckle, 1986). Viscosity was measured on ice cream mix samples during the processing week of every replication. Viscosity was measured at 7 C with a Haake Rotovisco Rv -12 viscometer equipped with a Haake NV spindle and cup (Elling and Duncan, 1996).

Firmness was determined on ice cream and butter samples to analyze if different fatty acid compositions lowered firmness on the samples. A universal testing machine (Instron Model

100, Instron, Canton, MA) was used to measure firmness (Matak, 1999). A 50 kg transducer with a 20% load range was used at a speed of 100 mm/min. Firmness measurements in butter were done in a temperature range between 4.2 and 5.5 ºC and reported as energy (joules). Firmness in ice cream was measured in a temperature range between -17.3 ºC- and -13.3 ºC.

Melting properties influence consumer acceptance of ice cream. For example, in warm climates a high melting point is desired (Abd El-Rahman et al., 1997 a). In ice cream the solid fat content is important to determine melting characteristics (Abd El-Rahman et al., 1997 a). An ice cream sample of every treatment and replication was weighed (110-120 g) and placed in a sieve Nº 10 to measure the rate of melting at approximately 22-25º C (Arbuckle, 1986). The time at which the first drop of ice cream dripped was recorded. Weight of melted ice cream was recorded every five min after the first drop of melted ice cream dripped.

**Sensory Analyses**

Sensory analyses were determined on the ice cream samples to back up the analytical methods related to oxidation and firmness. Analyses (paired comparison test, triangle test) were determined on the ice cream samples after 3 to 5 months of storage. Peroxide values and firmness were obtained for the tested samples. A third sensory test was assessed on ice cream samples stored after 7 to 9 months to determine oxidation flavor differences between the samples. The results were compared with peroxide values. The sensory tests were analyzed in the Department of Food Science and Technology with the cooperation of staff, students and faculty.

A paired comparison test was used to determine which sample was easiest to scoop. Three pairs of samples packaged in 4.6 L plastic containers were tempered on a freezer at -18º C. Twenty-four panelists were instructed to scoop the ice cream samples in a specific order. Each panelist completed the test individually but each test was completed from the same containers. The scooping action was explained to the panelists to be done from one extreme of the container to the end of it. Finally each panelist had to determine which ice cream sample was the easiest to scoop (Matak, 1999). The alpha value used was 0.05 (Meilgard et al., 1999). Samples were analyzed in duplicate.

A triangle test was used to measure overall difference. Three different pairs of samples were presented to 36 panelists, seated in individual booths, from the Food Science and Technology Department. Panelists were asked to taste the samples, compare them and determine which was the different sample. The alpha value used was 0.05 (Meilgard et al., 1999).

A paired comparison test was used to determine which ice cream sample presented a more pronounced oxidized flavor. Six panelists with experience in dairy products oxidation flavor analyzed the samples in triplicate for eighteen observations. The analysis took place in individual sensory booths and was done in three different sessions to avoid saturation. Panelists were encouraged to write observations of each sample. The alpha value used was 0.05 (Meilgard et al., 1999).

**Microbial Analyses**

Microbial analyses were assessed on the ice cream samples to verify production process and assure an efficient pasteurization step. Standard plate count and coliform count were used for monitoring the microbiological quality of the ice cream (Marshall, 1993).

Ice cream samples from each treatment and replication were thawed for no longer than 15 min (Richardson, 1985). Dilutions ($10^{-1}$, $10^{-2}$ and $10^{-3}$) in dairy dilution blanks made up of phosphate and magnesium chloride in distilled water (Marshall, 1993) were made. One milliliter of each dilution was transferred on aerobic count petrifilm (3M Petrifilm, Microbiology Products 3M Health Care, ST Paul MN) and coliform count petrifilm (3M Petrifilm, Microbiology Products 3M Health Care, ST Paul MN). The standard plate petri films were incubated at 32 C for 48 h, and coliform petri films were incubated at 32 C for 24 h. Psychrotrophic bacteria count plates were incubated at 7 C for 10 days on aerobic count petrifilm (Marshall, 19 93).

**Statistical Analyses**

Three different treatments were produced and replicated three times. At least two observations (duplicate) per analysis were done. For the interpretation and design of the statistical analyses, the Statistical Consulting Center at Virginia Polytechnic Institute and State University worked with the researchers. A randomized block design was used to evaluate for differences in data from chemical and physicochemical analyses. The software JMP® (SAS Institute, Cary, NC) was used to analyze the data. Tukey's test was used to determine mean differences when significant differences ($P<0.05$) were observed. Significance was determined at a alpha value of 0.05. Statistical contrasts were assessed on firmness and viscosity analyses.

Relationships between peroxide values and storage time were determined by calculating linear regression for each treatment using Microsoft Excel ‘2000 (Microsoft Corporation, Redmond, WA).

For the sensory analyses the responses were compared to the tables data presented in Meilgard et al. (1999). The overall difference results were compared to the data in Table T8 (Critical Number of Correct Responses in a Triangle Test, Meilgard et al., 1999). An alpha level of 0.05, â level of 0.20 at a $P_{max}$ level of 30% was used to analyze the responses.

The firmness and oxidative flavor results were compared to the data in Table T12 (Critical Number of Correct Responses in a Two-Sided Directional Difference Test, Meilgard et al., 1999). The scooping test (firmness) was analyzed using an alpha level of 0.05, â level of 0.05 at a $P_{max}$ level of 75%. The oxidation flavor test was analyzed using an alpha level of 0.05, â level of 0.40 at a $P_{max}$ level of 70% due to the limited number of panelists used for this test.

## RESULTS AND DISCUSSION

### Fatty Acid Profile of Milkfat.

Different studies (Ashes et al., 1997; Baer et al., 2001; Dhiman et al., 1999 a; Dhiman et al., 1999 b; Lawless et al., 1998; Noakes et al., 1996; Stegeman et al., 1992) show that milkfat fatty acid composition may be changed by altering forage type, amount and type of fat supplement (protected seeds, vegetable and fish oils, extruded or fullfat seeds) to alter fatty acid composition of the diet. Using the high-unsaturated safflower oil to alter milkfat composition resulted in the targeted change in milkfat composition in this study as well.

The addition of the high-oleic or high-linoleic oils to the treatment diets showed an effective change in the fatty acid profile of butter. The total percent of unsaturated fatty acids increased ($P<0.05$) for both treatments as compared to the controls (Table 3-4) (Figure A-1, Appendix A). The total percent of saturated fatty acids was higher in the control butter fat.

The concentration of short chain fatty acids (C4:0-12:0) did not vary significantly ($P>0.05$) among the treatments. The effect on different medium chain fatty acids was more variable. C12:0 and C14:0 did not vary significantly in the milkfat among the diet treatments. However,C16:0 decreased ($P<0.05$) in the milkfat from the high oleic dietary treatment (22.71g/100g fatty acid(fa.) and in the milkfat from high linoleic dietary treatment (21.12 g/100g fa), when compared to the milkfat from the control dietary treatment (28.83 g/100g fa). The decrease in concentration of C16:0 for the unsaturated treatments is of great importance for human health issues, specifically because its concentration in whole milk products is directly related to increases of low density lipoprotein (LDL) cholesterol in human studies (Noakes et al., 1996). In a previous study (Noakes et al., 1996), decreases in human total plasma cholesterol were related to modified fat products (milk and butter), in which increases in linoleic and oleic content (6.9% and 35.3% respectively) were observed as well as decreases in myristic and palmitic acid (6.7% and 15.5% respectively). Lin et al (1996b) also showed a decrease in palmitic acid content (from 27.2 to 20.9%) when feeding cows with calcium-protected high oleic sunflower oil (Lin et al., 1996b).

C18:1 (cis-9 18:1) increased by 23% for the milkfat ($P<0.05$) from the high-oleic dietary treatment, as compared to the control, as expected since the oleic acid was in a higher percentage in the diet. This fatty acid remained at levels around 18-20 g/100g fa, in the milkfat from control

Table 3- 4. Fatty acid profile of milkfat from cows fed control, high-oleic or high-linoleic diets (n=6)

| Fatty acid[1] | Diet | | | | | |
|---|---|---|---|---|---|---|
| | Control | SE[8] | Oleic[9] | SE | Linoleic[7] | SE |
| | (g/100g of total fatty acids) | | | | | |
| SC[6] | 15.61 | 0.27 | 16.07 | 0.19 | 15.79 | 0.40 |
| 12:0 | 2.88 | 0.06 | 2.82 | 0.05 | 2.83 | 0.11 |
| 14:0 | 10.17 | 0.14 | 9.94 | 0.10 | 9.70 | 0.21 |
| 16:0 | 28.83 [b] | 0.27 | 22.71[a] | 1.06 | 21.12 [a] | 0.65 |
| 18:0 | 12.74 | 0.39 | 13.86 | 0.38 | 13.98 | 0.96 |
| 18:1 c9 | 18.22 [a] | 0.32 | 22.45[b] | 0.41 | 19.92 [a] | 0.48 |
| 18:2 n-6 | 2.81[a] | 0.01 | 3.41[a] | 0.17 | 5.99 [b] | 0.09 |
| TVA t11[2] | 1.12 [a] | 0.03 | 1.10 [a] | 0.02 | 1.73 [b] | 0.04 |
| CLA c9,t11[3] | 0.62 [a] | 0.02 | 0.61 [a] | 0.17 | 1.00 [b] | 0.02 |
| Saturated[4] | 69.27 [b] | 0.46 | 64.11 [a] | 0.64 | 62.15 [a] | 0.41 |
| Unsaturated[5] | 30.72 [b] | 0.46 | 35.90 [a] | 0.63 | 37.85 [a] | 0.41 |
| PLC[7] | 4.87 [a] | 0.04 | 5.21 [a] | 0.19 | 8.38 [b] | 0.11 |

[1] Expressed as number of carbons: number of double bonds
[2]TVA= Transvaccenic acid (C18:1 t11)
[3]CLA= Conjugated linoleic acid (C18:2 cis9,t11)
[4] Saturated = saturated fatty acids from C4:0-C20:0
[5]Unsaturated= unsaturated fatty acids from C14:1-C22:6
[6]SC = Short chain fatty acids (C4:0-C10:0)
[7]PLC= Polyunsaturated long chain fatty acids (C18:2 w6 – C22:6 w3
[8]SE = Standard Error on duplicate samples for the three replications of each treatment
[9]Oleic= high-oleic treatment; linoleic= high-linoleic treatment
a,b= means within rows with different letters are significantly different ($P < 0.05$; n=6)

and high-linoleic dietary treatments (P>0.05). C18:2 w6 (linoleic acid) increased for the milkfat from the high-linoleic dietary treatment (P<0.05) when compared to the milkfat from the high-oleic and control dietary treatment, which remained constant.Having established that the dietary manipulation yielded the desired fatty acid modification, subsequent treatment reference in this study will be referred to as high-oleic milkfat and high-linoleic milkfat.

