EFFECTS OF CONTROLLED RELEASE PACKAGING IMPREGNATED WITH

MIXED TOCOPHEROLS ON OXIDATIVE DEGRADATION IN

MRE CHOICE SPREAD

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ABSTRACT OF THE THESIS

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MRE Choice Spread is a cheese spread variation containing large amounts of unsaturated lipids, which make lipid oxidation and development of rancidity the primary deterioration mode for this product. Lipid oxidation has traditionally been limited by adding antioxidants such as tocopherols directly to the food. However, the large amounts of antioxidant needed to provide the three year shelf life required by the military for MRE foods may cause phenol overloading, pro-oxidation, or browning. Controlled Release Packaging (CRP) addresses this problem by incorporating tocopherols in polymer films and releasing them into foods over time. Effectiveness of CRP in stabilizing model systems of pure unsaturated lipid has been demonstrated previously; this study was designed to extend testing of CRP technology to plant-scale applications that solve shelf life problems associated with a commercially processed real food system.

Control polymer films of PP-LDPE and CRP films of PP-LDPE impregnated with mixed tocopherols at 3000 ppm were produced by extrusion at Berry Plastics Corporation

(Chippewa Falls, Wisconsin). They were laminated, filled with Choice Spread, and sealed at Thermo Pac, LLC (Stone Mountain, Georgia). For comparison, half the control samples were flushed with nitrogen gas before sealing to provide an inert atmosphere inside the package. Choice Spread in the three kinds of packages were stored at 40°C for 15 weeks and at 60°C for 10 weeks for shelf life studies. Color (browning and fading) was quantified as L^* a^* b^* values using a colorimeter. Lipid peroxides, conjugated dienes, and aldehydes were measured to follow the progress of lipid oxidation.

Contrary to model system studies with oils and preliminary packaging studies with Choice Spread, control, nitrogen flushed and CRP packages showed similar browning and lipid oxidation during storage at both temperatures. This lack of apparent protection by CRP may be attributed to high levels of antioxidant (TBHQ) in Choice Spread that stabilize lipid oxidation during the storage period, overload the antioxidant system, and block both tocopherol access to active sites and protective effects of CRP. These results highlight the need to balance formulation and packaging and further customize Controlled Release Packaging to make it effective in stabilizing real food systems.

ACKNOWLEDGEMENT

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1. INTRODUCTION

Shelf life of foods is defined as the period of time from production and packaging to the point at which the product first becomes unacceptable under defined environmental conditions [1]. In the food industry, attaining a desired shelf life for any food commodity is of pressing concern as it is related to consumer satisfaction and economic profits. It depends on the food formulation, the packaging used and the environment in which the product is distributed and stored. The stability of the food is specifically affected by its formulation, processing techniques and conditions, while the packaging is responsible for maintaining the required internal environment and for protecting the food from the external environmental parameters such as temperature, oxygen, moisture, microbes, light and mechanical damages during distribution and storage.

MRE (Meal Ready to Eat) Choice Spread is a potato and cheddar flavored product with crunchy bacon flavored bits that can be consumed as a simple spread or as a soup by diluting it with water. It is a high fat product consisting of 62.5% lipids and 12.5% proteins. Although this product has been greatly appreciated by the soldiers for its taste and functionality and the NATICK Army RD&E Center has supported its introduction into the existing menus for the US Armed Forces, its shelf life is of major concern. The product has been formulated and processed to ensure microbial sterility and the MRE laminate packaging has been designed to maintain this condition throughout distribution and storage. However, the high content of unsaturated lipids contributed by the canola oil and cheese powders used in its formulation, makes the product very susceptible to oxidation even in the presence of small amounts of oxygen. Thus, lipid oxidation is the major deterioration mode limiting shelf life at ambient temperature, causing rancidity,

texture changes, browning and loss of nutritional value. A primary requirement for an MRE product is to have a shelf life of three years at 80°F and this, together with susceptibility of Choice Spread to oxidation, is limiting the launch of the product, especially keeping in mind the extreme conditions (battle fields in the Middle East areas) in which this product will be distributed, stored and consumed.

Lipid oxidation is a complex phenomenon that occurs in three stages: initiation, propagation and termination. Unsaturated lipids generate free radicals in the presence of oxygen and initiators, thereby triggering the chain reaction and propagation. In some cases, lipid oxidation can also transfer radicals to protein molecules, leading to protein co-oxidation, which greatly affects texture and nutritional quality of a product. Free radical chains terminate when radicals either recombine or undergo scission to form products such as alcohols, aldehydes, acids, ketones and low molecular weight compounds that contribute to the rancid odors and tastes associated with oxidation, as well as polymers that increase oil viscosity and affect food textures. In this way, important quality attributes such as taste, odor, color and texture of foods that determine a product's acceptability and shelf life are greatly affected by lipid oxidation. When lipid oxidation proceeds far enough, it can even make food inedible.

Common ways of inhibiting unwanted oxidation changes are using appropriate packaging to eliminate oxygen from the internal environment, using compounds like antioxidants to scavenge the free radicals, and avoiding prooxidants such as metals in the formulation. However, designing a package to exclude oxygen alone is insufficient to prolong the stability of MRE products and meet the shelf life requirement of three years. Thus, impermeable packaging is often used with addition of specific antioxidants or metal

chelators to the food formulation. Synthetic antioxidants such as BHT (Butylated HydroxyToluene) and BHA (Butylated HydroxyAnisole) have been used extensively to inhibit oxidation in various foods, both as direct food additives and in packaging. However, over the past few years, consumers have demanded more minimally processed foods stabilized with natural ingredients without sacrificing safety or shelf life. Tocopherol is one such natural stabilizer. Tocopherols are powerful antioxidants that act by competing with unsaturated lipid molecules for the free radicals formed during lipid oxidation, thus slowing it down. Tocopherols can be added directly to food formulations (referred to as instant addition) to enhance shelf life, but low concentrations will be insufficient to keep it stable for three years at 80°F and high concentrations may cause tocopherol to behave as a prooxidant, accelerating oxidation reactions instead of inhibiting them. Thus, although natural ingredients lend a clean label, they also pose some major problems for long term stabilization of lipid rich foods.

Controlled Release Packaging (CRP) is an innovative delivery system that can overcome the limitation of instant addition of antioxidants. Designed to be a functional packaging system where active compounds such as antioxidants are released from the package into the food over time, CRP attempts to ensure that the antioxidant levels in the food at any given point during storage are sufficient to quench the free radicals being formed due to lipid oxidation but are not high enough to cause prooxidation. Such benefits of CRP technology in prolonging shelf life have been previously established in model system studies comprised of unsaturated lipids such as linoleic acid. However, there is a gray area of unknowns in translating information from model system studies to applications in real food matrices. Few studies have been conducted to understand whether the inhibition

of lipid oxidation seen in model systems translates into extended shelf life in real foods with complex formulations and packaging parameters.

This research study evaluated the effects of CRP impregnated with mixed tocopherols in inhibiting oxidative degradation and associated reactions in a real food system -- MRE Choice Spread –in comparison to the same laminate packaging without antioxidants. The deterioration of the Choice Spread when subjected to accelerated shelf life conditions (elevated temperatures of 40°C and 60°C) was quantified in terms of color and lipid oxidation products such as conjugated dienes, peroxides and aldehydes. CRP was also compared with nitrogen gas flushing, which is commonly used in the industry to inhibit lipid oxidation by replacing the oxygen in the internal environment of the package with an inert gas.

The results indicated that the change in color of Choice Spread and progress of lipid oxidation followed a similar trend in control, nitrogen flushed and CRP packages. These results were not nearly as dramatic in terms of CRP effects as seen in model system studies and can be attributed to the overloading of system with antioxidants, as synthetic antioxidant- TBHQ was endogenously present in Choice Spread at 0.02% and the accessibility of tocopherol to lipid oxidation sites within the complex food matrix is of concern. An interesting observation was the ability of the CRP packages to retain texture and prevent formation of grittiness during the study period. Although, these quality aspects were not systematically quantified, it suggests the possible occurrence of protein co-oxidation indicating that CRP does have an effect on the system, opening up avenues for further research in the field of Controlled Release Packaging to make it commercially feasible.

2. BACKGROUND

2.1. MRE Choice Spread

2.1.1. Meal Ready to Eat (MRE)

MRE or 'Meal Ready to Eat' refers to self-contained, individually packaged ration in retort pouches developed by the Department of Defense, United States of America, to be used by its members where organized food facilities are not available, such as the battlefields.

Canned food rations previously given to soldiers were heavy to carry around, the food was not palatable and there was little variety in the menus, leading to incomplete consumption of the meal and loss of its nutrition. MRE pouches, as they are today (see examples in Figure 1), have continuously evolved over the years by incorporating latest technologies in food processing and packaging to provide better tasting meals and accompaniments with enhanced nutrition. The number of items on the menu has also expanded to include options to suit varied taste preferences [2].

The package used for retort pouches must be strong enough to withstand the pressures of airlifts and airdrops, provide a good barrier to oxygen, moisture and light and be and lightweight to allow portability. The package should also aid MREs in maintaining "high quality" of food for three years at 80°F and for six months at 100°F [3].

Figure 1: MRE Choice Spread pouches

2.1.2. Choice Spread

Choice Spread is a stable oil in water emulsion developed to provide a variation of cheese spread [4]. The formulation of Choice Spread is given in Table 1.

Table 1: Choice Spread formulation

Ingredients	Percentage (%)
76% Fat Cream Powder	30.9
Com Creamery Cheese Powder	11.6
Kerry Cheese Powder	4.8
Canola Oil	29
Non Fat Dry Milk	10.6
TIC Gums- Colloid Ultra Smooth	0.5
Incosity	1.5
Corn Syrup Solids	1.5
Baco's Art. Bacon Bits	3.9
Lecithin	2.9
Flavors & Seasonings	2.78
Dadex 21 (TBHQ)	0.02

A combination of cream and cheese powders was emulsified with dairy proteins and stabilizers to mimic a processed cheese product. This allows the emulsion to be easily diluted with water to be consumed as soup or consumed as a spread. The functionality of each ingredient is explained below to provide an insight into the complexity of this real food system and highlight sources of lipid oxidation.

- Cream Powder: It is the product obtained by removal of water only from pasteurized milk or cream or a mixture thereof, which may have been homogenized and contains 40-75% by weight of milk fat and not more than 5% by weight of moisture on a milk solids basis (CFR 131.149).

- Cheese Powder: It is made by spray drying cheese and water mixture to ensure not more than 5% moisture but same percentage of other components as the original cheese.

  Milk fat contains approximately 65% saturated, 30% monounsaturated and 5% polyunsaturated fatty acids and these powders contribute to the unsaturated fatty acids present in the final Choice Spread product.

- Canola Oil: The oil, obtained from a variety of rapeseed with very low erucic acid content (known to be toxic to humans), has approved GRAS Status by FDA. Canola oil contains 55% monounsaturated oleic acid, 25% linoleic acid, 10% alpha-linolenic acid and only 4% saturated fatty acids. Thus, it is a major contributor of the unsaturated fatty acids that make Choice Spread susceptible to lipid oxidation [5].

- Non Fat Dry Milk: Nonfat dry milk is the product obtained by removal of water only from pasteurized skim milk. It contains not more than 5% by weight of moisture and not more than 1.5 % by weight of milk fat unless otherwise indicated (CFR 131.125).

- Corn Syrup Solids: These solids contribute to the flavor profile of Choice Spread and aid in moisture retention and quick dissolution when diluted with water.

- Incosity (modified food starch), lecithin and gums are used as stabilizers to produce smooth and stable oil in water emulsion and thickeners to provide required viscosity.

- Bacon, cheese and roasted potato artificial flavors along with onion, garlic and black pepper powder seasonings, helped provide the desired flavor profile.

- TBHQ- Tertiary Butyl HydroQuinone, is an effective synthetic antioxidant used to prevent lipid oxidation and enhance shelf life.

All these ingredients are blended and processed using mixing and heat to ensure a homogenous product with desirable attributes. The minimum cook time specified for processed cheese by CFR is 65.5°C for 30 seconds. The product is then packaged, cooled and stored [4]. The label for MRE Choice Spread is shown in Figure 2.

> **Nutrition: (**Serving Size- 40g Soup/Spread)
> Calories 270Kcal, Fat 25g, Carbohydrate 7g, Protein 5g
>
> **Ingredients:** Cream powder, canola oil, cheese powder, nonfat dry milk, art. bacon bits, modified food starch, corn syrup solids, lecithin, artificial flavors, cellulose, xanthan and carrageenan gum, onion powder, garlic powder, black pepper, food grade antioxidant (TBHQ)

Figure 2: Choice Spread ingredient & nutrition label

2.2. Shelf life of Foods

2.2.1. Introduction to shelf life

"Shelf life is defined as the time period during which a food retains acceptable characteristics of flavor, color, aroma, texture, nutrition and safety under defined environmental conditions". The food formulation, the package used and the environment to which it is exposed all affect shelf life [1].

A food system is an extremely complex matrix with a number of reactions occurring simultaneously. For each food formulation, major deterioration reactions must be identified to estimate the feasible shelf life, while also keeping in mind marketing and logistics considerations. The packaging parameters (material, form, thickness etc.) and environmental parameters (temperature, oxygen, moisture and light) control the internal environment of the food and thus influence its deterioration, which may be physical, microbial or chemical [1, 6].

2.2.2. Physical Changes

Physical changes are most often caused due to mechanical damages that occur during harvesting, production, distribution, storage and usage of foods. Breaking of chips within a bag, bruising of fruits and vegetables during harvest or distribution and softening of cookies exposed to moist environments are all examples of physical abuse to a product. In most cases, the product is still safe for consumption but the shelf life is limited as the damage causes changes in sensory attributes (appearance, texture, taste and odor), making the food unacceptable to consumers. Importantly, physical changes such as the tissue disruption occurring during in bruising of fruits can trigger chemical or microbial reactions that make the product also unsafe for consumption [1, 7].

