THE APPLICATION OF CRANBERRY IN THE PRODUCTION AND QUALITY OF FRESH DAIRY PRODUCTS

by

STEPHEN TOMIUK

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ABSTRACT

The antioxidant activity of an ethanol extract prepared from cranberries was investigated using the oxygen radical absorbance capacity (ORAC) assay, as well as in a model food emulsion representative of dairy-based emulsions. The cranberry extract exhibited 348.31 ± 33.45 Trolox equivalents (TE)/g in the hydrophilic ORAC assay. In the model food emulsion consisting of 25% lipid stabilized by sodium caseinate, the extract was readily incorporated at the homogenization step until a pH of 5.6 was attained without causing emulsion destabilization. Under thermal conditions (50ºC), the lag phase prior to propagation of conjugated dienes (CD) in all cranberry emulsions at pH 5.6 was significantly extended relative to untreated controls ($P < 0.05$). The formation of secondary lipid oxidation products (hexanal and pentanal) in sunflower oil emulsions became significantly reduced ($P < 0.05$), but only after 64 hours of incubation. In contrast, the development of hexanal and pentanal in butterfat emulsions were significantly reduced ($P < 0.05$) over the entire incubation period. Increasing the level of cranberry extract in all emulsions beyond a final pH of 5.6 had an adverse effect on emulsion stability, and this was met with a drop in oxidative protection.

The acidity of the cranberry extract made it well-suited for use in a fresh cream cheese product, which requires the production of acid during manufacture. The cranberry extract was homogenized into a cream cheese premix consisting of milk and cream until a pH of 5.6 was reached. Cheese produced from 1.18 g extract/100 g premix had a light pink color that remained stable over 50 days of storage at 4ºC. The cheese curd was found to retain over 98% of phenolics from the extract after separation of the whey. The level of CD and the peroxide value in the final product were significantly lower ($P < 0.05$) as compared to the untreated control cream cheese, after two weeks of storage at 4ºC. This research shows for the first time that a cranberry extract rich in bioactives can provide color and flavor to fresh dairy products while also acting as a useful preservation agent.

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LIST OF ABBREVIATIONS

ABTS	2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate)
AAPH	2,2'-azobis(2-amidinopropane) dihydrochloride
AUC	Area under the curve
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CD	Conjugated dienes
ES	Emulsion stability
GAE	Gallic acid equivalents
HPLC	High performance liquid chromatography
H-ORAC	Hydrophilic oxygen radical absorbance capacity
L-ORAC	Lipophilic oxygen radical absorbance capacity
LC	Liquid chromatography
PV	Peroxide value
PUFA	Polyunsaturated fatty acids
RMCD	Randomized methylated cycoldextrin
SD	Standard deviation
TBHQ	tert-Butylhydroxyquinone
TE	Trolox equivalents
TEAC	Trolox equivalent antioxidant capacity

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1 INTRODUCTION

The shelf-life of a dairy product is best defined as "the time during which the product remains wholesome and exhibits no physical or organoleptic defects" (Muir, 1996). Synthetic food additives, such as BHA, BHT, or TBHQ, are common chain-breaking antioxidants (or stabilizers) used in food systems for the purpose of controlling against lipid oxidation (Decker, 1998). However, present day consumer demand for all-natural foods has prompted the food industry to look for more "label friendly" alternatives. Dairy products such as cream cheese, cottage cheese and yogurt, are examples of foods that exist as dispersions of fat throughout a continuous phase. The structural elements that make up fresh dairy products leave them susceptible to oxidative changes which may cause physical and chemical defects. At present, the addition of synthetic antioxidants to dairy products is not permitted and so, product stability relies primarily on the components present in the milk (Cuppett, 2001).

The commercial development of ingredients from plants to enhance the properties of foods for both nutritional purposes and for preservation is currently of major interest. Natural flavonoids may offer an alternative to protect lipids from oxidation in foods. Many of these flavonoids have been shown to inhibit lipid oxidation in meats, fish oil, lard and oil-in-water emulsions. For example, previous work has shown flavonoids to inhibit the oxidation of linoleic acid and cholesterol in an emulsion model system (Chen et al., 1996). Cranberries are a rich source of phenolic acids, anthocyanins, flavonol glycosides and proanthocyaninidins. Anthocyanins are responsible for color, and have been linked to the high radical scavenging ability of cranberries in a number of lipid oxidation models (Kahkonen and Heinonen, 2003). The stability of anthocyanins in food systems is dependent on pH, temperature, light, and oxygen, as well as the presence of proteins. Proanthocyanidins from cranberries have received recent attention due to the fact that they are polymeric, have strong anti-adhesion properties (Leahy et al., 2002, Howell et al., 2005), and can be readily partitioned into both aqueous and non-aqueous media (Lee et al., 2006).

The ability of phenolic-enriched extracts from berries to provide beneficial attributes to foods is believed to be due to the synergies and interactions that can occur between different classes of phenolics (Vattem et al, 2005). The practicality of isolating individual components from berries for functional usages in the food industry is limited by the complexity of isolation procedures, and the time and cost necessary to obtain purified fractions. Phenolic compounds provide antioxidant behavior while also contributing to characteristic flavors and odors associated with berries. The development of berry extracts that provide a multitude of factors may be highly desirable to food manufacturers that aim to produce value-added foods with unique characteristics. In the case of fresh dairy products, the inherent acidity of certain berries could be beneficial since acid production is required during production. The use of phenolics in dairy products has long been advocated not only for functional uses, but also for the need to create new flavorful and colorful dairy products that are otherwise plain in nature (O'Connell and Fox, 2001). An extract from cranberries, rich in phenolic compounds and organic acids, may act as a functional ingredient by improving the storage stability and aiding in the processing of fresh dairy products.

2 LITERATURE REVIEW

2.1 Manufacture and quality of fresh dairy products

Fresh dairy products are defined as those products formed by the coagulation of milk by acid or by a combination of acid and heat (Fox et al., 2000). The slow acidification of milk results in the formation of a curd which can then be manipulated in many different ways to give final products with desired physico-chemical characteristics. Fresh dairy products are generally ready for consumption immediately after manufacture. The most common varieties of fresh dairy products available in North America include cottage cheese, cream cheese, and ricotta (Lucey, 2004). Fresh dairy products represent an enormous economic value worldwide because they are relatively simple to produce, nutritious, and continuously being innovated with regard to flavor and added health benefits (O'Connell and Fox, 2001).

The quality and shelf-life of milk and dairy products is determined by many factors, from production and processing to distribution and retailing. The shelf-life of fresh cheeses is relatively short, ranging anywhere between 3 weeks and 3 months (Muir, 1996, Fox, 2004). For this reason, it is of utmost importance that these products be protected against environmental factors that could potentially cause product deterioration. The shelf-life of fresh dairy products begins with the quality of milk used and the subsequent processing steps which go into the manufacture of a specific product. In the case of cottage and cream cheese type products, shelf-life is highly influenced by the microbiological quality of the cream and milk, the microbial status of the other additives, as well as the final product pH (Muir, 1996, Lucey, 2004). Cottage cheese is manufactured by a process in which skim milk is acidified to form a curd, which is then annealed and coated with a cream dressing. The cream dressing is added together in some cases with fruit, herbs or spices (Muir, 1996). Cream cheese is made from homogenized, pasteurized cream which is acidified by the action of microbial cultures. It is structurally unique from other fresh dairy products in that it is spreadable, and often contains a much higher fat content. It is common practice to incorporate certain additives into cream cheese in order to obtain the desired physical characteristics, as well as prevent structural defects which may adversely affect

spreadability and mouthfeel. Fruits, herbs and spices are also often incorporated to provide products with unique flavors. The quality of cream cheese products can be compromised if additives are not properly treated before addition (Bot et al., 2007).

Due to the high fat content of cream cheese and other fresh cheeses, lipid oxidation may be a major factor affecting the quality of these cheeses. Lipid oxidation is a concern especially during long periods of storage and during transport where significant fluctuations in temperature can occur (Fedele and Bergamo, 2001). For fresh products, oxidation may lead to formation of various volatile carbonyl compounds which are responsible for off-flavors. The overall effect is rancidity, which is a term describing a wide variety of off-flavors and odors associated with oxidized fats and oils. It is a major deteriorative reaction in foods, leading to loss of quality in terms of color, flavor, texture and nutritional value (Downey, 1969).

Table 1. Characteristics of common fresh dairy products

Variety	Fat % (w/w)	Moisture % (w/w)	pH
Cream cheese			
Single	30	70	4.6
Double	14	60	4.6
Cottage cheese			
Whole milk	5	80	4.8
Low-fat	2	80	4.8
Queso Blanco	15	51	5.4
Ricotta			
Whole milk	13	72	5.8
Partly skim	8	75	5.8

*Adapted from Fox et al., 2000.

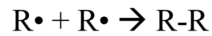
The chemical structure of lipids determines their susceptibility to lipid oxidation, with the number and position of double bonds being the primary determinant (McClements and Decker, 2000). Of fatty acids found in milk fat, 60-65% are saturated, and 25-40% are unsaturated. The fatty acids in milk fat are composed of approximately 2.3% palmitoleic acid (C16:1 n-7), 24.6% oleic acid (C18:1 n-9), and 2.8% linoleic acid (C18:2 n-6) with the last being the most susceptible to lipid oxidation (van Aardt et al., 2005). The oxidized flavor that can occur in milk and dairy products is generally caused by autoxidation of unsaturated fatty acids, mainly oleic, linoleic and linolenic acid associated with the phospholipids. Phospholipids and cholesterol are found mainly in the membrane of the fat globule, and collectively make up less than 1% of the lipid composition of milkfat (Downey, 1969).

2.2 Principles of lipid oxidation

Oxidation begins with the spontaneous abstraction of a hydrogen atom from an organic material. This is an endothermic reaction which demands a large activation energy supplied in the form of light or ionizing radiation, thermal energy (heat), the presence of metallic cations, enzymatic catalysis, or reactive singlet oxygen species produced by photosensitizers such as chlorophyll or myoglobin (O'Connor and O'Brien, 1994, Halliwell et al., 1995). Removal of hydrogen occurs at methylene groups adjacent to double bonds in unsaturated fatty acids. The homolytic cleavage of the covalent bond between hydrogen and the rest of the fatty acid results in the formation of free radicals (eq. 1). The unpaired electron found in the radical makes it extremely unstable, and causes it to react very quickly with other molecules or radicals to achieve a stable configuration. In the absence of oxygen, free radicals can recombine quickly thereby neutralizing themselves (eq. 2). Such reactions result in some random changes to the fatty acids, most of which are minimal from a sensory standpoint (O'Connor and O'Brien, 1994).

(1) $RH \rightarrow R. + H\bullet$

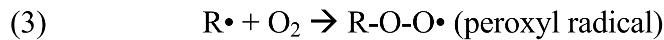
(2) $R\bullet + H\bullet \rightarrow RH$



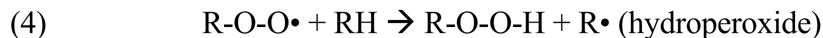
$R\bullet + R\bullet \rightarrow R\text{-}R$

$H\bullet + H\bullet \rightarrow H\text{-}H\ (H_2)$

In the presence of molecular oxygen, however, new radicals may form which are called peroxyl radicals (eq. 3). The reactions involving peroxyl radicals may proceed in several ways. The most likely event to occur is for the peroxyl radical to abstract a hydrogen atom from another fatty acid, since all fatty acids are abundant in hydrogen. This abstraction results in the formation of another fatty acid radical, and the generation of a new product called a hydroperoxide (eq. 4). Such a reaction is self-propagating, resulting in the continuous generation of unstable fatty acid radicals which may further react with one another. Hydroperoxides (eq. 4) and peroxides (eq. 5) are referred to as the primary products of lipid oxidation (O'Connor and O'Brien, 1994).



(3) $R\bullet + O_2 \rightarrow R\text{-}O\text{-}O\bullet$ (peroxyl radical)

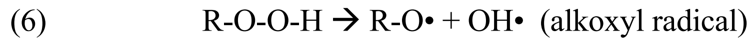


(4) $R\text{-}O\text{-}O\bullet + RH \rightarrow R\text{-}O\text{-}O\text{-}H + R\bullet$ (hydroperoxide)

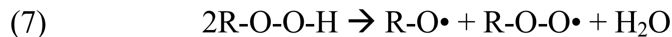
(5) $R\text{-}O\text{-}O\bullet + R\bullet \rightarrow R\text{-}O\text{-}O\text{-}R$ (peroxide)

The reaction between peroxyl radicals and other reactive species may also result in the formation of neutral, unreactive species. When such reactions occur, the propagation of radicals becomes interrupted. However, when the propagation of radicals exceeds reactions in which the neutral species are produced, there is an accumulation of peroxides. These peroxides are precursors to off-flavors and odors commonly associated with oxidative rancidity (Coupland and McClements, 1996).

Hydroperoxides are not chemically stable, and start to decompose especially as the concentration in the lipid system increases. The basic breakdown reaction of a hydroperoxide molecule results in the formation of an alkoxyl and hydroxyl radical (eq. 6).


(6) R-O-O-H → R-O• + OH• (alkoxyl radical)

The alkoxyl radical is very unstable, and may split in order to form a more stable, shorter chain aldehyde. Depending on the stability of the aldehyde molecule generated, it may subsequently be oxidized, giving carboxylic acids, or reduced, giving an alcohol. This is referred to as the monomolecular breakdown of peroxides. As lower molecular weight compounds form, changes in the flavor and odor become more pronounced until the lipid becomes unacceptable from a sensory standpoint. More complex reactions may also occur at higher peroxide concentrations. For instance, bimolecular reactions of hydroperoxides can occur (eq. 7), the net result being the formation of alkoxyl and peroxyl radicals. Alkoxyl radicals are transformed to acids, aldehydes, ketones or carboxylic acids as previously described, while the peroxyl radical prefers to abstract another hydrogen, thereby continuing the propagation of radicals.


(7) 2R-O-O-H → R-O• + R-O-O• + H_2O

2.3 Susceptibility of fresh dairy products to lipid oxidation

The susceptibility of a cheese product to oxidation may be highly influenced by its structural makeup. Cheese is often categorized as a food colloid because its structure is composed of finely divided particles of fat dispersed within a continuous medium (Dickinson and McClements, 1996, Coupland and McClements, 1996). Coagulation is accomplished either by gradual acidification of milk proteins, yielding fresh 'acid-cheeses'; or through the enzymatic action of rennet which causes the cleavage of bonds between amino acids along the protein chain (Herbert et al., 1999). Whether formed from the action of rennet or by acid coagulation, the structure of cheese can be seen as a continuous "mesh-like" network of casein that entraps fat and loosely binds water (Lucey, 2004). Cheeses are

essentially made up of three main components: water, fat and protein. Water may be found in free form within the channels of the protein network which enables it to act as a solvent for other cheese components such minerals, salts, and lactose. Water may also be bound loosely or tightly to the casein network to an extent that is dependent on the protein content (Everett and Auty, 2008).

The organoleptic characteristics of cheese are dependent firstly on the quality and treatment of the milk source and secondly, the raw materials which go into the manufacturing process (Muir, 1996). In the traditional manufacture of cheese, the milk is minimally treated. Many cheeses are made from non-pasteurized milk, as this process reduces much of the indigenous microflora found in milk that contributes to flavors and aromas. Many mesophillic and thermophillic organisms possess intracellular lipolytic activity. Though this may result in the development of beneficial flavor attributes in aged cheeses, enzymatic alterations to the lipids in fresh acid-cheese varieties may be undesirable to consumers (Fox et al., 2000). Ripened cheeses are also often made from non-homogenized milk, as homogenization has been shown to result in a softer, weaker curd with the potential for increased hydrolytic rancidity (Lucey, 2004).

In contrast to aged cheeses, many fresh acid-cheese varieties on the market today are manufactured from homogenized, pasteurized milk. Homogenization of milk prior to fresh cheese manufacture alters the composition of lipid droplet membranes, leading to increased interactions between milk fat and protein. The surface active components of milk, namely the casein and whey proteins, readily form an interface between the fat and aqueous phases during this process. Fresh cheeses made from homogenized milk have been shown to have reduced fat separation, improved consistency, increased whiteness and a reduction in whey separation (Lucey, 2004). The increased interaction between fat and protein is believed to reduce the formation of "pools" of fat that do not generally integrate well into the cheese matrix. This makes for a firmer cheese with less free oil formation which is often desirable for fresh products. Fat exists in fresh cheese either as small globules, aggregates of globules, or as large areas of fat that are most likely "pools" of free fat trapped within the protein matrix (Everett and Auty, 2008).

Homogenization is a critical step in the manufacture of fresh cream cheese products. It results in the fat being emulsified throughout a continuous protein phase which affects certain structural features of the cheese (Bot et al., 2007). The structure of cream cheese products is typical of emulsions, as milkfat droplets are dispersed throughout a continuous phase. As in most cheeses, the continuous phase consists of a protein-stabilized network. An interfacial layer, referred to as the filler phase, between the continuous phase and the fat droplets forms during processing. The textural properties of cream cheese are influenced by the gelling of milk proteins, the crystallization of the fat phase upon cooling of the product, and by the uniform dispersion of fat within the continuous phase by homogenization (Bot et al., 2006). As with many foods which exist as oil-in-water emulsions, the structure of cheese leaves it susceptible to lipid oxidation (Coupland and McClements, 1996).

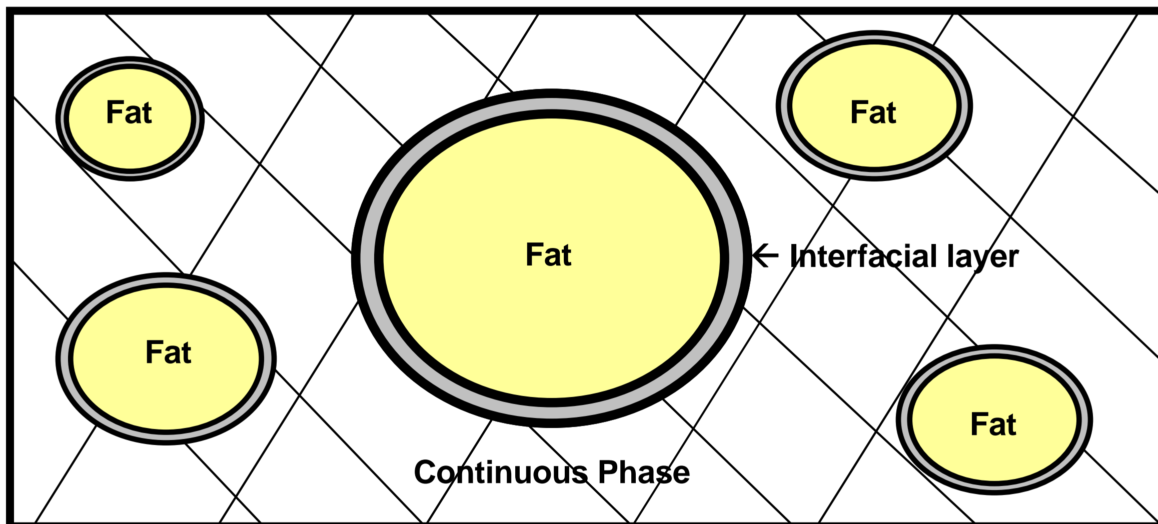


Figure 1. Schematic representation of a fresh cream cheese product as an oil-in-water emulsion. Adapted from Bot et al. (2007).

The oxidative stability of foods that exist as an emulsion has been extensively studied (McClements and Decker, 2000, Coupland and McClements, 2006). In order to limit oxidation in emulsions, fat globules must be protected from environmental factors which lead to oxidative deterioration. The molecules that surround the oil droplets must provide a barrier to molecular species that will initiate lipid oxidation in the droplets (Dickinson,

1999). Oxidation in food emulsions begins at the surface of the fat droplets when pro-oxidants are able to penetrate and diffuse through the protective membrane. The development of peroxyl radicals occurs at this interface. As oxidation proceeds, radicals migrate into the droplet, and decompose into rancid compounds (Coupland and McClements, 1996). Eventually, the oil phase in the emulsion may emit off-odors which affect the product quality as a whole.

Secondary oxidation products are formed when the lipid hydroperoxides decompose to alkanes, alkenes, aldehydes, alcohols, ketones, esters, and acids (Halliwell et al., 1995). These short chain compounds impart a variety of sensory characterisitics specific to cheese. Flavors and odors associated with these compounds, as well as subsequent polymerization, cyclomerization, and isomerization thereof, have been described as beany, fishy, painty, tallowy, rancid and oxidized (Kochhar, 1996). For instance, short chain chemicals such as n-hexanal, 2-octenal, 2-nonenal, 2,4-heptadienal and 2,4-nonadienal are the principal carbonyls contributing to the copper-induced 'cardboard'' off-flavor reported in milk (Forss et al., 1955). This flavor defect has been associated with the presence of n-hexanal in spray-dried whole milk (Hall and Lingnert, 1986). The ketone l-octen-3-one has been associated with a ''metallic'' off-flavor in dairy products (Stark and Forss, 1962, Bassette et al., 1986). This compound is detectable from a sensory standpoint at concentrations as low as 1 mg/kg (ppm) in butterfat (Shipe et al., 1978).

In dairy products, polyunsaturated fatty acids such as linoleic and linolenic acid associate primarily with phospholipids which orient themselves at the interface between fat and the continuous phases (O'Connor and O'Brien, 1994). For this reason, the presence of molecules at this interface capable of scavenging free radicals or sequestering metal ions play a vital role in limiting oxidation. The caseins of milk possess certain structural features that provide oxidative protection. Phosphoseryl residues have the capacity to sequester potential metal pro-oxidants (Gaucheron et al., 1996, Kitts, 2005). Metallic ions such as copper and iron are commonly present in dairy products and are found primarily in the continuous aqueous phase. Metal ions catalyze the breakdown of hydroperoxides that may form at the surface of the fat globule (O'Connor and O'Brien, 1994). Therefore it is

important to limit the amount of metal ions that come into contact with the surface of the droplet. Homogenization enhances the oxidative stability of milk by increasing the casein concentration at the fat droplet surface (Bot et al., 2007). The various surface hydrophobic regions found on casein may also allow for potential antioxidant functional side chain groups to align themselves at the oil-water interface. This is important in the scavenging of radicals that are formed as a result of oxidation (Huang et al., 1994).

2.4 Factors causing oxidation in fresh dairy products

2.4.1 Light

Light is known to initiate oxidation processes in foods, leading to losses of valuable nutrients, discoloration and formation of off-flavors from compounds such as aldehydes, ketones, methional and dimethyl disulphide (Bekbølet, 1990; Juric et al., 2003). Dairy products are often exposed to light during retail storage and display. Light exposure of cheddar cheeses decreased sensory acceptability after 3 days of storage due to the development of a pink color as a consequence of lipid oxidation (Kristensen et al., 2001). Several short chain organic acids and aldehydes derived from lipid breakdown products were found to have a significant effect on the physical and sensory attributes of fresh Samso cheese containing 28% fat (Juric et al. 2003). These products developed in the cheeses regardless of the packaging material, and when the cheeses were exposed to light over an extended period.

Cream cheese products are susceptible to deteriorative changes during storage, with light-induced oxidation as one of the major problems (Petterson et al., 2005.). The photooxidation of cream cheese has been shown to lead to the formation of pentanal and hexanal, which significantly affect most evaluated sensory attributes (Pettersen et al., 2005). For processed cheeses containing 31% fat, storage in light resulted in a sharp rise in the concentration of the volatile oxidation products octane, hexanal, heptanal, octanal and nonanal, compared to storage in the darkness after 14 days (Sunesen et al., 2002). Light-induced oxidation requires both oxygen, the presence of a UV light source and a

photosensitiser in order to occur (Mortensen et al., 2002). Dairy products are well known for being very sensitive to light, due to a high amount of the strong photosensitiser, riboflavin (vitamin B2) (Bekbølet, 1990).

2.4.2 Metal ions (pro-oxidant)

Metal ions are powerful catalysts for lipid oxidation, as they assist in the breakdown of hydroperoxides (O'Connor and O'Brien, 1994). Only trace amounts are required to increase the overall reaction rate of autoxidation, and this may come from contact with equipment or presence in raw materials used in the processing of food products. The difficulty in reducing the presence of transition metals in food systems arises due to the fact that they are ubiquitously present in food ingredients, water and equipment (Cuvelier et al., 2003). In the dairy industry, the use of stainless steel equipment has been adopted to minimize copper contamination (Downey, 1969). Metal ions are often found in packaging material, and the indigenous iron content of milk has been found to promote the development of oxidized flavour (Shipe et al., 1978). The catalytic role of metal ions may be significantly lowered by the presence of sequestering or chelating agents, which causes steric hindrance, thereby reducing the ability of metal to come into contact with the lipid phase where oxidation is initiated.

2.4.3 Temperature

Temperature is important only as a regulator of the rate of oxidative change. As the temperature increases, the rate of oxidative change increases, all other factors being constant (Fedele and Bergamo, 2001). Low temperature storage favors a slow rate of oxidative change. The formation of free radicals in processed cheeses was shown to depend more on temperature than on light exposure (Kristensen and Skibsted, 1999). Many fresh dairy products involve high processing temperatures. Temperature-induced changes in dairy products may also occur as a result of temperature fluctuations over long periods of storage, or during product transport. Increasing the storage temperature from 5 to 37ºC for processed cheese can result in significantly enhanced oxidation which was

apparent after a few days of light-exposed storage (Kristensen, 2001). Rising the storage temperature from 4ºC to 37ºC increased the concentrations of 2-propyl-1-pentanol, 2-hexanone, 2-octanone, 2-decanone, 2-tridecanone, octanal, nonanal and decanal in processed cheese (Sunesen et al., 2002).