Trans-vaccenic acid (TVA, C18:1 11 trans) and conjugated linoleic acid (CLA, C18:2 9 cis 11 trans; also called rumenic acid) increased for the high -linoleic milkfat (P<0.05) when compared to the high-oleic and control milkfat. The CLA levels obtained were 1 g/100g fa, while the control and high-oleic (rumenic) acid concentration varied around 0.61-0.62 g/100g fa (Table 3-4). Typical rumenic acid intake in humans is around 150 mg/day (Ritzenthaler et al.,2001). If daily human intake of dairy fat is estimated at 20 g/d and dairy products with a modified fatty acid profile like the one obtained in this study were to be consumed the daily rumenic acid intake should be around 200 mg/d. Consumption of rumenic acid delivered by other types of fat (beef fat) has not been taken into consideration. It can be observed that the hypothetical consumption of dairy products using our modified fat could successfully increase the ingested amount of rumenic acid, however values are not high enough to deliver anticarcinogenic properties (620 and 441 mg/d for men and women, respectively) (Ritzenthaler et al., 2001; Herbein, J., 2001 (personal communication)). Further transformation of TVA to rumenic acid has not been taken into consideration. Baer et al. (2001) mentions that, while the CLA concentration needed to have anticarcinogenic properties in humans is still under study, the estimated amount of CLA that may prevent cancer in humans is around 3g/d. Based on calculations considering concentrations of TVA and further transformation to CLA, they also mention lower CLA values of 1.33g/d. They assumed that 50% of TVA is transformed to CLA in the body.

Different studies have focused on increasing the CLA content due to its anticarcinogenic properties. Increase in CLA content in milk by addition of fish oils, and oils with high content of long chain fatty acids into the bovine diet, abomasal infusions of the CLA isomers and other methods has been demonstrated (Ashes et al., 1997; Baer et al., 2001; DeLuca and Jenkins, 2000; Dhiman et al., 1999 a; Dhiman et al., 2000; Lawless et al 1998; Noakes et al., 1996; Parodi, 1999). When comparing data between studies that added fish oil as fat supplement or cows fed with fresh pasture, CLA amount increases from 0.5% (untreated) to around 3% or more. It has

been shown that the best source of CLA is when cows are fed fresh pasture. Baer et al. (2001) showed that, by addition of menhaden fish oil, CLA concentration, as well as palmitic acid, increased significantly. Palmitic acid is related to increased blood levels of LDL-cholesterol (Noakes et al., 1996). In the present study, palmitic acid was successfully reduced by nearly 27% and CLA content was increased by 67% from 0.6g/100 g fa to 1.0g/100g fa. for the high-linoleic treatment.

**Changes in Physical Characteristics of Butter by Fatty Acid Modification**

The modified fatty acid profile resulted in variations in chemical and physical properties of the products. For instance, changes in butter firmness were observed (Figure 3-1). The butter samples with high-oleic and high-linoleic milkfat showed significant differences in firmness when statistically contrasted to the control butter ($P=0.0086$) (Table B-1, Appendix B). The control butter was firmer (10.54 J) than the high-unsaturated butters, specifically when compared to the high-oleic milkfat treatment (6.09) ($P<0.05$) (Figure.3-1). The high-linoleic butter firmness values were numerically lower than the control butter and very similar to the high-oleic butter values. It has been mentioned before that the fatty acid composition affects textural properties like firmness. In this case the control butter had a higher content of saturated fatty acids (C16:0 and C18:0) and a lower concentration of long chain unsaturated fatty acids (C16:1 and C18:1) and short chain fatty acids (C4:0 to C10:0) (Table 3-4). The fatty acid profile of normal milkfat yields a fat with a higher content of solid fat profile and higher melting point due to an increase in high-melting glyceride species (Kaylegian and Lindsay, 1995). In this study, the high-oleic butter had the highest content of 18:1 and short chain fatty acids; the firmness was decreased ($P<0.05$). However, when comparing the results of butter solid fat index (Figure 3-2) and dropping point, no significant differences were found among the treatments. A difference was expected due to the variations in the fatty acid profile, as mentioned before. According to Ashes et al. (1997), the solid fat content of milkfat of cow's feds full fat sobybean was between 50 to 60% at O C. The solid fat content of milkfat from cows fed protected canola seeds was lower (30-40% at O C) than milkfat of cows fed full fat soybeans. They observed a.variation of triglycerides C52 and C54 in both treatments but in a lower magnitude for the full fat soybean

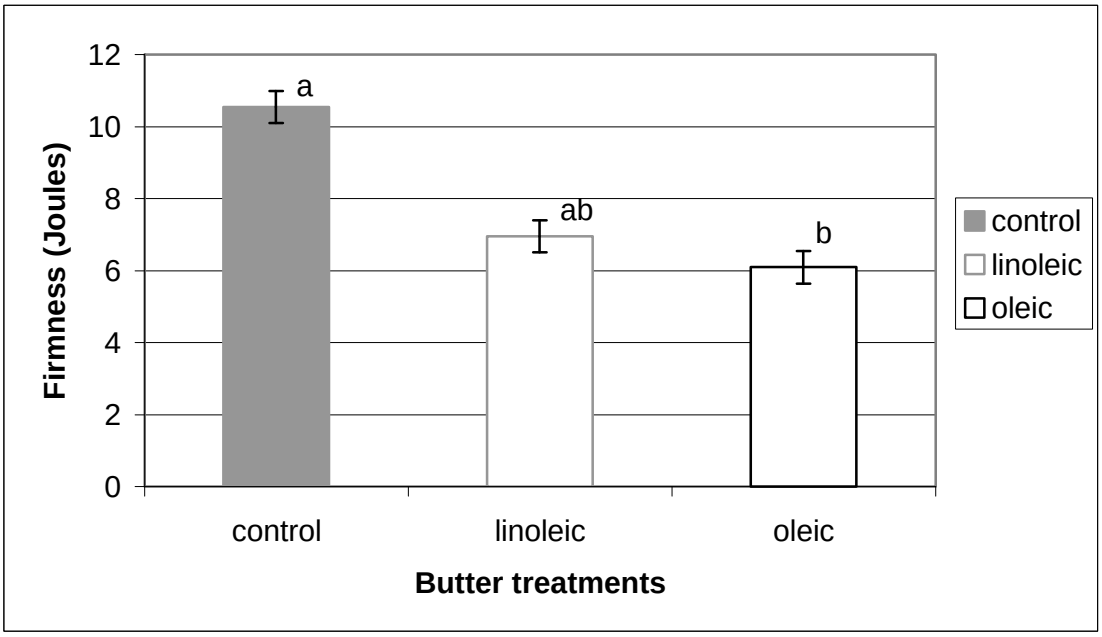


Figure 3-1. Butter firmness (joules) in a temperature range between 4.2-5.5 ºC.

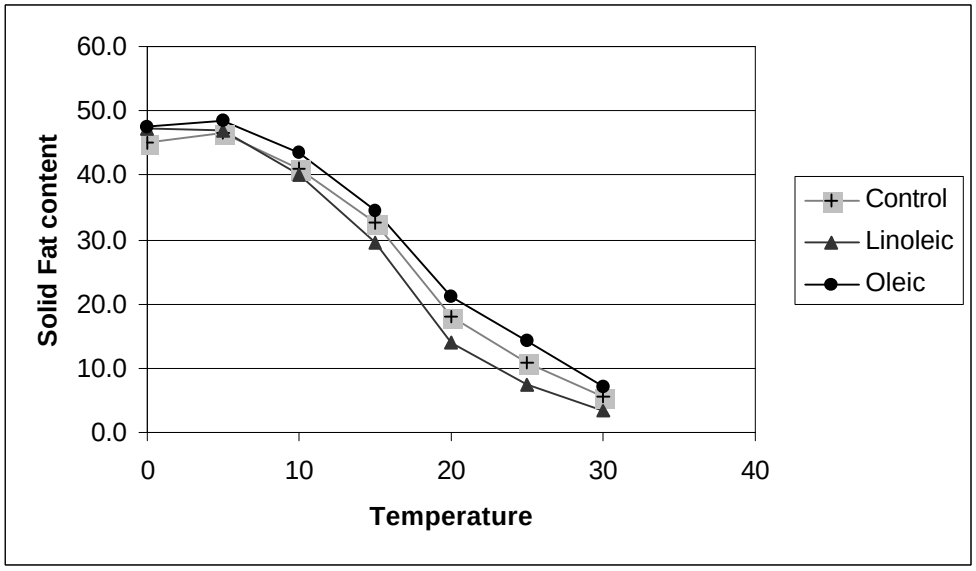


Figure 3-2. Butter solid fat index. No significant differences between the milkfat treatments were found (P>0.05)

milkfat (Ashes et al., 1997). In the present study the solid fat content of the three milkfat treatments ranged between 40-50% at O C.