2.2.3. Chemical Changes

In every food system, a number of chemical changes occur constantly between the food components under the influence of environmental parameters such as oxygen, temperature, light etc. and these changes typically reduce the shelf life of foods. Degradative reactions can be either enzymatic or non-enzymatic. Browning of apple slices occurs due to action of enzyme polyphenol oxidase when cells are broken and exposed to oxygen and affects the consumer acceptability. Foods rich in unsaturated fatty acids undergo oxidation in the presence of oxygen, leading to development of rancid odor and taste (as can be seen in old cereal), loss of functionality of vitamins and browning. Maillard browning that occurs between free amino acids and carbonyl groups can have effects similar to oxidation. Intrinsic characteristics of food such as pH and water activity strongly influence rates of these reactions [1, 7] and thus can be manipulated to extend shelf stability.

2.2.4. Microbial Changes

Microbial concerns are generally more in fresh and minimally processed foods and are observed in processed foods due to post process contamination (leaks in package, improper handling by the consumer etc.). Although this is not a concern for MRE Choice Spread, briefly introducing it is important for overall understanding of shelf life. The food formulation and its intrinsic characteristics (pH, water activity etc.) affect microbial growth and environmental parameters affect their survival. Chemical changes that alter intrinsic characteristics of foods are linked to microbial growth and survival and thus need to be understood and established to estimate the shelf life. The growth of microorganisms can lead to undesirable appearances, development of off-flavors, or even production of toxic compounds that can be fatal to humans. Processing methods like thermal treatment or high pressure processing can reduce pathogenic and spoilage microbial count significantly in foods and appropriate storage conditions can help maintain the sterility [8, 1, 7].

In MRE Choice Spread, processing eliminates microbial problems, but chemical changes remain a major concern. With the goal of limiting lipid oxidation, the most active chemical reactions, the effect of different types of packaging on these changes was studied.

2.2.5. Accelerated Shelf Life Testing

Shelf life for a product is typically estimated by storage studies during which the physical, microbial, chemical and sensory changes are monitored over time. Although investigating all these changes may be unnecessary for every product, it must be carried out at least

once on actual product rather than determining the major deterioration modes based solely on literature review and similarity to other food products. Identifying the main quality attributes that limit shelf life and setting critical limits or thresholds for them is the first step. Storage studies are then conducted to monitor the change in these attributes under different packaging and environmental conditions. However, this is extremely time-consuming and economically not feasible to test products under ambient conditions when a shelf life of three years is required [1].

To estimate or predict long term shelf life, Accelerated Shelf Life Testing (ASLT) applies external stresses such as elevated temperature, oxygen, light and moisture to speed up deterioration reactions and thus show the changes that will occur but over a shorter period of time. ASLT is useful for determining the shelf life of new products, in understanding effects of change in food formulation e.g. use of new preservative system, different processing methods and parameters e.g. switching from one kind of treatment to another and the change in packaging material or form e.g. reducing thickness of material to increase green aspect or reduce cost, on shelf life. This is an extremely useful practice even for products with short shelf lives since they can be tested and launched more quickly [1, 9, 10].

2.2.6. Chemical Kinetics

ASLT is based on the principle of chemical kinetics where the external factors behave as catalysts for the deterioration reactions. The products are tested to failure and the information obtained by quantifying the change in quality attributes determines the shelf

life under the stressed conditions. Assuming [Q] is the quality attribute, its decrease over time (t) is given by equation:

$$\frac{d[Q]}{dt} = -k[Q]^n$$

where k is the reaction rate constant and n is the order of the reaction.

Zero order reactions are independent of reactant concentration and depend instead on reaction conditions. The rate is thus constant over time. Zero order (n=0) reactions can be integrated between initial value (Q_0) and value at time t (Q_t) to obtain the following equation:

$$Q_t = Q_0 - kt$$

Sensory attributes and Maillard browning are zero order reactions and their degradation rates can be calculated and predicted using the above equation [10, 11].

In First Order (n=1) reactions, rates depend on concentrations of one reactant. In foods, quality attributes (which are derived characteristics) usually reflect changes in reactants, so may correspondingly follow first order degradation kinetics. On integration between initial value and value at time t, the equation describing the rate of change is:

$$\ln (Q_t / Q_0) = -kt$$

Reactions following exponential decay kinetics include loss of nutrients and pigments, protein losses and microbial growth [10].

Lipid oxidation is considered to be zero order reactions according to some sources [10, 11] and first order according to others as it is reactant dependent.

Temperature is the most common accelerating factor used in ASLT. The Arrhenius kinetic model predicts that the rate of a chemical reaction increases with increase in temperature according to the equation:

$$k_T = k_0 exp(-E_a/RT)$$

where k_0 is a constant, E_a is activation energy, R is ideal gas constant and T is temperature in Kelvin. Q_{10}, a term derived from the Arrhenius equation, is commonly used to explain the relationship between temperature and reaction rate constant and allow for easy prediction of reaction rates at different temperatures:

$$Q_{10} = \frac{\text{reaction rate constant at temperature (T + 10)}}{\text{reaction rate constant at T}}$$

The usual expectation is that reaction rates double with each 10 degree increase in temperature [12]. Consistent with this, accelerated shelf life testing conducted in this laboratory found the Q_{10} value for lipid oxidation in sunflower and vegetable oil to be 2 to 3 [13]. This principle is important in ASLT as it allows shelf life at different temperatures to be predicted by using information obtained at one specific storage temperature [1, 9].

2.2.7.   Limitations of ASLT

Although ASLT, is an extremely important tool in the food industry, it has certain limitations and disadvantages [6]. The increase in stress such as high temperatures may cause changes in the food system that would never occur at normal temperatures such as:

- Phase changes such as melting of fat

- Crystallization of carbohydrates

- Moisture loss

- Lowering solubility of gases in food

- Denaturation of proteins.

These effects affect other reactions and can cause over or under estimation of shelf life. Also, an increase in temperature may cause a significant change in reaction mechanism, in which case the Arrhenius equation cannot be employed.

Thus, the best practice is to use ASLT initially to estimate long term shelf life, then confirm this prediction by monitoring quality over the entire shelf life of a particular product under environmental conditions to which the product will be exposed in reality. Once this relationship between accelerated and normal conditions is established and understood for that product, ASLT can be used for further tests [14].

2.3. Lipid Oxidation

Lipid oxidation is a complex process in which unsaturated fatty acids in various lipid structures degrade in the presence of oxygen. It is the chemical reaction that most limits the long term shelf life of foods, producing off-flavors, odors, browning compounds, toxic products and degradation of vitamins and amino acids [15, 16].

2.3.1. Mechanism of Lipid Oxidation

Lipid oxidation is thermodynamically facile since the activation energy is 10-15Kcal/mole, as low as enzyme catalyzed reactions [13]! In simplified form, lipid

oxidation occurs as a free radical chain reaction in three stages: initiation, propagation and termination [17] with the main driving force for these reactions being the continuous abstraction of hydrogen atoms from new lipid molecules, generating new free radicals that keep the process going. The reactions that occur in each of the three stages are summarized below.

Initiation:

Catalysts such as metals, light and temperature initiate lipid oxidation by generating alkyl free radicals ($L^\bullet$) from unsaturated lipid molecules (LH). Redox-active metals, particularly iron and copper, are ubiquitous in foods; active at even trace levels (e.g. ppb), they mediate one-electron transfers to generate radicals in lipids. UV light lacks sufficient energy to actually break C-C bonds (except at wavelengths less than 190 nm), but it actively decomposes hydroperoxides to generate alkoxyl and hydroxyl radicals that react faster and less selectively, so accelerate and branch oxidation chains [18].

$$L_1H \quad \rightarrow \quad L_1^\bullet \tag{1}$$

Propagation

During the propagation stage, free radicals formed in initiation stages are transferred to other molecules by hydrogen abstraction to establish a continuous and self-perpetuating free radical chain reaction:

$$L_1^\bullet + O_2 \rightarrow L_1OO^\bullet \tag{2}$$

$$L_1OO^\bullet + L_2H \rightarrow L_1OOH + L_2^\bullet \tag{3}$$

$$L_2^{\bullet} \; + \; O_2 \; \rightarrow \; L_2OO^{\bullet} \tag{4}$$

$$L_2OO^{\bullet} + \; L_nH \; \rightarrow \; L_2OOH \; + \; L_n^{\bullet} \tag{5}$$

In early stages, new peroxyl radicals ($LOO^{\bullet}$) are generated and the first stable products, hydroperoxides accumulate. Peroxyl radicals, relatively slow and selective in their reactions, are the main chain carriers. However, in the presence of metals, UV light, or moderate heat, their hydroperoxide products decompose to yield more reactive alkoxyl and hydroxyl radicals which become the major reaction drivers in later oxidation stages [17].

$$L_nOOH \; \longrightarrow \; L_nO^{\bullet} \; + \; OH^{\bullet} \tag{6}$$

$$L_nOOH \; \xrightarrow{\text{Heat, UV} \atop \text{metals}} \; L_nO^{\bullet} + \; OH^- \quad \text{or} \quad L_nOO^{\bullet} + \; H^+ \tag{7}$$

$$L_nO^{\bullet} \; + \; L_3H \; \rightarrow \; L_nOH \; + \; L_3^{\bullet} \tag{8}$$

$$L_nOO^{\bullet} \; + \; L_3H \; \rightarrow \; L_nOOH \; + \; L_3^{\bullet} \tag{9}$$

$$L_nOOH \; + \; L_1OO^{\bullet} \; \rightarrow \; L_1OOH \; + \; L_nOO^{\bullet} \tag{10}$$

$$L_nOOH \; + \; L_1O^{\bullet} \; \rightarrow \; L_1OH \; + \; L_nOO^{\bullet} \tag{11}$$
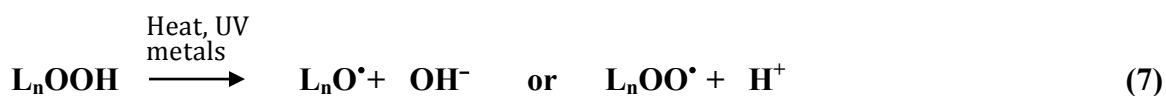
Termination

Oxidation reactions eventually slow down, forming non-radical products by recombination (Equation 12), cleavage to form smaller molecular weight compounds and volatiles (Equation13), or co-oxidation (transfer of free radical to non lipid molecules). The latter will be discussed in Section 2.3.2. The scission products formed during

termination are a major source of low molecular weight compounds that contribute to the rancid odors and flavors of oxidation.

$$L_n^{\bullet}/L_nO^{\bullet}/L_nOO^{\bullet} + L_n^{\bullet}/L_nO^{\bullet}/L_nOO^{\bullet} \rightarrow \text{Alkanes, Aldehydes, Ketones etc.} \qquad (12)$$

$$L_nO^{\bullet}/L_nOO^{\bullet} \rightarrow \text{Aldehydes, Ketones, Alcohols etc.} \qquad (13)$$

One thing to keep in mind is that there is no definite end point for oxidation as it is a set of many reactions that proceed in different ways rather than one reaction with a definite end product [17, 18].

In actual foods, lipid oxidation is much more complex than the simple scheme described above, involving a number of secondary reactions. The historical free radical pathway still provides the core reactions, but alternate pathways including internal rearrangements, addition and scission occur simultaneously and in competition with hydrogen abstraction, generating a much more complex mixture of products with different kinetics than predicted by the simple radical chain reaction. These alternate reactions are being investigated in detail [19] to develop a greater understanding of oxidation, improve approaches to monitoring lipid oxidation and inhibit occurrence of lipid oxidation in foods.

2.3.2.   Lipid Co-oxidation

A free radical formed during oxidation can abstract hydrogen from sources other than lipids, such as amino acids, proteins, carbohydrates, vitamins and pigments. Once the free radicals are broadcasted to these molecules the pathway followed is similar to lipid oxidation. The radicals formed can react with each other or lipid oxidation intermediates

to form co-oxidation products. Although it has not been investigated in the current research study, it is important to mention that monitoring only lipid oxidation products to measure levels of oxidation may provide an incomplete picture and could lead to underestimation [20] of oxidation effects.

The figure below briefly outlines lipid-protien co-oxidation and its effects on a food system.
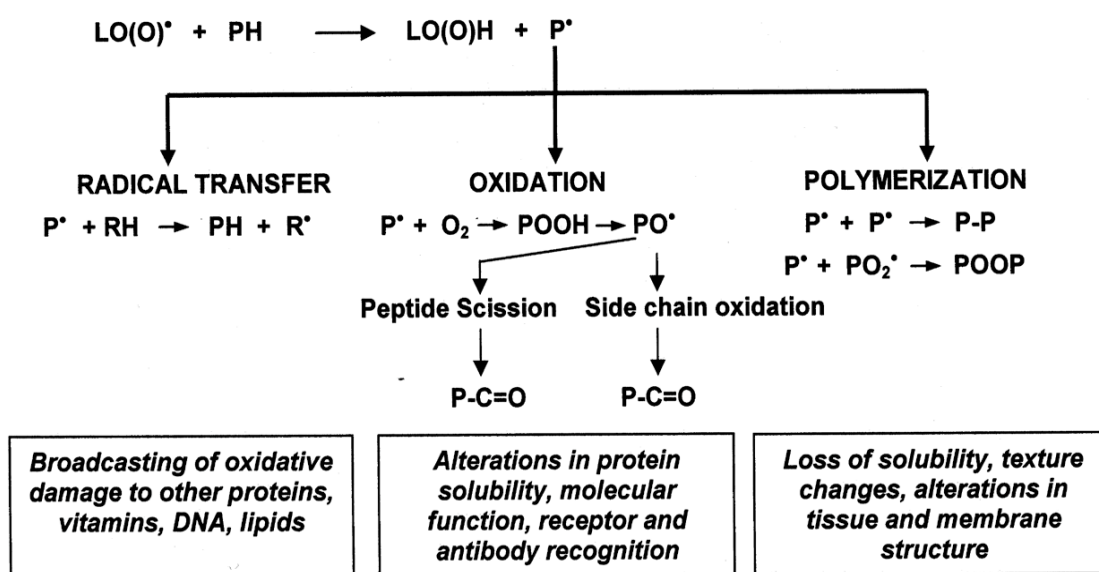
Figure 3: Protein co-oxidation & its effects (adapted form [20])

2.3.3. Effects of Lipid Oxidation on quality attributes

All major quality attributes of food i.e. appearance, texture, flavor, odor and nutritional quality are affected by lipid oxidation.