2.5 Strategies against lipid oxidation in dairy products

The storage instability of dairy products that occurs as a result of lipid oxidation is a concern to the dairy industry. The potential pro-oxidants affecting dairy products include oxygen and activated oxygen species, riboflavin and light, metals (copper and iron), and possible metallo-enzymes indigenous to milk. Various strategies to reduce lipid oxidation have been investigated, which include modifications to packaging materials, the use of synthetic antioxidant (BHA, BHT), and the addition of natural extracts from a variety of sources such as spices, root vegetables, and fruits (Mortensen et al., 2002, Soto-Cantu et al., 2008, Bandyopadhyay et al., 2008).

Innovative packaging materials have been used for cheese that block light (Mortensen et al., 2002; Sunesen et al., 2002), reduce oxygen transmission, or incorporate antioxidants such as butylated hydroxyl toluene (BHT) (Soto-Cantu, 2008). The transparency of different packaging material has been shown to affect the rate of lipid oxidation in cheeses displayed under fluorescent lighting (Hong et al., 1995, Andersen et al., 2006). Hexanal developed more quickly in cream cheeses stored in transparent trays than dark trays when exposed to fuorescent light (Pettersen et al., 2004). Higher oxygen transmission rates generally favor oxidative deterioration that causes off-odors and pink discoloration in cheese (Andersen et al., 2008). An antioxidant active-packaging material was developed consisting of a layer made of low-density polyethylene to which BHT was added. The diffusion of BHT from the active packaging material to the cheese was found to maintain the level of oxidation from 20 to 100 days of storage. However, it was found that in order to effectively reduce the odor associated with oxidation, a high level of BHT was required in the package, causing it to diffuse into the cheese at a level above the legal limit (Soto-Cantu et al., 2008).

The use of antioxidants, both natural and synthetic, has been examined in an extensive array of food systems. Primary antioxidants, or "chain-breaking" antioxidants, have the capacity to delay the initiation and propagation of oxidation by donating hydrogen to already formed radicals. In doing so, the antioxidant itself becomes a radical, but one which is much more stable and less effective at promoting oxidation (O'Connor and O'Brien, 1994, Rice-Evans et al., 1997). Chain-breaking antioxidants generally have a strong affinity for peroxyl radicals, but may also react with alkoxyl radicals in the latter stages of oxidation. The precise mechanism by which antioxidants prevent oxidation of dairy products is not known, but it has been suggested that they interrupt the chain reaction in autoxidation by capturing the free radicals necessary for hydroperoxide formation (Downey, 1969, O'Connor and O'Brien, 1994).

The use of phenolics to inhibit oxidative rancidity in a number of dairy products and dairy-based emulsions has been investigated. The effectiveness of natural antioxidants from beets, mint and ginger in reducing lipid oxidation as compared to the synthetic antioxidants, tertiary butyl hydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) under thermal treatment in an Indian dairy-type dessert was recently assessed (Bandyopadhyay et al., 2008). Mint, beet and ginger, as well as combinations thereof were found to inhibit lipid oxidation at a level comparable to BHA and BHT. In an emulsion model of sunflower oil stabilized by sodium caseinate, it was found that the most protective phenolic antioxidants were those compounds of lower polarity such as α-tocopherol and dodecyl gallate (Vellasco et al., 2004). This is due to the ability of non-polar antioxidants to position near the interface where oxidation occurs.

The most effective natural extracts in oil-in-water emulsions have been found to be those that contain antioxidant components that accumulate either in the droplets or at the oil-water interface (Coupland and McClements, 1996, McClements and Decker, 2000). Proteins such as casein that stabilize fat in complex food systems are often also capable of providing some protection against lipid oxidation. The effectiveness of proteins to provide oxidative protection is dependent on factors such as chelating properties, the presence of radical scavenging amino acids, as well as the interfacial film thickness (Villier et al.,

2005). At low pH, sodium caseinate was found to provide the highest oxidative protection to emulsions as compared to whey protein and soy protein isolates (Hu et al., 2003).

2.6 Cranberries as a source of antioxidants

2.6.1 Classes of phenolic components in berries

A diet rich in fruits and vegetables may reduce the risk of diseases caused by oxidative stress, such as cancer and cardiovascular disease (Vvedenskaya and Vorsa, 2004, Vattem et al., 2005). Berries contain a wide diversity phenolic phytochemicals, many of which have been studied in detail. Phenolics contribute to the color, taste, bitterness and odor found in most berries. Classes of phenolics are distinguished from one another based on structure, which may differ based on the number and substitution pattern of phenolic rings (Rice-Evans et al., 1997).

Phenolic acids are simplest in structure. In berries, the main phenolic acids present are hydroxylated derivatives of benzoic acid and cinnamic acid, which are commonly found bound to organic acids and sugar derivatives (Figure 2A). Flavonoids are more complex than phenolic acids, having the basic skeleton of diphenylpropanes ($C_6 + C_3 + C_6$) (Figure 2B). They are a complex and diverse class of compounds which are classified based on the different oxidation level of the central pyran ring. From this basic structure, individual flavonoids may differ based on the degree and type of glycosylation, and the presence of hydroxyl groups and methyl groups along the phenolic ring structure. Flavonoid monomers may condense into more complex molecules to form condensed tannins and proanthocyanidins (Figure 2C).

Phenolics have been attributed to many of the biological effects of berries, which include antioxidant activity, cholesterol reduction and in vitro anticancer effects. Recent research has determined the antioxidant activity of different phenolic compounds and attempts have been made to define the structural characteristics which contribute to these activities (Wilson et al., 1998, Seeram et al., 2004, Lee et al., 2006). Epidemiological evidence

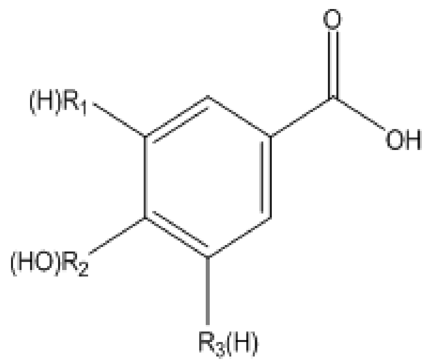
suggests that high consumption of flavonoids which are an important component of berries may provide protection against coronary heart disease, cardiac stroke, lung cancer, and stomach cancer (Vattem et al., 2005).

Table 2. Classes of phenolics commonly found in plants.

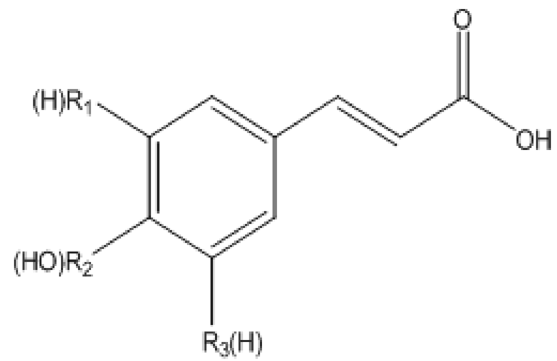
# C atoms	Basic skeleton	Phytochemical Class
6	C_6	Simple phenolics
7	C_6-C_1	Phenolic acid (hydroxybenzoic & coumaric acid derivatives)
15	C_6-C_3-C_6	Flavonoids
N	$(C_6$-C_3-$C_6)_n$	Condensed phenolics

A

p-hydroxybenzoic acid

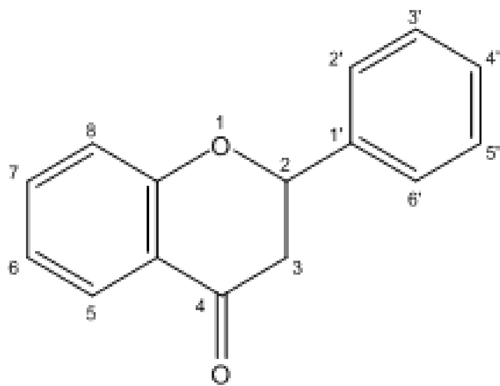


p-coumaric acid



B

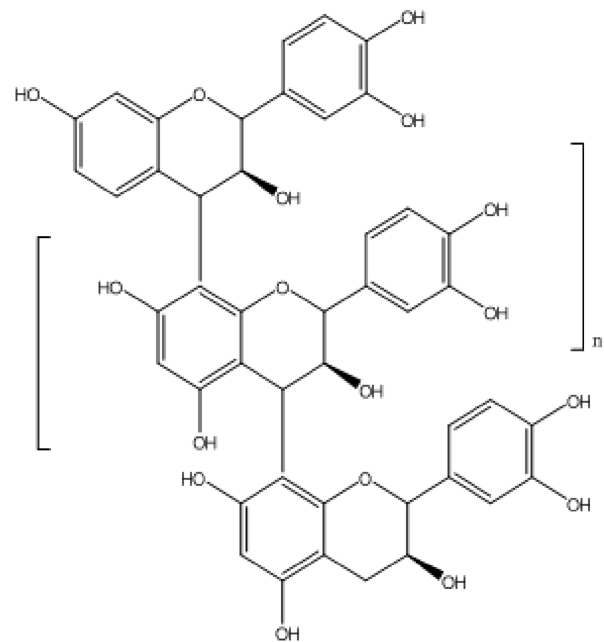
Basic structure



Flavonoids

C

Procyanidin



Condensed polyphenols

Figure 2. Classes of phenolics found in berries (A) phenolic acids (B) flavonoids (C) condensed phenolics. From Shahidi and Naczk (2003) and Gessner and Steiner (2005).

2.6.2 Phenolic composition of cranberry

Cranberries (*Vaccinium Macrocarpon*) are rich in phenolic acids, anthocyanins, flavonol glycosides, as well as proanthocyanidins. On a fresh weight basis, cranberries contain approximately 1 g/kg phenolic acids, mainly as hydroxylated derivatives of benzoic and cinnamic acids (Herrmann 1989, Shahidi and Naczk 1995). Most phenolic acids are present in bound form, as glycosides or esters. Caffeic acid and vanillic acid (Figure 4) were found to make up 4.4% and 6.4%, respectively, of total phenolics in American cranberries (*Vaccinium macrocarpon* cv. Ben Lear) (Zheng, 2003). The antioxidant activity of such simple phenolic acids has been associated to some extent with the number of hydroxyl groups positioned on the molecular structure.

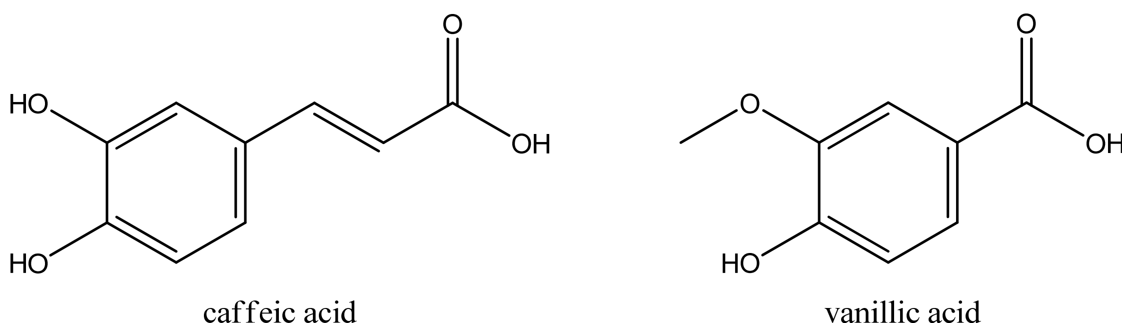
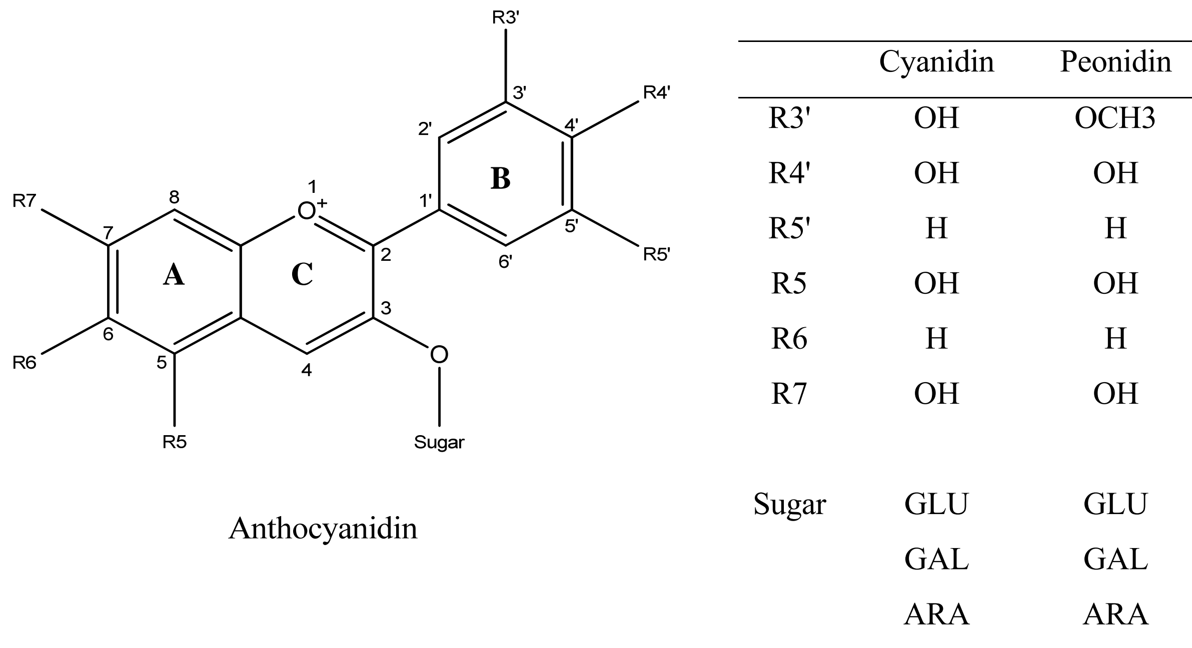


Figure 3. Common phenolic acids found in cranberries (*Vaccinum Macrocarpon*).

The predominant anthocyanins found in cranberries are 3-*O*-galactosides and 3-*O*-arabinosides of cyanidin and peonidin (Figure 5) (Zheng and Wang, 2003). Anthocyanins are water soluble pigments that give a red color common to the cranberry fruit. On a fresh weight basis, cranberries may contain anywhere between 190 and 533 mg/kg anthocyanins depending on cultivar, fruit size and shape, and pre- and postharvest conditions (Barrett et al., 2004). These are mostly found just beneath the skin of the cranberry fruit. The backbone structure of anthocyanins consists of an anthocyanidin molecule to which a sugar is bound (Figure 5). Glycosylation mainly occurs in the 3 position on the C ring with sugars such as glucose, rhamnose, xylose, galactose, arabinose, and fructose linked to the OH group. Glycosylation confers stability to anthocyanidins, which are inherently unstable

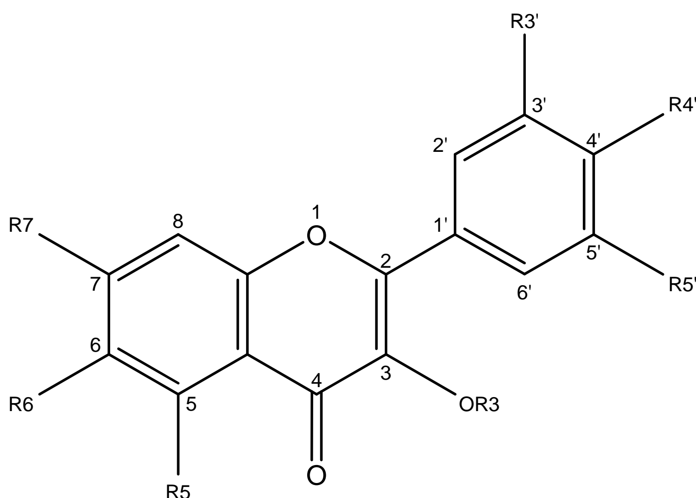
in water, and this stability results in color enhancement. The red color of cranberry fruit is due to the presence of four major anthocyanin pigments, namely cyanidin-3-galactoside, peonidin-3-galactoside, cyanidin-3-arabinoside, and peonidin-3-arabinoside, and the two minor anthocyanins, cyanidin-3-glucoside and peonidin-3-glucoside (Zheng and Wang, 2003).



	Cyanidin	Peonidin
R3'	OH	OCH3
R4'	OH	OH
R5'	H	H
R5	OH	OH
R6	H	H
R7	OH	OH
Sugar	GLU	GLU
	GAL	GAL
	ARA	ARA

Figure 4. Substitution pattern for major anthocyanins found in cranberries. Adapted from Zheng and Wang (2003) and Wang et al., (2007). GLU = glucose; GAL = galactose; ARA = arabinose.

Flavonols and related glycosides (Figure 5) have recently been identified in cranberries and spray-dried cranberry extracts (Vvedenskaya et al., 2004). Glycosides of quercetin, kaempferol, and myricetin have been related to number of important health benefits, including antioxidant activity exhibited by the affinity to scavenge the DPPH radical, and mediating the lipopolysaccharide (LPS)-induced inflammatory response by inhibiting cytokine production (Xagorari, et al., 2001). American cranberries rank among the highest in flavonol content among fruits and berries, especially with regard to quercetin, which ranges from 11 to 25 mg/100 g in the fresh fruit.



	Quercetin
R5	OH
R7	OH
R3’	OH
R4’	OH
R3	H, Sugar

Figure 5. Structure of flavonols found in cranberries. Adapted from Zheng and Wang (2003).

Proanthocyanidins belong to the class of phenolics known as condensed tannins. They are dimers, oligomers or polymers of flavan-3-ols. The different compounds that may arise within this group depend on the hydroxylation pattern on the phenolic rings. For example, procyanidins, which are the most predominant proanthocyanidin found in nature, are made up of units that have the 3',4'-dihydroxy pattern on the B ring. The difficulty in identifying and classifying these procyanidins arises due to the variations that can be found both in the degree and type of linkages between flavan-3-ol monomers. Whole cranberries have been found to contain approximately 17 μg/g of procyanidins on a dry weight basis. These components are mainly found in the skin or peel of the fruit (Prior et al., 2001). Prodelphinidins have also been identified in cranberries (Shahidi et al., 1995).

2.7 Cranberry phenolics against oxidation

2.7.1 Structure-activity relationships

The relative affinity of phenolic compounds to scavenge radicals is highly dependent on structure. It is the combination of various structural features that renders phenolics most effective in scavenging free radicals. Among the most important structural characteristics

are the positioning and number of hydroxyl groups, the degree of conjugation, as well as the degree of glycosylation pattern throughout the phenolic rings (Zheng and Wang, 2003). Plant polyphenols are multifunctional and can act as reducing agents, hydrogen donating antioxidants, and singlet oxygen quenchers (Rice-Evans et al., 1996). The affinity of any molecule to act as a reducing or hydrogen donating agent gives it the potential to scavenge free radicals formed during autoxidation. An antioxidant must be able to stabilize the newly formed radical on its own structure by delocalizing the unpaired electron. Its reactivity with other antioxidants, and propensity for metal sequestering activity also affects the capacity of molecules to behave as an antioxidant (Rice-Evans et al., 1997).

Flavonoids containing the *o*-dihydroxy structure in the B ring, that is, hydroxyl groups in the 3' and 4' position (Figure 6B), have been to shown to be highly effective at scavenging the radicals generated in the ABTS and ORAC assays. These hydroxyl groups allow phenolics to act as hydrogen or electron donors. When these compounds react with free radicals, the phenolic radicals produced are stabilized by the resonance effect of the aromatic nucleus. Electron delocalization around the aromatic nucleus is a key determinant of the antioxidant potency of the phenolic. There is strong evidence showing the importance of a 2,3 double bond in conjugation with a 4-keto group in the C ring for electron delocalization from the B ring (Figure 6A). This stabilization may also be further enhanced by the presence of hydroxyl groups located at the 3- and 5- positions on the A and C rings, respectively, combined with the presence of a 4-keto group (McClements and Decker, 2000, Rice-Evans et al., 1997).

There are few antioxidants which possess every characteristic shown to enhance antioxidant behaviour. Quercetin, a flavonol, is one which satisfies each requirement, and has been shown to exhibit the best radical scavenging potential among flavonoids (Rice-Evans et al., 1995). Different glycosides of quercetin have recently been identified in the American cranberry (Vvedenskaya et al., 2004). Cranberries also contain glycosides of myricetin and kaempferol (Porter et al., 2001).

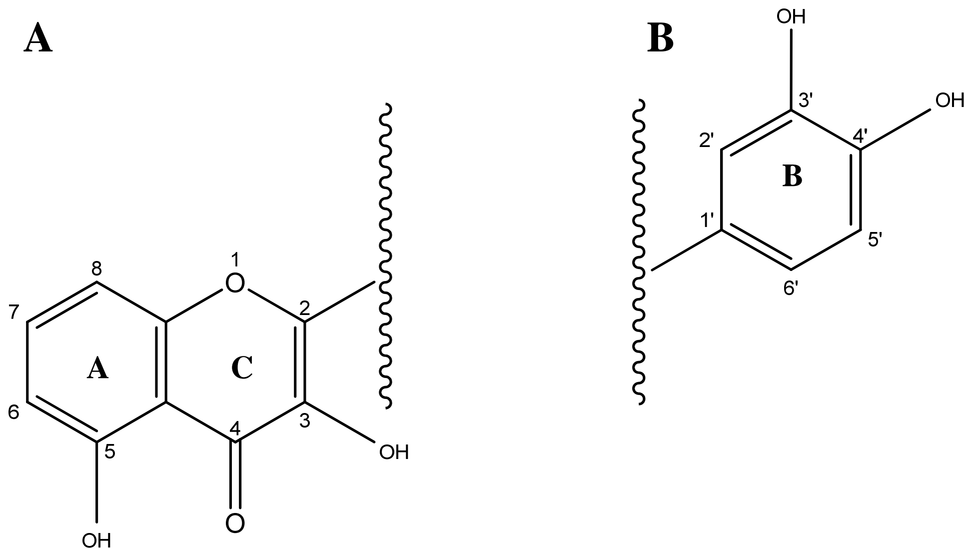


Figure 6. Structural features that enable phenolics to behave as strong antioxidants (A) hydroxyl groups at the 3- and 5- positions on the A and C rings with 4-keto function and 2,3 double linkage in the C ring (B) 3', 4' dihydroxy substituion in the B ring. Adapted from Rice-Evans et al., (1996).

The cyanidin backbone of anthocyanins found in cranberries contains the *o*-dihydroxy structure in the B ring, that is, hydroxyl groups in the 3' and 4' position. This *o*-dyhydroxy group may furthermore provide a point of attachment for transition metal ions. Peonidin has a methoxy group in the 3' position of the B ring with the 4'-hydroxy group, which has been shown to diminish the TEAC relative to cyanidin (Rice-Evans et al., 1996). The effect of different hydroxyl substitutions and degree of methylation on antioxidant properties of anthocyanins against peroxyl radicals using the ORAC assay have been subject to much discussion (Wang et al., 1997). In general, a trend shows that compounds with only one OH group in the B ring, such as peonidin, do in fact exhibit lower ORAC activities compared to a compound with 3′,4′-dihydroxy substitution.

The substitution of hydroxyl groups with sugars is generally believed to decrease the ability of phenolic compounds to delocalize electrons, thereby reducing radical-scavenging ability (Rice-Evans, 1997). Many of the phenolics that are found in pomace and other plant products exist in conjugated forms either with sugars or other moieties. This conjugation occurs via interaction with the phenolic hydroxyl groups. This is believed to reduce the

ability to function as effective antioxidants, since the availability of free hydroxyl groups on the phenolic rings is important for resonance stabilization of free radicals (Rice-Evans et al., 1996). Another possible explanation for this observation is that the steric hindrance caused by the presence of sugars reduces the availability of hydroxyl groups to scavenge radicals. Nevertheless, the effect of glycosylation on antioxidant potential of phenolics has been shown to depend on the structure of the phenolic backbone, the sugar moiety, and the nature of the pro-oxidant (Shahidi and Naczk, 2003). For instance, using the ORAC assay, it was shown that cyanidin-3-glucoside, and cyanidin-3-rhamnoglucoside had relatively greater antioxidant potency than both non-glycosylated cyanin and cyanidin-3-galactoside (Wang et al. 1997).

Proanthocyanidins have gained recent interest for potential health benefits, in particular the effect on cardiovascular disease (Prior et al., 2001, Porter et al., 2001, Kitts, 2006). Polymerization up to trimers increases an affinity to delocalize electrons thereby stabilizing newly formed radicals. However, this activity has been shown to decline upon further polymerization. Proanthocyanidins also inhibit the action of lipoxygenases and xanthine oxidase, which are two oxygen-metabolizing enzymes causing pro-oxidative effects in foods and living tissue (Reed, 2002). Reactions involving these enzymes are catalyzed by transition metal cofactors such as iron and copper. The *o*-dihydroxy group found on the B-ring of phenolics has metal-chelating capacity, and this capacity increases in proanthocyanidins with increasing polymerization.

The affinity of phenolics to behave as antioxidants is highly dependent on factors such as pH and the presence of other molecules such as sugars and proteins. The chemical form of anthocyanins is very much dependent on pH. At low pH (1-3), anthocyanins are heavily protonated, and the very stable flavylium cation predominates. In general, as pH is lowered, polyphenols have an increased metal reducing capacity (Sakihama et al., 2002). The reduced form of a metal such as iron (eg. ferrous ion) causes the rapid decomposition of peroxides into free radicals. Thus, the metal-reducing properties of phenolics may increase oxidative reactions.