Perhaps the variation of linoleic acid was not high enough to promote a high production of low melting triglycerides to observe significant differences in the solid fat content. However, the modification was significant enough to detect firmness differences.

Other studies (Ashes et al., 1997; Baer et al., 2001; Lin et al., 1996b; Stegeman et al., 1992) show that the modification of the fatty acid profile of milkfat by addition of unsaturated fat supplements in the bovine diet yields softer butters. Ashes et al. (1997) reported changes in churning temperature and time because the butter was soft. In the present research, special care had to be taken during the churning procedure of the unsaturated butters because they were very soft.

Slight differences in color between the treatments and the control were visually observed. The high-unsaturated butters were less yellow than the control butter; however differences between the high-oleic butter and high-linoleic butter were hard to see. Kaya (2000) related color change in butteroil due to oxidation reactions. At peroxide values higher than 10 meq/Kg the color of butteroil changed from yellow to light yellow, this color change was attributed to oxidation of chromophors (Kaya, 2000). In this study color variation was measured analytically in butter samples stored for 3-5 months at –24 C. The control butter had a higher b value (yellow) when compared to the high-oleic and high-linoleic butter (27.73, 18.75 and 19.44 respectively; $P<0.05$). The high-oleic butter had a higher L (white) value than the control butter (92.40, 87.39 respectively; $P<0.05$) but not significantly higher from the high-linoleic butter (90.95 ($P>0.05$). Color differences between the unsaturated treatments and control butter could be due to several factors such as color of supplemented oils, source of dietary fatty acids and oxidation reactions. Noakes et al. (1996) observed slight color differences in dairy products (milk, butter, cheese and ice cream) with a higher unsaturated fatty acid content.

**Oxidation Stability Of Milkfat In Ice Cream with Modified Fatty Acid Profile**

A disadvantage of a high content of unsaturated fatty acids is an increase in the risk of autoxidation and development of off-flavors (Ashes et al., 1997). In order to determine the influence of fatty acid profile in the oxidation stability of ice cream from the different treatments, peroxide values were measured during storage (-20 C) (Figure 3 -3). The peroxide value detected the amount of peroxides released by autoxidation reactions (Rossell, 1989). Ice cream peroxide

values were measured in three different storage periods: 0 months, 2 months and 3-5 months. Milk and cream peroxide values were measured during the processing period (0 months) to evaluate initial peroxide values of the main ingredients of ice cream and the influence in final product peroxide values. Milk and cream peroxide values were not significantly different among the treatments; however it was observed that cream peroxide values (1.3-1.7) were lower than those for the milks (5.5-6.7) (Table A-3, Appendix A). Decreased peroxide values in cream may have been due to additional heat processing to which creams were subjected. Hydroperoxides are very unstable and heat will accelerate their decomposition to secondary products (Badings, 1970).

Initial peroxide values measured in the three types of milkfats extracted from the ice cream mixes varied in a range between 2.91 to 4.12 meq of peroxide/Kg. The peroxide value increased numerically ($P>0.05$) after two months of storage for all the treatments; the control and high-oleic milkfats had lower peroxide values after 3-5 months of storage. A relationship between peroxide value and storage period was determined through regression analysis (Figure 3-3). The high-linoleic treatment showed to have the higher slope when compared to the other treatments.

Hydroperoxides increase slowly and, after reaching their highest concentration they decompose to secondary products and the peroxide value decreases (Figure 2-3). According to deMan (1999), a low peroxide value, especially after several months of storage, does not mean that oxidation has not taken place. In contrast to the values observed in control and high-oleic products, the peroxide values for the high-linoleic fat at 2 to 5 months of storage were higher (Table A-3 Appendix A). Free fatty acids (Table A-4, Appendix A) or acid values (Table A-5, Appendix A) also were measured but remained constant for milk, cream and ice cream through out the storage period. The values were low and indicated no hydrolytic rancidity occurred in the milk and products.

The increase in peroxide value at extended storage of high-linoleic milkfat ice cream may be attributed to the modified fatty acid composition. A higher percentage of unsaturated fatty acids was observed in the high-linoleic ice cream milkfat (Table A-1 Appendix A) (36.52g/100 g fa.) when compared to high-oleic ice cream milkfat (34.89g/100g fa.) and control ice cream milkfat (29.79g/100g fa).

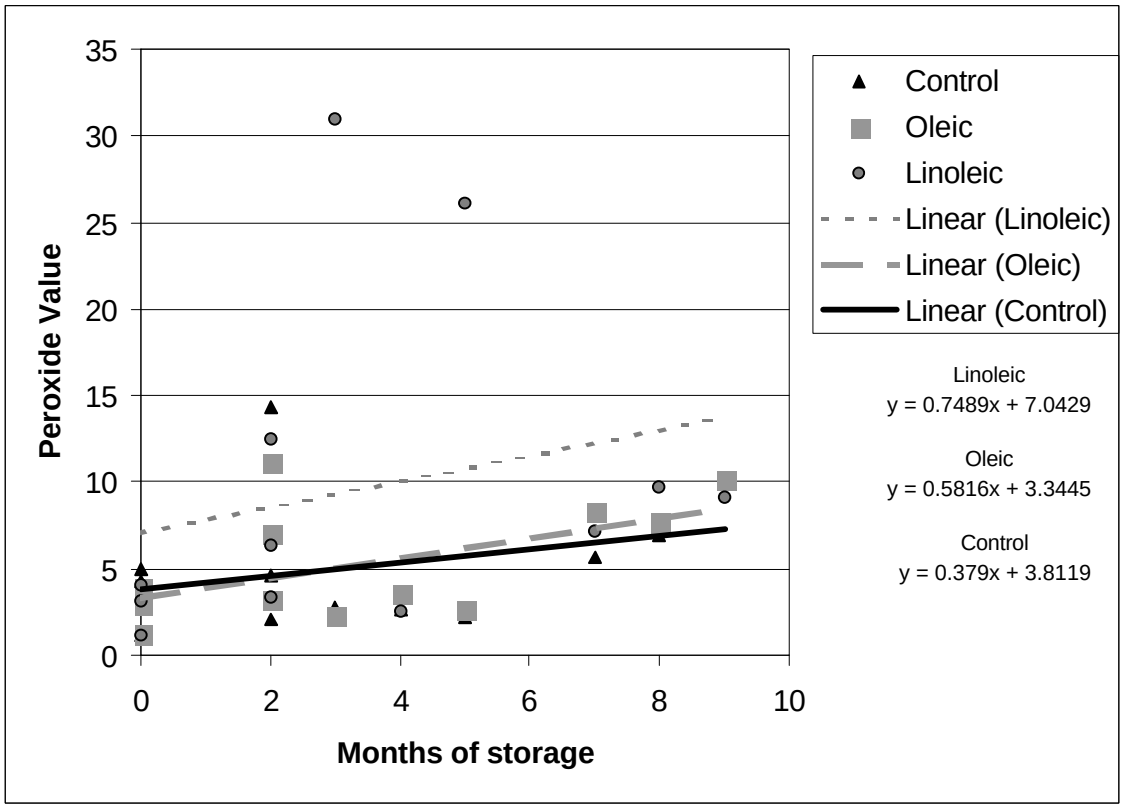


Figure 3-3. Relationship between ice cream peroxide values and storage time for every ice cream treatment. Storage periods (0,2,3,4,5,7,8,9 months). The different symbols represent the three ice cream treatments.

Most important is the higher percentage of polyunsaturated long chain fatty acids (Table A-1 Appendix A) (7.66g/100g fa.) in the high-linoleic ice cream milkfat when compared to high-oleic ice cream milkfat (4.76g/100g fa.) and control ice cream milkfat (4.37g/100g fa.).

The rate of autooxidation depends on the fatty acid composition, specifically on the degree of unsaturation (deMan, 1999). When comparing stearic (18:0), oleic (18:1 w9), linoleic (18:2 w6) and linolenic acid (18:3 w3), the ratio of relative rate of oxidation is estimated as: 1:100:1200:2500. This indicates that the presence of more unsaturated double bonds will increase the rate of oxidation (deMan, 1999).

Higher peroxide values also were found by Baer et al. (2001) in butterfat with a high content of unsaturated fatty acids (fish oil was added as supplement to the cows diet), after three months of storage (4 C) when compared to standard butterfat. Stegeman et al., (1992) showed no increase of peroxide values in butter between 0 and 3 months of storage (4 C). In that study sunflower or safflower seeds were added to cows diet to change fatty acid profile. However the linoleic acid content in the milkfat treatments (1.8-2.9g/100g f.a) was low when compared to the percentage of linoleic acid of our high-linoleic ice cream milkfat (5.62 g/100g f.a) (Table A-1, Appendix A). The higher linoleic acid percentage and presence of other polyunsaturated fatty acids influenced oxidation rate of frozen ice cream at extended storage. The high peroxide values in the high-linoleic ice cream at 3-5 months storage exceeded the reported threshold off-flavor is 20-50 meq/Kg (Hoffman, 1962). It was hypothesized then that a sensory difference might be observed. Sensory analyses, conducted at 7-9 months did not support that hypothesis.

Panelists with experience in oxidation flavors did not find significant difference between ice cream treatments ($P > 0.05$) (Table 3-.5). According to observations made by the panelists, most of the samples had an oxidized flavor, and differences in oxidized flavor intensity were not perceptible. The peroxide values for the milkfat treatments at the time of sensory (7-9 months) 7.51- 8.68 meq/Kg and no significant differences ($P>0.05$) between the treatments were found. Again, the variability in peroxide values over time suggests the difficulties using peroxide values as an indicator of perceptible oxidized off-flavor.