Color: A number of studies have reported that browning and color changes are commonly associated with oxidation and antioxidants like tocopherol when release from

LDPE films have shown to have enhanced color of fresh beef [21] and previous studies in our laboratory have shown better color protection in MRE Cheese Spread as seen in Figure 6.

Flavor (odor & taste): The volatile products generated during lipid oxidation have low thresholds for sensory detection (parts per billion) and thus oxidation of even a small amount of lipid will make the food unacceptable to the consumer. Most foods are considered fully rancid at 1% oxidation [22]. A variety of non-volatile products formed contribute to the rancid flavors associated with oxidation [23].

Texture: Lipid oxidation may often result in development of large molecular weight polymers that increase viscosity of edible oils and protein co-oxidation has been associated with texture changes [20].

Safety: Lipid oxidation products in some cases have been reported to be toxic or carcinogenic [24]. Compounds such as 4-hydroxy-2-alkenals and 4,5-epoxy-2-alkenals were shown to be toxic and may contribute to carcinogenesis, mutagenisis, Alzheimer disease and ageing. Oxidized fatty acids are capable of being absorbed by the intestine and can cause oxidative stress and atherogenesis by getting incorporated into lipoproteins [25, 26].

With increasing awareness of health benefits of unsaturated fats, negative press regarding synthetic food preservatives and iron fortification requirements, foods are becoming more susceptible to oxidation [27].

2.3.4. Monitoring Lipid Oxidation

Lipid oxidation mechanisms have been greatly studied in oils and fat to help understand factors that influence it and ways to control it [28]. However, the same does not apply to complicated food matrices and emulsions. As mentioned in Section 2.3.1 lipid oxidation can progress through various possible routes and to monitor just one reaction would provide an incomplete picture.

Measurements can be conducted to quantify the following[18]:

- Oxygen consumption

- Peroxide levels (primary oxidation product) that typically drives initial stages

- Conjugated dienes which are also primary oxidation products

- Aldehydes, alkenals and other secondary oxidation products

- Protein oxidation using SDS PAGE or protein solubility tests

- Volatile levels using GC-MS and any unknown non volatiles using LC-MS

Overall, depending on the food system being investigated, certain products are chosen to be marker compounds and monitored over storage studies. Conducting sensory studies in parallel to understand changes in aroma, taste, appearance and texture and to correlate the information with the chemical analyses will provide a more complete understanding.

2.3.5. Factors affecting Lipid oxidation

To develop strategies to counteract lipid oxidation, it is important to understand the factors that affect oxidation. These factors can be grouped into four categories:

1) Nature of Lipids: Studies have shown that as degree of unsaturation increases (number of double bonds), oxidative stability decreases [28, 18]. However, the number of double bonds is not directly proportional to oxidative stability as position of these bonds also affects the availability of hydrogen for abstraction. For example, cis-isomers are more readily oxidized when compared to their trans forms [18]. Overall, foods rich in saturated fats are less susceptible to oxidation when compared to those rich in unsaturated fats.

2) Surfaces: In bulk lipids like oils the surface area available for oxidation is much more when compared to oil in water emulsions where the lipid is trapped inside a hydrophobic core of droplets with emulsifies at the interfacial region [28, 18]. Moreover, solubility of oxygen in food oils is three times more than in water [29, 28] and thus is more readily present in bulk lipids. In emulsions, diffusion of oxygen to reach the core of droplets is of concern especially when present in low concentrations and availability of prooxidants (mostly hydrophilic) at the interface can alter the rate of lipid oxidation. The thickness of the interfacial layer in emulsions is also said to have an effect on rates of lipid oxidation [28, 30].

3) Presence of prooxidants and antioxidants: Prooxidants are compounds that initiate oxidation reactions or behave as catalysts during its propagation. They include metals, enzymes, amino acids, sugars etc. Metals such as iron and copper are major prooxidants as they are present in most raw materials. Enzymes, amino acids and sugars can act either as prooxidants or antioxidants, depending on their individual properties and environment [28]. Antioxidants inhibit oxidation by different mechanisms that have been discussed in Section 2.4.2.

4) Environment: Parameters such as temperature, light, oxygen, water activity and pH have a bearing on lipid oxidation. Increased temperatures provide greater energy for branching reactions during processing or storage of foods leading to increased oxidation. Both UV and visible light can initiate production of free radicals and cyclic products breakdown under its influence producing off-flavors, odors and co-oxidation. Similar to most reactions, lipid oxidation is also enhanced in foods with high water activity but it is equally enhanced at very low levels as well as the water layer present is very thin, exposing lipids to deteriorative reactions. pH of the food is also an important consideration especially in emulsions as it affects the polarity of lipids and partitioning of reactive species. Thus foods should be carefully formulated to ensure minimum oxidation effects [28].

2.3.6. Approaches to inhibiting lipid oxidation

Based on the mechanism of lipid oxidation and the various factors that affect it, a number of approaches for stabilization can be used. In most cases, due to the complexity of this deterioration mode a combination of tools targeting different aspects of lipid oxidation work most effectively. Controlling the initiation of lipid oxidation is a straightforward approach that will decrease the starting load of free radicals and may include[18] approaches mentioned below.

- Exclude oxygen by using vacuum or nitrogen gas flushing during processing of the food and its storage.

- Reduce level of unsaturation in a food by using alternative lipids and ingredients.

- Eliminate light exposure using opaque barrier packaging

- Formulating the food product to have appropriate pH and water activity

- Eliminate any source of metals by using high purity ingredients, which have been stored properly. This may prove to be economically unfeasible on a large scale in which case metal chelators such as EDTA, Citric Acid and emulsion surface charge modifiers to lend it a positive charge can be used to make them unavailable for oxidation [28].

Once initiated lipid oxidation can be inhibited by using antioxidants that quench the free radicals being formed preventing further propagation or oxidation. These can inhibit both primary reactions that generate hydroperoxides or secondary reactions that produce aldehydes, ketones and smaller molecular weight compounds.

2.4. Antioxidants

2.4.1. Introduction and advantages

Antioxidants are defined as "substances that in small quantities will prevent or greatly retard oxidation of easily oxidisable materials such as fats". However over time the definition has broadened to include a large number of compounds whose protective activity may not be directly due to its antioxidative effect [31, 32].

Natural compounds have gained immense momentum in the last few years owing to the negative stigma associated with synthetic additives mainly due to its effect on human health on consumption. Synthetic antioxidants such as Butylated HydroxyToluene (BHT) and Butylated HydroxyAnisole (BHA) have been used in the past but studies have shown that BHT may cause internal and external hemorrhaging at high doses and even death on

high consumption in mice and guinea pigs [33]. This has pushed research to be focused on evaluating compounds from natural sources for antioxidant activity. Most of these natural antioxidants also provide additional health benefits such as having anti-carcinogenic and antitumor activities [34].

Similar to lipid oxidation, antioxidant action is also complex in nature and its effectiveness depends on a number of factors listed below.

1) System: Antioxidant effectiveness depends on the physical state of a system and its components. Depending on the polarity, chemical structure and interaction with emulsifiers, antioxidants are partitioned and localized in the aqueous, hydrophobic or the interfacial regions of a colloidal system [35-37]. Evidence for this is shown in studies where the inhibition constant for alpha tocopherol was noted to be 100 times lesser in a aqueous systems relative to chlorobenzene as they are most functional in lipophilic environments and hydrogen bonding with water molecules reduces its activity [38, 39] while the antioxidant effect of Trolox (carboxylic acid derivative of alpha tocopherol) was much more effective in bulk oils with low surface to volume ration [27]. This can be explained by the affinity of Trolox to be oriented at the oil-air interface where early oxidation occurs whereas in emulsions such polar antioxidants could behave as metal reducers and become prooxidants. The partitioning of the antioxidants also depends on the pH, temperature, lipid substrate and surfactants used [32].

2) Nature of Lipids: The degree of unsaturation and physiochemical state of the lipid affects the antioxidant effectiveness. Different antioxidants are compatible with different types of lipids such as triacylglycerols, phospholipids and fatty acids as they lend

different colloidal properties to the system. Foods mainly consist of triacylglycerols that are less polar compared to linoleic acid, which is commonly employed as a model system becoming a reason for difficulty in translation of information from a model to real food system. Trolox exhibited enhanced activity in a linoleic acid emulsion when compared to triacylglycerol emulsion, both emulsified with Tween 20 as it partitions into the aqueous phase in the latter. Moreover, phenolic antioxidants can have antioxidant or prooxidant activities depending on the substrate and oxidation conditions [40].

3) Emulsifier: The charge of emulsifiers can affect antioxidant action in emulsions. Antioxidants, which exist as anions in neutral solutions, are ineffective in emulsions with Sodium Dodecyl Sulphate (SDS) as emulsifier as its negative charge causes repulsion [41].

4) Oxidation Conditions: High temperatures, oxygen concentration, presence of initiators alter the efficacy of antioxidants depending on their mode of action as will as explained in the next section [32].

2.4.2. Mechanism of Antioxidant Action

Mechanistically antioxidants are defined to be radical acceptors [42]. Based on their mode of action, antioxidants are of two classes. The first class of antioxidants is of two types: primary and secondary. Primary antioxidants work by quenching free radicals and forming stable radicals themselves such as phenolic compounds while secondary antioxidants enhance the activity of the primary antioxidants by regenerating radical forms of the primary or reducing radical load of the system such as reducing agents, organic acids and sulfur compounds [43, 22]. The second class consists of preventive

antioxidants that prevent introduction of new free radicals and reduce rate of initiation reactions by chelating metals like iron and copper that act as catalysts for oxidation or by quenching the singlet oxygen species. EDTA and citric acid are examples of metal chelators commonly employed in food formulations while carotenoids and tocopherol are effective in quenching reactive oxygen species [32, 43].

Primary or chain breaking antioxidants (A) compete with the substrate to interact with the free radical species formed in oxidation as shown below either as electron donors (equation 1 and 2) or as electron acceptors (equation 3) [15, 32].

$$L^{\bullet} + AH \rightarrow LH + A^{\bullet} \tag{14}$$

$$LOO^{\bullet} + AH \rightarrow LOOH + A^{\bullet} \tag{15}$$

$$L^{\bullet} + A^{\bullet} \rightarrow L^{**} + AH \tag{16}$$

where L^{**} is a lipid molecule with a new double bond

Antioxidants can also inhibit decomposition reactions of primary lipid oxidation products by reacting with alkoxyl radicals that form aldehydes and other compounds that contribute to rancid flavors and odors.

$$LO^{\bullet} + AH \rightarrow LOH + A^{\bullet} \tag{17}$$

The radical $A^{\bullet}$ is not reactive like $L^{\bullet}/ LO^{\bullet}/LOO^{\bullet}$ species as it capable of delocalizing unpaired electrons to stabilize the radical and not propagate the chain reactions [43, 44, 18]. They are also capable of forming non radical, termination products as shown below.

$$LOO^{\bullet} + A^{\bullet} \rightarrow LOO\text{-}A \tag{18}$$

$$LO^\bullet \;+\; A^\bullet \;\rightarrow\; LO\text{-}A \qquad\qquad\qquad\qquad\qquad (19)$$

However, in complex matrices of real foods, antioxidant mechanisms overlap where scavenging, chelating and other types of inhibition occur simultaneously [15, 45].

2.4.3. Tocopherol as an antioxidant

2.4.3.1. Introduction

Tocopherols are commonly found in oil seeds, nuts, vegetables such as peas, beans and carrots and green parts of higher plants. Tocopherols contain a head with two rings (phenolic and heterocyclic) and a phytyl tail. The homologues of tocopherol α, β, γ and δ all have saturated tails and differ in the number and position of methyl substituents as shown below in Figure 4 [46, 47].
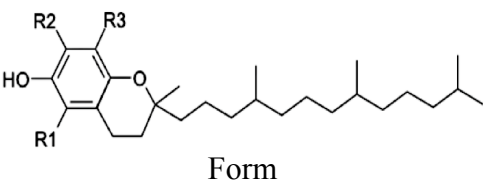
Form	R1	R2	R3
Alpha (α)	CH_3	CH_3	CH_3
Beta (β)	CH_3	H	CH_3
Gamma (γ)	H	CH_3	CH_3
Delta (δ)	H	H	CH_3

Figure 4: Homologues of tocopherol

Olcott and Emerson first studied the antioxidant activity of tocopherol, as they observed that unsaponifiable material in vegetable oils showed antioxidant properties and γ-

tocopherol exhibited greatest protective effect towards oxidation of lard and olive oil. As most antioxidants known at that time were phenolic nature, they speculated tocopherol to be phenolic, which was confirmed later through analysis of α-tocopherol decomposition products [48].

A number of natural antioxidants such as phenolics, carotenoids, essential oils and plant extracts have been studied in bulk oils [37]. Phenolic compounds such as tocopherol are capable of readily scavenging lipid peroxyl radicals by donating hydrogen atoms and forming a stable radical by electron delocalization or intramolecular hydrogen bonding [32]. They have been effective in inhibiting oxidation in both foods and biological systems [47]. Tocopherol is an important lipid soluble antioxidant *in vivo,* referred to as Vitamin E and provides defense against oxidative stress that causes free radical generation in the human body. Its functions also have extended to gene regulation, prevention of atherosclerosis, cancer and coronary diseases [49-51].

2.4.3.2. Mechanism of action

It has been demonstrated that tocopherol is consumed during oxidation by a previous study where tocopherol was added to linoleic acid and migly oil and measured after 90 days at 23°C. Less than 5% degradation of tocopherol was recorded in migly oil which is completely saturated and non reactive while greater degradation was recorded in highly unsaturated and reactive linoleic acid [13] suggesting that the molecule participates in the reactions.