2.7.2 Biological and food systems

The presence of anthocyanins, quercetin and proanthocyanidins in cranberries provides a rich phenolic profile capable of protecting the body against oxidative reactions which lead to the development of cardiovascular disease and various cancers (Neto, 2007). A whole cranberry extract was shown to limit the oxidation of low-density lipoproteins (LDL) separated from the blood of male subjects. Oxidation of the LDL was promoted by incubating samples at 37ºC with varying levels of cranberry extract. Thiobarbituric acid reactive substances (TBARS) and LDL electrophoretic mobility were reduced in a dose dependant manner, and it was concluded that inhibition was a function of the total polyphenol content of the cranberry extract (Wilson et al., 1998). The inhibitory activity of cranberry extracts against various tumor cell lines has also been investigated. A recent study of six anthocyanin-rich fruits including blueberries and cranberries found that aqueous methanol berry extracts inhibited proliferation of human oral, prostate, breast, and colon cancer cell lines in a dose-dependent manner (Seeram et al., 2004). Quercetin and its glycosides have recently shown significant antitumor properties, particularly against breast cancer, leukemia and human colon adenocarcinoma cells (Neto, 2007).

Proanthocyanidins recovered from cranberries are the subject of recent work investigating potential risk reduction effects against cancer and cardiovascular disease. Cranberry proanthocyanidins have been shown to be effective at reducing oxidation in both an LDL and liposomal model system. They have the capacity of binding to chylomicrons and very low-density lipoproteins (Porter et al., 2001). They are bulky in size, and less polar than phenolics that contain bulky sugar side-groups. This property makes the proanthocyanidins more lipophillic than other phenolics (Lee et al., 2006). Cranberries were shown to score the highest antioxidant score in the lipophillic ORAC assay among over 20 fruits and berries (Wu et al., 2004).

Many oxidative reactions occur in food systems which will cause deleterious effects in food quality. The utilization of a cranberry powder to inhibit lipid oxidation processes in mechanically separated turkey (MST) and cooked ground pork has been investigated (Lee

et al., 2006). A commercially obtained cranberry powder was fractionated to generate fractions rich in phenolic acids, anthocyanins, flavanols, or proanthocyanidins. These fractions were then individually assessed for the ability to inhibit lipid oxidation in MST and ground pork by monitoring the inhibition of thiobarbituric acid reactive substances (TBARS) formation. Though it was found that the flavonol-rich fractions were most effective in retarding TBARS formation and rancidity development in MST during 14 days of storage, it was concluded that cranberry antioxidants should be enhanced by using appropriate carriers for directing antioxidants into membrane phospholipids.

2.7.3 Assessing antioxidant activity

The ability of molecules to behave as antioxidants can be tested under many different conditions. The formation of radicals can be initiated by a number of independent factors. For this reason, no single test or assay exists that can fully take into account all variables which may lead to the onset of oxidation for a given system. Rather, most assays for antioxidant capacity focus on only one such variable. For instance, assays based on non-site specific Fenton reactions test the ability of a molecule to sequester metal ions such as copper or iron (Kitts, 2005). However, this assay gives less emphasis on the affinity to directly scavenge harmful peroxyl or hydroxyl radicals, which may be equally important depending on the system.

The ABTS assay has been extensively used to determine the ability of an antioxidant to scavenge the synthetic 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical. It is useful in determining the activity of hydrophilic and lipophilic antioxidants. The oxygen radical absorbance capacity (ORAC) assay is superior to other similar methods because it uses the area-under-curve (AUC), which combines both inhibition time and degree of free radical scavenging by an antioxidant into a single quantity (Cao et al., 1995). The peroxyl radical (ROO•) generated in ORAC from the hydrolysis of 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH) is a common free radical found in the body and has been used in other antioxidant activity assays. It is slightly less reactive than OH• and thus possesses an “extended” half-life of seconds instead of nanoseconds. The ORAC assay

is commonly used for determining the total antioxidant activity of fruits (Wang et al., 1996). It has been used in the measure of antioxidant activities of all traditional antioxidants including ascorbic acid, R-tocopherol, β-carotene, glutathione, bilirubin, uric acid, melatonin, and flavonoids (Cao et al., 1997).

3 RESEARCH OBJECTIVES AND HYPOTHESES

The objective of this study was to incorporate cranberries into the production of fresh dairy products with the aim of extending the shelf-life and providing desirable color and flavor attributes to the products. Fresh dairy products are an example of foods which exist as dispersions of fat throughout a continuous phase. The structural elements that make up these products can leave them susceptible to oxidation reactions that cause physical and chemical defects. Cranberries are a rich source of phenolic phytochemicals that have shown the potential to act as antioxidants in a number of different biological and food systems. Cranberries are also an excellent source of organic acids, sugars, vitamin C and possess a beautiful red color due to the high anthocyanin content. Since fresh dairy products require acid in their production, the acidity provided by cranberries in the form of organic acids could make cranberries well-suited for use in product manufacture. Cranberries may also provide flavor and color to fresh dairy products such as yogurt and cream cheese that are continuously being innovated with new flavors.

The first objective of this work was to assess the antioxidant behavior of a cranberry extract, rich in phenolics and organic acids, in a model emulsion system representative of fresh dairy products. This would enable subsequent food formulation experiments to be assessed by using a standardized extract with known antioxidant potential in a structurally relevant food system. Using chemical and biophysical information gathered from a defined model system, the next objective was to utilize the cranberry extract in the manufacture of a value-added cream cheese product. Experiments in the study were conducted based on the following hypotheses:

1. A standardized cranberry extract will provide the required acidity that is expected using typical acidulants to manufacture fresh dairy products.
2. The oxidative stability of lipids in oil-in-water emulsions is enhanced by the presence of phenolics found in cranberries.
3. A cranberry extract, standardized for its phenolic content and antioxidant potential, provides a value-added fresh cheese product with an extended shelf-life.

4 MATERIALS AND METHODS

4.1 Materials

Frozen cranberries were purchased from a local supermarket and kept frozen at -20ºC until use. Technical grade ethanol for extraction of phenolics was obtained from Commercial Alcohols Canada (Toronto, Ont.). Phosphoric acid (HPLC grade), methanol (HPLC grade), formic acid and hydrochloric acid were obtained from Fisher Scientific (Nepean, Ont.). Sephadex LH-20 was purchased from Amersham Biosciences (Uppsala, Sweden) and Zorbax C-18 columns obtained from Agilent Technologies (Mississauga, Ont.). Quinic, malic, citric, ascorbic acids used as standards for HPLC analysis were obtained from Sigma–Aldrich Canada Ltd. (Oakville, Ont.). Gallic acid standard and ferrous sulfate heptahydrate were obtained from Sigma-Aldirch Canada Ltd. AAPH (2,2′-azobis (2-amidinopropane) dihydrochloride) was obtained from Wako Chemicals USA (Richmond, VA). Trolox and 2′,7′-dichlorofluorescin diacetate (DCFH-DA) were purchased from Sigma–Aldrich Canada Ltd..

Spray-dried sodium caseinate and sunflower oil (non-stripped) was obtained from Sigma–Aldrich Canada Ltd.. Standards for gas chromatography (hexanal, pentanal and 4-heptanone) were obtained from Fluka (Buchs, Switzerland). Iso-octane and 2-propanol (ACS grade) were purchased from Fisher Scientific. Pasteurized, non-homogenized milk and full-fat cream were obtained from local supermarkets. Freeze-dried cultures for cheese-making were obtained from Danisco Canada Ltd. (Scarborough, Ont.).

4.2 Preparation of cranberry extract

Frozen cranberries were blended 1:1 (w/v) with 80% (v/v) ethanol using a Waring blender for 5 min. The slurry was transferred to an Erlenmeyer flask, and extracted for several hours. The extract was filtered through a Buchner funnel using Whatman filter paper (No. 1) and rinsed twice with 25 mL of 80% ethanol. The filtrate was stored at 4°C. The filter cake was transferred to a new Erlenmeyer flask and was re-extracted overnight with 150 mL of 80% ethanol using an orbital shaker at 400 rpm (Innova 4000, New Brunswick Scientific, NJ). The filter cake was extracted for a third time with 80% ethanol for 1 hour and all filtrates were pooled together. The ethanol in the pooled filtrate was removed under vacuum at 35°C. The residue was then freeze-dried and kept at 4 °C. This procedure was repeated for three separate batches of cranberries to obtain three extracts (n=3).

4.3 Characterization of the cranberry extract

4.3.1 Total phenolics

The total content of phenolics in the freeze-dried cranberry extract was determined using the Folin-Ciocalteu assay (Singleton et al., 1999). A standard solution of gallic acid was diluted with distilled deionized water (ddH$_2$O) to give concentrations of 20, 40, 60, 80 and 100 mg/L. To a 96-well microplate was added 20 μL of each standard or appropriately diluted cranberry extract. Folin−Ciocalteu reagent (previously diluted 10-fold with distilled water) was added at a level of 100 μL, the samples mixed and allowed to stand at room temperature for 5 min; 80 μL of sodium bicarbonate (75 g/L) solution was then added to the mixture. After 10 minutes in the dark at 22 °C, absorbance was measured at 765 nm. A standard curve of gallic acid concentration versus absorbance was obtained using the standard solution dilutions (Appendix A). Results for the cranberry extract are expressed as gallic acid equivalents (GAE).

4.3.2 Total anthocyanins

Total anthocyanins were determined using the pH differential method (Wrolstad and Giusti, 2001). The freeze-dried cranberry sample was first dissolved in ddH_2O to give a 10% (w/v) stock solution. This solution was diluted in potassium chloride buffer (KCl, 0.025 M, pH 1.0) and the absorbance spectrum was measured to determine λ_{max}. The dilution factor was such that the absorbance at λ_{max} did not exceed 1.2 units. Using this factor, the sample was then diluted in 0.4 M sodium acetate (pH 4.5). Sample absorbancies in both buffers were read at 510 and 700 nm against a blank cell containing ddH_2O. The absorbance (A) of the diluted sample was then calculated as follows.

$$A = (A_{\lambda\ vis\text{-}max} - A_{700})_{pH\ 1.0} - (A_{\lambda\ vis\text{-}max} - A_{700})_{pH\ 4.5}$$

The concentration of anthocyanin pigment in the sample was calculated using the following formula:

$$\text{Monomeric anthocyanin pigment (mg/litre)} = (A \times MW \times DF \times 1000)/(\varepsilon \times 1)$$

Anthocyanins were calculated on the basis of cyanidin-3-glucoside, where the molecular weight (MW) was 449.2 g/mol with a molar absorptivity (ε) of 26,900.

4.3.3 Phenolic profile by HPLC

Characterization of the cranberry extract was done according to the method described by Porter et al. (2001), with some modifications. The freeze-dried cranberry extract was dissolved to 10% w/v in 1 mL methanol and loaded onto a 60-g Sephadex LH-20 column (i.d. = 2.5 cm, length = 50.0 cm) equilibrated with 20% (v/v) methanol. About 500-mL of 20% aqueous methanol (v/v) at 1.5 mL/min was used to elute non-phenolic cranberry constituents such as organic acids and carbohydrates. Subsequently, 500 mL of 60% aqueous methanol (v/v) was used to elute cranberry flavanols and anthocyanins (F1). Finally, 100% methanol was used for the elution of proanthocyanidins and non-polar

phenolics (F2). Fractions were concentrated under vacuum by rotary evaporation at 35ºC and subsequently lyophilized. Freeze-dried powders were re-dissolved in 100% methanol and filtered through a 0.45 μM nylon membrane-filter. HPLC analyses for the identification of individual phenolics was performed using an Agilent 1100 HPLC system (Agilent Technology 1100 series, Palo Alto, Ca), equipped with quaternary pumps, autosampler and a diode array detector. Phenolics separation was performed using a Zorbax RX-C18 column (5 μm, 4.6 mm × 250 mm) at 30°C. Mobile phases constituted of 2% formic acid in 100% methanol (A) and 2% formic acid water (B) at a flow rate of 1 ml/min. The gradient condition started with 23% A, linearly increased to 24.3% A at 15 min, then to 50% A at 20 min and 55% A at 25 min.

4.3.4 Non-polar phenolics and proanthocyanidin content

The concentration of non-polar phenolics and proanthocyanidins in the cranberry extract was determined by measuring the total phenolics in the 100% methanol fraction, F2 (as prepared in the previous section), using the Folin-Ciocalteu assay. Results were expressed as gallic acid equivalents (GAE). The presence of proanthocyanidins in F2 was confirmed by the acid-butanol assay (Gessner and Steiner, 2005). Fraction F2 was diluted to an appropriate concentration in 500 μL of ddH_2O. To this was added 7 mL of a solution consisting of 700 mg of ferrous sulfate heptahydrate dissolved in 50 mL of concentrated hydrochloric acid and adjusted to 1 L using n-butanol. The initial absorbance was measured at 550 nm. The sample was then heated in a water bath at 95ºC for 50 minutes, after which time it was allowed to cool to room temperature, and the absorbance measured again at 550 nm. The presence of proanthocyanidins was confirmed by an increase in absorbance observed due to the acid-butanol reaction.

4.3.5 Organic acids by HPLC

Organic acids in the freeze-dried extract were determined by AOAC method 986.13. The freeze-dried extract was reconstituted in ddH_2O to give a 1% (w/v) solution. The extract was first run through a Sep-Pak C18 disposable cartridge that had been pre-conditioned

with 10 mL of 50% (v/v) acetonitrile solution. The first 4-5 mL of cranberry solution passing through the Sep-Pak was discarded. The remaining sample was collected, and filtered through a 0.45 µM nylon membrane-filter prior to LC analysis. HPLC analyses was performed using an Agilent 1100 HPLC system (Agilent Technology 1100 series, Palo Alto, Ca), equipped with quaternary pumps, autosampler and a diode array detector. The stationary phase consisted of two reverse phase Zorbax RX-C18 columns used in tandem. The mobile phase consisted of 0.2 M KH_2PO_4 buffer, pH 2.4, with a flow rate of 0.8 mL/min. In order to identify and quantify the organic acids present in the extract, standard solutions were prepared by accurately weighing 0.200 g ACS grade organic acids (quinic, malic, citric and ascorbic) and dissolving separately in 100 mL of ddH_2O using volumetric flasks. Standard curves based on peak response for each acid were prepared by injecting 5-20 µL of each standard. Calibration plots for each organic acid are shown in Appendix B.

4.3.6 Titratable acidity and pH

Measurement of pH for all samples was carried out using a Corning Pinnacle 530 Basic pH Meter (Nova Analytics Corporation, CA). Titratable acidity was determined using AOAC method 942.15. The cranberry extract was reconstituted in ddH_2O to give a slightly colored dilute solution. The solution (100 mL) was titrated with 0.1*N* alkali using 0.3 mL of phenolphthalein indicator.

4.3.7 Antioxidant activity by ORAC

The hydrophilic oxygen radical absorption capacity assay (H-ORAC) was followed as previously described (Kitts and Hu, 2005). Briefly, cranberry extracts and a range of Trolox standards were appropriately diluted in phosphate buffer (50 mM, pH 7.0) added in triplicate to a 96-well plate (Nunc, Fluorescent microplate). To this was added 60 µL of 20 nM fluorescein, and plates were incubated at 37 °C for 15 min. The peroxyl radical initiator, AAPH, was added to a final concentration of 12 mM and fluorescence (Ex = 485 nm, Em = 527 nm) was continuously taken for 60 min (Fluoroskan Ascent FL, Labsystems). In the lipophilic ORAC assay (L-ORAC), the lipophilic components of the

cranberry extract were extracted using hexane followed by a further dilution in 7% randomly methylated cyclodextrin (RMCD) in 50% acetone. Solutions were subsequently shaken at 400 rpm for one hour protected from exposure to light. Phosphate buffer (75 mM, pH 7.0) was added up to 90 μL in the microplate, and to this was added 10 μL of the lipid soluble component (dissolved in 7% RMCD), to give a final mixture volume of 100 μL. Trolox standard and fluorescein were maintained consistent to that used for the H-ORAC assay. The final concentration of AAPH however, was adjusted to 18 mM rather than 12 mM and the measurement time was extended to 100 minutes instead of 60 minutes. The blank constituted of 90 μl of phosphate buffer and 10 ul of 7% RMCD. Data transformation for both H-ORAC and L-ORAC was performed according to Davalos, Gomez-Cordoves, and Bartolome (2004). The ORAC values were expressed as μmoL Trolox equivalents (TE)/g extract.

4.4 Behavior of the cranberry extract in a model food emulsion

4.4.1 Emulsion preparation

Sodium caseinate was the sole emulsifying agent used to formulate emulsions. A 4% (w/w) sodium caseinate and a 2% (w/w) cranberry extract stock solution were prepared separately in ddH_2O. The lipid phase consisted either of sunflower oil or dehydrated butterfat. Anhydrous butterfat was prepared from fresh butter by melting at 45°C and centrifuging at 650*g* for 20 min at 40°C (Yost and Kinsella, 1993). The upper anhydrous layer of butterfat was siphoned off and stored at 4°C until needed.

Stock solutions of sodium caseinate and cranberry were combined and distilled water added (where necessary) so that the final concentration of sodium caseinate was 2% (w/w with aqueous phase) and the final concentration of cranberry extract in the emulsion ranged from 0 to 1.0% (w/w with the aqueous phase) prior to emulsion formation. The aqueous phase was then combined with the appropriate lipid phase and emulsion formation was accomplished by homogenizing for 30 seconds using an Ultra Turrax T50 Homogenizer (Janke & Kunkel) operating at 4000 rpm/min. These emulsions were kept for emulsion

stability (ES) analysis using the turbidimetric method (4.3.2). The amount of cranberry added to the emulsion was formulated to give emulsions with a final pH ranging between 5.3 and 6.0.

For droplet size distribution and oxidation experiments, an extra high-pressure homogenization step was employed to form emulsions that were physically stable over the course of an extended incubation period. Emulsions were formed using a two-stage high-pressure homogenizer operating at 40 and 400 bar in the first and second stage, respectively. For oxidation studies, the cranberry extract was either incorporated as part of the ingredients during the homogenization steps (as above), or added dropwise as a dilute solution to emulsions immediately after formation. In either case, predetermined amounts of the cranberry stock and sodium caseinate stock were measured to ensure that all emulsions contained sodium caseinate at a level of 2% (w/w with the aqueous phase), 25% (w/w) fat, and contained the cranberry extract at a concentration ranging from 0 to 1% (w/w with the aqueous phase). Control emulsions were prepared either without the extract (pH 6.7), or with the addition of a pre-determined amount of dilute citric acid (5% w/v) to obtain emulsions with the same final pH as treatments.

4.4.2 Emulsion stability

Emulsion stability (ES) is the ability of the emulsion to remain stable against coalescence. ES was evaluated after emulsion formation using the Ultra Turrax T50 Homogenizer (Janke & Kunkel) by a turbidity method similar to that reported by Pearce and Kinsella (1978). Emulsions were poured into a biuret and samples were taken at timed intervals from the bottom by opening the valve at the base. Emulsion samples were diluted 1/500 in 0.1% SDS, and the absorbance at 500 nm was measured. Six emulsions were prepared for each treatment, and ES was determined over 60 minutes

Table 3. General design of emulsion experiments.

Analysis	Emulsion lipid component	Emulsion preparation	Level of cranberry extract (%w/w with aqueous phase)	pH	Method
Emulsion stability	25% Sunflower oil	Ultra Turrax Homogenizer	0	6.7	Turbidimetry
			0.25	6.0	
			0.5	5.6	
			1.0	5.3	
Particle size distribution	25% Sunflower oil	High-pressure homogenization	0	6.7	Laser diffraction (Malvern Mastersizer)
			0.25	6.0	
			0.5	5.6	
			1.0	5.3	
Lipid oxidation at 50ºC	25% Sunflower oil or 25% Butterfat	High-pressure homogenization	0	5.6	Conjugated dienes Headspace volatiles
			0.5	5.6	

4.4.3 Measurement of particle size distribution

A Malvern Mastersizer 2000 (Malvern Instruments Ltd, c/o Pharmacy Department, University of British Columbia) was used to determine the volume-surface average diameter ($d_{3,2}$) for each emulsion. Particle size distribution was presented as volume percentage vs. droplet diameter. Analysis was carried out within 1 hr of initial preparation. Measurements were performed in triplicate on each emulsion and averaged.

4.5 Determination of lipid oxidation in emulsion model

4.5.1 Headspace analysis

Aliquots (2.5 mL) of emulsions were distributed in 22.4-mL headspace vials sealed with polytetrafluoroethylene/silicon septa and aluminum crimp seals. Vials were incubated in the dark at 50°C in an orbital shaker set at 75 rpm. The appearance of hexanal and pentanal as markers of oxidation was followed by static headspace GC. An internal standard of 4-heptanone was used to confirm the retention times of hexanal and pentanal. Vials containing emulsions were thermostated at 60°C for 10 min in a Tekmar 7000 headspace autosampler. After a 3 minute pressurization with helium as the carrier gas, the headspace was injected during an interval of 0.10 min through the transfer line set at 115°C to the gas chromatograph (Hitachi). The gas chromatograph was fitted with a DB-5 column (J&W Scientific, Folsom, CA) (30m × 0.25 mm × 0.25 μm). The initial temperature of the oven was maintained at 35°C for 2 min, then increased to 80°C at a rate of 5°C/min, and to 220°C at a rate of 20°C/min. The FID was set at 250°C. Hexanal and pentanal were identified by comparing the retention time of the peaks at with those of authentic reference compounds. Concentrations of aldehydes were expressed in mg/kg of emulsion from peak areas using a standard curve made from standard hexanal or pentanal added to emulsions (Appendix C). Measurements were performed at least twice for each emulsion sample and averaged. Emulsions were prepared in duplicate or triplicate.

4.5.2 Conjugated dienes

Conjugated dienes (CD) were measured according to a modified version of the AOCS Official Method Ti:64 (Cuvelier et al., 2003, Kiokias et al., 2006). Following analysis for secondary products, the emulsion sample (20 µl) was diluted to 10 ml with a mixture of isooctane/2-propanol (2:1 v/v) and vortexed for 1 min. The absorbance was measured at 233 nm by using a UV–Vis scanning spectrophotometer (Unicam Helios, Spectroncic Unicam EMEA, Cambridge, UK). A filtration through Macherey-Nagel filters (25 mm, pore 0.2 µm) was applied just before the measurement to remove protein from the sample and thereby diminish its spectrum interference in this region. The amount of CD (mmol/kg oil) in the oxidizing emulsions was calculated using the following formula:

$$CD\ (mmol/kg\ oil) = ((A / (b \times \varepsilon)) \times 1000) / C$$

CD was calculated on the basis of linoleic acid which has a molar absorptivity (ε) of 27,000. The absorbance (A) was determined at 232 nm using a pathlength (b) of 1 cm. The initial oil concentration (C) in the isooctane/2-propanol mixture was expressed in kg oil/L.

4.6 Behavior of the cranberry extract in a fresh cheese product

4.6.1 Cream cheese production

The formulation of a fresh cream cheese product is shown in Figure 7. The cranberry ingredient was incorporated into the cheese premix, which consisted of skim milk and full fat cream. The cranberry extract was added as a dilute 5% solution, and skim milk powder added at a level of 0.1 g/mL for every extra mL of cranberry solution added to the mix. Ingredients were homogenized so that the final fat content of the milk mixture was 11% (full-fat cream cheese) and the final pH was 5.6. A pre-determined amount of reconstituted skim milk was added to the control cheese in order to ensure that the fat and protein content of the cheese-milk was consistent with the treatment. The pre-acidified cranberry-formulated milk and the control milk were then inoculated with starter culture, and gelation

was allowed to take place until a final pH of 4.6 was reached. The curd was then whisked, and drained at room temperature in cheese cloth until the final moisture content was less than 60%.