According to Table 2-3, a low peroxide value does not mean that oxidation has not taken place, especially after such long storage time.

Table 3-5. Two-sided paired comparison of oxidation off-flavors in ice cream (n=6 panelists)

| Samples | # of Agreeing Responses/ Total # of Responses | Period 1 | Period 2 | Period 3 |
|---|---|---|---|---|
| | Number of agreeing responses[4]/ total responses | | | |
| OL[1] | 14/36 | 3/12 | 3/12 | 8/12 |
| LC[2] | 18/36 | 7/12 | 4/12 | 7/12 |
| CO [3] | 21/36 | 7/12 | 5/12 | 9/12 |

[1]OL: oleic vs. linoleic

[2]LC: linoleic vs. control

[3]CO: control vs. oleic

[4] The number of agreeing responses from 36 independent observations at a p=0.05 to obtain significant difference is 25. The number of agreeing responses from 12 independent observations at a p= 0.05 to obtain significant difference is 10(Meilgard et al., 1999).

**Physicochemical and Textural Properties of Ice Cream Related to Modified Fatty Acid Profile**

A variation in the fatty acid profile could influence milkfat textural properties also. Solid fat content, liquid fat content, crystallization behavior, and melting behavior will vary depending on fatty acid profile, distribution on the triglyceride backbone and on processing conditions (Fox and McSweeney, 1998; Kaylegian and Lindsay, 1995). Fat in ice cream determines several properties including texture. The variation of the fatty acid profile in the milkfat used to produce the different ice cream treatments (10% fat) could have influenced melting behavior and textural properties of the ice creams.

Melting behavior in ice cream is a quality parameter that could influence consumer acceptance (Arbuckle, 1986). An optimum melting quality of ice cream is when ice cream starts to melt within 10-15 min. Fast melting in ice cream is often related to variations in freezing point and is an undesirable quality. Ice cream mix stability will influence the texture and other quality attributes of the frozen ice cream. The stability of the mix depends on the fat stability (emulsion) and protein stability (colloid) (Arbuckle, 1986). Fat stability is influenced by temperature and subsequent fat globule behavior. Solid fat and liquid fat ratio of the globule change according to variations in temperature, fat content and processing conditions and this influences the way globules are grouped and dispersed (Arbuckle, 1986). Freezing also affects fat stability by inducing agglomeration of fat globules. Agglomeration of fat globules can behave differently depending on factors like protein stability, melting point of fat, temperature, emulsifier, stabilizer, salts content and agitation (Arbuckle, 1986). This process will influence the final texture and body of ice cream (Arbuckle, 1986).

Viscosity, firmness and melting point of ice cream were measured to determine if the variation in the fatty acid profile of milkfat influenced fat behavior in ice cream texture. In an effort to monitor differences in oxidation, texture and sensory quality in the processed products, processing conditions were controlled as possible to limit variability in product differences. Ice cream mix composition documented that there were no gross compositional differences other than from the milkfat modification (Table A-6, Appendix A).

Microbial analyses showed that the product was produced under sanitary conditions, then microbial counts were below detectable limits. There were some observations of sandy texture

among the ice creams produced. No logical association with the milkfat modification can be identified. Such effects influenced the overall difference test completed by an untrained panel at 3-5 months of storage (Table C-1, Appendix C).

Ice cream mixes as well as yogurt and some cheeses have been classified as possessing a viscoelastic nature (Velez-Ruiz and Barbosa, 1997), which is an intermediate state between solid and liquid behavior. Ice cream texture is primary related to physical properties of cell walls. The cell walls are influenced by size of fat globules, distribution of air cells, and physical properties of protein- emulsifier film absorbed on the fat globule. Other factors that might affect ice cream viscosity are related to processing procedures such as homogenization and storage period (Velez-Ruiz and Barbosa, 1997).

In the present research, viscosity was measured in ice cream mixes at 7 C. Hysteresis curves (Figure 3-4) were plotted to compare the viscoelastic nature of ice cream milkfat with different fatty acid profile. The three ice cream mix treatments followed the same trends when applying increasing, and then decreasing shear rates. The apparent viscosity increased while shear rate increased, remaining almost constant for the last shear rate (1384.96 $s^{-1}$) (Figure 3-4) (Table B-2, Appendix B). The ice cream mixes exhibited lower apparent viscosities when the shear rates applied were decreasing. When the mix was exposed to stress, the aggregated fat globules broke down, creating a shear-thinning mix (Prentice, 1992). The obtained shear-thinning mix did not regain the same apparent viscosity values from the first readings (Table B-2, Appendix B).

Apparent viscosities (Figure 3-4) for the control ice cream mix were statistically higher ($P= 0.0003$) than those for the high-oleic and high-linoleic treatments based on statistical contrasts. It is known that viscosity of milkfat depends to a great extent on the solid to liquid fat ratio (Kaylegian and Lindsay, 1995). Control fat had a lower content of unsaturated fatty acids (Table 3-4) when compared to the unsaturated treatments. A higher percentage of long chain unsaturated fatty acids and short chain fatty acids would influence the fat to have lower melting point (Kaylegian and Lindsay, 1995). However the solid to liquid fat ratio based on solid fat index of the milkfat treatments (Figure 3-2) were not different from each other ($P>0.05$). Viscosity of ice cream mix does not only depend on functionality of fat, but on the interaction with the other ingredients and processing conditions (Arbuckle, 1986). The type of the fat globule arrangement (clumps or chains), the solid to liquid fat ratio of the fat in the emulsion, the

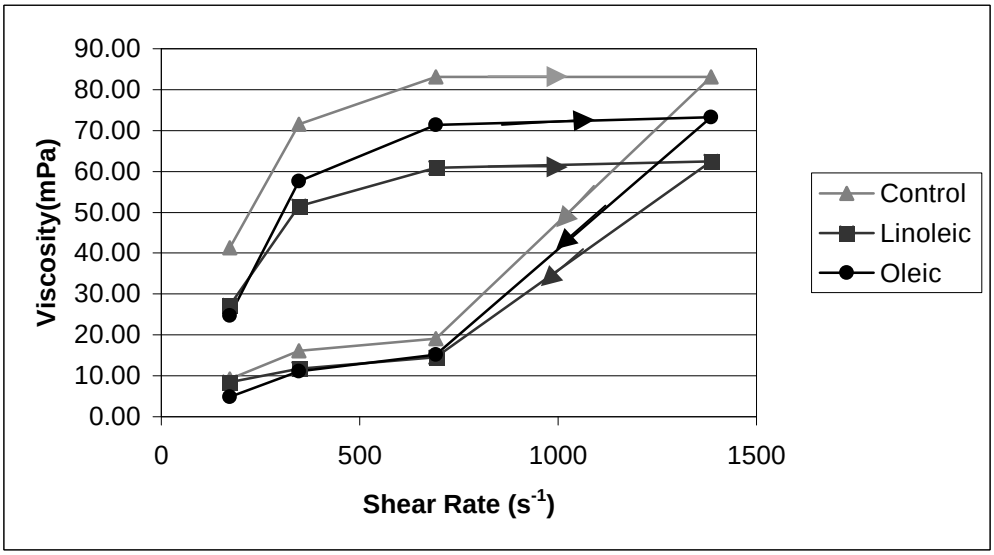


Figure 3-4. Hysteresis curve for ice cream mixes. (Control; high-oleic= oleic; high-linoleic= linoleic).

interaction with the stabilizer and the other main ingredients and also the temperature used during the viscosity measurements may have influenced observed rheological properties of the ice cream mix treatments (Arbuckle, 1986).

Im and Marshall. (1998) obtained lower viscosity values for ice cream formulated with canola and soybean oil due to differences in sizes and dispersion of the fat globules, as compared to the control milkfat. Temperature and concentration of fat were held constant (Im and Marshal, 1998). Literature has shown that when measuring viscosity in ice cream mixes, different factors (method, temperature, effect of the different phases and measurements of the ice cream or ice cream mix) play important roles when it comes to determine the type of rheological behavior observed (Velez-Ruiz and Barbosa, 1997).

Event though viscosities differences were found in the ice cream mixes, no differences ($P>0.05$) were found in firmness or melting range of ice cream treatments. Firmness was measured in ice cream at a temperature range between –17.3 and –13.3 ºC. Variation in temperature during firmness testing may have altered firmness to an extent that variation in the data due to milkfat composition could not be assessed. Other research (Matak, 1999) has shown that firmness values in ice cream vary significantly with a small variation in temperature. When the ice cream mix is being frozen, an increase in solidified fat occurs as well as an increase in firmness (Prentice, 1992). The solid fat index (Table B-3, Appendix B) assessed on the milkfat treatments did not show any differences on solid fat content between the treatments at temperatures between O and 35 C nor did dropping points of the milkfat treatments ($P>0.05$). Melting rate of ice cream was measured at 22-25ºC. No significant differences were found. The high-linoleic ice cream had the shortest ($P>0.05$) time recorded for the first ice cream drip to occur (14.75 min) when compared to control (16.60 min) and high-oleic (17.6 min). Fat content of ice cream treatments (10%), similar solid fat indexes and temperature were factors that may have influenced textural properties of the frozen ice cream treatments to be similar.