It functions typically as a chain breaking antioxidant [27, 32]. The bulky groups and aromatic structure of tocopherol lend it the ability to delocalize electrons and be a

powerful chain breaker [43]. The first step in its action is conversion from tocopherol to tocopheroxyl radical by donating one hydrogen atom to peroxyl or alkoxyl radicals (seen in equation 1 and equation 2). Evidence of formation of these radicals is available from electron spin resonance and electron double resonance studies [52, 53].

$$LOO^\bullet \ + \ T\text{-}OH \ \rightarrow \ LOOH \ + \ TO^\bullet \tag{20}$$

$$LO^\bullet \ + \ T\text{-}OH \ \rightarrow \ LOH \ + \ TO^\bullet \tag{21}$$

The tocopherol in polar protic solvents donates a hydrogen atom or electron while in non-polar lipophilic solvents, the tocopheroxyl radical reacts mainly by radical-radical coupling reactions with its own radicals or those formed as oxidation intermediates to form dimers (equation 22) [47]. The tocopheroxyl radical after stabilizing peroxyl and alkoxyl radicals can form stable dimers, peroxides and alkyl derivatives that regenerate the antioxidant [27].

$$TO^\bullet \ + \ TO^\bullet \ \rightarrow \ TO\text{-}TO \tag{22}$$

In some cases like low oxygen pressure, tocopherol can directly react with alky radicals [47].

$$L^\bullet \ + \ T\text{-}OH \ \rightarrow \ LH \ + \ TO^\bullet \tag{23}$$

Various studies have demonstrated the antioxidant effects of tocopherol. 0.05% α-tocopherol has shown to increase the oxidative stability of lard 15 fold and that of soybean oil 1 fold [37]. α-tocopherol can behave as a prooxidant or antioxidant depending on the test system used, the concentration, oxidation time [54, 55, 47] and thus

its use must be carefully regulated. When present in high concentrations the following prooxidation reactions may occur:

$$LH + \alpha\text{-}TO^\bullet \rightarrow L^\bullet + \alpha\text{-}TOH \tag{24}$$

$$LOOH + \alpha\text{-}TO^\bullet \rightarrow LOO^\bullet + \alpha\text{-}TOH \tag{25}$$

Tocopherol can also act to reduce metals by donation of electrons, which in turn can initiate oxidation reactions [47].

$$TOH + M^{(n+1)+} \rightarrow TO^\bullet + H^+ + M^{n+} \tag{26}$$

$$TO^\bullet + M^{(n+1)+} \rightarrow TO^+ + M^{n+} \tag{27}$$

A study conducted on antioxidant activity of α-tocopherol on rapeseed oil showed that activity was highest at a concentration of 43mg/Kg and further increase did not have any beneficial effect and even led to shorter induction period compared to lower concentrations of α-tocopherol used [56].

2.4.3.3. Homologues of tocopherol

Different homologues of tocopherol have been studied for antioxidant effectiveness solely or in combination. α-tocopherol is the most effective of the four homologues in inhibiting oxidation in foods [55, 47]. The chemical structure of tocopherols support hydrogen donating power in the order: α > β > γ > δ[57] as β, γ and δ forms lack *ortho* methyl groups. Increase in methyl substituents on the ring not only increases antioxidant activity but also its lipophilic properties making the α-form effective. However recent research has established that the effectiveness of these homologues in *in vitro* systems

like foods depends not only on its reactivity towards hydroperoxy and other free radicals but also its participation in a number of side reactions, which are affected by light, temperature, type of lipid, presence of prooxidants and concentration of tocopherol employed [47]. Tocopherols at a concentration of 0.01-0.2% when used in olive oil showed that γ and δ forms alone and in combination were more effective than α alone or in combination with γ and δ forms [58]. The enhanced activity of γ-tocopherol over the α-tocopherol can be attributed to the formation of dimerizable compounds that are effective as antioxidants. Studies in methyl linoleate have suggested that when mixed tocopherols are employed, α-tocopherol acts first followed by decomposition of γ-form and then δ-form once the α-form is consumed [47].

A number of external factors also affect the antioxidant action as mentioned before. Studies have reported the order of antioxidant activity to be $\alpha > \beta > \gamma > \delta$ under low-mild temperatures and $\alpha < \beta < \gamma < \delta$ at higher temperatures. Tocopherols operate best in highly unsaturated systems as with increase in degree of unsaturation, the competition with fatty acids increases for oxidation. Low pH in the presence of transition metals was reported to increase lipid peroxide decomposition and hence antioxidant action [47].

2.4.3.4.   Synergistic action of Antioxidants

A combination of α-tocopherol and other antioxidants like ascorbic acid, citric acid etc. was shown to be more effective than using one by itself. This synergism can be either as the ascorbic acid reduced the tocopheroxyl intermediate to tocopherol molecule or the ascorbic acid behaves as a metal chelator [47, 43]. Tocopherols used in combination with other antioxidants such as ascobyl palmitate, phosphatidyl ethanolamine and rosemary extract have synergistic antioxidant effects in fish oils [59, 47, 37].

2.5. Controlled Release Packaging

2.5.1. Active Packaging

One of the basic functions of packaging is to protect food and maintain optimum quality during shelf life. Traditionally, a package provided protection against environmental factors such as dirt, light, microbes and prevented contamination and was limited in its ability to extend shelf life. Active Packaging is an innovative system that provides protection beyond just being a barrier to the external environment and involves interactions between the package, the internal environment and food to maintain optimum quality of the food product [60-62]. It has been defined as a type of packaging that changes the condition of the package to extend shelf life or improve safety or sensory properties while maintaining the quality of foods. These include oxygen scavengers, moisture absorbers, ethylene absorbers, flavor releasing systems, films with antimicrobials and antioxidants, etc. [62, 63].

Permeable sachets with iron have been used as oxygen scavengers to absorb residual oxygen through iron oxidation reactions. These sachets are found in packages of fresh pasta, meat products, bread, cheese, coffee, nuts and potato chips. Other oxygen scavengers include ascorbic acid, enzymes such as glucose oxidase, unsaturated acids and immobilized yeast. Besides oxidation, by removal of oxygen they also prevent growth of aerobic microorganisms and molds [63]. However, there are dangers of possible contamination of food with the sachet contents when in contact. Due to this reason its application in liquid foods is hazardous as there could be spillage of sachet contents into the food. To prevent such a situation, iron powder was incorporated in LDPE and

effectively absorbed oxygen but imparted an undesirable flavor to the food product [62]. Another disadvantage of removal of oxygen is that it may favor the growth of anaerobic microorganisms such as *Clostridium Botulinum*. In industry, vacuum packaging or inert gas flushing has been used to eliminate oxygen. However practically it is impossible to remove all traces of oxygen especially in liquid and semi-solid foods as they have minimal headspace [64, 63]. An answer to such problems is Controlled Release Packaging,

Controlled Release Packaging (CRP) is a type of active packaging that is designed to release active compounds such as antimicrobials or antioxidants at a required rate from the package to the food to protect the food from deterioration. This concept has been borrowed from the pharmaceutical industry where CRP has demonstrated to be successful for drug delivery applications [65-68] and studies have shown great potential in protection of food. In food applications, films with antimicrobials like sorbate, benzoic acid, nisin and antioxidants like BHT and BHA have been successfully tested [69, 67, 70].
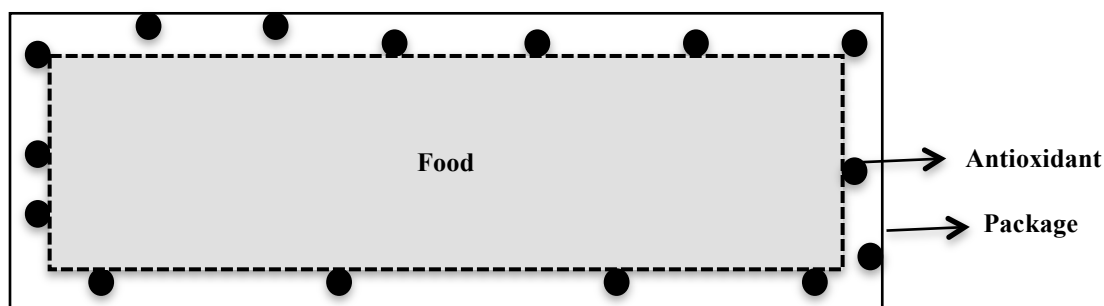

Figure 5: Controlled Release Packaging

2.5.2. Benefits of CRP

The benefits of CRP in theory have motivated further research in its development and commercial application to provide shelf life extension. The preservatives added to the food formulation directly (instant addition), are consumed in oxidation and microbial growth that are continuous reactions that increase exponentially. Once the added antioxidant is consumed or degraded, protection ceases and the foods deteriorate quickly. CRP will release these active compounds at a required rate to replenish the molecules that have been consumed and ensure a pre determined amount is present at all times, allowing for protection over a longer period of time. Induction period of linoleic acid at 40°C was extended from 4.3 days obtained by instant addition of 3000ppm of tocopherol to 6.8 days by incorporating the same in Polypropylene (PP) films [67].

Another advantage of CRP is that active compounds when used in high concentrations to provide long term stabilization, may reverse their behavior to exhibit prooxidant activity as mentioned in Section 2.4.3.2. By releasing small amounts of antioxidant, this limit beyond which an antioxidant behaves as a prooxidant is not crossed. Also, the amount of active compound required in CRP is much lesser to provide the same degree of protection. Moreover the disadvantages of instant addition in terms of interaction of active compounds with other ingredients in the formulation are not of concern when employed in a CRP system [71].

CRP has a dual benefit of not only protecting the food but also the package in development as the antioxidants function as stabilizers during polymer processing [67] and the active compounds are in turn protected by the package from degradation when subjected to extreme processing conditions such as extrusion [71].

Using systems like CRP may also provide economical benefits as steps involved in processing of foods sensitive to oxidation such as removal of oxygen and flushing with inert gases can be eliminated [60].

2.5.3.   Mechanism of CRP

Controlled Release Packaging is governed by transport phenomena. For active compounds like tocopherols that are non-volatile, contact with food layer is required to be effective, where as volatile compounds like sesamol diffuse into the headspace. Release of active compounds from the polymer occurs in three steps [72]:

1) Diffusion within the polymer

2) Mass transfer across food-package interface

3) Diffusion within the food

The release of BHT from High Density Polyethylene (HDPE) and α-tocopherol from Ethylene Vinyl Acetate (EVA) is said to follow first order kinetics with the presence of food inside having a major effect as observed in a study on oatmeal [73, 74]. Fick's first law of diffusion drives diffusion within the polymer and food and is governed by the equation:

$$F = -D\,dC/dx$$

where F is the rate of transfer per unit area, D is the diffusion coefficient, C is the concentration of diffusing substances and x is distance diffused.

Mass transfer across the interface is driven by the concentration gradient of the active compound and depends on polarity and solubility in the food. But food formulation, its phase, contact properties and the polymer material all affect the release of active compound [71, 75].

It was seen that α-tocopherol did not release into test media in Polypropylene (PP) films but was readily released from Low Density Polyethylene (LDPE) indicating the importance of choosing the right polymer for CRP applications. Studies in the laboratory have shown that tocopherol release to food simulant- ethanol, was slowest in HDPE and fastest from LDPE [72, 75] that can be explained by the greater crystalline character as the tight packing slows down diffusion. This allows for polymer blends to be used to achieve the desired release rate and ensure long term stabilization.

2.5.4.   Antimicrobial CRP Systems

Model system studies: Sorbic acid, chlorine dioxide and plant extracts have been successfully incorporated to exhibit antimicrobial activity [69]. Nisin incorporated films have shown to suppress growth of Gram positive bacteria that are responsible for food spoilage [76]. Films incorporated with a chlorinated phenoxy compound and chlorine dioxide are biocidal films being commercially marketed [77].

Real food studies: Films with potassium sorbate have been tested on real foods such as American processed cheese and mozzarella cheese [62, 78]. Shelf life of fresh fruits can be extended by using a commercial antifungal coating with chitosan [77].

2.5.5.   Antioxidant CRP Systems

Model system studies: The incorporation of α-tocopherol into LDPE films has shown to inhibit oxidation of a linoleic acid emulsion stored in contact with the film [74]. Ascorbic acid has been incorporated to scavenge oxygen in head space in cans and bottles making it a good candidate [60].

Real food studies: BHT and BHA incorporated HDPE has shown to protect cereals against oxidation [70]. CRP with antioxidants has shown to be effective in oatmeal cereal and vegetable oils [79, 74]. Release and effect of BHT, BHA and α-tocopherol has been studied in dry milk products [80, 81]. Paperboard coated with 3% α-tocopherol showed moderate protection against lipid oxidation in milk cream (37-38% crude fat) versus the control board [70]. Tocopherols have proved to be successful in protecting meats and various edible oils from oxidation [55, 40, 46, 47, 21, 82, 83, 58, 37].

3. RATIONALE AND OBJECTIVES

Choice Spread is a type of processed cheese spread formulated with cream powders, cheese powders, dairy proteins and emulsifiers to form stable oil in water emulsion. It contains a high percentage of unsaturated fats that are susceptible to oxidation, making it the major deterioration mode for long term stabilization of Choice Spread. In another study, long term shelf life of processed cheeses stored at ambient temperature was similarly limited by Maillard browning and lipid oxidation [84]. Hence, this soup-spread product must be stabilized for successful manufacture, distribution and storage with appearance, flavor and nutrition intact over the three year shelf life required for inclusion in existing menus of the US Armed Forces.

Addition of antioxidants to food formulations inhibits lipid oxidation and keeps products stable. However, literature has clearly established the disadvantages of instant addition of these compounds for long term stabilization as high concentrations are required and at such high concentrations the antioxidant can exhibit prooxidant activity. Controlled Release Packaging is an innovative system that can be employed to deliver these active compounds to the food to overcome limitations of instant addition by providing a specific amount of antioxidants as and when it is required to inhibit the oxidation reactions and avoid prooxidation.

In the past, synthetic antioxidants such as BHT and BHA were used in controlled release applications. The release of such antioxidants from package to food has raised consumer concerns regarding safety on consumption, leading the way to incorporation of natural substances as they have low toxicity and high effectiveness [60]. Moreover, studies have

also confirmed that the release of BHT is too fast for long term stabilization as it is a small molecule with greater diffusivity in the polymer. Tocopherol, a natural antioxidant and a form of Vitamin E has GRAS status when used as a chemical preservative according to good manufacturing practices (21 CRF Part 182, Subpart D, Sec 182.3890 Tocopherols) and has excellent solubility in polyolefins [63] making it feasible to be incorporated into a Controlled Release Packaging system. Tocopherol is also well accepted by consumers as it lends a clean label to products unlike BHT and BHA. Studies have shown that the migration of α-tocopherol from package into oil in water emulsion (o/w) is more than into a water in oil (w/o) emulsion due to the hydrophobic properties of tocopherol [75] making tocopherols a suitable candidate for use in Choice Spread.