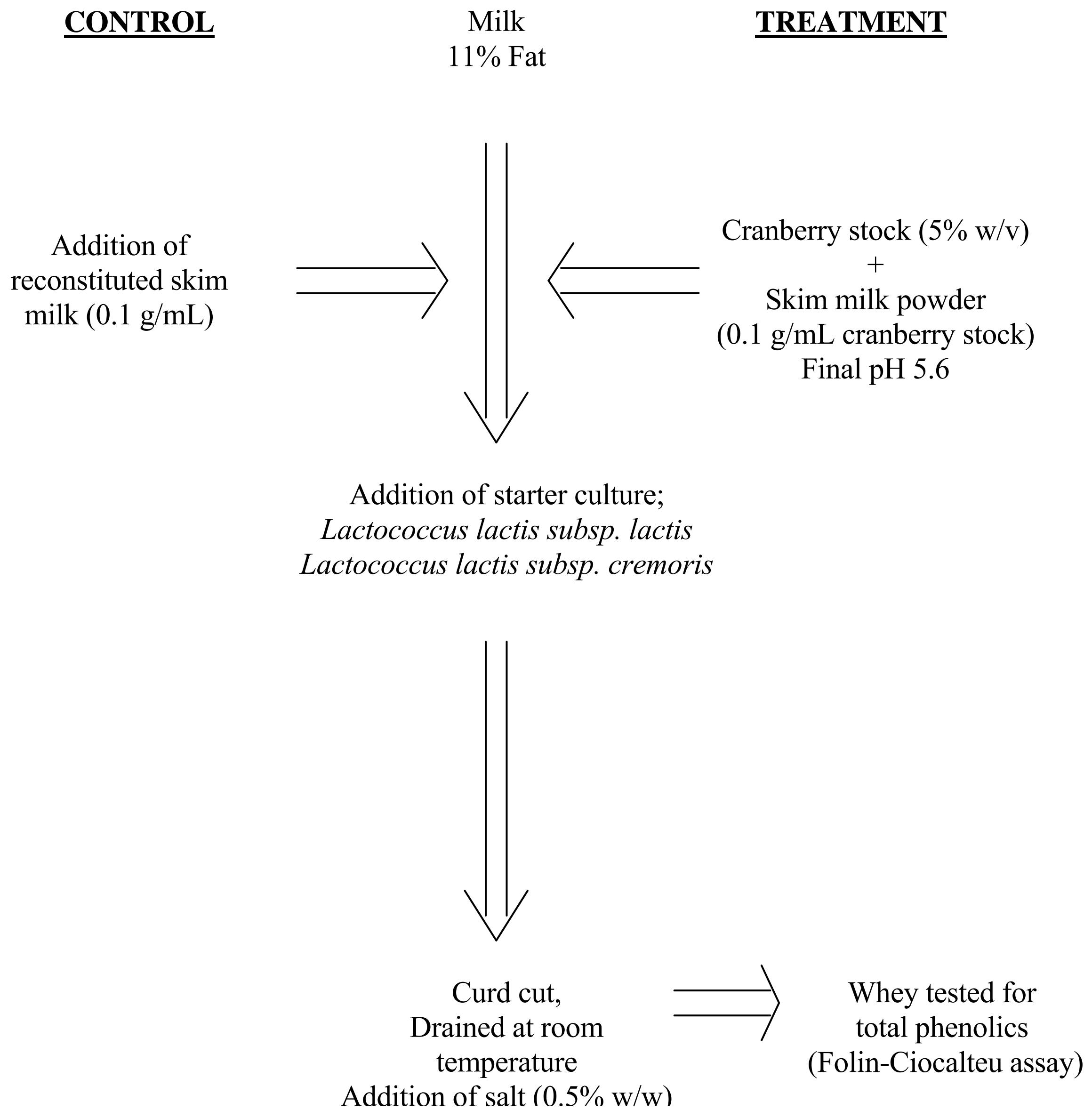


Figure 7. Manufacture of fresh cream cheese product containing cranberry extract

4.6.2 Fat content

Total fat in the cream cheese product was determined using the Mojonnier method (AOAC Official Method 989.05). Weighed samples (1 g) were mixed with 10 mL of H_2O until smooth, and this was transferred to a Mojonnier extraction flask. To the sample flask was added 1.5 mL of NH_4OH, followed by 3 drops of phenolphthalein indicator to help sharpen the visual appearance of the interface between aqueous and ether layers. Following this, 10 mL of ethyl ether was added, the flask stoppered with a cork and shaken vigorously for 1 minute. Petrolem ether was then added at a level of 25 mL and the flask shaken vigourously for 1 minute. Ether and aqueous layers were then allowed to separate, and the upper ether layer was transferred to a dry, pre-weighed glass beaker. A second extraction was carried out by adding ethyl ether (5 mL) and petroleum ether (10 mL) successively to the sample flask and proceeding as above. The pooled ether layers were evaporated under nitrogen, and the beaker containing fat weighed. A blank sample was also run in which the cheese sample was replaced by 10 mL of water. This was done to minimize errors caused by residue. Reagent blanks should be less than 0.0020 g residue. Total fat in the cheese was calculated as percent fat by weight.

4.6.3 Ionic calcium

Ionic calcium was determined directly using an Orion Calcium Plastic Membrane Half-Cell ISE with a single junction reference electrode (Thermo Scientific, Waltham, MA). Calibration of the electrode was carried out each time before analysis by preparing a calibration curve from serially diluting 0.1 M (4008 ppm) $CaCl_2$ calcium calibration standard (Appendix D). Results were expressed in ppm Ca^{2+}.

4.6.4 Color

Color analysis was performed using HunterLab LabScan XE with Easy Match QC Software (Hunter Associates Laboratory, Inc., VA). A 1-inch opening was placed on the plate until the line indicator aligned. The reflectance value in the software was set to 1.00 inch. Prior to testing, the machine was standardized with black and white tiles. Cream cheese samples were spread evenly over disposable plastic Petri dish. The black cover was placed onto the sample dish and the Lab value was read. The Lab values of three cheeses per treatment were measured using the same procedure to obtain triplicate values.

4.7 Determination of lipid oxidation in fresh cheese product

4.7.1 Peroxide value

Twenty gram samples of cream cheese were incubated at 4ºC in thermoformed plastic cups and sealed using plastic wrap. Peroxide value (PV) of the product was tested using procedures from AOAC Official Methods 965.33: Fats and Oils (AACC, 1969). Lipid content of the cheese was extracted according to method described by Kristensen et al. (2001). The sample (2.5 g of cheese) was transferred to a beaker. Chloroform–methanol (2:1 v/v; 50 mL) was added and the mixture was homogenised with an UltraTurrax Homogeniser (Jankel & Kunkel IKA-Labortechnik, Staufen, Germany). $CaCl_2$ solution (1.0 mM) was added at a level of 10 mL and the mixture was shaken vigorously for 15 s. The mixture was centrifuged at 500 g for 30 minutes at room temperature and the lower layer transferred to an evaporation flask. Chloroform (30 mL) was added to the upper layer, and the mixture was homogenised for 60 s and then again centrifuged at 500 g for 30 min. The resultant lower layer was again added to the evaporation flask where the lipids were dried under vacuum at room temperature. Two cheese samples per combination were withdrawn for lipid oxidation analysis at each time.

PV testing of sample was done by weighing 2.00 ± 0.05 grams of room temperature extracted lipid into Erlenmeyer flask. Blank determinations were carried out using water instead of fat. An aliquot of 30 ml of acetic acid and chloroform (3:2, v/v) mixture and 0.5 ml saturated KI were added to the flask and the flask was continuously swirled for

exactly 1 minute. Next, 30 ml ddH_2O was added to the flask. The sample was titrated with 0.002 *N* sodium thiosulphate using 1% starch solution as an indicator until the blue colour disappeared. The final volume of titrant in the burette was recorded. The peroxide value (milliequivalents of active oxygen per kg of sample) was determined using the formula:

$$PV = (V \times T \times 1000) / m$$

V is the number of mL of the sodium thiosulphate solution used for the titration, corrected to take into account the blank test, *T* is the exact normality of the sodium thiosulphate solution used, and *m* is the mass (in grams) of the test protion.

4.8 Statistical analysis

Freeze-dried cranberry extracts were prepared in triplicate (n=3). Characterization analyses were carried out for each extract and repeated at least twice. For analysis of emulsion stability, particle size, and lipid oxidation, emulsions were prepared at least in duplicate (n=2) for control and treatments, respectively. Statistical differences between control and treatments for particle size and emulsion stability were determined using the Student's *t* test with a significance level set at $P < 0.05$. Lipid oxidation experiments over the set incubation period for emulsions were repeated at least twice in order to obtain sufficient data points for determination of the lag phase and propagation phase of oxidation. Measurements of CD and headspace volatiles were repeated at least twice for each emulsion. Cream cheeses were prepared in triplicate (n=3) for treatment and control. Statistical differences between control and treatment for CD, PV, hexanal and pentanal were determined using a two-way ANOVA (GraphPad Prism 5) with a significance level set at $P < 0.05$, followed by Bonferrini *t* test for pairwise comparison ($P < 0.05$).

5 RESULTS

5.1 Preparation and characterization of a cranberry extract

5.1.1 Extraction of phenolics

The extraction of phenolic compounds from cranberries using ethanol resulted in a yield of 9.64 ± 0.75 grams of freeze-dried extract per 100 grams of whole cranberries (n=3). The extract was readily reconstituted into water or methanol, indicating that it consisted mainly of soluble solids. Soluble solids present in cranberries have been found to range between 8-12% (w/w) depending on cultivar and stage of ripening (Celik et al., 2008). The extract had a brilliant red color and a flavor typical of cranberry fruit (personal observation).

Table 4. Total phenolics, anthocyanins, proanthocyanins and organic acids present in freeze-dried cranberry extract [1]

Component	Freeze-dried extract
Total phenolics (mg GAE/g extract)	42.16 ± 4.21
Anthocyanins (mg cyanidin-3-glucoside/g)	4.09 ± 0.20
Proanthocyanidins / non-polar phenolics (mg GAE/g)	5.41 ± 0.56
Organic acids (mg/g extract)	
Quinic	64.30 ±1.61
Malic	59.11 ± 0.57
Citric	86.85 ± 11.94
Ascorbic	6.17 ± 0.01

[1] Results expressed as mean ± SD for extracts prepared in triplicate.

5.1.2 Phenolic and organic acid content

Freeze-dried extracts of cranberries were characterized for total phenolics, anthocyanins, proanthocyanidins, and individual organic acids (Table 4). The phenolic content of the extract was found to be 42.16 ± 4.21 mg/g extract. This corresponds to approximately 4-5% (w/w) of the extract. Total anthocyanins were quantified in the whole extract. The anthocyanin content was 4.09 ± 0.20 mg/g extract (n=3), which corresponds to approximately 10-12% of total phenols. The major organic acids found in the cranberry extract were identified and quantified using HPLC. Prior to HPLC analysis, the extract was reconstituted in ddH_2O at a concentration that ensured the peak responses for all major organic acids were within the linear ranges of the calibration plots (Appendix C). Citric acid was identified as being the most abundant organic acid, followed by quinic and malic acid (Table 4). Organic acids made up approximately 22% (w/w) of the ethanolic extract. The extract also contained a small amount of ascorbic acid. The pH of a 10% (w/v) aqueous solution of the cranberry extract was 2.84 ± 0.06, with a titratable acidity ranging from 8-9% (w/w) (n=3).

The extract was characterized for its phenolic profile using HPLC. The extract was first separated into different classes of phenolics based on polarity using an LH-20 column. Anthocyanins were eluted with 60% methanol on the LH-20 column to obtain Fraction 1 (F1). The solvent was evaporated under vacuum, and the concentrated fraction lyophilized into a brilliant red powder. Individual anthocyanins were separated from F1 using HPLC, revealing five distinct peaks at 520nm (Figure 8). Related times for individual peaks separated by HPLC corresponded to main anthocyanins found in cranberries, as previously reported (Zheng and Wang, 2003), namely cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-galactoside, and peonidin-3-arabinoside. Pure standards for each anthocyanin were not available to confirm this result. A second cranberry fraction (F2) was eluted from the LH-20 column with 100% methanol. Lyophilization of F2 yielded a yellow-brown powder. The separation of components from F2 using HPLC showed distinct peaks at 280 nm, likely due to the presence of proanthocyanidins and phenolics of low polarity (Figure 9). A total phenolic measurement was carried out on F2 in order to determine the content

of proanthocyanidins and non-polar phenolics in the extract (Table 4). The phenolics determination for F2 showed that approximately 12-13 wt% of total phenolics in the extract were found in F2. The presence of proanthocyanidins in F2 was confirmed using the acid-butanol assay. Hydrolysis of this fraction with acid-butanol caused a pink color to develop which absorbed at 500 nm. Monomer, dimers, and A-type trimers of procyanidins have been previously identified in the 100% methanol fractions of cranberry extract and juices using HPLC/MS (Prior et al., 2001).

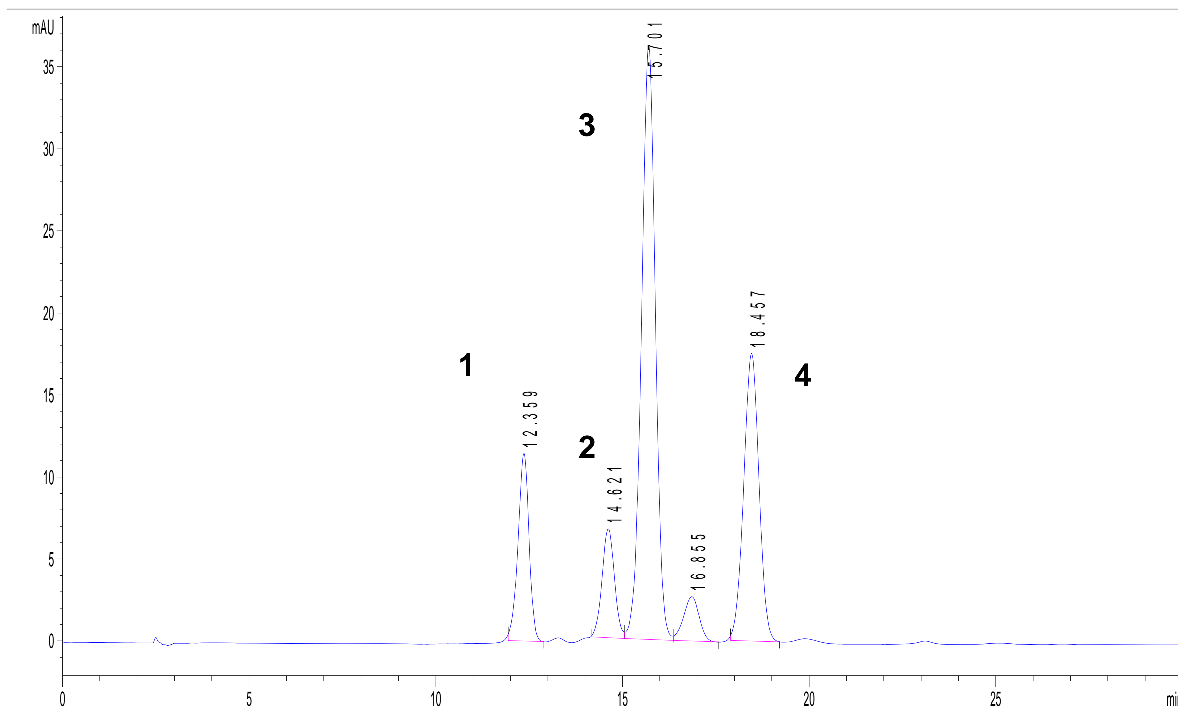


Figure 8. HPLC chromatogram at 520 nm for 60% methanol fraction (F1) obtained from the freeze-dried cranberry extract. (1) cyanidin galactoside (2) cyanidin arabinoside (3) peonidin galactoside (4) peonidin arabinoside, according to individual retention times (Zheng and Wang, 2003)

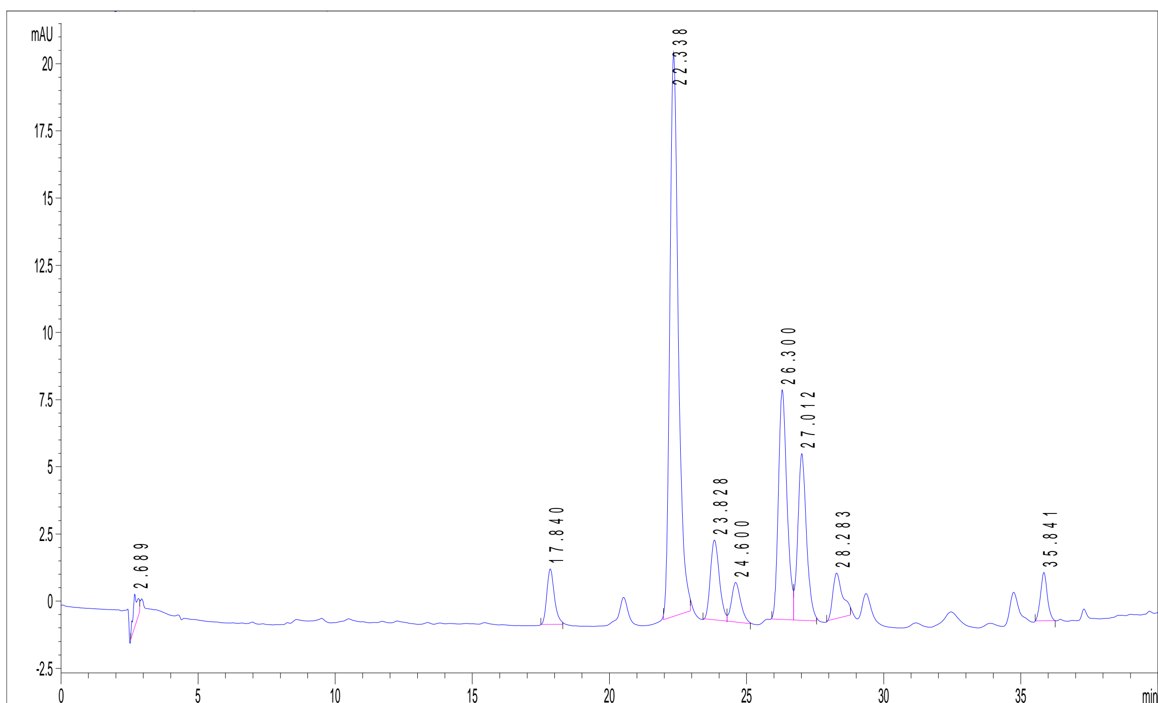


Figure 9. HPLC chromatogram at 280nm for 100% methanol fraction (F2) obtained from freeze-dried cranberry extract. Peaks were not identified due to lack of standards.

5.1.3 Antioxidant activity in the ORAC assay

The freeze-dried cranberry extract was assessed for antioxidant activity *in vitro* using the ORAC assay (Table 5). Both hydrophilic ORAC (H-ORAC) and lipophillic ORAC (L-ORAC) assays were performed in order to fully establish the antioxidant capacity of the cranberry extract against a peroxyl radical generator. The extract was found to exhibit 348.31 ± 33.45 µmol of Trolox equivalents (TE)/g of sample in the H-ORAC assay. In the L-ORAC assay, lipophilic components were extracted from the cranberry extract using hexane. Lipophilic components were found to contribute an antioxidant capacity 11.02 ± 0.85 µmol of TE/g of extract. Fractions F1 and F2 were found to exhibit a high total antioxidant capacity in the H-ORAC test.

Table 5. Antioxidant activity of the whole cranberry extract, F1 and F2 in the H-ORAC and L-ORAC assay [1]

	H-ORAC (μmol of TE/g)	L-ORAC (μmol of TE/g)
Whole extract	348.31 ± 33.45	11.02 ± 0.85
F1	5109.34 ± 143.76	ND
F2	3398.23 ± 92.68	ND

[1] Values represent mean ± SD (n=3). H-ORAC = hydrophillic oxygen radical absorbance capacity; L-ORAC = lipophillic oxygen radical absorbance capacity. ND – not detectable.

5.2 Behavior of the cranberry extract in a model emulsion system

5.2.1 Effect on emulsion formation and particle size distribution

An oil-in-water emulsion model was used to determine the effects of a cranberry extract on the ability of casein to form stable emulsions. The model was designed to represent a fresh dairy product, composed of 25% fat stabilized by caseins, the major proteins present in dairy products. Emulsions were formed by blending all ingredients (including the cranberry extract) using an UltraTurrax blender operating at 4000 rpm/min. The level of cranberry extract added was based on a final emulsion pH, as summarized in Table 6.

Table 6. Final pH, emulsion droplet size, and proportion of active cranberry ingredient for emulsions containing three levels of cranberry extract

	Cranberry extract (g/100g emulsion)	Final emulsion pH	Droplet size, (μm)[1]	Phenolics (mg/100g emulsion)	Anthocyanins (mg/100g emulsion)	Proanthocyanidins (mg/100g emulsion)
CTL	0	6.7	0.261±0.024[a]	ND	ND	ND
T1	0.1875	6.0	0.294±0.017[a]	7.91	0.767	1.014
T2	0.375	5.6	0.284±0.024[a]	15.81	1.533	2.028
T3	0.75	5.3	0.363±0.032[b]	31.62	3.067	4.058

[1] Values given for emulsion droplet size represent mean ± SD (n=3). [ab] Different letters indicate significant differences ($P < 0.05$) for droplet size between emulsions. ND – not detectable

Emulsion stability (ES), as measured by the loss in absorbance of emulsions at 500 nm, decreased over time for all emulsions formed using a Ultra Turrax T50 Homogenizer (Janke & Kunkel) (Figure 10). The rate at which the absorbance decreased relative to the control over 60 minutes was significantly greater ($P < 0.05$) in emulsions to which the cranberry was added to a pH of 5.3, but not pH 6.0 or 5.6 (Table 7). There was an increasing loss in turbidity with decreasing pH, though emulsions were still relatively stable at pH 5.6 compared to the control.

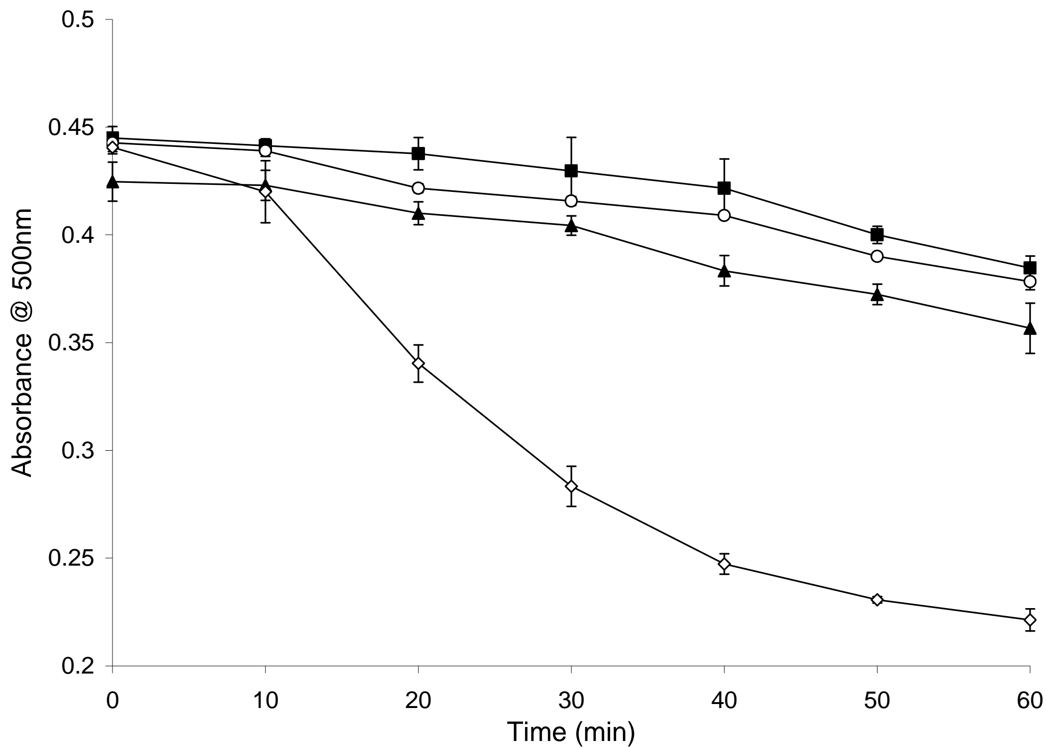


Figure 10. Emulsion stability based on loss in turbidity observed over 60 minutes at 500nm for 25% sunflower oil-in-water emulsions formed at pH 6.7 (control, ■), and containing cranberry extract to a final pH of 6.0 (○), 5.6 (▲) and 5.3 (◊). Each point represents the mean ± SD for six emulsions (n=6).

Table 7. Rate of change for loss in absorbance at 500nm for emulsions containing different levels of cranberry extract [1]

Sample[2]	ΔAbsorbance/hour
Control	0.060 ± 0.010[a]
T1	0.066 ± 0.001[a]
T2	0.072 ± 0.006[a]
T3	0.244 ± 0.003[b]

[1] Results expressed as mean ± SD (n=6). [2] T1, T2 and T3 correspond to 25% sunflower oil-in-water emulsions with the addition of cranberry extract to a final pH of 6.0, 5.6 and 5.3, respectively. [ab] Different letters shows statistical differences ($P < 0.05$) between each emulsion.

In order to further assess the effect of the standardized cranberry extract on the ability of casein to form stable emulsions, a two-step high-pressure homogenization step was employed. The extract was incorporated with the ingredients similar to that repeated in the emulsion stability experiment. A droplet size distribution measurement was used to determine the homogeneity of emulsions that included the addition of cranberry extract. A loss in homogeneity of droplet diameter immediately after emulsion formation was interpreted as the destabilization of the emulsions. No significant difference was detected in the average droplet size between the control emulsions and emulsions treated to pH 6.0 and 5.6, respectively, when the cranberry extract was incorporated as an ingredient prior to high-pressure homogenization (Table 6). The particles were uniformly distributed and showed no sign of fat aggregation, indicating that emulsions were initially stable. Lowering the pH further to 5.3 caused the emulsions to destabilize. The particle size distribution for emulsions at pH 5.3 showed clear signs of fat coalescence and droplet aggregation due to the presence of much larger particles (Figure 11). Particle size analysis of emulsions formed under high pressure showed that the droplet size distribution was uniform for emulsions to which the cranberry ingredient was added up to a final pH of 5.6 (Figure 11).