Sensory analyses on firmness of ice cream were measured also to support the analytical firmness results. Differences in firmness between the ice cream treatments, based on scooping tests, were not found ($P>0.05$) (Table 3-6). The temperature at which the scooping test was completed was approximately –18 ºC. At this temperature, ice creams samples were very hard

Table 3-6. Paired Comparison of firmness, based on ease of dipping, in ice cream with natural and modified fatty acid profiles

| Samples | Oleic vs Linoleic | | Control vs Oleic | | Linoleic vs Control | |
|---|---|---|---|---|---|---|
| | Number of times the treatment was chosen[1] /total responses | | | | | |
| Period 1 | 20/24 | 4/24 | 10/24 | 14/24 | 16/24 | 8/24 |
| Period 2 | 11/24 | 13/24 | 12/24 | 12/24 | 13/24 | 11/24 |
| Period 3 | 1/24 | 23/24 | 19/24 | 5/24 | 12/24 | 12/24 |
| Total | 32/72 | 40/72 | 41/72 | 31/72 | 41/72 | 31/72 |

[1]Number of agreeing answers to obtain significant difference based on72 observations at a p=0.05 is 45 (Meilgaard et al., 1999)

and difficult to scoop according to some observations made by the panelists. The temperature at which average composition ice cream should be dipped is –13.3  C (Arbuckle, 1986).

## CONCLUSIONS

Manipulation of the cow's diet resulted in a milkfat with a modified lipid profile. The high-oleic milkfat had a fatty acid profile that could contribute to a better nutritional value because of lower concentration of saturated fatty acids; the oxidative behavior was similar to the control treatment. Butter with high-oleic concentration was less firm when compared to the control milkfat. Spreadability properties of the high-oleic butter at low temperatures may be improved. These qualities suggest that high-oleic milkfat has potential for improving product value and maintaining quality. High-linoleic milkfat had a higher content of CLA and TVA potentially making it more valuable than the standard milkfat or a high-oleic milkfat from a nutritional point of view. Oxidation in frozen storage suggesting that ice cream processed from modified milkfat may have quality problems. Nevertheless, the high-oleic and high-linoleic milkfat had an improved and more desirable fatty acid profile than the control milkfat.

Modification of the fatty acids could be manipulated in a way in which not only a healthier profile could be achieved but one that could benefit chemical and physical and sensory properties of milkfat. Solutions, such as, addition of antioxidants to prevent lipid oxidation will be needed to control product flavor if high-linoleic milkfat is utilized in dairy products.

## ACKNOWLEDGEMENT

The authors express their appreciation to Walter Hartman, Harriet Williams, Kim Waterman, Wendy Wark and Juan Loor for their technical support on this project. Also to the Wisconsin Center for Dairy Research for their help with the solid fat index analyses.

Special appreciation is expressed to Virginia Agriculture Council for funding the present research

## REFERENCES


Abd El-Rahman., A.M, Madkor., S.AS., Ibrahim, F.S., and Kilara, A. 1997 a. Physical characteristics of frozen desserts made with cream, ahydrous milk fat, or milk fat fractions. J Dairy Sci. 80: 1926-1935.

Abd El-Rahman, A.M., Shalabi, S.I., Hollender, R., and Kilara, A. 1997 b. Effect of milk fat fractions on the sensory evaluation of frozen desserts. J. Dairy Sci. 80: 1936-1940.

Aigster, A., Sims, C., Staples, C., Schmidt, R., and O'Keefe, S.F. 2000. Comparison of Cheeses made from milk having normal and high oleic fatty acid compositions. J.Food Sci 65(5) 920-924.

AOAC,1997. Official Mehthods of Analysis, 16$^{TH}$ ed. Association of Official Analytical Chemists, AOAC Internat. Gaithersburg, MD.

Arbuckle.W.S. 1986. Ice Cream, Fourth Edition.Van Nostrand Reinhold, New York.

Ashes, J.R., Gulati, S.K., and Scott, T.W. 1997. Potential to alter the content and composition of milk fat through nutrition. J. Dairy Sci. 80:2204-2212.

Badings, H.T. 1970. Autoxidation processes of fatty acids. Ch2,3 in Cold storage defects and their relation to the autoxidation of unsaturated fatty acids. Netherlands Milk and Dairy Journal 24 (3/4):151-176.

Baer, R.J., Ryali, J., Schingoethe, D.J., Kasperson, K.M.,Donovan, D.C., Hippen, A.R.,and Franklin, S.T. 2001. Composition and properties of milk and butter from cows fed fish oil. J. Dairy Sci. 84: 345-353.

Bayourthe, C., Enjalbert, F., and Moncoulon, R. 2000. Effects of different forms of canola oil fatty acid plus canola meal on milk composition and physical properties of butter. J. Dairy Sci. 83:690-696.

Bligh, E.G and. Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. Can. J. Biochem. Physiol. 37:911-917.

DeLuca, D.D and Jenkins, T.C. 2000. Feeding oleamide to lactating jersey cows.2. Effects on nutrient digestibility, plasma fatty acids and hormones. J. Dairy Sci. 83:569-576.

DeMan,J.M. 1999. Lipids. Ch 2 in Principles of Food Chemistry, p 33-81. Aspen Publishers, Inc., Maryland.

Dhiman, T.R., Anand, G.R., Satter, L.D., and Pariza, M.W. 1999 a. Conjugated linoleic acid content of milk from cows fed different diets. J. Dairy Sci 82:2146-2156.

Dhiman, T.R., Helmink, E.D., Mcmahon, D.J., Fife, R.L., and Pariza, M.W. 1999 b. Conjugated linoleic acid content of milk and cheese from cows fed extruded oilseeds. J. Dairy Sci. 82:412-419.

Dhiman. T.R., Satter, L.D., Pariza, M.W., Galli, M.P., Albright, K., and Tolosa, X. 2000. Conjugated linoleic acid (CLA) content of milk from cows offered diets rich in linoleic and linolenic acid. J. Dairy Sci. 82:412-419.

Elling, J., Harris, J., and Duncan, S.E. 1995. Incorporation of modified butteroil into high-fat dairy products: ice cream manufactured with reduced-cholesterol reformulated cream. Dairy, Food and Environmental Sanitation 15(12); 738-744.

Focant, M., Mignolet, E., Marique, M., Clabots, F., Breyne, T., Dalemans, D., and Larondelle, Y. 1998.The effect of vitamin E supplementation of cow diets containing rapeseed and inseed on the prevention of milk fat oxidation. J. Diary Sci. 81:1095-1101.

Fox, P.F and McSweeney, P.L.H.1998. Dairy Chemistry and Biochemistry. Blackie Academic and Professional, New York.

Goff, D.H., and Hill,A.R. 1993. Chemistry and physics. In Dairy Science andTechnology Handbook, vol. 1. Hui, Y.H. (ed), p.50-54. VCH, New York.

Grundy, S.M. 1997.What is the desirable ratio of saturated, polyunsaturated, and monounsaturated fatty acids in the diet. Am. J. Clin. Nutr. 66(suppl): 988S.

Hawke, J.C. and Taylor, M.W. 1995. Influence of nutritional factors on the yield, composition and physical properties of milkfat. Ch. 2 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.37-88. Chapman & Hall., Great Britain.

Hoffman, G. 1962. Vegetable oils. Ch. 12 in Symposium of Foods: Lipids and their Oxidation, Schultz, H.W (ed), p. 215-226. Avi Publishing Company, Inc., Westport, Connecticut.

Im, Ji-S and Marshall, R. 1998. Effects of homogenization pressure on the physical, chemical and sensory properties of formulated frozen. Food Sci. Biotechnol. 7(2):90-94

Jenkins, T.C. 1998. Fatty acid composition of milk from holstein cows fed oleamide or canola oil. J. Dairy Sci. 81: 794-800.

Kaya, A. 2000. Properties and stability of butter oil obtained from milk and yoghurt. Nahrung 44(2): 126-129.

Kaylegian, K.E., and Lindsay, R.C.1995. Handbook of Milkfat Fractionation Technology and Application. AOCS Press., Champaign, IL.

Kramer, J.K.G., Fellner, V., Dugan, M.E.R., Sauer, F.D., Mossoba, M.M. and Yurawecz, M.P. 1997. Evaluating acid and base catalysts in the methylation of milk and rumen fatty acids with special emphasis on conjugated dienes and total trans fatty acids. Lipids 32:1219.

Lawless, F., Murphy, J.J., Harrington, D., Devery, R. and Stanton, C. 1998. Elevation of conjugated cis-9, trans-11-octadecadienoic acid in bovine milk because of dietary supplementation. J. Dairy Sci. 81:3259-3267.

Lin, M-P., Staples, C.R., Sims, C.A., and O'Keefe, S.F. 1996a. Modification of fatty acids in milk by feeding calcium- protected high oleic sunflower oil. J. Food Sci. 61(1): 24-27.

Lin, M.P., Sims, C.A., Staples, C.R., and O'Keefe, S.F. 1996b. Flavor quality and texture of modified fatty acid high monoene, low saturate butter. Food Research International 29 (3-4):367-371.

Marshall, R.T (ed.). 1993. Standards Methods for the Examination of Dairy Products. 16th ed. American Public Health Association, Washington, D.C.

Matak, Kristen.1999. Lactose Hydrolysis by Fungal and Yeast Lactase: Influence in Properties of 20% Reformulated Cream. Master Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

McDonald, I.W. and Scott, T.W.1977. Food of ruminant origin with elevated content of polyunsaturated fatty acids. World Rev. Nutr. Diet. 26:144-207.

Meilgaard, M., Civille, G.V., Carr, B.T.1999. Overall difference tests. Ch.6 in Sensory Evaluation Techniques, 3 ed., CRC Press, P: 60-73, New York.

Molkentin, J. 1999. Bioactive lipids naturally occuring in bovine milk. Nahrung 43(3):185-189.

Noakes, M., Nestel, P.J., and Clifton, P.M. 1996. Modifying the fatty acid profile of dairy products through feedlot technology lowers plasma cholesterol of humans consuming the products. Am. J. Clin. Nutr. 63: 42-46.

Otero, M., Rodriguez,T., Camejo,J., Regalado,O.L., and Fung,V. 1999. Uso de grasa marina y aceite vegetal en helado. Alimentaria 299:61-63.