Mixed tocopherols have been chosen in this study instead of a specific homologue as the range of polarities and antioxidant actions will make the system more effective against oxidation in a complex real food where a number of reactions are occurring simultaneously. This was indicated in a study where a mixture of α, γ, δ tocopherols were shown to better protect lard from oxidation when compared to either homologue alone [83].

A major question that arises as a packaging system impregnated with tocopherols is developed is the stability of tocopherols in extruded polymers as they are subjected to very high temperatures and shear stress. Studies have indicated that a subsequent portion of tocopherol incorporated in polymers remains in the final plastic films after extrusion and is capable of interacting with the food [85, 86] due to its heat stability. 100% of the incorporated tocopherol was extracted from biodegradable Poly Lactic Acid (PLA) films using methylene chloride stressing on its possible incorporation into green packaging

[87]. It is just not enough that the compound is present but also that the antioxidant activity be maintained to be functional as CRP system. Antioxidant assays have confirmed that more than 90% of the activity of tocopherol is retained upon processing of films [22]. Studies have shown that tocopherol degrades and forms dimers, trimers, quinones and aldehydes during extrusion but since a number of these products are commonly present as metabolites of Vitamin E in human plasma, they are non-toxic and safe to consume if present in the package [88, 22, 82].

Another advantage of incorporating tocopherols in a packaging system is that it helps retain the physical properties of films without compromising its structural integrity [13]. A laboratory study has shown that although tocopherol reduced tensile strength of the film by 10% due to its plasticizing effect and slightly reduced its transparency, change was within tolerable and acceptable limits [86]. It acts as a stabilizer during polymer processing [60, 67] by preventing free radical reactions that occur under high heat and shear of extrusion and prevents production of volatile compounds contributing to off odors. Tocopherols in CRP can also possibly quench oxygen that may be present at contact surfaces adding another level of protection to prevent initiation of lipid oxidation reactions.

Research in our laboratory, has shown that there is an optimum release rate for tocopherol from package to the food system to be most effective for a particular application and anything above or below will result in a smaller induction period for oxidation and unnecessary economic expenses [13]. Although LDPE films with α-tocopherol have demonstrated the ability to stabilize linoleic acid [89], laboratory studies have shown that PP films released tocopherol in a more slow and sustained manner to

better inhibit oxidation of linoleic acid when compared to LDPE that provides fast release. Using a blend of PP and LDPE provided a more suitable moderate release rate to protect cheese spread from oxidation [13] making this combination effective for a CRP system where long term stabilization is required. Also, as temperature increased when accelerated conditions were employed in storage studies, the release rate was increased as oxidation reactions occur much faster and require more tocopherol to suppress it. An earlier study on MRE Cheese Spread has shown that in CRP with 3000ppm of tocopherol, 1238ppm of tocopherol had released into the spread over 12 weeks at 40°C and prevented browning and texture deterioration. Sensory results obtained for the storage study conducted on MRE Cheese Spread indicated that cheese spread packaged in tocopherol films of 75%PP - 25%LDPE had higher sensory scores when compared to the control and CRP samples retained color much better as shown below [13].

Figure 6: Browning of MRE Cheese Spread after 6 months at 40°C (adapted from [13])

Based on all these results, 3000 ppm of tocopherol was incorporated in a 79% PP and 20.7% LDPE tocopherol film and used as the active layer in the MRE laminate packaging for Choice Spread.

Despite studies conducted under the umbrella of Controlled Release Packaging, in 2003 the *Food Science and Biotechnology* group reported "sufficient knowledge to characterize the chemical and biological response of antioxidant-impregnated food packaging is lacking. Further research on antioxidant-impregnated food packaging will provide a better understanding of the inhibition of lipid oxidation and will allow the shelf life of food products to be prolonged" [90]. This study focuses on translating the success obtained in CRP technology in previous model system studies to a real food application on a commercial scale. It investigates in the detail the effects of MRE laminate packaging impregnated with tocopherols in inhibiting lipid oxidation in Choice Spread and protecting its color, which has been noted to be limiting its shelf life.

Based on the rationale the main objective of this research is to:

Evaluate the effects of Controlled Release Packaging impregnated with mixed tocopherols on browning and oxidative degradation in a real food system- MRE Choice Spread.

Three sub-objectives were formulated to address the above.

Sub-objective 1: Evaluate the effect of CRP with mixed tocopherols in delaying *browning* associated with oxidation in MRE Choice Spread by monitoring color change.

Sub-objective 2: Evaluate the effect of CRP with mixed tocopherols in stabilizing *lipid oxidation* in MRE Choice Spread in comparison to a control by monitoring oxidation products.

Sub-objective 3: Establish the practical feasibility of CRP with mixed tocopherols by comparing it to *nitrogen flushing* in stabilizing lipid oxidation and delaying browning in MRE Choice Spread. Nitrogen flushing is a common method used to replace oxygen in the internal environment of the package to prevent initiation of oxidation.

These sub-objectives were achieved by conducting a storage study under accelerated conditions as described in Section 4.

4. EXPERIMENTAL DESIGN

4.1. Overall Design of Experiment

4.1.1. Preliminary Study

To determine whether CRP will cause any changes within the Choice Spread system, a preliminary screening study was conducted where the control and CRP packages were placed in a 60°C incubator and at ambient temperature for 6 weeks. During the initial weeks, methods for fat extraction and monitoring lipid oxidation were being developed and tested. Lipid oxidation products- peroxides and conjugated dienes were quantified after 5 and 6 weeks of incubation. Changes in quality attributes were recorded as casual observations by fellow colleagues and tabulated.

4.1.2. Shelf life study

Shelf life of MRE Choice spread packaged in three different types of MRE packaging:

1) Control: Polypropylene (PP)-Low Density Polyethylene (LDPE) films filled with Choice Spread and sealed

2) Nitrogen flushed: PP-LDPE films filled with Choice Spread and flushed with nitrogen gas before sealing and

3) CRP: PP-LDPE films impregnated with 3000ppm mixed tocopherols, filled with Choice Spread and sealed without nitrogen flushing

was studied under accelerated conditions of 40°C and 60°C. 40°C is ambient conditions for MRE food products during distribution and storage in many combat areas, especially

in Middle East areas. While 60°C was shown in the preliminary study to readily cause burning, it was included in the study because foods stored in these areas often reach these stress temperatures during storage, sometimes for extended periods. Both of these elevated temperatures cause deterioration reactions in food to occur at faster rates and allow changes to be seen over shorter periods rather than waiting for the entire shelf life at ambient temperature (around 25°C).

The general equation relating accelerated shelf life temperature to equivalent shelf life at room temperature is:

$$\text{Time at test T} = \text{Time at room temperature} / Q_{10} \exp[(T_{test}-T_{RT})/10]$$

Thus, for 6 weeks at 40°C and 60 °C and assuming that oxidation doubles in rate for every 10 degrees increase in temperature, equivalent shelf life at room temperature would be 17 and 68 weeks, respectively [91, 1].

The steps involved in this study are depicted in the flow diagram shown in Figure 7. Control, CRP and nitrogen flushed samples were placed in incubators set to maintain constant temperatures of 40°C and 60°C. Samples were withdrawn for analyses every week from the 60°C incubator and every 10 days initially, then every two weeks later on from the 40°C incubator. Color of the samples was measured using a colorimeter. Lipids were extracted from Choice Spread and analyzed for oxidation by conjugated dienes, hydroperoxides (primary products) and aldehydes (secondary products) to understand the progression of lipid oxidation reactions. Extraction of lipid from the samples was conducted in duplicate and all analyses were conducted in triplicates to eliminate differences due to sample heterogeneity.

| Received three variables of MRE Choice Spread from Thermo Pac LLC | → | Put one set of each in 60°C incubator and another set in 40°C incubator | → | Removed one pack of each variable from 60°C incubator every 7 days and 40° C incubator every 14 days |

Extracted the fat from Choice Spread using ASE, Solvent Evaporator and Vacuum Oven in duplicate ← Measured color of Choice Spread in pack using a colorimeter & recorded images using a digital camera

Quantified peroxides and aldehydes content in extracted fat using Saftest^{TM} Analysis in triplicates

Quantified conjugated dienes in extracted fat using a UV Spectrophotometer in triplicates
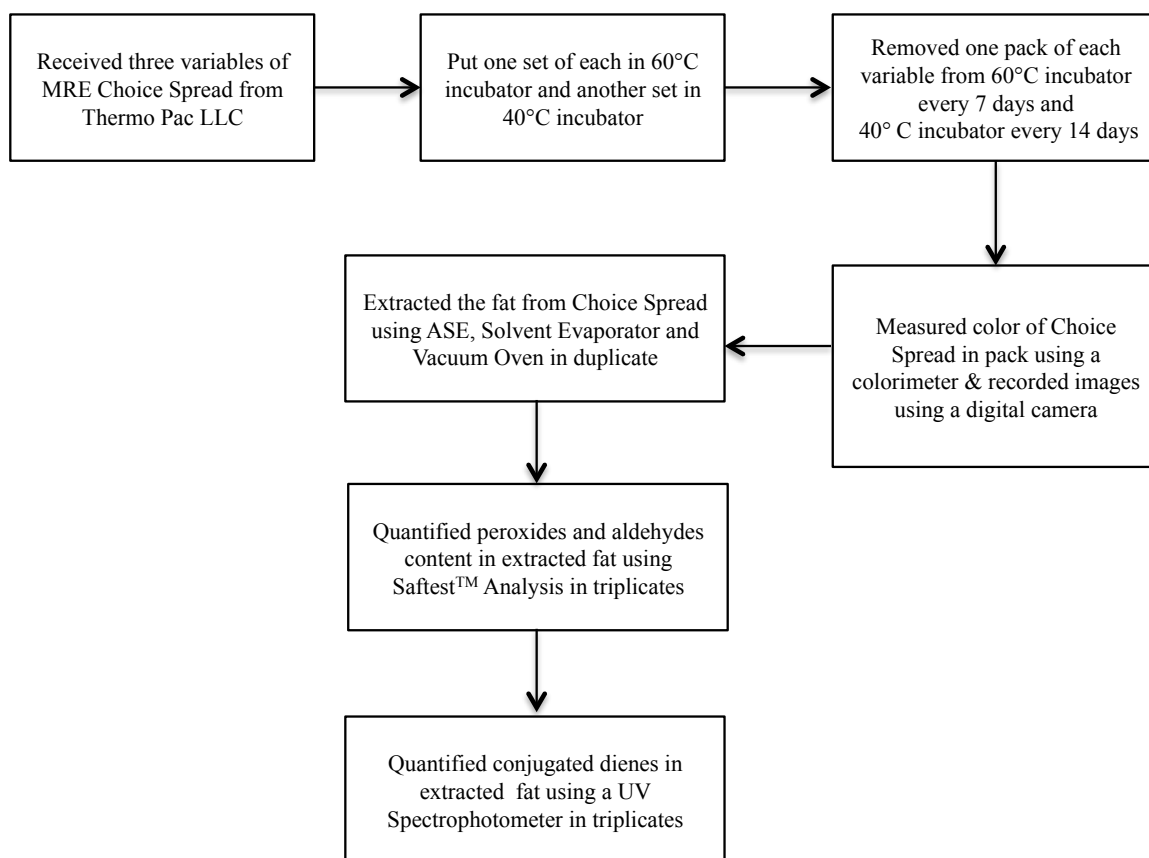
Figure 7: Flow diagram of experimental design

Data obtained for color change and lipid oxidation products was plotted against time to understand the effects of the three kinds of packaging on Choice Spread quality over the study period. In particular, the goal of collecting this data was to determine whether CRP with tocopherols provided benefits over the control or nitrogen flushed samples in delaying browning and oxidative degradation.

4.2. Materials

4.2.1. Mixed Tocopherols

Mixed tocopherols were purchased from Cargill with composition: α-tocopherol: 10%, β-tocopherol: 5%, γ-tocopherol: 65% and δ-tocopherol: 20%.

4.2.2. CRP films and Packages

CRP films composed of 79% Polypropylene (PP) and 20.7% Low Density Polyethylene (LDPE) polymer resins containing mixed tocopherols were extruded using a single screw extruder at Berry Plastics Corporation in Chippewa Falls, Wisconsin, USA. 3000 ppm mixed tocopherols were premixed with the polymer resins, then films were formed by a simple process of melting and mixing the polymer resin at a die temperature of 430°F and screw speed of 70 rpm during extrusion, then casting it (expelling through a die) to form a film. For MRE applications, these films were laminated onto aluminum foil to increase barrier properties (Figure 8). Films were produced on commercial scale to obtain uniformly distributed tocopherol and repeatable film properties. Control films were produced in a similar manner without any tocopherols.
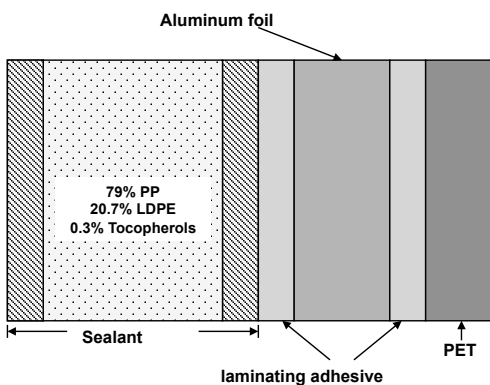

Figure 8: Cross section of CRP Film with mixed tocopherols

Laminated CRP films containing 3000 ppm mixed tocopherols were fabricated into pouches. Each pouch was filled with approximately 40g of commercially processed Choice Spread and sealed at Thermo Pac LLC, Stone Mountain, Georgia. Control packages were produced using the same films without any tocopherols and nitrogen flushed samples were produced by flushing the internal environment of the control package with nitrogen gas before sealing.

4.2.3. MRE Choice Spread

Choice Spread was formulated as specified in Table 1 (cream and cheese powders, canola oil, nonfat dry milk, stabilizers, artificial flavors and seasonings such as onion powder, garlic powder, black pepper, TBHQ) blended and heat processed to ensure a homogenous product at Thermo Pac LLC, Stone Mountain, Georgia. The product was filled into respective packages as described above, then cooled and stored until use in shelf life studies. These samples were provided by NATICK and Berry Plastics Corporation to us for further analysis.