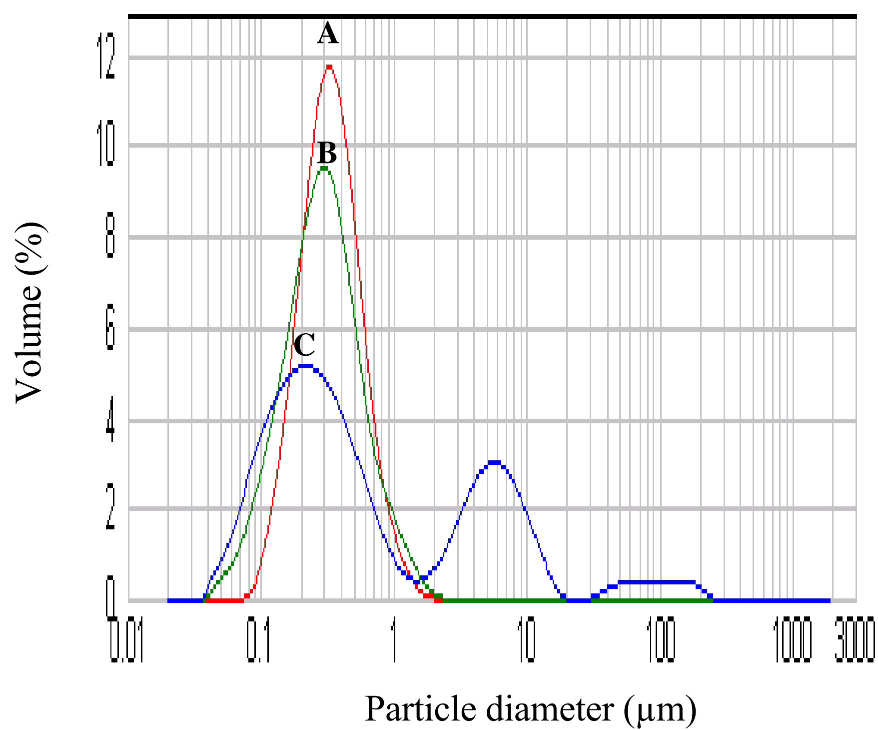


Figure 11. Particle size analysis for emulsions immediately after formation by high-pressure homogenization for (A) control emulsion, (pH=6.7) (B) emulsion containing 0.5% (w/w with aqueous phase) cranberry extract (pH=5.6), and (C) emulsion containing 1.0% (w/w with aqueous phase) cranberry extract (pH 5.3).

5.3 Effect of the cranberry extract on lipid oxidation in model emulsion

5.3.1 Effect on 25% sunflower oil emulsions

Oxidation experiments were initially carried out in emulsions to which cranberry was added to a final pH of 5.3 or 5.6. For each treatment, the standardized cranberry extract was incorporated into a 25% sunflower oil emulsion either (A) mixing the extract in after emulsion formation, or (B) along with the ingredients during emulsion preparation. In each case, measures were taken to ensure that the final concentration of sodium caseinate was 2% (w/w with the aqueous phase). The development of conjugated dienes (CD) was monitored for each emulsion incubated in the dark at 50ºC in an orbital shaker. It was found that oxidation occurred more rapidly, and to a significantly greater extent ($P < 0.05$) in emulsions at pH 5.3 than at pH 5.6 after 22 hours (Figure 12, Table 8). It was also found that emulsions in which the ingredient was incorporated after homogenization had significantly higher levels of CD ($P < 0.05$) in the first 22 hours of incubation for a given pH (Figure 12, Table 8). Because the incorporation of the extract to a pre-formed emulsion did not provide as strong a positive effect, all further oxidation experiments were focused on emulsions where the ingredient was incorporated at the homogenization step. It was also decided that further oxidation experiments were to be carried out in emulsions to which the cranberry extract was added to a final pH of 5.6.

Oxidation was monitored in emulsions over a 90 hour incubation period. This was found to be the optimum time beyond where emulsions became physically unstable at 50ºC. For the control emulsion, a pre-determined amount of 5% (w/v) citric acid solution was carefully added to the aqueous phase prior to homogenization so that the final pH of the emulsion was adjusted 5.6 (same as the treatment). Emulsions were prepared in duplicate and sampled at different times during the incubation. Careful attention was paid to obtaining samples during the propagation phase of lipid oxidation. Oxidation was monitored by measuring CD, hexanal and pentanal in all emulsions.

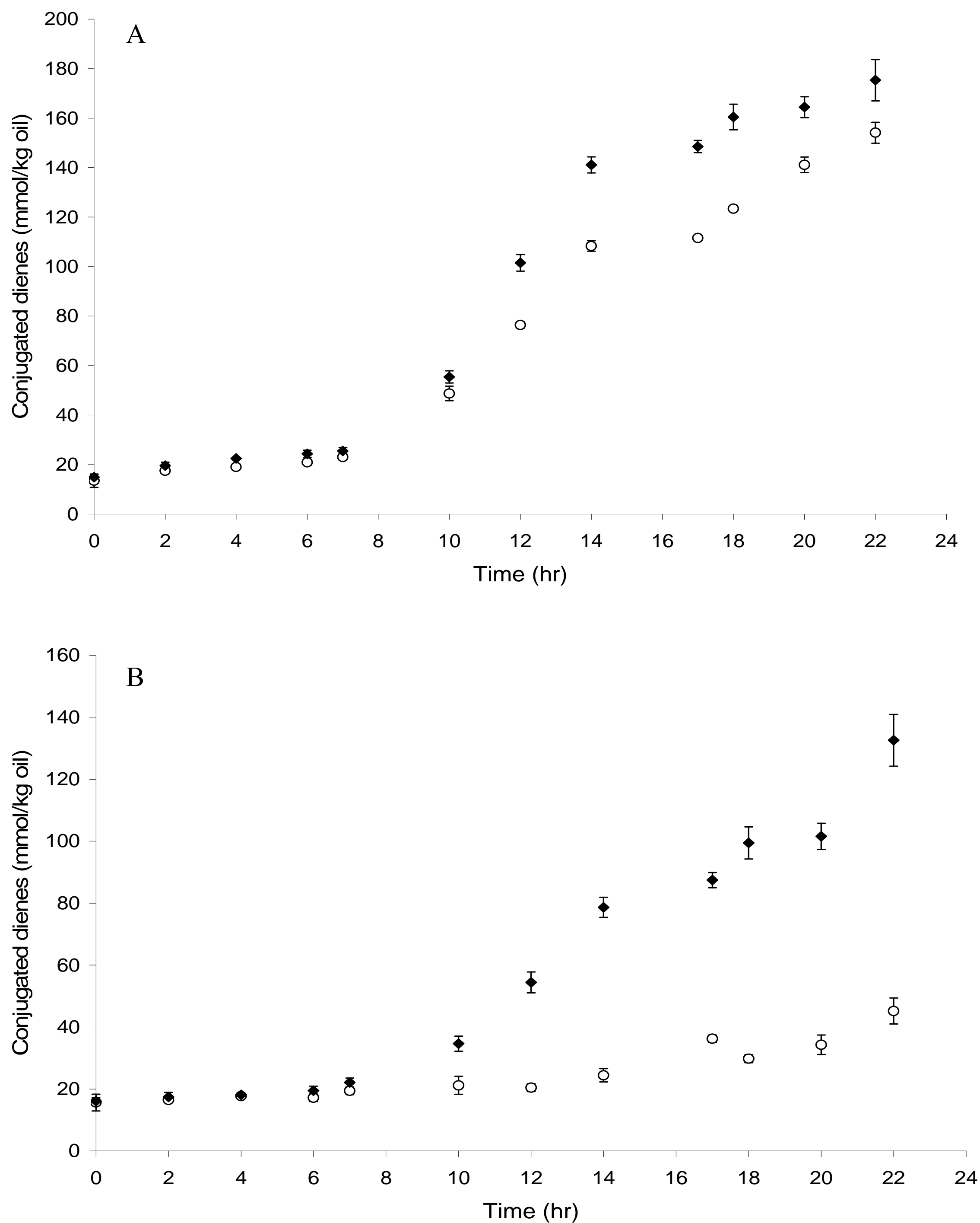


Figure 12. Production of conjugated dienes in sunflower o/w emulsions stored in the dark at 50ºC for 24 hours at (A) pH 5.3, and (B) pH 5.6. Cranberry extract was incorporated by addition of a 2% w/w stock solution to a pre-formed emulsion (♦), or by addition of the stock solution with ingredients prior to emulsion formation (○). Each point represents mean ± SD for emulsions prepared in triplicate.

Table 8. Comparison of conjugated diene (CD) formation in emulsions with two modes of incorporating the standardized cranberry extract [1]

	Conjugated dienes (mmol/kg oil)			
	pH 5.3		pH 5.6	
Time (hr)	+	-	+	-
0	13.50 ± 1.87[a]	14.88 ± 2.11[a]	15.63 ± 2.72[x]	16.10 ± 1.03[x]
4	19.03 ± 2.09[a]	22.43 ± 3.01[a]	17.70 ± 0.31[x]	18.10 ± 0.35[x]
10	48.74 ± 4.32[a]	55.43 ± 4.23[b]	21.19 ± 2.93[x]	34.65 ± 2.44[y]
14	108.33 ± 3.21[a]	141.09 ± 4.16[b]	24.44 ± 2.12[x]	78.65 ± 3.24[y]
18	123.43 ± 5.43[a]	160.44 ± 6.32[b]	29.78 ± 1.26[x]	99.43 ± 5.17[y]
22	154.10 ± 6.32[a]	175.32 ± 5.32[b]	45.19 ± 4.21[x]	132.57 ± 8.32[y]

[1] Incorporation of standardized cranberry extract into emulsions to a final pH of 5.3 or 5.6 was accomplished either by (+) homogenization of the extract with emulsion ingredients; or (-) addition of dilute cranberry solution to emulsions after homogenization. [abxy] different letters show significant differences ($P < 0.05$) in CD within a row for each pH level.

The generation of CD in sunflower oil emulsions is shown in Figure 13. The emulsion containing standardized cranberry extract had significantly lower CD ($P < 0.05$) than the control after approximately 6 hours of incubation. The cranberry ingredient significantly extended ($P < 0.05$) the lag time prior to propagation of primary products from 7.95 ± 0.80 hours in control emulsions to 15.09 ± 1.32 hours for emulsions containing cranberry extract (Table 9 & Figure 13). These values were calculated by determining the time point along the curve where the slope significantly changed for both control and treatment (Appendix E). The maximum amount of CD that formed in both control and treatment, however, was not significantly different, at 186.39 ± 3.88 and 181.01 ± 3.93 mmol/kg oil, respectively (Table 9).

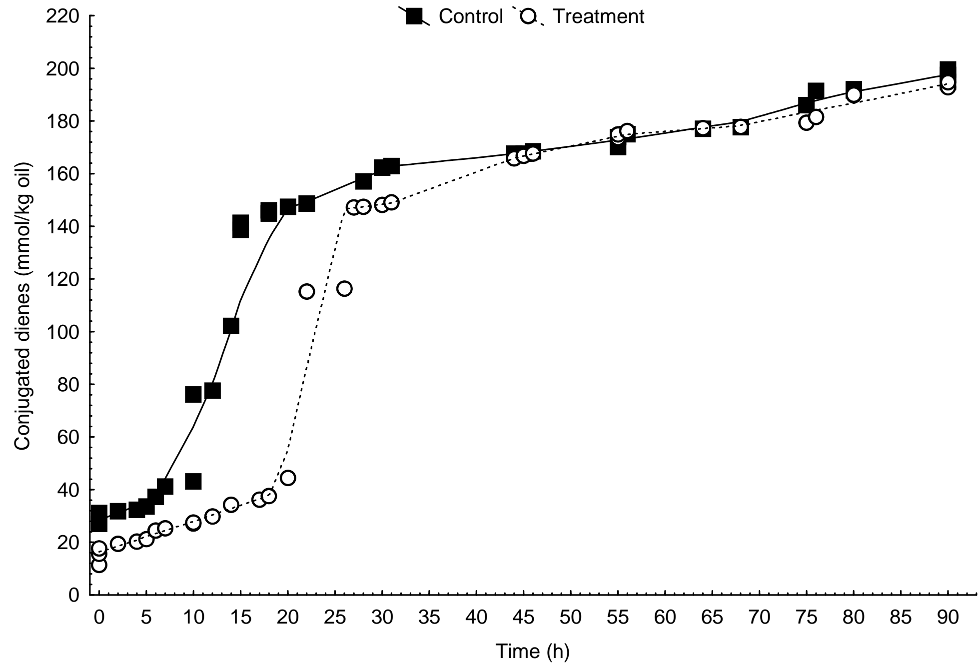


Figure 13. Production of conjugated dienes in sunflower o/w emulsions (pH 5.6) over 90 hours stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

The formation of hexanal in sunflower oil emulsions was similar in both control and treatment until 64 hours, when hexanal levels were noted to be significantly lower ($P < 0.05$) in the emulsions containing the cranberry extract (Figure 14). The development of pentanal showed a similar trend, though it developed to a much lesser extent than hexanal (Figure 15).

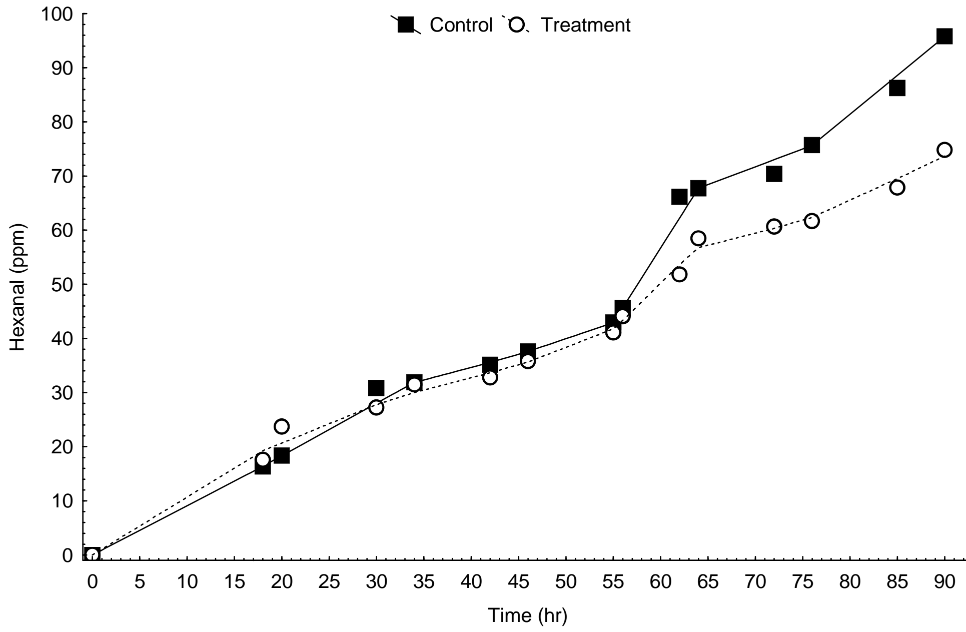


Figure 14. Production of hexanal in sunflower o/w emulsions (pH 5.6) stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

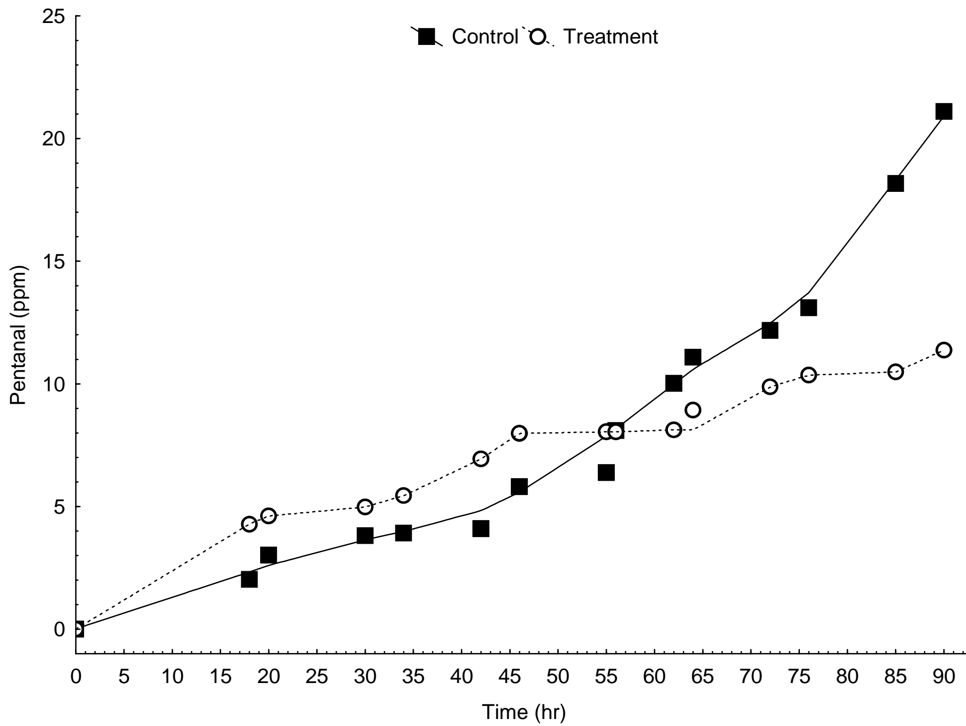


Figure 15. Production of pentanal in sunflower o/w emulsions (pH 5.6) stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

5.3.2 Effect on 25% butterfat oil emulsions

The generation of CD developed to a lesser extent in the butterfat emulsions compared to the sunflower oil emulsions, likely a result of the greater proportion of saturated fatty acids in the butterfat. The presence of the cranberry extract in the butterfat emulsions also significantly extended ($P < 0.05$) the lag time prior to propagation of primary products from 9.67 ± 1.68 to 13.74 ± 1.21 hours (Table 9 & Figure 16). The level of CD was significantly reduced ($P < 0.05$) in butterfat emulsions containing the cranberry extract over a 90 hour incubation period with the cranberry extract. The incorporation of cranberry into the butterfat emulsions also greatly reduced the formation of hexanal and pentanal (Figure 17 & 18).

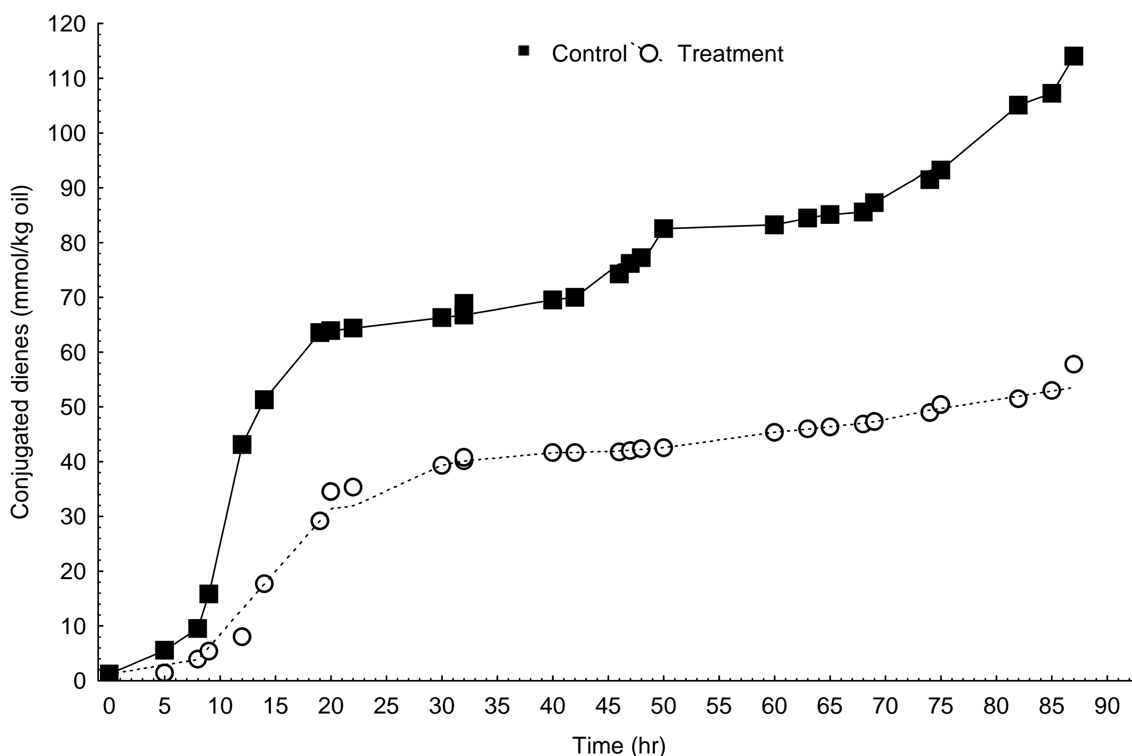


Figure 16. Production of conjugated dienes in butterfat o/w emulsions (pH 5.6) stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

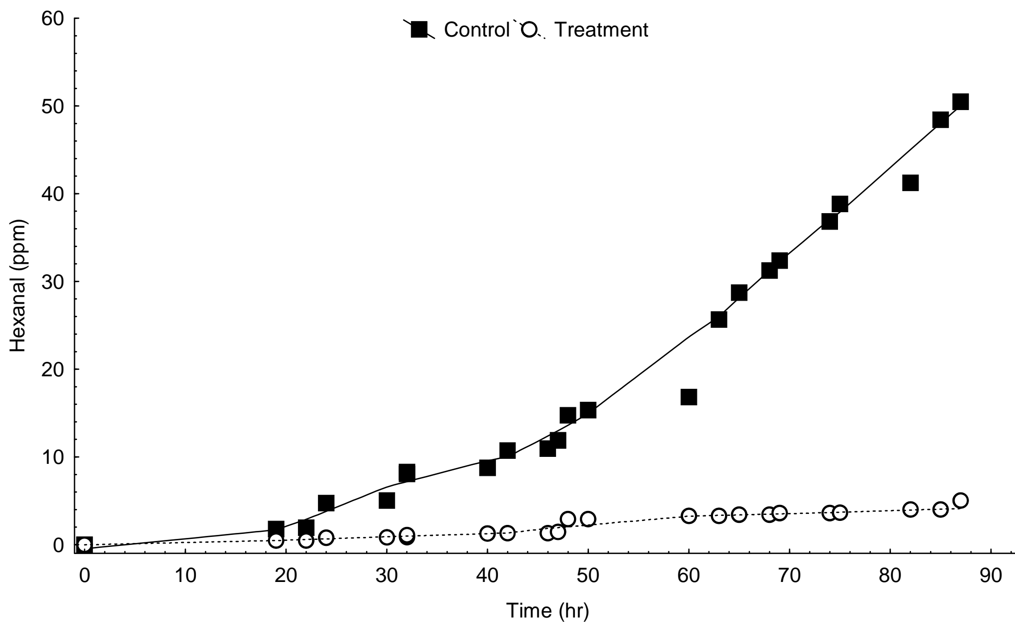


Figure 17. Production of hexanal in butterfat o/w emulsions (pH 5.6) stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

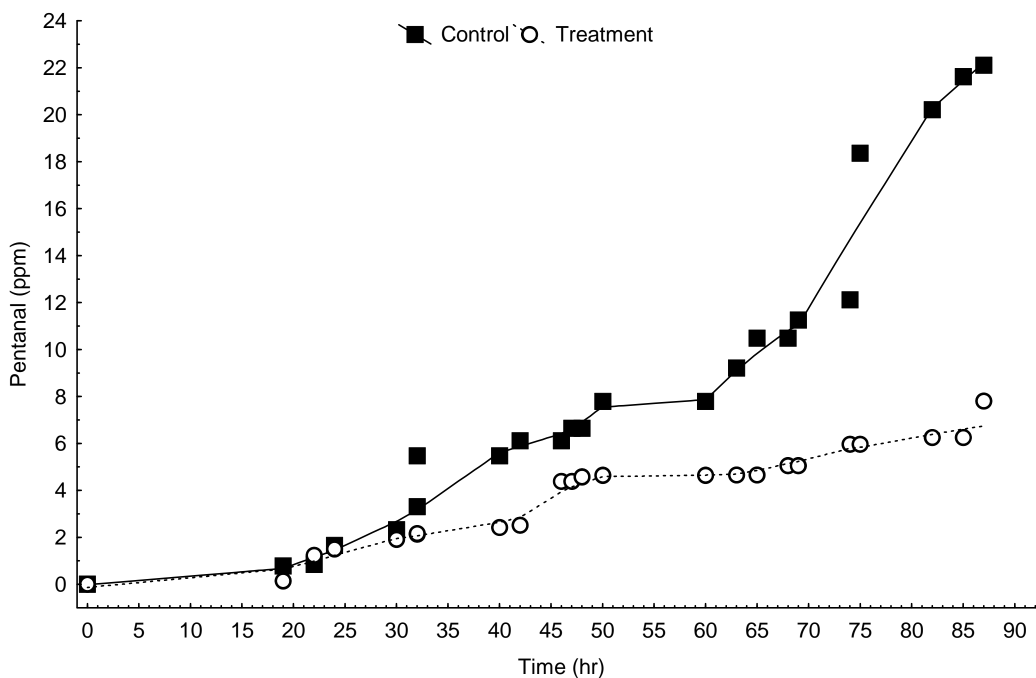


Figure 18. Production of pentanal in butterfat o/w emulsions (pH 5.6) stored in the dark at 50ºC for control emulsion (■) and emulsion containing cranberry extract (○). Each point represents a duplicate measurement.