Parodi, P.W. 1999. Conjugated linoleic acid and other anticarcinogenic agents of bovine milk fat. J. Dairy Sci. 82:1339-1349

Prentice, J.H, 1992. Dairy Rheology. A Concise Guide. Hui, Y.H (ed). VCH Publishers, Inc., New York.

Richardson, G.H. 1985. Standard Methods for the Examination of Dairy Products.15$^{TH}$ Edition. American Public Health Association, Maryland.

Ritzenthaler, K.L., McGuire, M.K., Falen, R., Shultz, T.D., Dasgupta, N., and McGuire, M.A. 2001. Estimation of conjugated linoleic acid intake by written dietary assessment methodologies underestimates actual intake evaluated by food duplicate methodology. J. Nutr. 131: 1548-1554.

Rossell, J.B.1989. Measurement of rancidity. Ch.2. In Rancidity in Foods, Allen, J.C and Hamilton, R.c (Ed.), p.23-30. Elsevier Science Publishers LTD., USA.

Scott, L. 1999. The effect of milk fat melting properties on chemical and physical freezing point and dipping characteristics of Ice Cream. Master Thesis, Virginia Polytechnic Institute and State University, Blacksburg, VA.

Stegeman, G.A., Baer, R.J., Shingoethe, D.J., and Casper, D.P. 1992. Composition and flavor of milk and butter from cows fed unsaturated dietary fat receiving bovine somatotropin. J Dairy Sci. 75: 962-970.

Velez-Ruiz, J., and Barbosa, G.V. 1997. Rheological properties of selected dairy products. Crit Rev Food Sci Nutr 37(4):311-359.

Walstra. P. 1995. Physical chemistry of milkfat globules. Ch 4 in Advanced Dairy Chemistry: Lipids, Fox.P.F. (ed), p.131-178). Chapman & Hall, Great Britain.

# APPENDIX A

# CHEMICAL ANALYSES OF LIPIDS

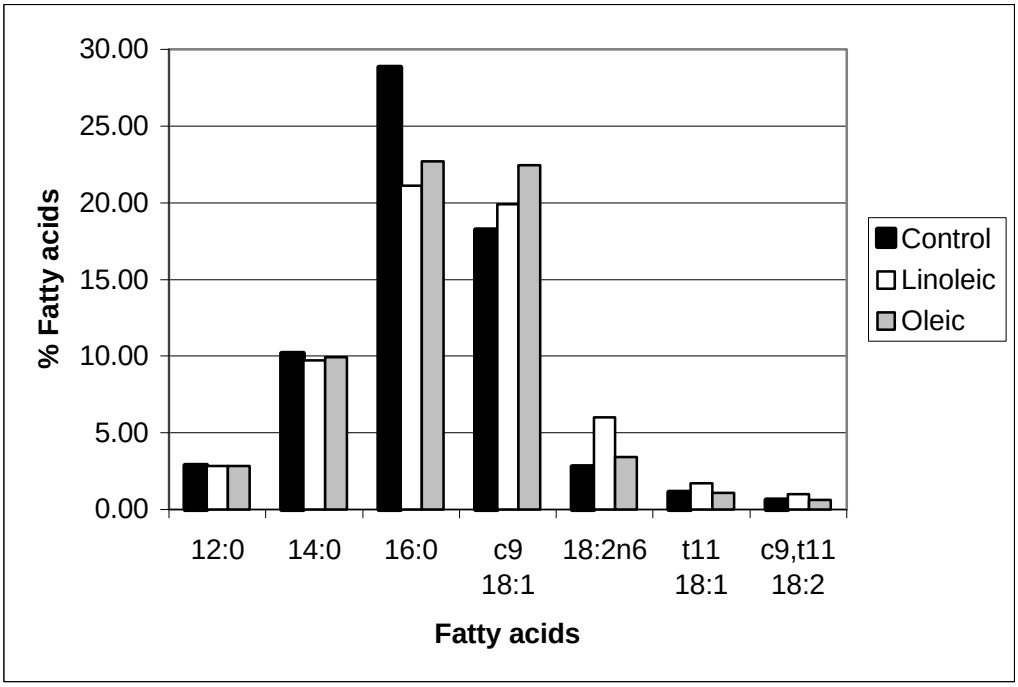


Figure A-1. Fatty acid profile of milkfat form cows fed normal, high-oleic and high-linoleic diets

Table A-1. Fatty acid profile of milkfat cows fed normal, high-oleic and high- linoleic diets (milkfat extracted from ice cream stored at –24 C)

| Fatty acid[1] | Diet | | | | | |
|---|---|---|---|---|---|---|
| | Control | SE[6] | Oleic[7] | SE | Linoleic[7] | SE |
| | (g/100g of fatty acids) | | | | | |
| SC[4] | 15.61 | 0.27 | 16.07 | 0.19 | 15.79 | 0.40 |
| 12:0 | 2.82 | 0.06 | 2.79 | 0.03 | 2.81 | 0.08 |
| 14:0 | 10.09 | 0.12 | 9.94 | 0.08 | 9.84 | 0.14 |
| 16:0 | 28.98 | 0.27 | 23.17 | 1.06 | 21.87 | 0.65 |
| 18:0 | 13.02 | 0.13 | 13.79 | 0.33 | 13.85 | 0.09 |
| 18:1 c9 | 18.22 | 0.33 | 22.45 | 0.41 | 19.92 | 0.48 |
| 18:2 n-6 | 2.81 | 0.01 | 3.41 | 0.17 | 5.99 | 0.09 |
| TVA t11[2] | 1.12 | 0.03 | 1.10 | 0.02 | 1.73 | 0.04 |
| CLA c9,t11[3] | 0.62 | 0.01 | 0.61 | 0.17 | 1.00 | 0.02 |
| Saturated | 69.27 | 0.46 | 64.11 | 0.64 | 62.15 | 0.41 |
| Unsaturated | 30.72 | 0.46 | 35.90 | 0.63 | 37.85 | 0.41 |
| PLC[5] | 4.87 | 0.04 | 5.21 | 0.19 | 8.38 | 0.11 |

[1] Expressed as number of carbons: number of double bonds
[2]TVA= Transvaccenic acid ($C_{18:1}$ t11)
[3]CLA= Conjugated linoleic acid ($C_{18:2}$ cis9,t11)
[4]SC = Short chain fatty acids
[5]PLC= Polyunsaturated long chain fatty acids ($C_{18:2} - C_{22:6\ n-3}$
[6]SE = Standard Error on duplicate samples for the three replications of each treatment
[7]Oleic= high oleic treatment; linoleic= high linoleic treatment

Table A-2 Total fatty acid profile milkfat cows fed normal, high-oleic and high- linoleic diets

| Fatty acids | Control | Oleic | Linoleic |
|---|---|---|---|
| | g/100g of fatty acids | | |
| 4:0 | 6.822 | 6.942 | 6.677 |
| 6:0 | 2.630 | 2.773 | 2.737 |
| 8:0 | 1.343 | 1.443 | 1.445 |
| 10:0 | 2.702 | 2.772 | 2.772 |
| 12:0 | 2.818 | 2.788 | 2.808 |
| 14:0 | 10.085 | 9.942 | 9.842 |
| 14:1 | 0.778 | 0.902 | 0.930 |
| 15:0 | 1.012 | 0.817 | 0.842 |
| 16:0 | 28.980 | 23.168 | 21.865 |
| t 16:1 | 0.367 | 0.307 | 0.355 |
| c 16:1 | 1.537 | 1.327 | 1.192 |
| 17:0 | 0.642 | 0.482 | 0.482 |
| 18:0 | 13.022 | 13.790 | 13.848 |
| t4 18:1 | 0.020 | 0.065 | 0.032 |
| t5 18:1 | 0.018 | 0.047 | 0.028 |
| t6,t7 18:1 | 0.283 | 0.697 | 0.457 |
| t9 18:1 | 0.207 | 0.413 | 0.340 |
| t10 18:1 | 0.312 | 0.495 | 0.540 |
| t11 18:1 | 1.028 | 0.972 | 1.568 |
| t12,c7 18:1 | 0.390 | 0.493 | 0.652 |
| t13,c6 18:1 | 0.875 | 0.857 | 1.210 |
| c9 18:1 | 17.572 | 21.657 | 18.993 |
| c11 18:1 | 0.602 | 0.612 | 0.567 |
| c12 18:1 | 0.735 | 0.648 | 1.243 |
| c13 18:1 | 0.203 | 0.265 | 0.193 |
| t16 18:1 | 0.395 | 0.333 | 0.492 |
| 19:0 | 0.003 | 0.042 | 0.022 |
| c15 18:1 | 0.258 | 0.200 | 0.212 |
| t9,t12 18:2 | 0.017 | 0.018 | 0.018 |
| c9,t12 18:2 | 0.013 | 0.000 | 0.000 |
| t9,c12 18:2 | 0.017 | 0.027 | 0.040 |
| t11,c15 18:2 | 0.080 | 0.022 | 0.018 |
| 18:2n6 | 2.560 | 3.183 | 5.623 |
| t9,c15 18:2 | 0.028 | 0.023 | 0.017 |
| 20:0 | 0.153 | 0.157 | 0.138 |
| 18:3n3 | 0.418 | 0.335 | 0.338 |
| c9,t11 18:2 | 0.547 | 0.532 | 0.910 |
| t10,c12 18:2 | 0.008 | 0.000 | 0.020 |
| c9,c11 18:2 | 0.033 | 0.027 | 0.023 |
| t11,t13 18:2 | 0.018 | 0.003 | 0.005 |
| other t18:2 | 0.042 | 0.033 | 0.045 |
| 20:3n3 | 0.100 | 0.107 | 0.133 |
| 22:1 | 0.023 | 0.022 | 0.032 |
| 20:4n6 | 0.142 | 0.153 | 0.182 |
| 20:5n3 | 0.022 | 0.013 | 0.017 |
| 22:4n6 | 0.027 | 0.033 | 0.027 |
| 22:5n3 | 0.092 | 0.067 | 0.067 |
| 22:6n3 | 0.027 | 0.007 | 0.005 |