4.3. Experimental Procedures

4.3.1. Lipid Extraction

0.5 g of the choice spread was weighed and mixed with Hydromatrix diatomaceous earth to disperse it homogeneously. Hydromatrix also binds water present in samples, thereby maintaining constant ability of the organic solvents to dissolve lipids. The sample mixture was transferred to an Accelerated Solvent Extractor (ASE) stainless steel cell, tapped into place and any remaining space was filled with Hydromatrix to avoid solvent

channeling. ASE parameters used for extraction of lipids from Choice Spread are presented in Table 2; the solvent was chloroform.

Table 2: Parameters used for ASE extraction of lipids from Choice Spread

Solvent	Chloroform
Temperature	40°C
No. of cycles	2
Purge time	180 sec
Static time	6 min

Solvent plus extracts flowing out of the extractor cell were collected in 250 ml bottles sealed with serum caps, then transferred to pre-weighed round bottom flasks and placed on a rotary evaporator. Evaporation of chloroform was completed in 15 minutes under vacuum, yielding a film of yellow color viscous liquid. The round bottom flask was then placed in a vacuum oven, covered with a Kim wipe to prevent any contamination from the external environment and then dried under vacuum for one hour to remove all traces of solvent and moisture. The final lipid extract obtained was golden yellow in color. The flask with the lipid extract was weighed to calculate the amount of fat recovered from the sample and ensure that there has been greater than 90% recovery.

4.3.2. Analyses of oxidized lipids

Identifying a specific path for oxidation in complex food matrices is difficult because a number of reactions occur simultaneously and antioxidants could be protective against one or more of these reactions. Thus, literature has suggested that monitoring both

primary (peroxides, conjugated dienes) and secondary oxidation products (carbonyls) will provide a better picture of the effect of antioxidant systems on the food product [32, 18].

4.3.2.1. Conjugated dienes

20 μl of each of the various samples were transferred to test tubes. 1800 μl of isooctane was added to each test tube and vortexed. The sample was transferred to a quartz cuvette and the absorbance at 234 nm was read in a Varian Cary-50 Bio UV-Vis Spectrophotometer. All analyses were conducted in triplicates.

Optical absorbance was converted to mM conjugated diene by Beer's Law, using the molar extinction coefficient of 29,500 l/mol-cm for iso-octane. Values were further converted to mmol/mol triacylglycerol (TAG), the major component of the extracts, using an average molecular weight of 885 for TAGs of mixed oleic and linoleic acid. The calculations involved are as follows.

- OD = Absorbance at 234 nm read from UV-Vis Spectrophotometer
- According to Beer-Lamberts Law, O.D. = E * Conc * l

  where E is molar extinction coefficient, E = 29500 l/mol-cm in iso-octane and l is cell path length that is 1 cm

- Concentration (A) = OD/E*l $A = \dfrac{OD}{29,500}$ mol/l or mmol/ml solution

- In 2.5 ml of sample, X g of extracted fat was dissolved,

  In 20 μl of sample, $Y = \dfrac{X\,g * 20\,\mu l}{2.5\,ml}$ g of fat is present

- As 20 μl of sample was added to 1.8 ml in the analysis, total = 1.82 ml

  Wt of oil/ ml reaction solution = $\dfrac{Y\,g\,fat}{1.82\,ml\,solution}$

- As average molecular weight of mixed 18:1/18:2 TAG= 885 g

- Z= $\dfrac{(Y/\ 1.82)}{885}$ mol TAG/ml solution

- Concentration (mmol CD/mol TAG)= $\dfrac{A\ \text{mmol}}{Z\ \text{mol TAG}}$

4.3.2.2. SafTest^{TM} Analysis for lipid hydroperoxides

Lipid hydroperoxides were determined using the PeroxySafe Test (standard version) from MP Biomedicals, Solon, OH. 25 µl of the various samples (lipid extract dissolved in the SafTest^{TM} Prep Reagent at different dilutions) were transferred to test tubes. 1000 µl of Reagent A, 100 µl of Reagent B and 160 µl of Reagent C were then added to all the test tubes sequentially. De-ionized water was used as the blank. All tubes were capped, vortexed and incubated for 15 minutes at room temperature. Sample tubes were then placed in the SafTest optical analyzer, the absorbance at 570 nm was recorded and results were reported in terms of (Meq/Kg sample) based on comparison to a cumene hydroperoxide standard curve. These values were converted to mmol/mol TAG using an average molecular weight of 885 for TAGs with linoleic acid and oleic acid as the major components. All measurements were conducted in triplicates.

The calculations involved are as follows.

- A= Concentration obtained in Meq LOOH/Kg sample from instrument, where sample is 25 µl of SafTest^{TM} Prep Reagent with trace amounts of LOOH dissolved

- As average molecular weight of mixed 18:1/18:2 TAG= 885 g

  1 Kg fat = 1000 g fat, In 1 Kg of fat= (1000/885) = 1.13 mols TAG

- Sample dilution = weight of fat/ml of SafTest^{TM} Prep Reagent

- Concentration (mmol LooH/mol TAG)= $\dfrac{\text{A * Sample Dilution}}{1.13}$

4.3.2.3. SafTest^{TM} Analysis for lipid aldehydes

Lipid aldehydes were determined using the AlkalSafe Test from MP Biomedicals, Solon, OH. 70 µl of samples (lipid extract dissolved in the SafTest^{TM} Prep Reagent at different dilutions) at different dilutions were transferred to test tubes. 1000 µl of Reagent A and 250 µl of Reagent B were then added to all the test tubes. De-ionized water was used as the blank. The tubes were capped, vortexed and incubated for 20 minutes at room temperature; the tubes were then transferred to the SafTest optical analyzer, the absorbance at 550 nm was measured and results were reported as nmol/ml, calculated from a standard curve prepared from the kit standards. All measurements were conducted in triplicates. The calculations involved are as follows.

- A= Concentration obtained in nmol/ml solution from instrument

- Total reaction solution= 70 µl sample + 1000 µl A + 250 µl B

$$= 1320\ µl = 1.32\ ml$$

In total reaction solution, A * 1.32 nmols of aldehydes is present

- In 2.5 ml of Sample, X g of extracted fat was dissolved,

In 70 µl   Y= $\dfrac{\text{X g * 70 µl}}{2.5\ ml}$ g of fat is present

- Hence   B= $\dfrac{\text{A* 1.32 nmol of aldehydes}}{\text{Y g of fat}}$

$$= \dfrac{(A*1.32/1000000)\ \text{mmol of aldehydes}}{\text{Y g of fat}}$$

- 1 kg fat contains 1.13 mols TAG, 1 g fat contains (1.13/1000) mols TAG

- Concentration (mmol /mol TAG) = $\dfrac{B \text{ mmol of aldehydes}}{(1.13/1000) \text{ mol TAG}}$

4.3.3. Color

The color of the Choice Spread was measured using a Konica Minolta Colorimeter to quantify the color change (in terms of L^* a^* b^* values) that occurred during storage. L^* represents the lightness value and ranges from 0-100, a^* represents red vs green color and ranges from positive (red) to negative (green) values and b^* represents the yellow (positive) vs blue (negative) color, also ranging from positive to negative values as shown in Figure 9. On opening the package, six sets of readings were taken in different positions to cover the entire surface area of Choice Spread.

Figure 9: L^* a^* b^* color scale (adapted from www.hunterlab.com)

5. RESULTS

5.1. Lipid Extraction

The recovery of lipid from samples incubated at 40°C and 60°C is presented in Figures 10 and 11 respectively. As more than 90% of the lipid was extracted in almost all cases, it validates the parameters chosen in the ASE (Section 4.3.1) and shows that very little of the oil was bound to molecules or trapped in matrices. Contrary to expectations, there was a trend toward increased oil recovery with extended incubation time and yields were generally highest for the nitrogen-flushed samples. This is consistent with increased oiling off or phase separation noted with the samples and it also is evidence that oxidation was not polymerizing the lipid or crosslinking it to macromolecules.
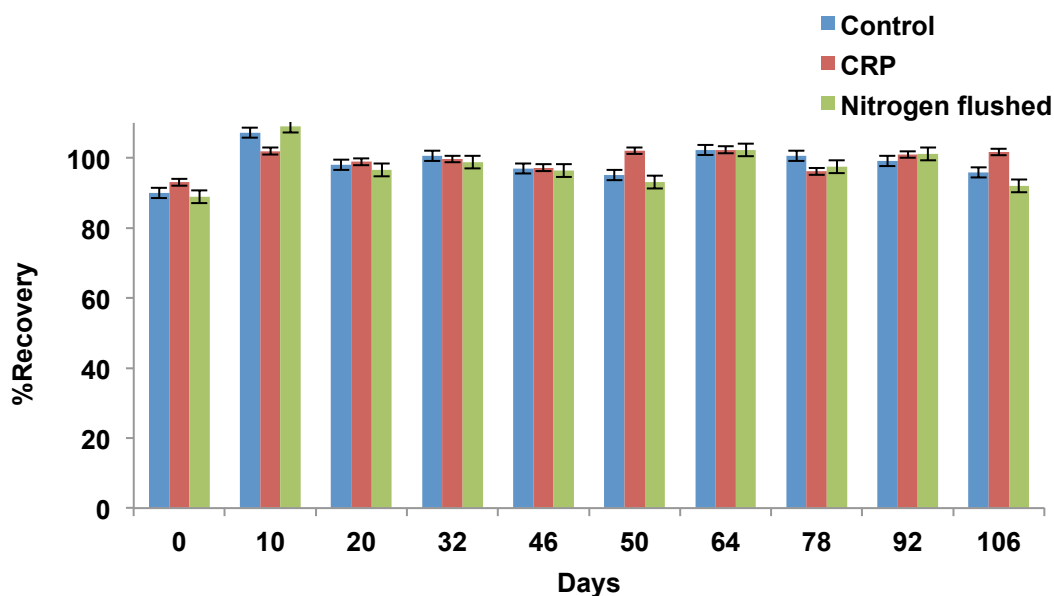

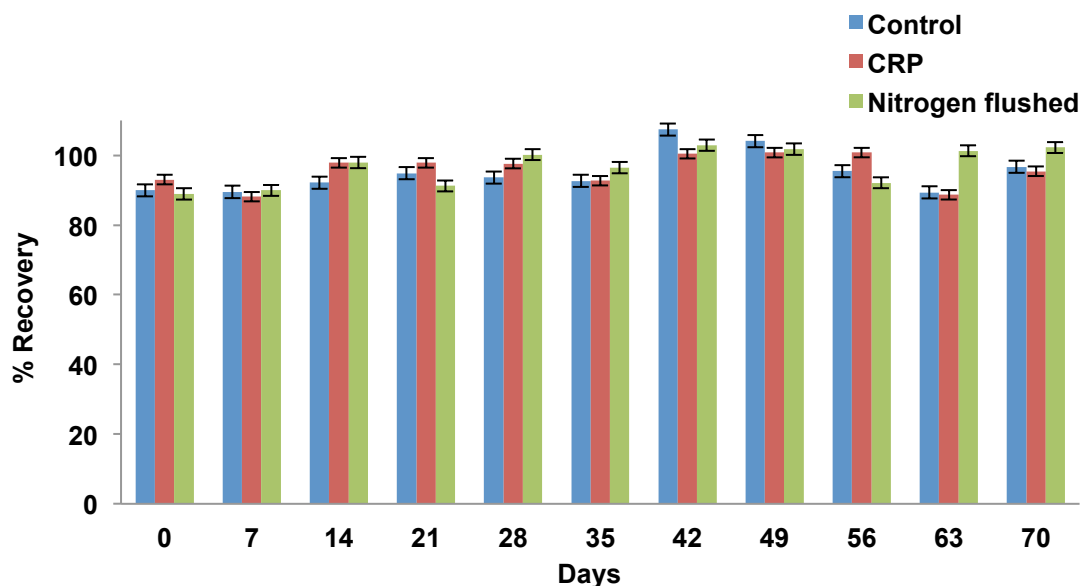Figure 10: Recovery of lipid from samples incubated at 40°C

Figure 11: Recovery of lipid from samples incubated at 60°C

5.2. Preliminary Study

5.2.1. Observed Sensory Characteristics

A preliminary screening study was conducted to gauge the magnitude of antioxidant effects of CRP on Choice Spread and provide guidance for the main storage study. Samples of Choice Spread in MRE packages composed of CRP films were incubated for 5 and 6 weeks at ambient temperature and 60°C and analyzed for rancidity and lipid oxidation. Informal sensory evaluation (untrained panel of lab members) found that color changes (browning) and texture stiffening associated with rancidity were lower in CRP packages than with control packaging (Table 3). These characteristics were consistent with observations made in MRE processed cheese packed in CRP-tocopherol packaging (unpublished data, Yam-Schaich group at Rutgers University and Natick RD&E Center).

Table 3: Observed sensory characteristics in preliminary study

Samples	Color	
	Control	CRP
Ambient Temperature: Week 5	Cream yellow	Cream yellow
Ambient Temperature: Week 6	Cream yellow	Cream yellow
60°C: Week 5	Medium brown	Lighter brown
60°C: Week 6	Dark Brown	Medium Brown
	Texture	
Ambient Temperature: Week 5	Firm	Firm
Ambient Temperature: Week 6	Firm	Firm
60°C: Week 5	Semi-firm	Mostly firm
60°C: Week 6	Semi-firm	Mostly firm
	Odor	
Ambient Temperature: Week 5	Creamy/cheesy	Creamy/cheesy
Ambient Temperature: Week 6	Creamy Cheesy	Creamy/cheesy
60°C: Week 5	Burnt odor	Burnt odor
60°C: Week 6	Strong burnt odor	Strong Burnt odor

Differences between the treatments became more evident with even longer storage. After 14 weeks of storage, the CRP samples maintained their natural flavor while the control samples had pronounced off-flavors (Table 4). Even more striking, the control samples developed a notable sandy grittiness while samples with CRP packaging retained a

smooth, creamy texture (Figure 12). This provides clear evidence that CRP has positive

effects on sensory qualities of Choice Spread, even if mechanisms are not identified.