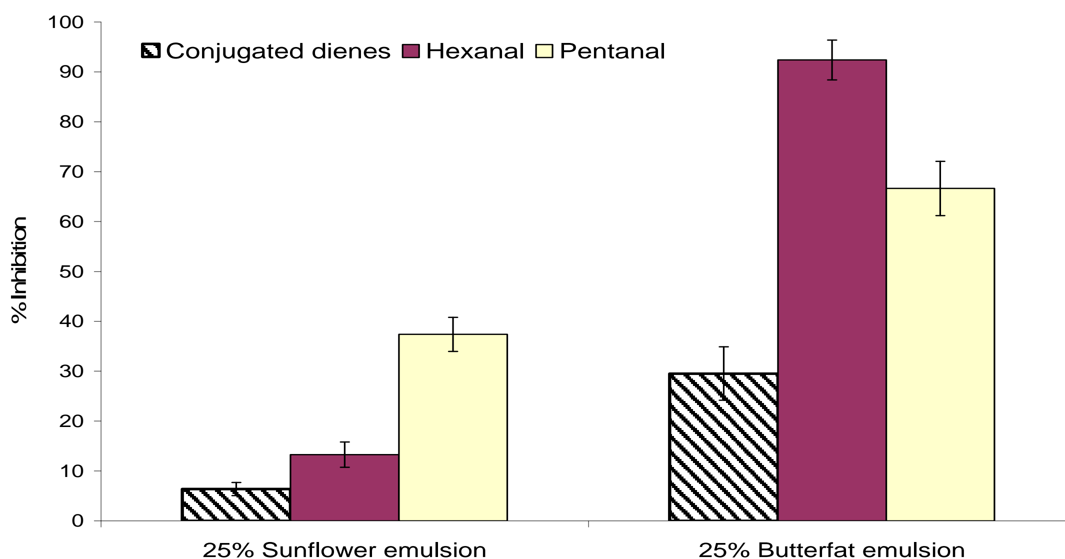


Figure 19. Antioxidant activity of the cranberry extract in different food models as percent inhibition of primary and secondary products as calculated using areas under the curve (AUC). Bars represent %inhibition ± SD.

A summary of the lipid oxidation reaction processes that occurred in both sunflower and butterfat emulsions in summarized in Table 9. The absolute amounts of CD and hexanal were significantly higher ($P < 0.05$) in sunflower oil emulsions. The cranberry extract, however, was more effective as an antioxidant in the butterfat emulsions in terms of the extent to which primary and secondary products were inhibited relative to the control (Figure 19).

Table 9. Comparison of oxidation between sunflower and butterfat emulsions

Emulsion type		pH	Lag phase (hr)	Maximum CD (mmol/kg oil)	Maximum Hexanal (ppm)	Maximum Pentanal (ppm)
SFO	Control	5.6	7.95±0.80[a]	186.39±3.88[a]	86.22±4.02[a]	18.16±3.48[b]
	T1	5.6	15.09±1.32[b]	181.01±3.93[a]	74.79±5.09[a]	11.37±4.49[a]
BF	Control	5.6	9.67±1.68[a]	84.20±6.35[b]	45.26±7.37[b]	21.32±0.98[b]
	T1	5.6	13.74±1.21[b]	59.23±3.00[a]	3.37±1.81[a]	5.52±2.72[a]

[ab] Different letters show significant difference ($P < 0.05$) between control and treatment within a column for each type of emulsion. SFO = sunflower oil (25%) emulsion; BF = butterfat (25%) emulsion.

5.4 Behavior of cranberry extract in a fresh cream cheese product

5.4.1 Effect on fat, moisture and ionic calcium

The behavior of the standardized cranberry extract in a freshly formulated dairy product was investigated. It was established in the emulsion model that the cranberry ingredient possessed the ability to effectively limit lipid oxidation in a simple system consisting of water, fat and casein. The ingredient was incorporated into a cheese formulation to further establish how it would affect the physicochemical characteristics of a dairy product stored under normal refrigerated conditions. Cream cheese was selected as the fresh cheese product. The main factors that were assessed were the color stability, the effect on ionic calcium solubilization, and inhibition of generation of lipid oxidation products over the course of a 50 day storage trial at 4ºC.

The cranberry ingredient was incorporated into the cheese premix, which consisted of milk and full fat cream, until a final pH of 5.6 was reached. Ingredients were homogenized so that the final fat content of the premix was 11% w/w (full-fat cream cheese) for both control and treatment formulations. Characteristics of the premix used for cream cheese manufacture are summarized in Table 10. The pre-acidified cranberry-formulated milk and the control milk were inoculated with starter culture, and gelation was allowed to take place until a final pH 4.6 was reached. The curd was then whisked, and drained at room temperature in cheese cloth to a final moisture content of 60%. The final characteristics of the cream cheese are shown in Table 11.

Table 10. Characteristics of milk premix used for the manufacture of cream cheese product

	Fat (%w/w)	Cranberry extract (g/100g milk)	pH	Phenolics (mg/100g milk)
Control	11	--	6.7	--
Treatment	11	1.18	5.6	49.6

Table 11. Final characteristics of control cheese and cheese made with the incorporation of cranberry [1]

	Control	Treatment
Fat (% w/w)	30.1 ± 0.4	30.3 ± 0.7
Moisture (%w/w)	59.12 ± 1.17	58.97 ± 1.98
Salt (w/w %)	0.5	0.5
pH	4.61	4.64
Ionic calcium (ppm)	29.0 ± 0.4[a]	60.0 ± 1.2[b]

[1] Results expressed as mean ± SD for triplicate cheeses. [ab] Different letters show significant difference ($P < 0.05$) for ionic calcium between control and treatment.

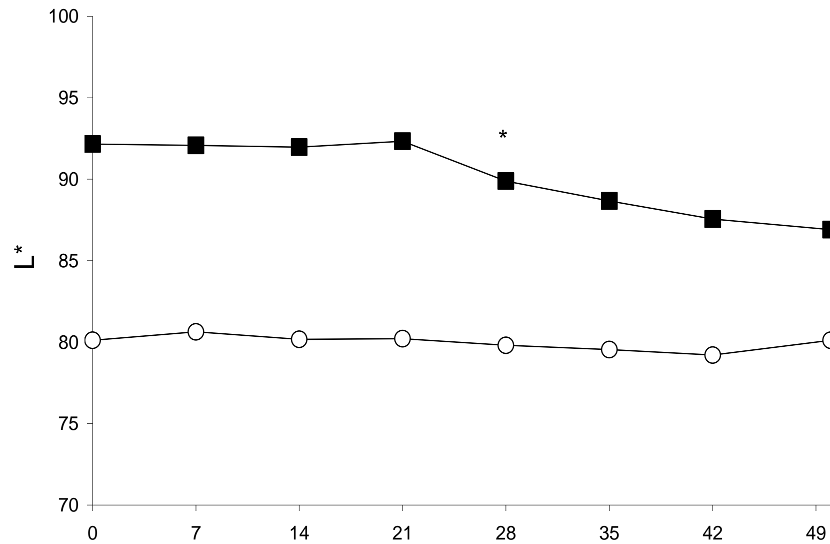
It was determined that over 98% of phenolics added to the cheese mix were retained in the curd by measureing the phenolic content of the separated whey fraction. Under the same manufacturing conditions, no difference was observed in the final fat content of the control and treatment cream cheeses. Salt was added to the curd in order to maintain the final pH of the product. It was found that the pH dropped slightly over the course of incubation for all cheeses, never falling below 4.45. The concentration of calcium in ionic form was found to be significantly higher ($P < 0.05$) in cheeses containing the cranberry ingredient.

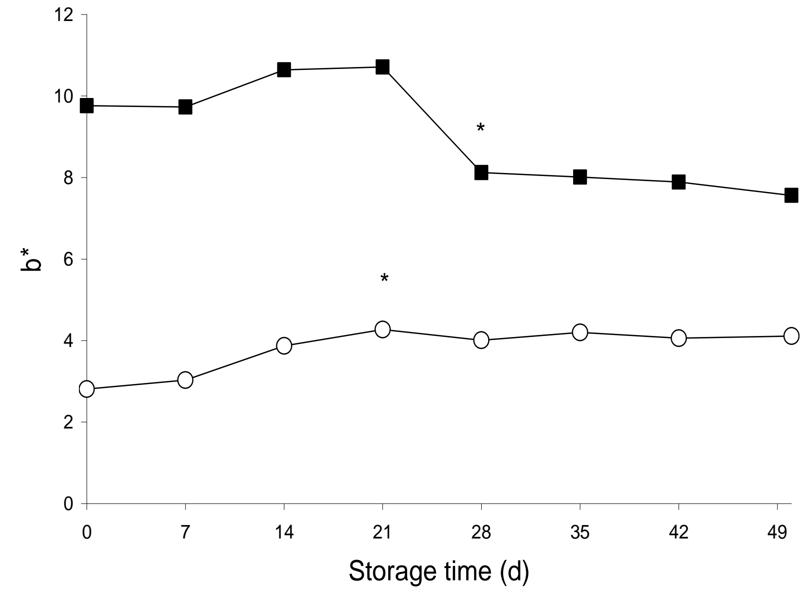
5.4.2 Color

Color was monitored using a HunterLab to measure the L*a*b* score. The L*-value (lightness) significantly decreased ($P < 0.05$) after 28 days for the control cheese, but remained constant for cheeses containing the cranberry extract over 50 days (Figure 20). There was a slight decrease in the a*-value (red – green) for cranberry cheeses, indicating a loss in redness. The a*-value was much lower to begin with for the control cheese, and significantly decreased ($P < 0.05$) after 28 days which could be a possible sign of some oxidative changes taking place. The b*-value (yellow – blue) significantly decreased for the control cheese, but increased ($P < 0.05$) for the cranberry cheese after 28 days of storage. The increase in b*-value observed for the cranberry cheese indicates a slight loss on the blue scale. The anthocyanins were quite stable over the 50 day incubation period, as no large changes in any of the color values was observed.

5.5 The effect of cranberry extract on lipid oxidation in fresh cheese

A significant difference ($P < 0.05$) in PV was observed between control and treatment starting after 14 days of storage at 4ºC. A clear decrease in PV occurred for cheeses containing the cranberry extract. A small increase in CD was observed for control cheeses at 14 days, and this became more significant at 28 days of storage, after which time CD content remained elevated in the control. The amount of CD that developed, however, was very low. The storage conditions were representative of normal product storage, as opposed to accelerating oxidation by means of a catalyst or high temperature. Thus, the low CD values indicate that free radical formation did not reach a point of propagation under the storage conditions in either the control or treatment in this experiment. Nevertheless, the lower level of CD observed with the cranberry extract indicates that free radicals were indeed scavenged over the incubation period tested herein.





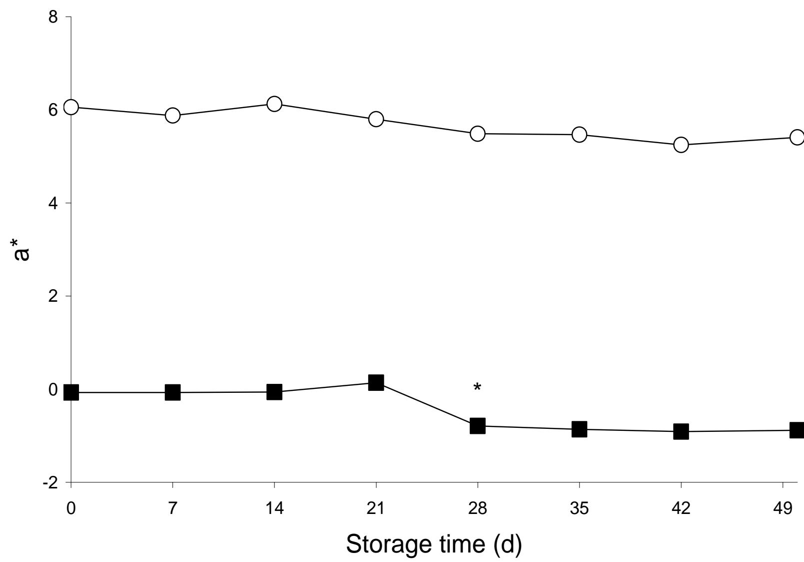


Figure 20. Color scores for control (■) and treatment (○) cream cheese incubated over 50 days at 4ºC. * shows time where scores significantly changed ($P < 0.05$) for treatment or control.

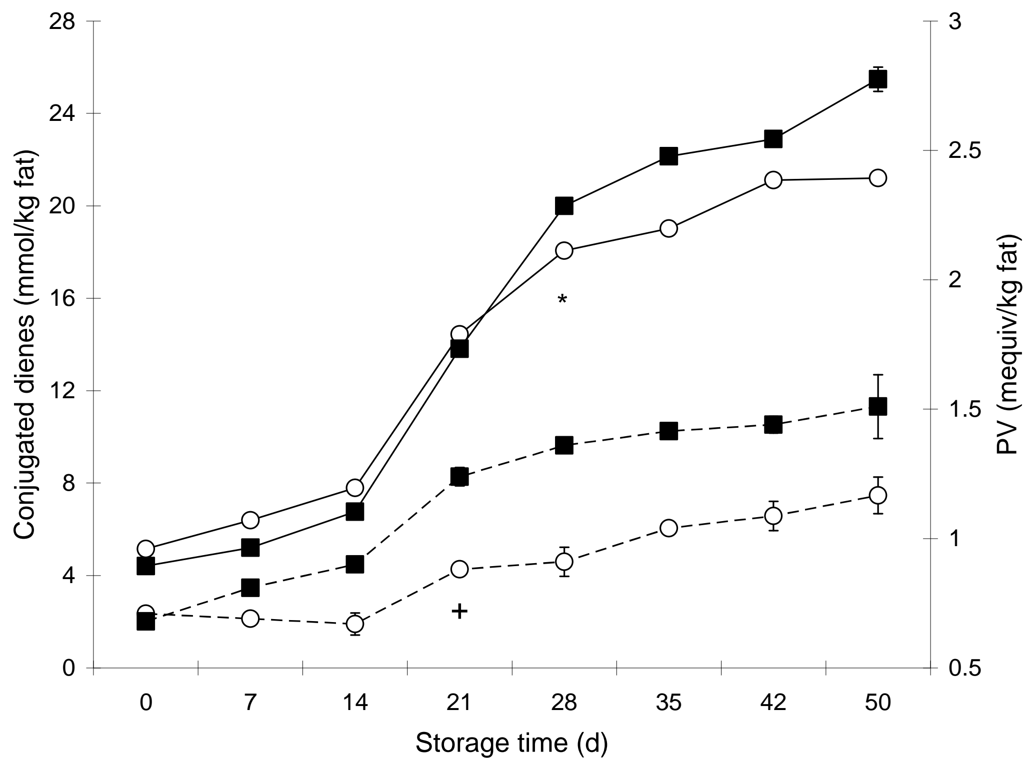


Figure 21. Temporal pattern of CD (—) and PV (---) development in control (■) and treatment (○) cream cheese stored for 50 days at 4ºC. *+ shows time where scores significantly changed ($P < 0.05$) between treatment and control.

Table 12. Comparison of lipid oxidation in control and treatment cream cheeses

	Maximum CD (mmol/kg fat)	Maximum PV (milliequivalents O_2/kg fat)
Control	25.48 ± 1.77^{b}	1.50 ± 0.10^{b}
Treatment	21.20 ± 0.44^{a}	1.67 ± 0.06^{a}

[ab] Different letters show significant difference ($P < 0.05$) between control and treatment. CD = Conjugated diene; PV = Peroxide value.

6 DISCUSSION

6.1 Preparation and characterization of the cranberry extract

6.1.1 Extract preparation

Preparation of a phenolic-enriched standardized cranberry extract was accomplished using ethanol as the extraction solvent. For extracting phenolics from fruit and vegetable matrices, water and ethanol have been shown to be very effective solvents (Meireles, 2008). For fruits such as red currant, black currant and grapes, the recovery of anthocyanins was 2, 3 and 10 times higher, respectively, in 70% ethanol than pure water (Lapornik et al., 2005). In the design of the extraction process, it is important to consider the type of solvent, the solvent-to-sample ratio and the time of extraction. These factors are highly dependant on the desired purity of the phenolic-enriched product. By extracting the cranberries several times over with aqueous ethanol, a heterogeneous mixture was obtained that not only contained water-soluble components, but also components of lipophillic character. Ethanol was selected as the solvent due to the potential food application of the extract. A total of 9.64 ± 0.75 g of extract was obtained from 100 g of whole frozen cranberries. This yield was expected, as the total soluble solids content of cranberries generally ranges between 9-12% (w/w). (Girard and Sinha, 2006, Barrett et al., 2004)

Aqueous alcohols remain the most common solvents for extracting phenolics, in particular anthocyanins, from plant materials (Shi, 2006). Concentrating extracts at lower temperatures in the absence of oxygen minimizes the destabilization of the phenolics during processing (Shahidi et al., 1995, Kahkonen et al., 2001). The anthocyanin and proanthocyanidin content of the extract indicated that these classes of phenolics were effectively separated from the whole berry. Whole berries have been found to contain between 190 and 533 mg/kg anthocyanins (Barrett et al., 2004). The total yield of anthocyanins from 100 g of cranberries was approximatey 40 mg, which falls well within the expected range. For proanthocyanidins, the total yield from three extracts was between 50-60 mg for every 100 g of fresh berry. Proanthocyanidin content in mature cranberries

may differ depending on variety, but is usually within the range of 40-120 mg/100 g fresh weight. The content of anthocyanins and proanthocyanidins in cranberries depends highly on fruit development and maturation (Vvedenskaya and Vorsa, 2004).

Separation of individual phenolic classes in the cranberry using an LH-20 column was done to best characterize the phenolic components present in the extract. The presence of major anthocyanins in cranberries was confirmed using HPLC, as these color pigments strongly absorb light at 520 nm (Markakis, 1982, Mullen et al., 2002). The non-polar substances separated from the extract showed minimal color. When this fraction (F2) was subject to acid hydrolysis under high temperature, a pink color developed. This is likely due to the cleavage of proanthocyanidins to cyanidin and delphinidin, which are known to absorb light in the visible range (Gessner and Steiner, 2007).

There is a recent body of evidence showing that the consumption of cranberry as a whole may in fact be more beneficial to health than simply consuming individual phytochemical constituents (Seeram et al., 2004, Vattem et al., 2005). Nevertheless, many studies have sought to elucidate the relative contributions of individual phenolic constituents for a specific function. Isolated compounds from cranberry have been studied for possible contributions to reduce the risk of cardiovascular diseases (Reed, 2002, Yan et al., 2002, Wilson et al., 1998,). Enriched fractions of cranberry anthocyanins and phenolic acids reduced intracellular H_2O_2-induced damage and inhibited oxidation of cell membrane fatty acids (Youdim et al., 2002). In another study, a total cranberry extract was compared against its phytochemical constituents for anti-proliferative effects against human tumor cell lines (Seeram and Nair, 2002). It was found that a fraction containing all classes of polyphenols most effectively enhanced anti-proliferative activity relative to its individual phytochemicals, suggesting synergistic or additive anti-proliferative interactions of the anthocyanins, proanthocyanidins, and flavonol glycosides within the cranberry extract. The synergies and interactions between cranberry components may be important with regard to overall functionality within a given food system. In most cases, isolating different cranberry constituents is a timely and costly practice and may not be practical depending on the desired application.

6.1.2 pH, titratable acidity and organic acids

The most abundant acid found in the cranberry extract was citric acid. The use of organic acids as adjuncts for starter cultures in the manufacture of fresh dairy products offers certain advantages. Citric acid and other acids such as phosphoric and hydrochloric have found a number of applications in the manufacturing of dairy products such as cottage cheese and ricotta. Acidulants serve a wide range of functions, among which includes flavor, microbial prevention and an affinity to reduce processing time, which in the dairy industry may translate into improved efficiencies (Berry, 2006). They allow for rapid and consistent processing time, improve moisture retention and offer precise pH control. Other important attributes offered by acidulants relate to the fact that they require no aging and will improve economics due to the elimination of culture expense and variability. A 10% (w/v) solution of the cranberry extract had a pH of 2.56 and a titratable acidity of 8.79%. This acidity was useful to enable the partial acidification, or pre-acidification of the milk to a level that is required in combination with starter cultures.

The presence of ascorbic acid in the cranberry extract, albeit low in proportion to other organic acids, was significant. From a nutritional standpoint, the presence of ascorbic acid may be beneficial since dairy products are very low in Vitamin C content. At low pH, ascorbic acid has an increased metal reducing capacity (Niki, 1991). In the presence of metal ions, ascorbic acid has been shown to increase the rate of lipid oxidation in food emulsions since the reduced form of certain transition metals (iron, copper, etc.) are strong pro-oxidants (Frankel, 1996).

6.1.3 Antioxidant activity of cranberry extract in ORAC

The cranberry extract was found to exhibit antioxidant activity in both the hydrophilic and lipophilic ORAC assays. In order to obtain an accurate total ORAC value for the extract, both lipophilic and hydrophilic fractions were measured. Due to the complexity of many biological and food systems, the ability of antioxidants to function in both an aqueous and lipophilic environment can be of great significance. On a fresh weight basis, cranberries

have been found to have the highest H-ORAC value among fruits, at 92.56 umol of TE/g dry weight (Wu et al., 2004). In the same study, cranberries also scored the highest in the L-ORAC assay, having a value of 2.00 ± 0.38 umol of TE/g dry weight. Concentrating the phenolics present in cranberry and freeze-drying produced an extract that had H-ORAC and L-ORAC values of 348.31 ± 33.45 and 11.02 ± 0.85 μmol of TE/g, respectively. This is a strong indicator that components found in the cranberry extract exhibited strong antioxidant behaviors in both environments; a result that is attributed to the difference in polarity of different phenolics found in cranberries.

The antioxidant activity exhibited by the cranberry extract in the H-ORAC assay is attributed to a large number of hydrophilic phenolic compounds present in the extract. Phenolic acids, anthocyanins, and flavonol glycosides make up the more polar fraction of cranberry phenolics (Shahidi and Naczk, 2003). The anthocyanins in cranberries are mainly 3-glucosides of anthocyanidins. There is conflicting evidence as to the effect of sugar molecules on antioxidant potential of phenolics. Results have been shown to differ based on the assays used, as well as environment in which the phenolics were tested. For instance, glycosylation has been shown to reduce the antioxidant activity of various anthocyanidins as measured by aqueous radical trapping capacity assay (Rice-Evans et al., 1996). In the ORAC assay, however, glycosylation of cyanidin to cyanidin 3-glucoside increased the activity, but glycosylation of malvidin to the corresponding 3-glucoside decreased the activity, with pelargonin and its 3-glucoside having a similar response (Wang et al., 1997). Glycosylation stabilizes anthocyanins and imparts polarity, making the phenolics more soluble in water. The high H-ORAC value obtained for the cranberry extract can in large part be attributed to the rich anthocyanin content. The presence of ascorbic acid may have also increased the antioxidant ability of the extract in the H-ORAC assay. Ascorbic acid has been shown to scavenge superoxide radicals, as well as act as a synergist with tocopherol for the reduction of lipid peroxyl radicals (Nandi and Chetterjee, 1987, Niki, 1991). However, due its highly polar structure, ascorbic acid has shown little effectiveness as an antioxidant in emulsion systems (Frankel, 1996, Ueda et al., 1985).

Proanthocyanidins are relatively less polar than anthocyanins and flavonol glycosides due to the fact that they are composed of non-glycosylated aglycone monomers which have a low degree of hydroxylation (Guyot et al., 2001). The non-polar character of proanthocyanidins could account for the antioxidant activity exhibited in the L-ORAC assay in the present study, due to an affinity to partition into a non-aqueous environment. The affinity of phenolics from cranberries to partition between lipophillic and hydrophilic phases is a very desirable characteristic for retarding lipid oxidation in foods that exist as fat dispersions, such as fresh dairy products. Non-polar antioxidants have been shown to exhibit strong antioxidant potential relative to polar phenolics and ascorbic acid in oil-in-water emulsions (Cuvelier et al., 2003, Frankel et al., 1996). This is due to the association of phenolics with the lipid phase in emulsions which allows for the scavenging of radicals in lipids as they develop. The potential shown by the polar and non-polar phenolics in the cranberry extract to scavenge the AAPH radical makes it a suitable antioxidant in oil-in-water emulsions.

6.2 Behavior of cranberry extract in a model food system

6.2.1 Emulsifying properties

The emulsifying properties of caseins are important for the manufacture of fresh dairy products. In emulsions, certain conformational changes to caseins cause emulsions to break which may lead to creaming, flocculation, coalescence and oiling-off (Dickinson et al., 1996). Caseins have good emulsifying characteristics due to an amphiphilic structure. However, this structural nature is easily manipulated by variables such as pH, ionic strength, and the presence of other components which can bind the molecules thereby modifying physico-chemical characteristics (Dalgleish, 1997). The inherent acidity of the cranberry extract prepared in this study could have accounted for certain changes in the structural stability of the emulsions produced herein. For example, emulsion destabilization was evident for all emulsions produced at pH 5.3; a finding that was visibly noted by the coalescence of fat at the surface of the emulsion immediately after formation. Incorporation of the cranberry extract with the ingredients prior to emulsion formation gave

the most stable emulsions. Initial experiments showed that this mode of incorporation produced more stable emulsions at higher cranberry concentrations than when the extract was added to a pre-formed emulsion.

It is well-established that acid has the ability to curdle milk protein upon contact, and for this reason, acid should be appropriately diluted or released slowly in a system containing caseins (Lund et al., 2000, Fox et al., 2000). Reactions such as aggregation or flocculation, which lead to creaming and possibly coalescence, need to be avoided. Instability during emulsion formation occurs if there is insufficient surfactant to cover the entire oil-water interface created by the homogenization process. The main individual protein components coexisting at the oil–water interface in many dairy-based colloids are αs1-casein and β-casein (Euston and Hirst, 2000). The structure of adsorbed casein monolayers over flat hydrophobic surfaces has been shown to substantially differ for the two major caseins over the pH range 5.5–7.0 (Dickinson, 1999). Adsorbing protein will spread to cover the maximum area, but if there are gaps in the interfacial layer, oil droplets will coalesce, decreasing the total surface area, until it is totally covered by the available surfactant. Coalescence is likely to occur during or immediately after homogenization, but may continue for some time in a viscous product (Dalgleish, 1997).