Table A-3. Peroxide values (meq of peroxide/Kg sample) of milk, cream, and ice cream during the processing period (0 months) and ice cream during 2 months of storage and 3-5 months of storage (Period when sensory analyses took place)

| Product | Control | SE[1] | Oleic | SE | Linoleic | SE |
|---|---|---|---|---|---|---|
| | | | Peroxide Value[2] | | | |
| Milk (0 months) | 6.37 | 0.65 | 6.74 | 0.83 | 5.51 | 0.62 |
| Cream (0 months) | 1.35 | 0.20 | 1.28 | 0.16 | 1.74 | 0.25 |
| Ice cream (0 months) | 4.12 | 0.52 | 2.91 | 0.45 | 3.25 | 0.46 |
| Ice cream (2 months) | 8.06 | 2.24 | 7.77 | 1.36 | 8.10 | 1.62 |
| Ice cream (3-5 months) | 2.54 | 0.12 | 2.83 | 0.26 | 22.04 | 4.34 |
| Ice Cream (7-9 months) | 7.51 | 0.81 | 8.66 | 0.51 | 8.68 | 0.57 |

[1]SE= Standard error of the mean
[2]Peroxide Value= miliequivalents of hydroperoxide/kg of sample

Table A-4. % Free fatty acids of milk, cream, and ice cream during the processing period (0 months) and ice cream during 2 months of storage and 3-5 months of storage (Period when sensory analyses took place

| Product | Control | SE[1] | Oleic | SE | Linoleic | SE |
|---|---|---|---|---|---|---|
| | | | [2] % Free fatty acids | | | |
| Milk (0 months) | 0.12 | 0.00 | 0.09 | 0.01 | 0.12 | 0.01 |
| Cream (0 months) | 0.05 | 0.00 | 0.05 | 0.01 | 0.05 | 0.01 |
| Ice cream (0 months) | 0.10 | 0.01 | 0.08 | 0.01 | 0.09 | 0.00 |
| Ice cream (2 months) | 0.10 | 0.01 | 0.08 | 0.00 | 0.08 | 0.00 |
| Ice cream (3-5 months) | 0.12 | 0.01 | 0.09 | 0.00 | 0.11 | 0.01 |

[1]SE= Standard error of the mean
[2] % Free fatty acids= percentage of free fatty acids expressed as oleic acid

Table A-5. Acid value of milk, cream, and ice cream during the processing period (0 months) and ice cream during 2 months of storage and 3-5 months of storage (Period when sensory analyses took place)

| Product | Control | SE[1] | Oleic | SE | Linoleic | SE |
|---|---|---|---|---|---|---|
| Acid Value[2] | | | | | | |
| Milk (0 months) | 0.24 | 0.01 | 0.18 | 0.01 | 0.25 | 0.01 |
| Cream (0 months) | 0.10 | 0.01 | 0.10 | 0.01 | 0.09 | 0.01 |
| Ice cream (0 months) | 0.20 | 0.02 | 0.17 | 0.01 | 0.18 | 0.01 |
| Ice cream (2 months) | 0.20 | 0.01 | 0.15 | 0.00 | 0.16 | 0.00 |
| Ice cream (3-5 months) | 0.24 | 0.02 | 0.18 | 0.01 | 0.22 | 0.02 |

[1]SE= Standard error of the mean
[2]Acid value= free fatty acids expressed as acid value

Table A-6 Proximate compositions of ice cream mixes during processing period (0 months)

| Proximate Composition | Control | SE[1] | Linoleic | SE | Oleic | SE |
|---|---|---|---|---|---|---|
| | | | % | | | |
| Fat | 10.3 | 0.4 | 10.3 | 0.0 | 9.9 | 0.3 |
| Protein | 7.8 | 0.4 | 7.5 | 0.2 | 7.7 | 0.6 |
| Ash | 0.9 | 0.0 | 0.9 | 0.0 | 0.9 | 0.1 |
| Total Solids | 35.0 | 0.9 | 36.1 | 0.6 | 36.8 | 0.6 |
| Moisture | 65.0 | 0.9 | 63.9 | 0.6 | 63.2 | 0.6 |

[1]SE= Standard error of the mean

Table A-7 Microbiological Analyses

| | Control | | | Linoleic | | | Oleic | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | **ESPC** | | | | | |
| Period | A | B | C | A | B | C | A | B | C |
| Coliforms | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| Psychotrophs | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg | Neg |
| Standard plate count | Neg | Neg | 20 | 10 | 10 | 15 | 10 | 10 | 25 |

Neg= no growth was observed:<100ESPC

# APPENDIX B

# PHYSICAL ANALYSES

Table B-1. Butter and ice cream firmness expressed in joules measured at specific temperature ranges.

| Product | Firmness mean (Joules) | SE |
|---|---|---|
| Butter (4.2-5.5 ºC) | | |
| Control | 10.54 | 0.45 |
| High-oleic | 6.09 | 0.40 |
| High-linoleic | 6.95 | 0.37 |
| Ice cream (-17-(-13.3ºC)) | | |
| Control | 1.25 | 0.27 |
| High-oleic | 1.15 | 0.26 |
| High-linoleic | 0.74 | 0.12 |

Table B-2. Apparent viscosity (milli pascals) of ice cream mixes at increasing and then decreasing shear rates

| Shear rate (s^-1) | Control | SE | Oleic | SE | Linoleic | SE |
|---|---|---|---|---|---|---|
| | | | Apparent Viscosity (mPa) | | | |
| 173 | 41.34 | 12.44 | 24.66 | 4.88 | 27.31 | 10.27 |
| 346.24 | 71.48 | 10.60 | 57.56 | 5.92 | 51.42 | 8.91 |
| 692.5 | 83.08 | 9.16 | 71.34 | 4.89 | 60.92 | 8.06 |
| 1384.96 | 83.18 | 7.89 | 73.24 | 3.60 | 65.52 | 6.51 |
| 692.5 | 19.16 | 2.33 | 15.18 | 1.14 | 14.50 | 2.00 |
| 346.24 | 16.14 | 2.69 | 11.04 | 1.41 | 11.74 | 2.20 |
| 173 | 9.16 | 2.99 | 4.88 | 0.62 | 8.44 | 1.88 |

Table B-3. Dropping points and solid fat index of the butteroil treatments

| Treatment | Control | SE[1] | Linoleic | SE | Oleic | SE |
|---|---|---|---|---|---|---|
| Dropping point ( C) | 23.5 | 0.4 | 24.4 | 0.9 | 24.5 | 0.1 |
| Temperature ( C) | Solid Fat content % | | | | | |
| 0 | 45.2 | 0.8 | 47.3 | 2.1 | 47.7 | 2.6 |
| 5 | 46.5 | 0.5 | 46.9 | 1.2 | 48.6 | 1.9 |
| 10 | 41.1 | 0.1 | 40.2 | 1.6 | 43.4 | 2.3 |
| 15 | 32.6 | 1.2 | 29.6 | 0.8 | 34.5 | 2.1 |
| 20 | 18.2 | 2.1 | 14.1 | 0.7 | 21.2 | 2.7 |
| 25 | 11.0 | 2.1 | 7.5 | 0.7 | 14.2 | 2.5 |
| 30 | 5.7 | 1.1 | 3.5 | 0.4 | 7.0 | 1.5 |

# APPENDIX C
# SENSORY ANALYSES

Table C-1. Responses of overall difference test (Triangle Test)

| Samples | Oleic | Linoleic | Control | Oleic | Linoleic | Control |
|---|---|---|---|---|---|---|
| | Number of times the treatment was chosen /total responses | | | | | |
| Rep 1 | 20/24 | 4/24 | 10/24 | 14/24 | 16/24 | 8/24 |
| Rep 2 | 11/24 | 13/24 | 12/24 | 12/24 | 13/24 | 11/24 |
| Rep 3 | 1/24 | 23/24 | 19/24 | 5/24 | 12/24 | 12/24 |
| Total | 32/72 | 40/72 | 41/72 | 31/72 | 41/72 | 31/72 |

**Overall difference**

Significant difference was found in the overall difference test (triangle test) between the control and oleic ice cream as well as the control and linoleic ice cream at a $p = 0.05$.

Since the objective of the triangle test was to determine if an overall difference existed, the difference found by the panelists can not be related to a single characteristic like flavor. Several characteristics like texture, color, odor and flavor, may have contributed to the difference.

In this specific case, sandiness and iciness were present in some of the ice cream samples and this influenced some panelists in determining the overall difference. The production of large ice crystals can be due to temperature difference between the product and the freezing environment, area of the product, heat transfer coefficient and way of hardening (Goff,2000). Recrystallization processes are responsible of the production of large crystals in ice cream (Sutton et al., 1996). Sandiness is formed by the presence of large insoluble lactose crystals. It is related to the glass transition temperature of sucrose-lactose and effect of water on glass transition temperature, which is also related to ice recrystallization (Szczesniak, 1998). According to this author the glass transition temperature of ice cream is around –34 ºC, while the

temperature in storage freezers is around -20ºC. this temperature difference will influence in the development of sandiness. The larger the temperature difference, the higher the probability that the ice cream would develop sandiness. Stabilizers are used to reduce the crystal growth but some researchers think that they would only influence the organoleptical perception of the crystals (Trgo et al., 1999).