Table 4: Sensory changes in Choice Spread after prolonged storage at 40°C and 60°C

	Color		Texture		Flavor
	40°C	**60°C**	**40°C**	**60°C**	**40°C & 60°C**
Control	Cream yellow	Burnt brown	Very gritty	Firm, crumbly & dry	Clear off-flavor & burnt odor
CRP	Cream yellow	Burnt brown	Hint of grittiness	Smooth & moist	Cheesy/ creamy ideal flavor & burnt odor

A: 60°C Control   **B: 60°C CRP**   **C: 40°C Control**   **D: 40°C CRP**

Figure 12: Choice Spread samples after 14 weeks storage

5.2.2. Lipid oxidation

5.2.2.1. Conjugated Dienes

Conjugated dienes contents of lipid extracts diluted in iso-octane were determined from

the absorbance at 234 nm. Conjugated dienes were higher in control samples than in the

samples packaged in tocopherol-CRP for both Week 5 and Week 6; the protection was

more pronounced at 60°C than at ambient temperature (Table 5). These results demonstrate that CRP with tocopherol was able to slow oxidation and decomposition in the Choice Spread.

Dienes content in Week 6 samples were higher than in Week 5 at ambient temperature, indicating that oxidation was still increasing. In contrast, conjugated dienes decreased in samples at 60°C, more in the controls than in CRP samples. This most likely results from thermal decomposition of the hydroperoxides at the elevated temperature, but also suggests that the tocopherols released from CRP packaging may stabilize intermediates and prevent loss of the diene structure, e.g. by donating a hydrogen to an alkoxyl radical to form a hydroxyl lipid (lipid alcohol), thereby preventing scission of the alkoxyl radicals to aldehydes.

Table 5: Conjugated dienes in lipid extracts from Choice Spread incubated for 5 and 6 weeks

Conjugated Dienes (µM)				
Dilution	**Ambient temperature**		**60°C**	
Week 5	**Control**	**CRP**	**Control**	**CRP**
1:20	7.46	7.06	17.48	8.44
1:40	3.56	3.60	6.68	4.53
1:80	1.66	1.49	4.08	2.52
Week 6				
1:20	8.30	8.29	10.81	8.27
1:40	6.08	4.78	4.74	3.37
1:80	1.37	1.21	2.22	2.02

5.2.2.2. Hydroperoxides

In contrast to the conjugated dienes, peroxide values of spread packaged in CRP with tocopherols were higher than the control and they decreased from Week 5 to Week 6. While this may appear to indicate lack of antioxidant protection by the CRP packaging, it may also indicate that the hydroperoxides are decomposing in the control packaging while they are being stabilized in the CRP packaging. The latter can in fact be an antioxidant action since the alkoxyl radicals generated in hydroperoxide decomposition are active propagators of lipid oxidation chains. This possibility can be verified in future studies by analyzing carbonyl products that result from hydroperoxide decomposition.

Table 6: Peroxide contents in lipid extracts from Choice Spread incubated for 5 and 6 weeks

Peroxide concentration (absorbance at 570 nm)				
Dilution	Ambient temperature		60°C	
Week 5	Control	CRP	Control	CRP
1:20	0.185	0.240	0.062	0.071
1:40	0.154	0.162	0.048	0.061
1:80	0.131	0.150	0.0352	0.018
Week 6				
1:20	0.042	0.0312	0.0015	0.0042
1:40	-*	0.0102	-*	-*
1:80	-*	-*	-*	-*

[1]*-below detection limit

5.3. Shelf life Storage Study

The preliminary study indicated that the CRP does interact with Choice Spread in a way that protects sensory attributes and inhibits lipid oxidation. Based on these observations, a more extensive shelf life study was designed to elucidate and quantify the effect of controlled release of tocopherol in slowing down oxidative degradation and browning and to compare its effect to nitrogen flushing of the packaged food. Choice Spread in three different kinds of packages were incubated at 40°C and 60°C (accelerated conditions). The samples incubated at 60°C were analyzed every 7 days and those incubated at 40°C were analyzed every 10 days initially and 14 days later on since the reactions were slow.

5.3.1. Sub-objective 1

To address the first sub-objective, browning in Choice Spread samples was compared in control packaging vs CRP with tocopherols at both 40°C and 60°C.

5.3.1.1. 40°C Storage Study

Negligible change in lightness as represented by the L^* values occurred when Choice Spread was stored at 40°C for 32 days; however, with longer incubation there was a slight drop in L^* consistent with some browning (Figure 13). Color differences between types of packaging were negligible.

Similar results were evident in b^* values, which remained constant for the first 20 days the decreased about two points (Figure 14). This loss of yellow is also consistent with browning. Again, differences between types of packaging were negligible.

In contrast, a* values (redness) showed considerable variability both over time and between packaging (Figure 15). Red color appeared to be cycling, much as lipid oxidation products do and may reflect some oxidation of pigments. At all times, a* values were higher for the CRP packaging, indicating greater protection of color.
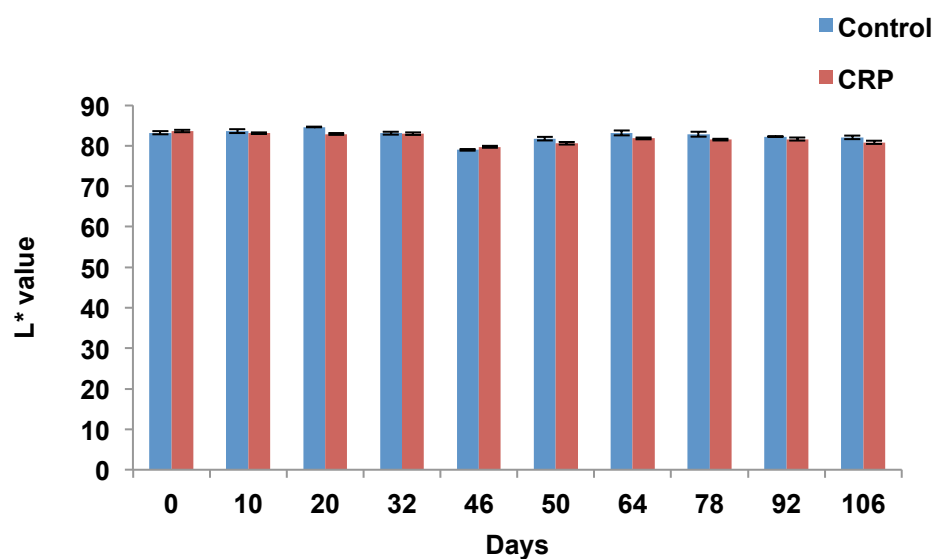

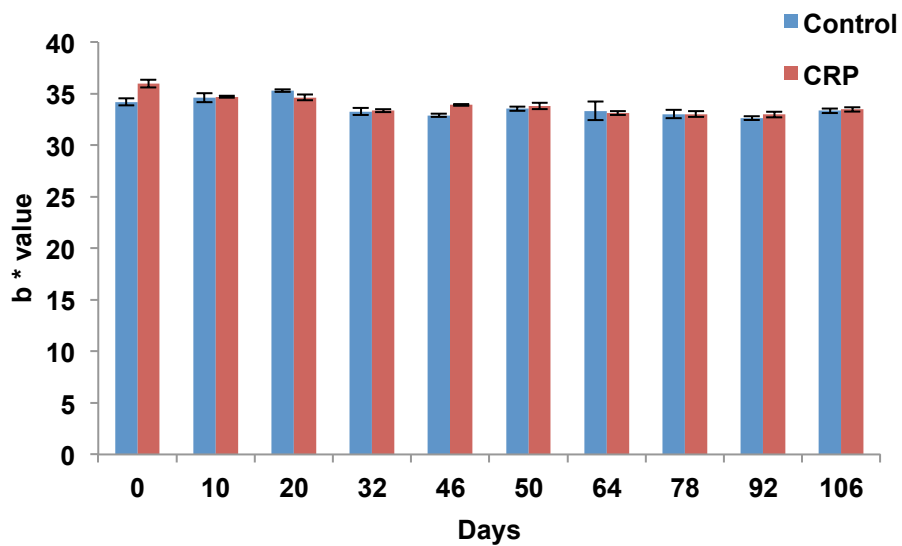Figure 13: Change in L* value of Choice Spread over 106 days at 40°C
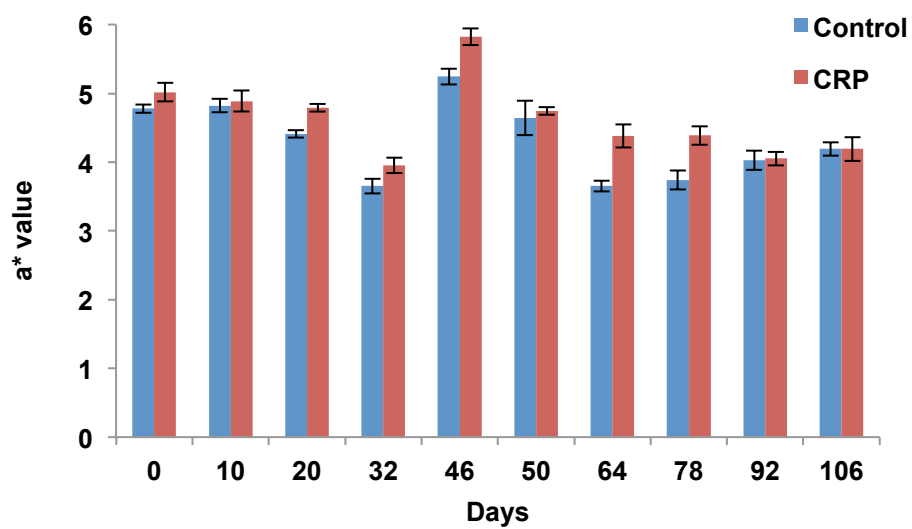
Figure 14: Change in b* value of Choice Spread over 106 days at 40°C

Figure 15: Change in a* value of Choice Spread over 106 days at 40°C

5.3.1.2.   60°C Storage Study

At 60°C, the browning reactions commenced almost immediately during storage of Choice Spread and samples increased in darkness and redness over time.

L^* values decreased continually over the entire incubation time, ending up at less than half the starting values. By the end of the storage period, the samples definitely had a burned appearance and the low L values reflect this. Once again, b^* values followed this pattern, although they remained elevated for 28 days before declining. In contrast, a^* values increased for 35 days, then decreased; at all times a^* values for the CRP samples were higher than the control, supporting a role for tocopherols released from CRP in protecting food color. That L^* values did not change with packaging suggests that the most likely point of effect is inhibiting oxidation of plant pigments rather than blocking non-enzymatic Maillard browning.
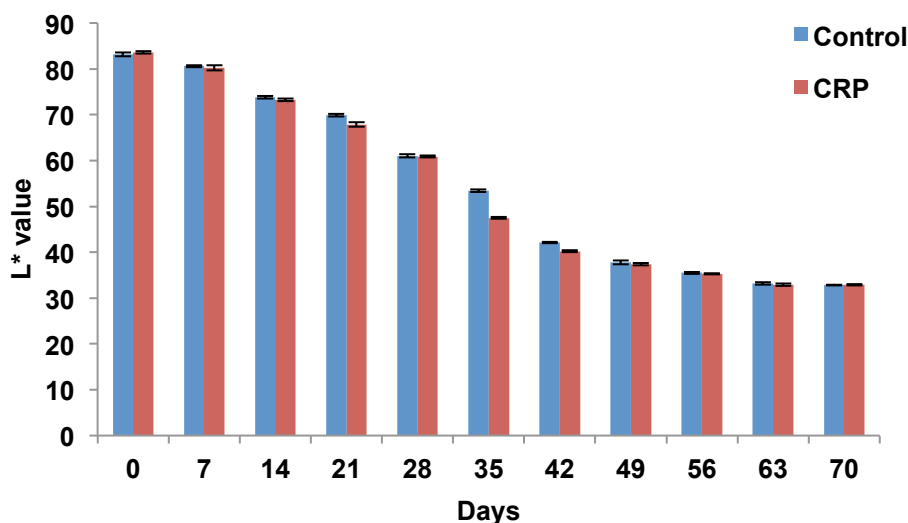

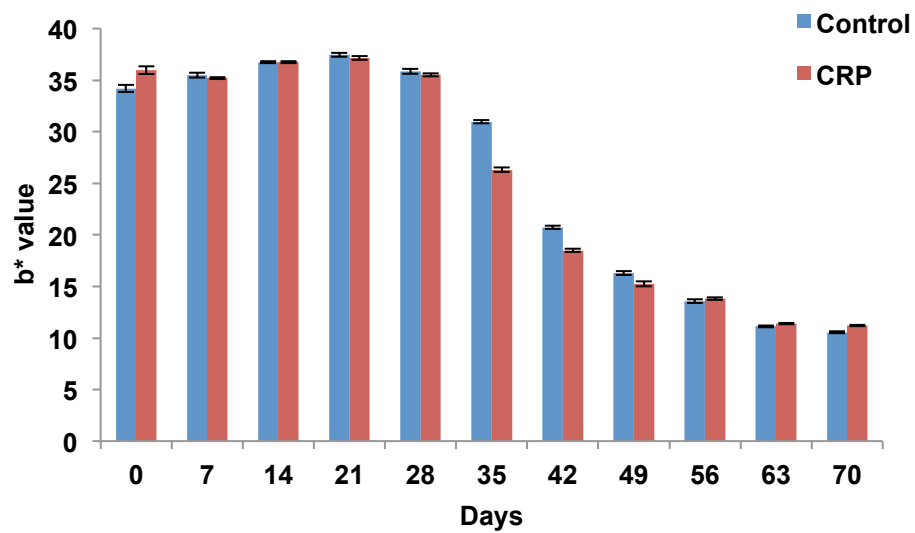Figure 16: Change in L^* value of Choice Spread over 70 days at 60°C

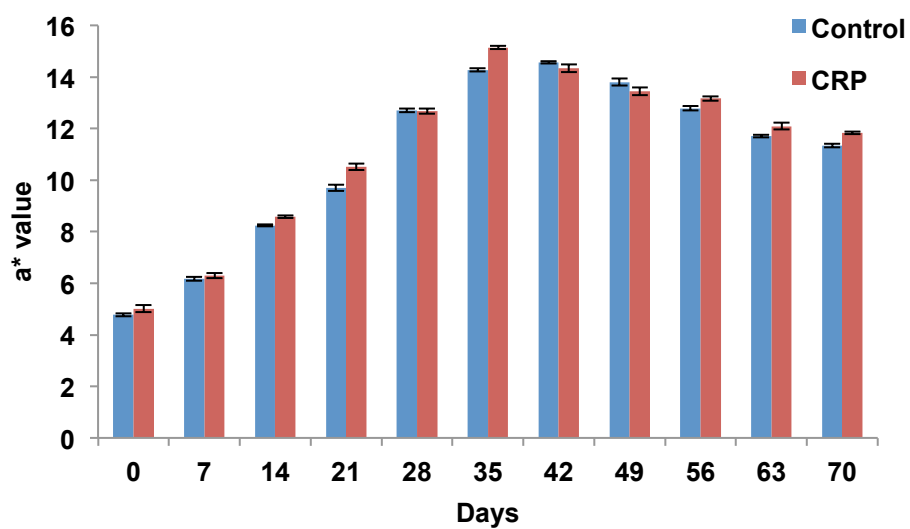Figure 17: Change in b* value of Choice Spread over 70 days at 60°C

Figure 18: Change in a* value of Choice Spread over 70 days at 60°C

5.3.2.   Sub-objective 2

To address the second sub-objective, lipid oxidation products in control samples and CRP packages with tocopherols were compared at both 40°C and 60°C.