Instability of emulsions is usually reflected by a change in consistency or a loss of homogeneity (Agboola and Dalgleish, 1996). The capacity of sodium caseinate to emulsify fat and produce stable emulsions has been studied over a wide range of pH and protein concentrations (Hung and Zayas, 1991, Dickinson et al., 1997). Previous findings have found that the stability of caseinate-stabilized emulsions is sensitive to pH under quiescent conditions (Agboola and Dalgleish, 1996). For a given protein concentration, a decrease in emulsifying capacity and stability was observed when the pH was lowered from 6.0 to 5.2 (Hung and Zayas, 1991). The emulsifying capacity of caseinate, defined as the ability to emulsify a certain volume of oil, also decreased as pH increased from 1.5 to 3.5, and increased between pH 5.5 and 7.0 (Mohanty et al., 1988). These results were attributed to the lower solubility of casein at or near its isoelectric point; thus the likely cause for the

observed instability of emulsions produced when the pH was lowered to 5.3 with the cranberry extract.

The presence of phenolics in the extract could also have an impact on the stability of emulsions (Siebert et al., 1996). Various interactions between phenolic compounds and proteins have been shown to affect protein functionality (Spencer et al., 1988). The interactions of phenolic acids with proteins have been shown to stabilize protein structure (Suryaprakash et al., 2000, Spencer et al., 1988). Phenolics can precipitate proteins, particularly proline-rich proteins, such as casein. Precipitation may occur when hydrophobic interactions between polyphenols and the protein sufficiently form at the surface of the complex; in this manner, polyphenols may in fact raise the isoelectric point of caseins (Spencer et al., 1988, Luck et al., 1994). It has been proposed that this property could potentially be exploited in the utilization of phenolics as additives in cheese and acid casein (O'Connell and Fox, 2001). To be noted is that processes that cause the formation of dense protein aggregates that precipitate instead of forming a gel should be avoided in cheesemaking. The combination of phenolics and acids present in the standardized cranberry extract may have led to casein precipitation at pH 5.3, which adversely affected the stability of the casein-based emulsions.

6.3 Lipid oxidation in model emulsion

The activity of antioxidants in food and biological systems is dependent on a multitude of factors, including the colloidal properties of the substrate, the conditions and stages of oxidation, and the localization of antioxidants in different phases (McClements and Decker, 2000, Viljanen et al., 2004). Particle size analysis and emulsifying stability results obtained herein, showed that stable emulsions were obtained by incorporating the cranberry extract at the homogenization step and at a concentration of 0.5% (w/w with aqueous phase), thus yielding emulsions with a final pH of 5.6. The development of conjugated dienes (CD) occurred more rapidly in emulsions at pH 5.3 where reduced physical stability was evident. This result could be explained by fat rising to the surface when emulsion particles are destabilized, thereby increasing the exposure to oxygen and other external factors.

Based on preliminary results, accelerated oxidation studies focused on emulsions that contained the cranberry extract after a homogenization step at a pH of 5.6. This pH corresponded to a highest level of cranberry that could be incorporated into the model system without adversely affecting the physical properties of the emulsion. Homogenization of the cranberry with the emulsion also ensured an even distribution of the extract throughout the emulsion to a final pH. Due to the importance of final pH on the quality of fresh dairy products, the addition of the cranberry extract prior to homogenization was found to be more appropriate in obtaining a desired final pH. The alternative of adding the cranberry extract after product manufacture, in which case the pH would then be altered, was not satisfactory to produce a quality product.

For the control emulsion, a pre-determined amount of 5% (w/v) citric acid solution was added to the aqueous phase prior to homogenization, so that the final pH of the emulsion was also set at 5.6. Oxidation experiments were carried out at 50ºC over a 90 hour incubation period, which was determined to be a suitable temperature-time combination for oxidation reactions to occur at an appreciable rate, while at the same time the emulsions remained physically stable. The measurement of secondary oxidation products was important since primary products may not necessarily indicate the presence of off-odors and flavors associated with the product.

6.3.1 Effect on 25% sunflower oil emulsion

The cranberry extract effectively prevented peroxyl radical formation by prolonging the induction phase of lipid oxidation in the sunflower oil emulsion model system. Previous research has shown caseinate-based emulsions containing sunflower oil to be a useful model system for yogurt and dairy products (Kiokias et al., 2004). Sunflower oil contains a relatively high proportion of unsaturated fatty acids. The significant increase in the time taken for propagation of CD formation with the cranberry treatment showed a potential for employing cranberry antioxidants to scavenge lipid radicals in a model system that was highly susceptible to oxidative deterioration. However, the fact that the level of CD in the cranberry emulsion reached the same concentration as the control after approximately 30

hours indicates that the antioxidants were exhausted over the prolonged incubation time. Various phenolic antioxidants acids have shown a similar mode of action towards primary oxidation products in other model systems containing a high degree of unsaturated fatty (Cuvelier et al., 2003, Lethuaut et al., 2002).

The exhaustion of cranberry-derived antioxidants was also shown by the fact that a significant decrease in hexanal occurred only after approximately 64 hours of storage, the time before which the formation was very similar between treatment and control. The generation of hexanal at higher concentrations than pentanal is further evidence that hexanal was the major secondary product of lipid oxidation in this emulsion model. Pentanal levels were also slightly lower in the cranberry treatment, again, only after approximately 64 hours. Hexanal has proved to be a useful marker for the oxidative decomposition of n-6 PUFAs (Abdalla and Roozen, 1999). Hexanal and pentanal develop as a consequence of the breakdown of linoleic acid (C18:2).

The small positive effect observed with inhibition of secondary products of lipid oxidation is related to the antioxidant synergy and regeneration effects of certain polyphenols found in cranberries. Quercetin, a primary flavonone in cranberries (Neto, 2007), acts synergistically with other phenolics, as well as α-tocopherol at prolonging the induction phase of peroxidating methyl linoleate in homogeneous solutions (Pedrielli and Skibsted, 2001). Though the underlying mechanism is not entirely clear, the affinity of quercetin to regenerate other antioxidants reflects its reducing power for prolonged antioxidative protection over a long period of incubation. To be noted is that emulsions herein were made using non-stripped sunflower oil that likely contained α-tocopherol. The regeneration phenomenon may explain why secondary product development was limited despite the high proportion of phenolics that were expended during the propagation phase of oxidation.

6.3.2 Effect on 25% butterfat oil emulsion

In butterfat emulsions, CD developed to a lesser extent compared to sunflower emulsions over similar incubation periods for the control and cranberry-treated samples. The fact that a higher proportion of fatty acids associated with the lipids in butterfat are saturated compared to sunflower emulsions explains this observation. Phospholipids likely accounted for less than 2% of the fat phase in the butterfat model system. Phospholipids in butterfat are high in poly-unsaturated fatty acids (PUFAs) and are likely to associate closely with the interfacial protein layer due to an amphiphillic structure. This positioning leaves PUFAs susceptible to oxidative deterioration, since oxidation is known to take place at the surface of the lipid droplet in emulsions. The cranberry extract, when added to the emulsion significantly reduced the formation of CD in emulsions containing butterfat after around 14 hours of oxidation. A particular behavior of the cranberry extract in the butterfat emulsions that was different than in sunflower oil emulsions was the relatively slow rate of oxidation and no apparent lag phase, which ultimately limited the total amount of CD generated over the 90 hour incubation period.

The incorporation of cranberry into the butterfat emulsions greatly limited the formation of hexanal. Since primary products formed to a lesser extent in fat-containing emulsions high in saturated fatty acids, it is possible that a lesser amount of phenolics was expended in the scavenging of peroxyl radicals formed at the early stages of lipid oxidation, compared to the highly unsaturated sunflower oil. Therefore, a higher number of phenolic antioxidants may have been available to scavenge alkoxyl and hydroxyl radicals that develop at later stages of oxidation as peroxides begin to breakdown. As a result, hexanal and pentanal development was effectively suppressed in butterfat emulsion containing the cranberry extract. This result is significant due to the volatile nature of both hexanal and pentanal, which are known to contribute to flavors and odors characteristic of oxidative rancidity (Downey, 1969, Kochar, 1996, Petterson et al., 2005).

6.4 Proposed mechanism of the cranberry extract

6.4.1 The partitioning of phenolics between phases

Characterization of the cranberry ingredient showed that it possessed antioxidant activity in both the lipophillic and hydrophilic assay. The effectiveness of antioxidants in delaying the onset of oxidation in o/w emulsions has been shown to depend strongly on the polarity of the antioxidant (McClements and Decker, 1996, Rice-Evans et al., 1997, Abdalla and Roozen, 1999). The initiation of lipid oxidation occurs near the interface between lipid and water in emulsions (Jacobsen et al., 2001). Propagation of primary lipid oxidation products, such as hydroperoxides, occurs at the oil droplet surface of emulsions, and the migration of these reactive species throughout the droplet causes further deleterious reactions as the development of secondary products proceeds (Chen et al., 1999).

The cranberry extract provides oxidative stability to food emulsions if it scavenges free radicals that have been formed at the lipid droplet surface, and prevents further interactions of radicals with lipids when in an immediate vicinity. Non-polar molecules are located predominantly in the oil phase, polar molecules in the aqueous phase, and amphiphillic molecules at the interface. The cranberry extract contains phenolics capable of partitioning between all three phases of both emulsions containing butterfat and sunflower oil. Glycosylated phenolics such as anthocyanins and flavonol derivatives are more polar and thus, more soluble in the aqueous phase. The non-polar phenolics in the cranberry extract have a higher propensity to partition into lipid phases.

Lipid oxidation in food emulsions is accelerated when pro-oxidants come into contact with the surface of dispersed lipids (McClements and Decker, 2000). Protection from pro-oxidants may occur throughout all three phases, and is highly influenced by the ingredients which make up the emulsion. Radicals in foods may form from the action of enzymes, metal catalysts, or other inducers such as light and heat. Metal catalysts are mainly found in the aqueous phase, and their solubility is increased as pH is lowered (Mei et al., 1997). Metals such as iron become a concern when they interact with dispersed lipids. The polar

antioxidants in the cranberry extract may reduce lipid oxidation in emulsions if they can chelate metal ions in the aqueous phase, thereby reducing the contact with the lipids. The affinity of anthocyanins to chelate metal ions has been reported (Kuhnau, 1976, Satue-Garcia et al., 1997). Molecules of cyanidin and delphinidin, with two ortho-hydroxyl groups form complexes with copper (Satue-Garcia et al., 1997). However, this affinity may be reduced as pH is lowered due to a reduction in the ionization of hydroxyl groups on the B-ring which causes less complexation between polyphenols and metals.

Non-polar phenolics are more effective as antioxidants in oil-in-water emulsions, due to the ability to position at the interface where oxidation occurs (Johnson and Williamson, 2003). Increasing the concentration of antioxidants in the organic phase increases the antioxidant activity in oil-in-water emulsions (Huang et al., 1997). Flavonol aglycones found in cranberries have been shown to readily incorporate into lipophilic phases of food systems (Vellasco et al., 2004). This class of compounds from cranberry was most effective in inhibiting lipid oxidation in mechanically separated turkey and cooked pork, and was attributed to the characteristic low polarity (Vellasco et al., 2004). Pure flavones, flavonols and proanthocyanidins from a variety of plants inhibited oxidation of β-carotene in a linoleic acid system stabilized by Tween 20 (Skerget et al., 2004). Proanthocyaninidins have lower polarity, though the bulkiness of these polymeric molecules may limit their affinity to collect at the interface of the emulsion.

The ionized form of an antioxidant is much more polar than the non-ionized form and therefore will have a greater affinity in aqueous solutions (Schwarz et al., 1996). Charged antioxidants have a greater tendency to partition into the water phase of biphasic systems while uncharged counterparts have a higher tendency to partition into organic phases. The charge, and thus the solubility of phenolics as well as proteins are altered by pH. For anthocyanins, structural transformations occur around its pKa values (Mateus and de Freitas, 2008). At pH 7, the anionic quinonoidal base (A-) predominates whereas as pH 4 is approached, anthocyanins are mainly present in the form of flavylium cations (AH+). The lowering of the pH to 5.6 may affect the partitioning of anthocyanins due to electrostatic forces between negatively charged caseins and positively charged

anthocyanins. Caseins maintain a net negative charge at a pH above its isoelectric point. These forces allow for a higher proportion of anthocyanins to be associated at the interfacial layer of the emulsions, and thereby provide better protection to the emulsified lipids.

Despite the polarity imparted by sugar groups, the anthocyanins have been shown to act as strong antioxidants in emulsions of methyl linoleate oxidized in the dark at 40ºC (Kahkonen and Heinonen, 2003). Various anthocyanins from plant extract have effectively reduced metal-induced oxidation in a liposomal model system (Satue-Garcia et al., 1997). Anthocyanin-protein binding has been suggested as an underlying mechanism for antioxidant activity by favoring the access of the antioxidant to the lipids and improving protection against oxidation (Satue-Gracia et al., 1997, Kahkonen and Heinonen, 2003).

6.4.2 Protein – polyphenol complexation

The homogenization of the cranberry extract into the emulsion with the rest of the ingredients provided the most effective protection against lipid oxidation in this study. This protection, however, was absent when the extract was added to a pre-formed emulsion to pH 5.6. It is well-established that homogenization increases the inter-facial surface area and will alter the composition of the droplet membrane. Casein, which is originally dispersed in the aqueous phase, adsorbs to the droplet surface forming a protective layer. Homogenization will thus allow for a high degree of interactions between polar phenolics and caseins to take place. These interactions cause polar phenolics, such as anthocyanins, to come into close proximity with the oil-water interface due to protein-polyphenol interactions.

The binding of polyphenols to proteins is principally a surface phenomenon that has a very strong dependence on molecular size and conformational flexibility of the polyphenol component (Spencer et al., 1988). The cranberry extract contained a diverse class of phenolic compounds with different degrees of polarity. Principally, five potential types of interactions of phenolics and proteins can be proposed: hydrogen bonding, π-bonding, and

hydrophobic, ionic, and covalent linkages (Spencer et al., 1988). The presence of proline along the polypeptide sequence of casein will attract the aromatic rings of phenolics, generating hydrophobic interactions that allow for the phenolics to associate with the protein. Proline residues are fairly evenly distributed throughout the amino acid sequence of caseins, imparting hydrophobic regions to the molecules (Swaisgood, 1996). This facilitates a strong complexation with polyphenols, due to hydrophobic bonding between aromatic side chains of the protein and the aromatic nuclei of the polyphenol. The complex formed through these interactions will be reinforced by hydrogen bonds between hydroxyl groups found on the phenolic rings and polar groups in the region of the hydrophobic pocket (Spencer et al., 1988). The interaction of protein−polyphenol complexes is both reversible and irreversible depending on pH, temperature, and protein and flavonoid concentrations.

Complex formation is usually strongest just below the isoelectric point of proteins where the protein-protein electrostatic repulsion is minimized (Hagerman and Butler, 1981). The functional properties of milk proteins have been shown to be affected by the presence of polyphenols (Spencer et al., 1988, Luck et al., 1994, O'Connell and Fox, 1996). Complex phenolics readily interact with caseins, and this will cause precipitation or an alteration of the isoelectric point of the proteins if sufficient hydrophobic surfaces on the complex are formed (Shahidi et al., 1995). The cranberry extract was incorporated so as to avoid precipitating the caseins, either from the action of the acid present or by the phenolics. At pH 5.6, emulsions were physically stable under quiescent conditions. This observation correlates with previous findings that have shown that incorporation of phenolics from various berry juices into o/w emulsions containing sodium caseinate can be accomplished without compromising the emulsion stability (Viljanen et al., 2004). Interactions of β-lactoglobulin with (+)-catechin actually improved the foam stability of an o/w emulsion (Sarker et al., 1995). Grape seed extract provided antioxidative protection to whey protein stabilized oil-in-water emulsions without reducing the physical stability of the emulsions (Hu et al., 2004). In addition, the interactions of certain phenolic acids with proteins have been shown to stabilize protein structure (Suryaprakash et al., 2000).

Much of the interest in protein-polyphenol complexation has revolved around its effects on bioavailability of phenolics for further metabolism in the body (Almanjano et al., 2007). For instance, black tea, which is rich in catechins, is of particular interest, since the addition of milk is common practice. From a health standpoint, there is conflicting evidence concerning whether bioavailability of phenolics is reduced, enhanced, or unaffected by complexation with milk proteins (Reddy et al., 2005, Lorenz et al., 2007). With respect to shelf-life and preventing oxidation in complex food systems, the effect of complexation between food components may be beneficial. In the case of dairy products, where fat is dispersed throughout a continuous matrix of casein, the positioning of phenolics at the oil-water interface is a key factor in preventing lipid oxidation. Thus, the degree of complexation between phenolics and the casein matrix may have an impact on the shelf-life of the product.

6.5 Product development

6.5.1 Effect of cranberry extract on physical properties of cream cheese

Cranberry extract was incorporated into the cheese premix to a final pH of 5.6. The mix consisted of milk and cream standardized to give a full-fat cream cheese product with 30% fat (w/w). From the emulsion study, information was obtained with regards to the behaviour of the extract at this level in a similar system. The amount of extract required to achieve a pH of 5.6 was higher in the cream cheese product than with the model emulsion. This is likely due to the buffering capacity of milk proteins and salts that were not present in the model system. Nevertheless, homogenization of the cranberry extract into the cheese premix to give a pH of 5.6 produced milk emulsions that were uniform with regard to droplet size.

The use of organic acids in conjunction with traditional starter cultures in the manufacture of dairy products has been advocated. Organic acids provide further control over the level of acid produced, and will reduce the time required for acidification (Berry, 2006). The control cheese was acidified solely with a starter culture consisting of *Lactococcus lactis*

subsp. lactis and *Lactococcus lactis subsp. cremoris*. It was found that the time required to attain the final pH of 4.6 in these cheeses was longer as compared to the pre-acidified cranberry cheese. Organic acids have been shown to improve texture and moisture retention in mozzarella cheese. The exact mechanism is not fully understood, but it has been suggested that perhaps the effect of organic acids on the final calcium content of cheese may affect its texture (Keller et al., 1974).

Fresh dairy products are generally very low in calcium. This is because the insoluble calcium associated with the casein micelle becomes fully solubilized as the pH is lowered to 5.2. As a consequence, most of the calcium is lost during the draining of the whey protein. There have been strategies that have looked into means of fortifying fresh cheeses with calcium, among which includes the addition of calcium chloride after cheese manufacture (Lucey, 2004). The level of ionic calcium in the cranberry cheese was found to be significantly higher than the control in this study. This occurred due to the presence of citric and quinic acids in the extract, which are know calcium sequestering agents. Though increased free calcium may be beneficial from a nutritional standpoint, it may also have an impact on the texture of fresh dairy products. Calcium chloride is added to fresh products such as cottage cheese to improve the firmness of the curd and reduce moisture loss. For cream cheese, increased firmness may not be desirable, as it may cause a reduction in the spreadability of the final product. Adding salt, however, is beneficial in reducing syneresis of the curd, which is a common defect in cream cheese and other spreadable products.

The addition of the cranberry extract prior to curd formation and whey draining led to some loss of phenolics during the draining step. However, it was found that almost 99% of phenolics were retained in the curd, which is a strong indicator of the complexation which took place between casein and phenolics. The presence of phenolics in the whey fraction may be of concern due to the fact that the whey fraction is collected and used in the manufacture of other products, such as whey protein isolates and dairy based ingredients. The processing of dairy products is a very efficient process in terms of making maximum use of the by-products that are generated. The question remains as to whether the presence

of phenolics in the whey by-products would interfere at other usages planned for this fraction. A phenolic recovery system from the whey would perhaps be required.

6.5.2 Color

The use of anthocyanins as natural food colorants has been extensively investigated (Markakis, 1982). High stability of anthocyanins is observed in acidic aqueous solutions, and color stability has been found to be greatest at pH values below 3. Anthocyanins become less stable as pH increase above 3.5, and therefore use as a colorant in medium to low acid foods is limited. The color of anthocyanins at low pH (1-3) is generally an intense red; under these conditions, the flavylium cation predominates. In slightly acidic conditions (pH 4-6), the red color may fade and shift to blue pigments as the deprotonation of flavylium cation occurs (Shahidi and Naczk, 1995). The color of anthocyanins may also degrade as a consequence of hydrolysis reactions during processing and storage. In natural media, however, anthocyanins display more color intensity as a result of co-pigmentation phenomena. Co-pigmentation of anthocyanins with other molecules is believed to have certain stabilizing properties. Quercetin and associated glycosides have been shown to co-pigment very readily with anthocyanins (Asen et al., 1971). Co-pigments do not necessarily contribute color, but rather interact with the anthocyanins themselves causing the flavylium structure to be much less reactive towards water (Markakis, 1982). Certain co-pigments found in the cranberry extract may have allowed for more of the anthocyanins to remain in its colored form despite the fact that the pH of the product was around 4.6. The color stability over 50 days of cold storage in a fresh cream cheese remained stable with regard to lightness and redness. To maintain the stability of the color in the cream cheese product during the incubation period was important because it corresponded to the time period when the cream cheese product would usually be consumed. Further analysis would be required to determine the color stability for products which may have a longer shelf-life.

6.6 Effect of cranberry extract on lipid oxidation in fresh cheese product

The cranberry extract significantly lowered the PV and reduced the development of CD in the freshly formulated cream cheese product. Cream cheese can be regarded as an acid-stabilized emulsion gel (Bot et al., 2007). At pH 4.6, the caseins are near the isoelectric point, and thus possess little to no charge (Swaisgood, 1996). This neutral charge will prevent charged metal ions from coming into close proximity with the fat phase. It has been shown that lipid oxidation occurs much more readily in emulsions where proteins are in a charged state (Mei et al., 1998). This is partly due to the attractive forces between charged metal species which bind near the interface between fat and the continuous phase. The complexation of phenolics with caseins may also neutralize some of the charge found on the caseins. Phenolics contained in the interfacial layer are capable of preventing further oxidative reactions. Superior antioxidant action of phenolics occurs when electrostatic interactions concentrate the antioxidants at the surface of the dispersed lipids. For instance, the antioxidant activity of ascorbic acid (negatively charged) increases dramatically in the presence of positively charged lipid micelles, and the antioxidant activity of Trolox (negatively charged) is higher in the presence of positively charged phospholipid liposomes (Barclay and Vinqvist, 1993). The decrease in primary oxidation products may be due to the combination of a neutrally charged casein matrix the close proximity of polyphenols near the surface of the lipid droplets.

7 CONCLUSION

The optimal condition tested for the incorporation of the standardized cranberry extract into an emulsion stabilized by sodium caseinate involved homogenization of the ingredient into the matrix of ingredients to yield a final emulsion pH of 5.6. Below this pH, emulsion characteristics, such as heterogeneity of emulsion droplets and stability were compromised. This is likely due to the presence of organic acids in the extract and the result of polyphenol-protein complexation which caused caseins to destabilize and precipitate. This precipitation could be detrimental for fresh cheese varieties where gel formation by slow acidification is a key processing step. The poor emulsion stability (as shown by the particle size analysis) that resulted from bringing the pH to 5.3 using the cranberry extract also resulted in a faster lipid oxidation rates. Furthermore, homogenization of the cranberry with the rest of the ingredients to a given pH resulted in improved protection against lipid oxidation relative to adding the cranberry extract to the emulsion subsequent to homogenization. The results from the emulsion models showed the potential of the cranberry extract to prevent lipid oxidation in systems designed to represent fresh dairy products.

The protection against lipid oxidation occuring at a pH of 5.6 in the emulsion models was attributed to the diverse class of phenolic compounds present in the cranberry extract. The cranberry extract exhibited antioxidant activity in both H-ORAC and L-ORAC assays. Oxidative protection also occurred in a fresh cream cheese product when the extract was incorporated into the milk used to formulate the cream cheese to a pH of 5.6. Various flavonol aglycones and proanthocyanidins have low polarity, and thus associate more closely with the lipophillic phase of foods that exist as dispersions of fat. This positioning provides a certain degree of protection for the lipid phase against the degradation due to free radical-induced oxidation that is initiated close to the lipid-oil interface. The complexation of the more polar compounds such as phenolic acids and anthocyanins with caseins also provided protection at the oil-water interface in the emulsion model, as the association of phenolics with the caseins allowed for a higher concentration of phenolic phytochemicals at this interface. Casein-polyphenol complexation occurs most strongly

around the isoelectric point of casein, which explains the decrease in oxidation seen in the cream cheese product. These events could prolong the shelf-life and improve the quality of fresh dairy products during times of storage and consumer consumption.

Acidification of milk to pH 5.6 using the cranberry extract provided a cheese product with a stable color and limited lipid oxidation over a 50 day of storage period. Increasing the concentration of extract beyond this level potentially destabilized caseins prior to cheese manufacture and would thus compromise the quality of the final cheese product. Slow acidification to the isoelectric point of casein can be achieved with the aid of starter cultures. It should be noted that the optimal pH for the action rennet is 5.6, and therefore the cranberry extract could have potential application at lowering the pH to a desirable level in the making of rennet cheeses.