Figure C-1

Human Subjects Forms for Sensory Evaluation

Protocol for Projects of Sensory Evaluation

If the project involves sensory evaluation, please complete the following questions about the project to assist you and the Institutional Review Board in determining the risk level of the project.

Definition: Sensory evaluation is the evaluation of food or other substances by the senses including taste, touch, smell, sight and hearing.

Check all that apply:

1. The procedure for sensory evaluation in this project involves:
   __X___ Tasting in the mouth (includes tests where the panelist is instructed to spit it out)
   _____ Substances applied to the skin
   _____ Substances smelled for odor components
   _____ Substances evaluated by sound when chewed
   __X___ Substances evaluated by visual senses

2. The product/s to be evaluated are:
   __X___ Made entirely of ingredients approved by FDA for consumption or application under approved conditions of processing
   _____ Made of ingredients approved by FDA but not approved for the use in the project (e.g. heating of aspartame, fat substitutes approved only as an emulsifier).
   _____ Made partially or entirely of experimental ingredients pending FDA approval.
   _____ Made partially or entirely of experimental ingredients not approved for human consumption or topical use
   _____ Made from materials from or altered by biotechnology

3. The processing or preparation of the product is:
   __X___ By usual approved good manufacturing or preparation practices for that food or topical product.
   _____ By experimental procedures including non-good manufacturing practices. Briefly describe the procedures.

4. The packaging of the product includes:
   __X__ Processing or storage in FDA-approved packaging materials.
   _____ Processing or storage in packaging materials not approved by FDA.

5. Describe the storage protocols for the product that are necessary to maintain the product in safe condition.

Ice cream mix was pasteurized and prepared in sanitary conditions. Microbiological analyses are going to be assessed to assure its safety. Ice cream is going to be stored at –20 °C.

6. If microbiological cultures are a part of the food processing or preparation procedure, describe what cultures will be used, if they will be active on consumption, and give evidence that these cultures are known to be safe for human consumption.

   No microbiological cultures are used

7. Allergies

   ___X__ Are any ingredients to be used potentially allergenic as consumed or by topical application? If yes, describe. Have panelists been made aware of these ingredients?

   Lactose present in the ice cream could affect lactose intolerant patients.

When you have completed this form, indicate the risk level to the panelists of this project. Complete the appropriate form; for "not at risk", the Certificate of Exemption form; for "at minimal risk", the Request for Approval form.

Virginia Polytechnic Institute and State University
Informed Consent for Participation in Sensory Evaluation

Title of Project: **Evaluation of quality properties of butter, butteroil and ice cream with a higher content of linoleic and oleic acid.**

Principal Investigator: Dr. Susan E. Duncan, Ph.D.,R.D., Associate Professor

I. THE PURPOSE OF THIS PROJECT
The purpose of this research is to determine if different fatty acid profiles (highly unsaturated) influence flavor quality of ice cream.

II. PROCEDURES
There will be __2__ sessions over a period of __1___ weeks involving about __10___ minutes at each session. You will be presented with approximately __18___ samples. As a panelist, it is critical to the project that you attend to the session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

_____
_____
_____

III. BENEFITS/RISKS OF THE PROJECT

**Your participation in the project will provide the following information that may be helpful. Increased levels of polyunsaturated and monounsaturated fatty acids may influence in chemical, physical and sensory properties of this type of product. There is limited information concerning the effect of milkfat modification on dairy product quality.**

**You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have unknown food allergy or lactose intolerance.**

IV. EXTENT OF AN0NYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by code for analyses and in any publication of the results.

V. COMPENSATION
Please accept a piece of candy as a appreciation for your participation in this project.

VI. FREEDOM TO WITHDRAW

It is essential to sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and

becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subject's review of the Department of Food Science and Technology.

VIII. SUBJECT'S RESPONSIBILITIES

I know of no reason I cannot participate in this study, which will require sensory evaluation of ice cream

_____

Signature/Date

Please provide address and phone number so investigator may reach you in case of emergency or schedule changes.

Address _____

Phone _____

----------(tear off)----------

IX. SUBJECT'S PERMISSION (provide tear off for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation in this project.

I know of no reason I cannot participate in this study

_____

Signature/Date

Should I have any questions about this research or its conduct, I should contact:

Dr. Susan Duncan 231-8657
Principal Investigator
Faculty/ Phone

Sonia Gonzalez
Graduate student

David Moore (540)231-6077
Chair, IRB/Phone for Research Division

**OUTLINE FOR PROTOCOL TO ACCOMPANY IRB REQUEST**

JUSTIFICATION OF PROJECT

*Coronary artery disease and cancer are related to high saturated fat diets. Some dairy products like ice cream and butter have been related to these diseases due to the high-saturated fatty acid content and therefore have been less consumed over the past years. Different studies have shown that the fatty acid profile of milkfat can be modified by changing the diet of the cows and improving in this way its nutritional value (less saturated and more polyunsaturated fatty acids). The influence of this modification on physical and chemical properties of the product has to be measured to assure its quality and consumer acceptance.*

PROCEDURES

Ice cream mix was prepared from fresh, wholesome ingredients, pasteurized at 155°C for 30 minutes. Microbiological analyses were done to document sanitary conditions. Samples were frozen at –20°C and stored under this temperature until the date of the analysis.

Products will be evaluated at -15°C to -18°C at which no safety risk is involved. There would be two sessions over a period of one day involving 10 minutes approximately. The session consists in a Paired Comparison test to measure differences in texture through an easy of dipping test.

Panelists will be chosen from the food science department. They will complete a human consent form and the evaluation will be anonymous. No identification of individual panelists will take place in the reporting.

*Another investigator of the project who will have contact with the human subjects is Kim Waterman (laboratory technician of the Food Science Department).*

RISKS AND BENEFITS

No identifiable risks have been found in this research. Some risk may be involved if the panelist has food allergy or lactose intolerance. The participation will be anonymous.

CONFIDENTIALITY/ANONYMITY

Panelists will be assigned coded scorecards previously written by the investigators.

*Researchers (student, advisors) may be aware of individuals responses but will protect the identity in any written or oral communication regarding the project.*

Figure C-2

**SCORECARD**

**TRIANGLE TEST**

JUDGE NUMBER:_____
PRODUCT: Vanilla Ice cream

There are three sets of triangle test. Test the samples in the order presented and identify the odd sample. Expectorate samples and rinse your mouth with water. After concluding the first triangle test continue with next one.

| | CODE | CHECK ODD SAMPLE |
|---|---|---|
| TRIANGLE TEST 1 | _____ | _____ |
| | _____ | _____ |
| | _____ | _____ |

PASS THE TRAY AND WAIT FOR THE SECOND SET OF SAMPLES

| | | |
|---|---|---|
| TRIANGLE TEST 2 | _____ | _____ |
| | _____ | _____ |
| | _____ | _____ |

PASS THE TRAY AND WAIT FOR THE THIRD SET OF SAMPLES

| | | |
|---|---|---|
| TRIANGLE TEST 3 | _____ | _____ |
| | _____ | _____ |
| | _____ | _____ |

THANK YOU! THE TEXTURE ANALYSIS IS TAKING PLACE IN THE DAIRY PROCESSING LAB. PLEASE GO DOWNSTAIRS AND COMPLETE THE ANALYSIS

THANK YOU FOR YOUR TIME

Figure C-3

SCORECARD FOR DIRECTIONAL PAIRED COMPARISON TEST

| EASE OF DIPPING ICE CREAM |
| --- |

JUDGE NUMBER:_____

PRODUCT: ICE CREAM SAMPLES

This test is going to be conducted in the dairy laboratory and you should complete the scoresheet, while you are at the freezer.

The scooping action has to be done from one extreme of the container to the end of it.

Dip approximate one scoop of each ice cream sample and indicate which sample is easiest to dip.

You should scoop the first sample indicated in the scoresheet.

You must make a choice

Sample codes :

Easiest to dip: _____

Sample codes:

Easiest to dip:_____

Samples codes:

Easiest to dip: _____

THANK YOU FOR YOUR TIME!

Figure C-4

SCORECARD FOR DIRECTIONAL PAIRED COMPARISON TEST

OXIDATION FLAVOR IN ICE CREAM

JUDGE NAME:_____

PRODUCT: ICE CREAM SAMPLES

INSRTRUCTIONS:

You will receive three trays of samples with two sets of samples at a time. Please make sure you finish all six pairs.

Taste each pair from left to right and determine which sample has the strongest oxidation flavor within each pair.

Circle the code of the sample that is more oxidized. Please write any additional observations on flavor and texture of each sample.

Please make sure to come back today at _____ and at _____.

**SET 1** **OBSERVATIONS**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

**SET 2**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

SCORECARD FOR DIRECTIONAL PAIRED COMPARISON TEST

OXIDATION FLAVOR IN ICE CREAM

Circle the code of the sample that is more oxidized. Please write any additional observations on flavor and texture of each sample.

**SET 3** **OBSERVATIONS**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

**SET 4**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

SCORECARD FOR DIRECTIONAL PAIRED COMPARISON TEST

| OXIDATION FLAVOR IN ICE CREAM |
| --- |
| |

Circle the code of the sample that is more oxidized. Please write any additional observations on flavor and texture of each sample.

**SET 5** **OBSERVATIONS**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

**SET 6**

SAMPLE CODE: _____ _____

SAMPLE CODE: _____ _____

**THANK YOU FOR YOUR TIME!**

# VITAE

Sonia Gonzalez Artola was born on November 3, 1973 in San Salvador, El Salvador. She graduated from the German School in El Salvador. She graduated in 1998 from Universidad Centroamericana “Jose Simeon Canas” (UCA) in El Salvador with a B.S. in Agricultural Chemistry. In the fall of 1999, she began working towards a Master of Science degree in Food Science at Virginia Polytechnic Institute and State University.