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APPENDICES

APPENDIX A – Calibration curve for determination of total phenolics

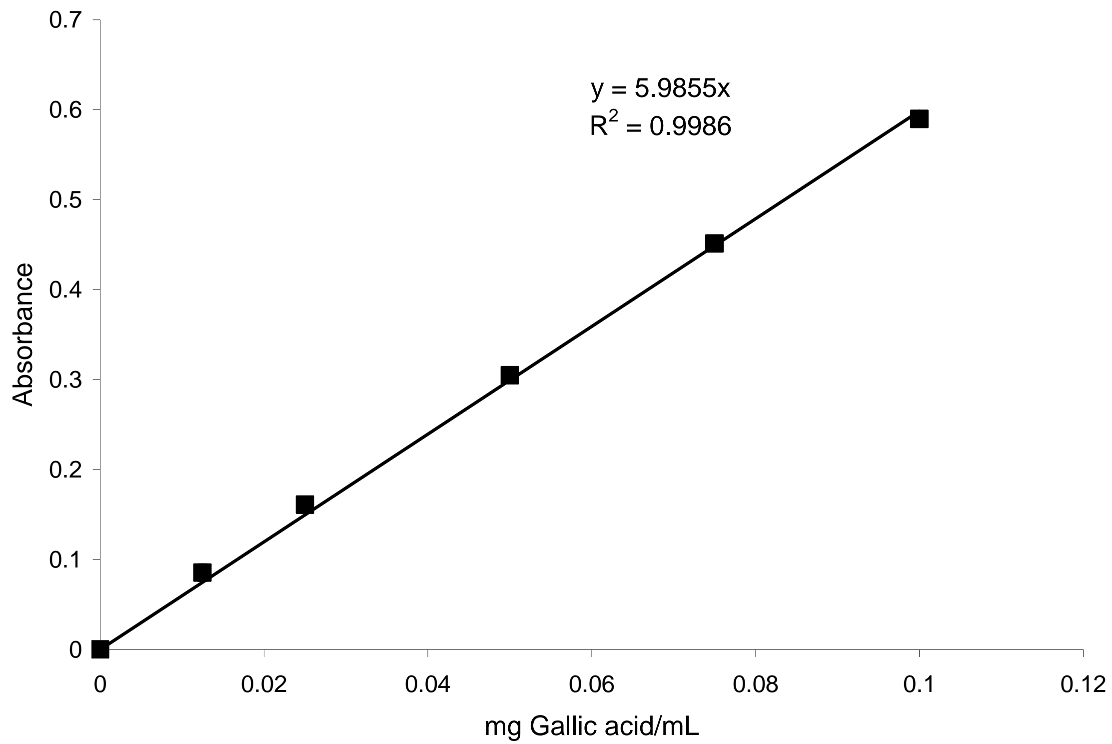


Figure 22. Standard curve for gallic acid using the Folin-Ciocalteu assay for total phenolics. Each point represent mean for triplicate samples.

APPENDIX B. Calibration plots for individual organic acids found in cranberries using HPLC

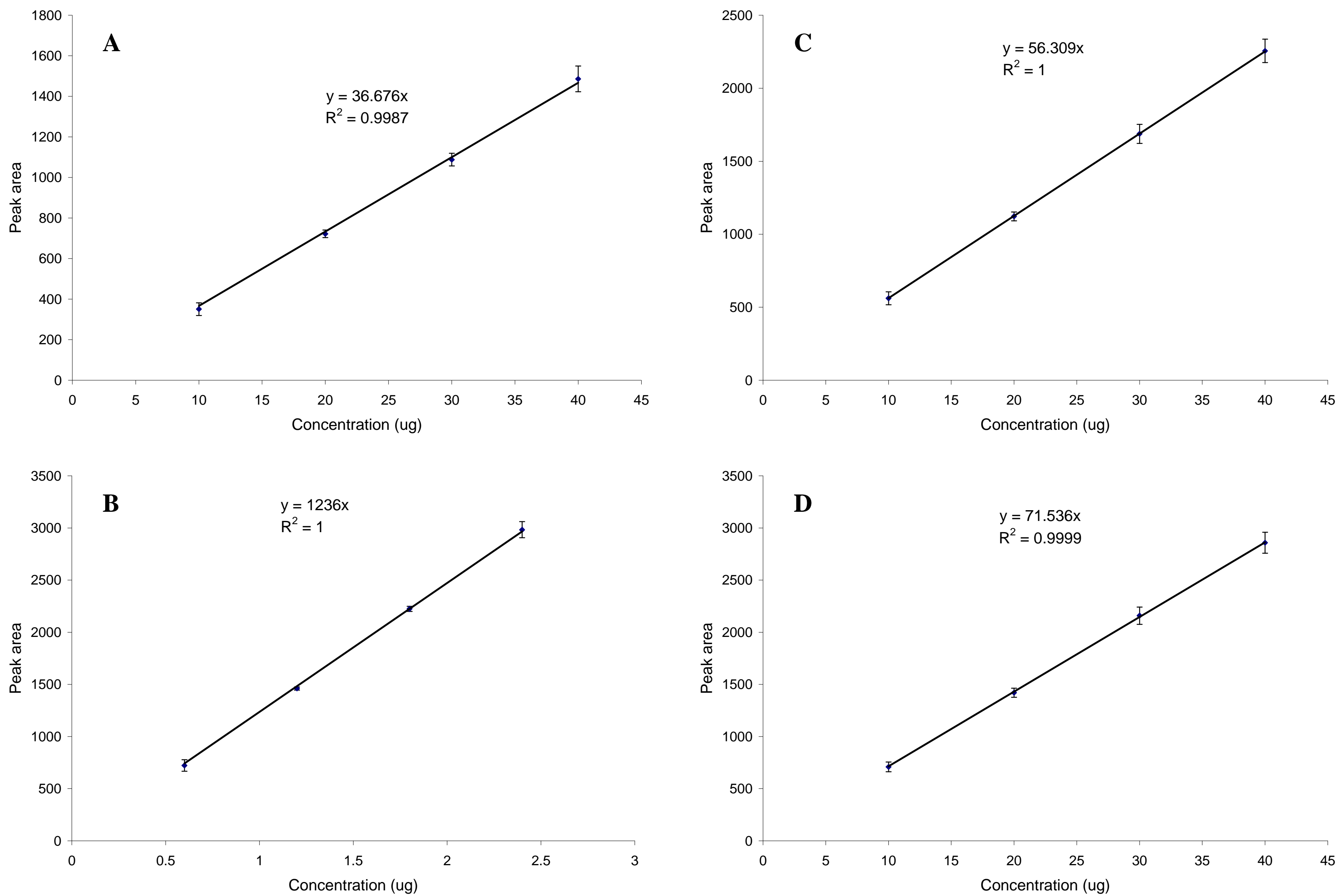


Figure 23. Calibration plots of peak response against concentration for individual organic acids using HPLC. (A) quinic acid; (B) ascorbic acid; (C) malic acid; and (D) citric acid. Each point represents mean ± SD for three calibrations.

APPENDIX C – Calibration plots and chromatograms for headspace analysis of oil-in-water emulsions containing standard hexanal and pentanal

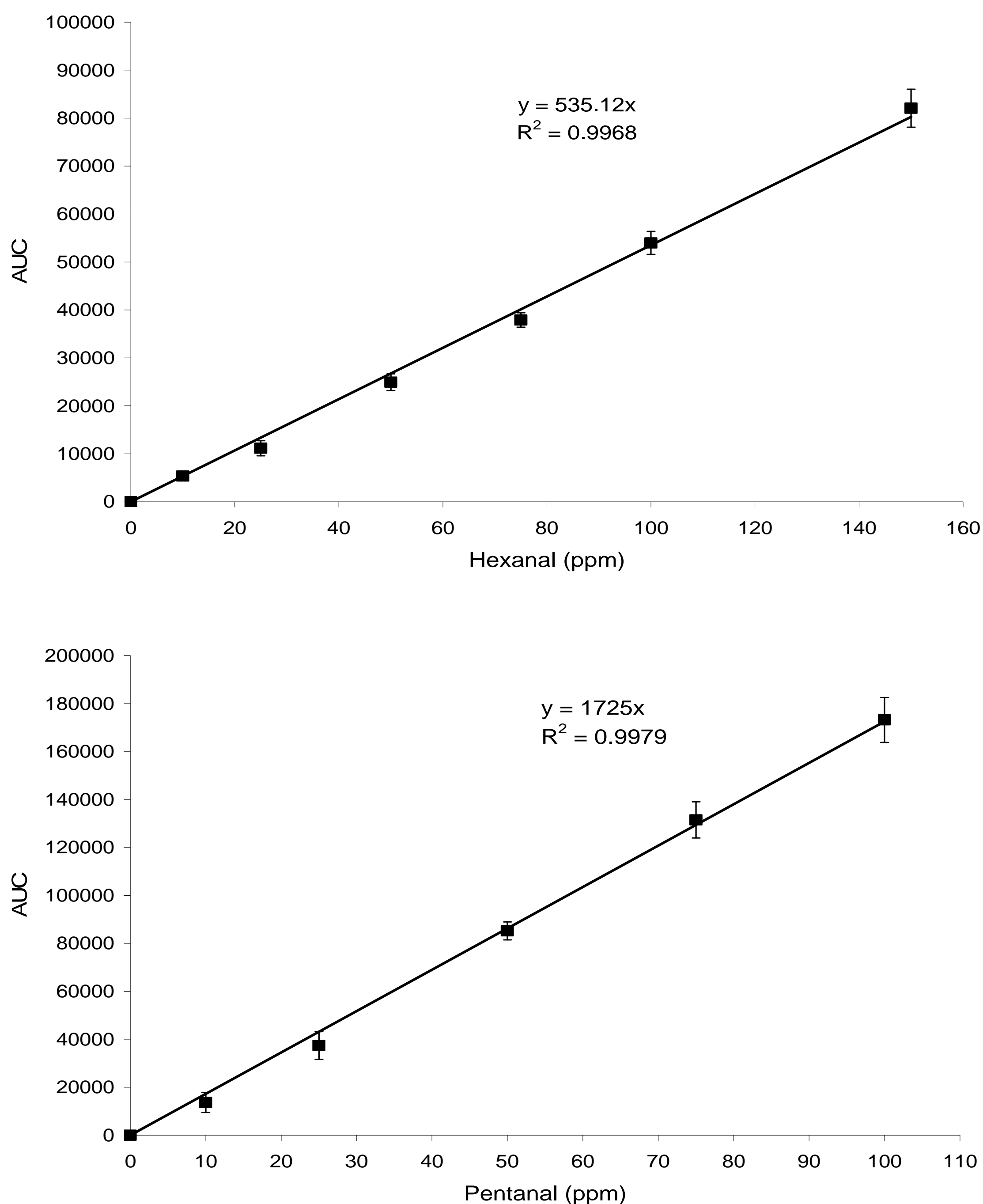


Figure 24. Calibration plot of GC response (AUC) for (A) headspace hexanal, and (B) headspace pentanal in emulsions containing 25% fat, stabilized by 2% (w/w with the aqueous phase) sodium caseinate. Analysis was performed in 22.4 mL headspace vials containing 2.5 mL of emulsions containing varying levels of standard hexanal. Each point represents mean ± SD (n = 6). AUC = Area under the curve.

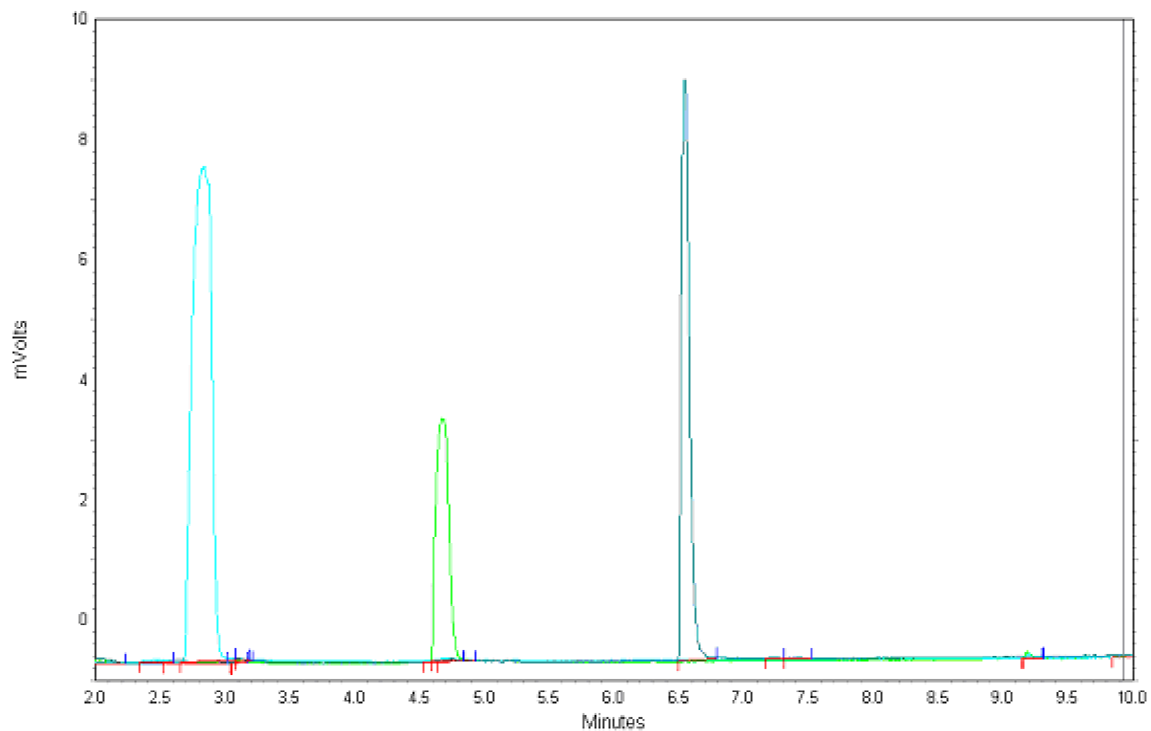
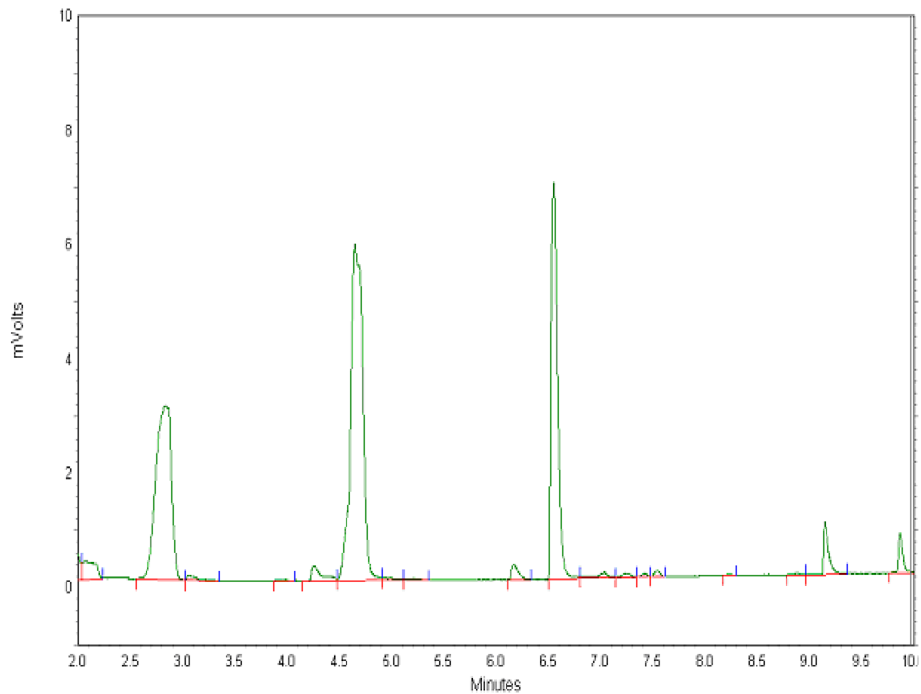
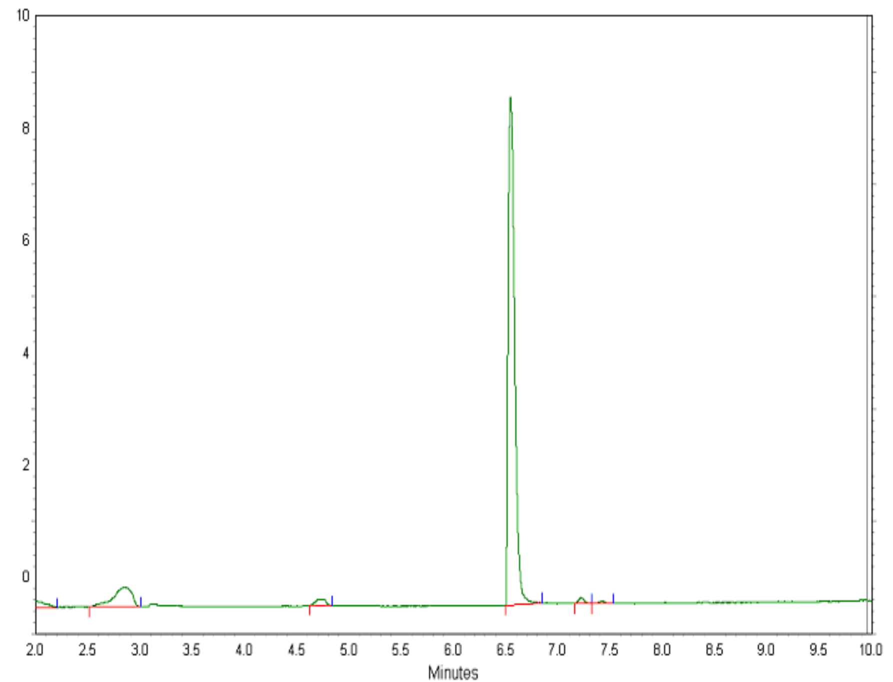


Figure 25. Typical peaks for standards used for GC analysis (A) Pentanal, 50 ppm; (B) Hexanal, 50 ppm; and (C) 4-heptanone, 50 ppm.





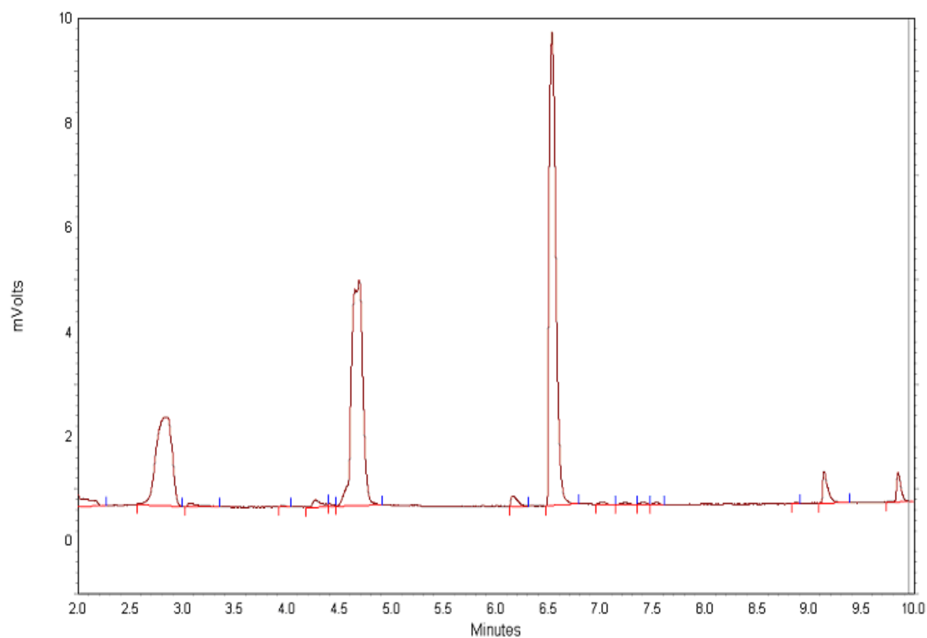


Figure 26. A GC separation of headspace volatiles during accelerated oxidation at 50ºC after 90 hours. Emulsions consist of 2% sodium caseinate containing (A) 25% sunflower oil (control, pH 5.6); (B) 25% sunflower oil + cranberry extract to a final pH of 5.6; and, (C) 25% butterfat + cranberry extract to a final pH of 5.6.

Appendix D – Typical calibration curve for calcium ion selective electrode (ISE) response versus concentration of calcium ion (Ca^{2+}).

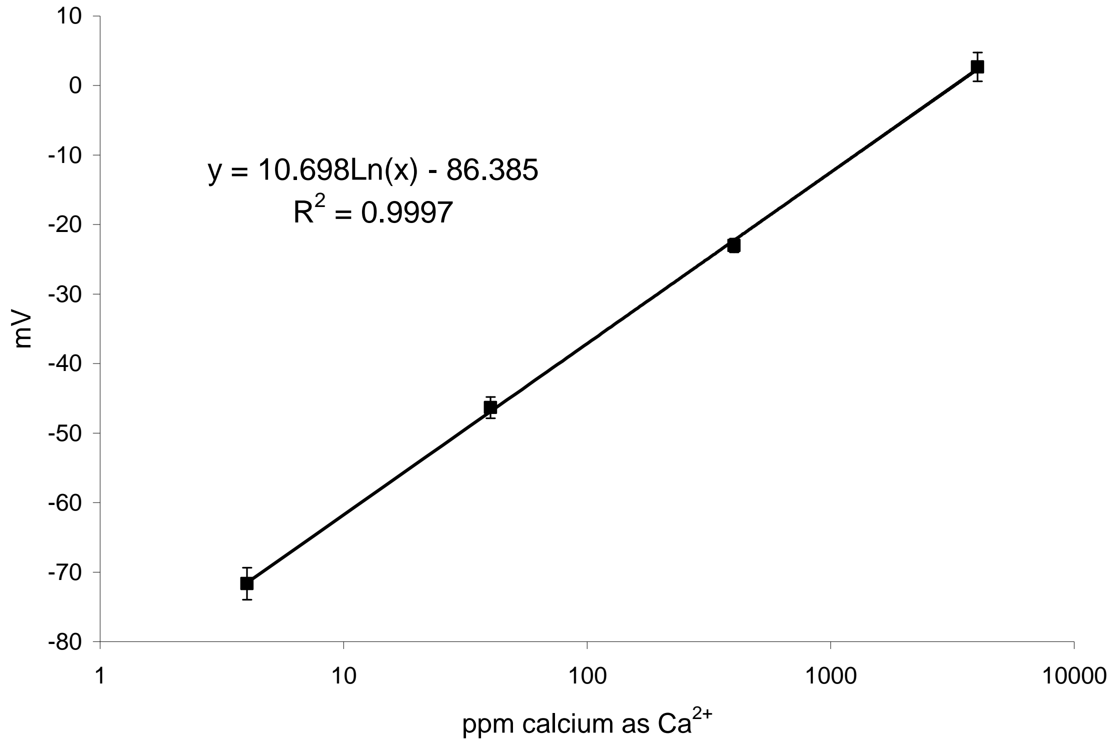


Figure 27. Direct calibration plot for Orion 9320BN calcium electrode (Thermo Scientific, Waltham, MA) using standard calcium (Ca^{2+}) solutions. Each point represent mean ± SD for solution prepared in triplicate.

APPENDIX E. Calculation of lag phase prior to propagation development of primary oxidation products

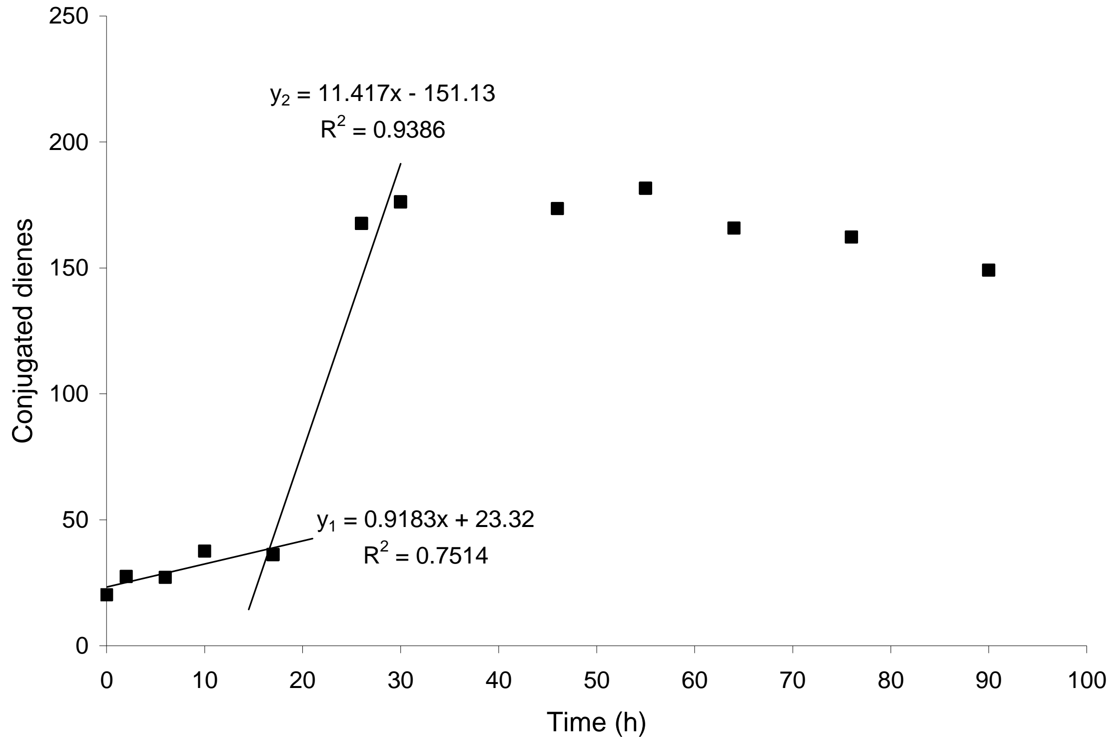


Figure 28. Graphical representation of the change from lag phase to the propagation phase for primary lipid oxidation products in a sunflower oil emulsion. Each point represents mean CD for duplicate sunflower oil emulsions containing standardized cranberry extract to a final pH of 5.6..

Calculation of lag time

$Y_1 = Y_2$

11.417x – 151.13 = 0.9183x + 23..32

x = 14.36 hours