5.3.2.1.   40°C Storage Study

Peroxide values remained low (generally <1) throughout the storage period at 40°C. For both controls and CRP samples, hydroperoxides initially increased, reached a peak, then decreased to very low values (Figure 19). PVs in control samples peaked earlier than in CRP samples and the peak is lower than for CRP (1 for controls vs 2 for CRP). While these results could be construed as showing ineffectiveness of CRP in these systems, they also may indicate protection in slowing both the formation and decomposition of lipid hydroperoxides. Another possibility that cannot be eliminated is that the high PV for CRP at 55 days is an analytical error.
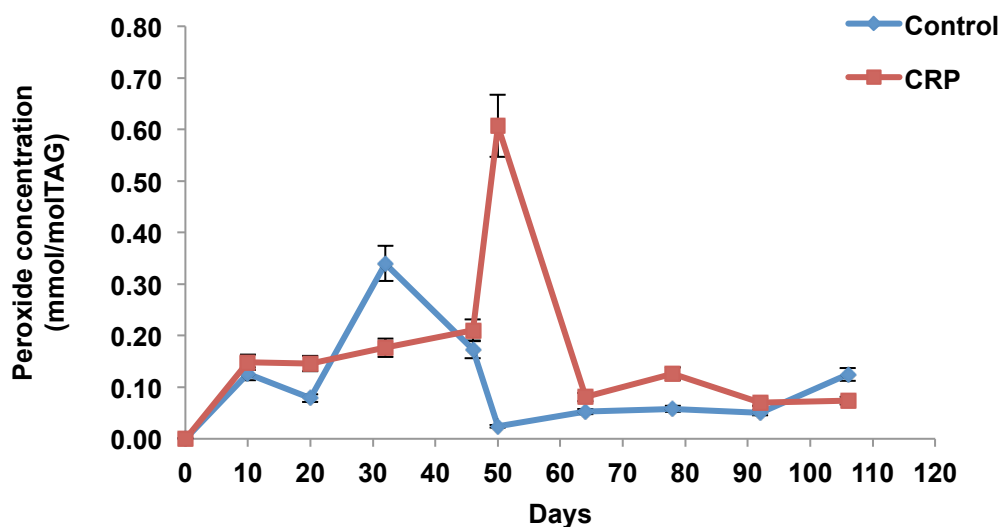

Figure 19: Change in peroxide concentration over 106 days at 40°C

Conjugated dienes (Figure 20) remained within a range and aldehydes (Figure 21) remained at very low values throughout the incubation period and both showed no differences between packaging.
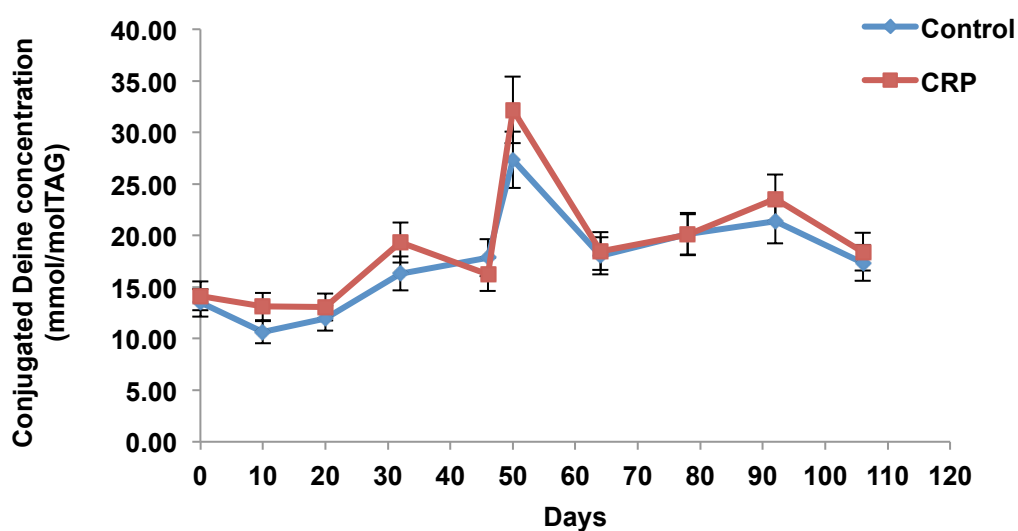

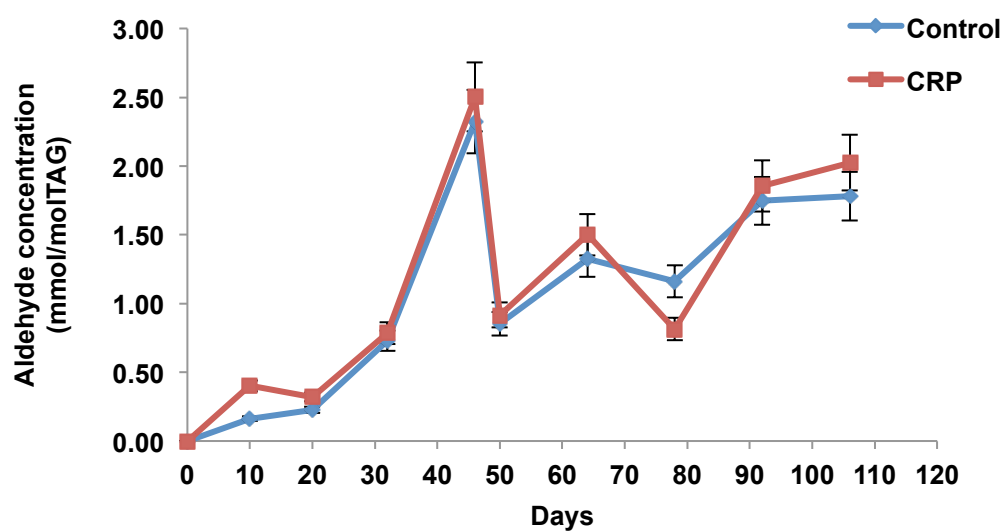Figure 20: Change in conjugated dienes concentration over 106 days at 40°C

Figure 21: Change in aldehydes concentration over 106 days at 40°C

5.3.2.2. 60°C Storage Study

Marker compounds such as peroxide, conjugated dienes and aldehydes concentrations do not have fixed profiles since their production and degradation into other compounds is a continuous process in complex food matrices. However, following these three product classes together can sometimes reveal shifts between stages of lipid oxidation, e.g. decomposing one product into another.

Results showed no substantial difference between packaging for any lipid oxidation product (Figures 22-24). This was disappointing since it did not reproduce earlier observations of CRP protection. A surprising and interesting point, however, was that expected patterns of lipid oxidation development were not observed with these samples and conditions. Conjugated dienes, the first stable chemical change during lipid oxidation, were constant until 20 days and then began increasing to a peak at 50 days (Figure 22).
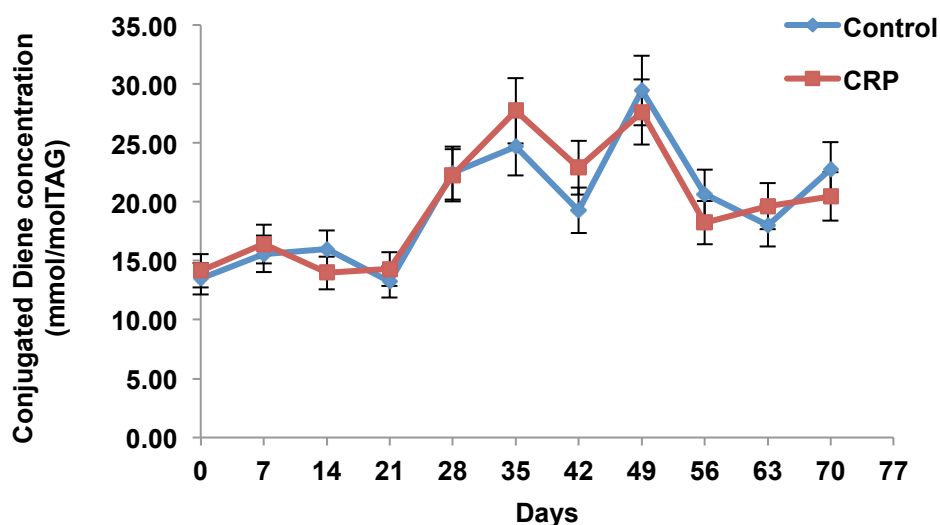

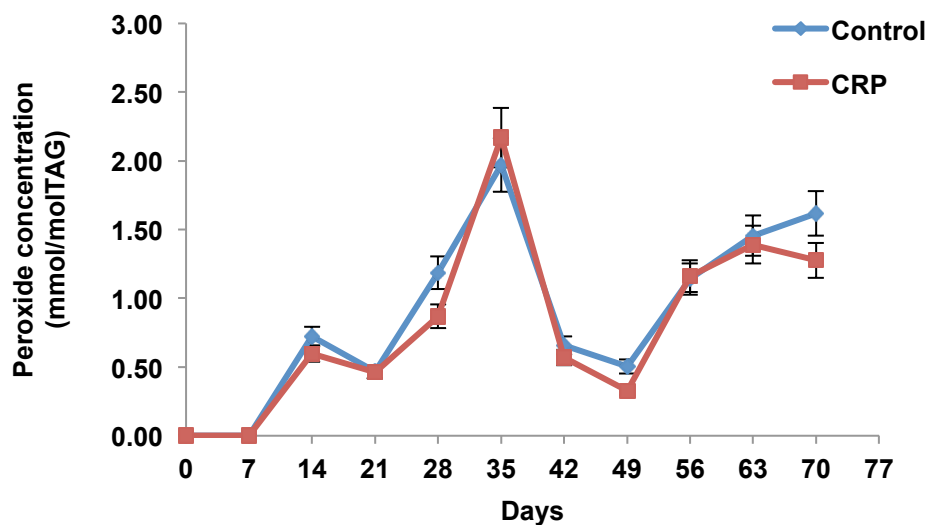Figure 22: Change in conjugated dienes concentration over 70 days at 60°C

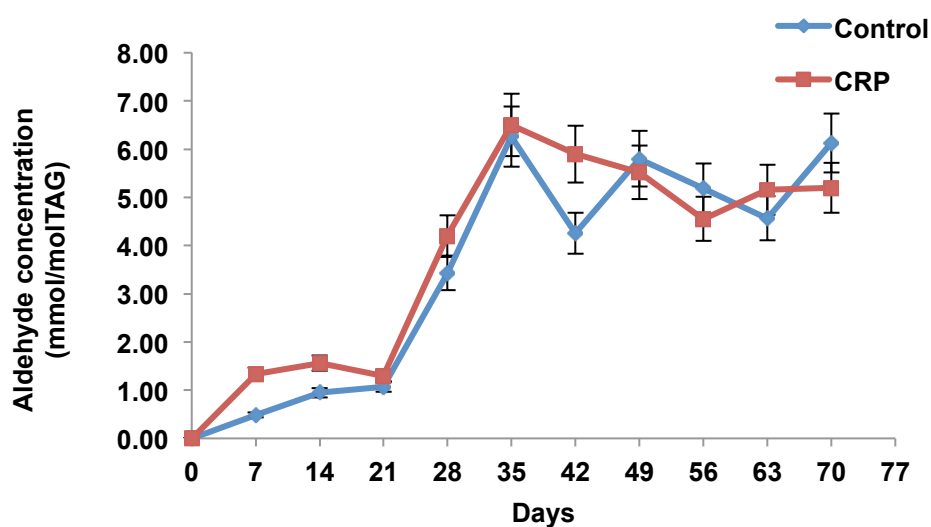Figure 23: Change in peroxide concentrations over 70 days at 60°C

Figure 24: Change in aldehydes concentration over 70 days at 60°C

Hydroperoxides developed in parallel with conjugated dienes, but in this study they peaked earlier, at 35 days, dropped to low values and then began to dramatically increase again after 50 days. Aldehydes, primarily generated by chain scissions on either side of

alkoxyl radicals, usually lag behind hydroperoxides decomposition. In these samples, aldehydes paralleled hydroperoxides in production but remained elevated when hydroperoxides decreased.

The differences in levels of oxidation products (20-30 for conjugated dienes, ~2 for hydroperoxides and ~6 for aldehydes, expressed as mmol/mol TAG for each) provides some perspective for these product patterns and some insight into oxidation processes in the Choice Spread:

a) That conjugated dienes are 10-fold greater than hydroperoxides and remain elevated after hydroperoxides decomposition equally in both types of packaging indicates that there are strong lipid oxidation initiators that are not affected by antioxidants in either the formulation or delivered from the packaging.

b) That conjugated dienes are 10-fold greater than hydroperoxides in concentration and remain elevated even when hydroperoxides decompose indicates that the Choice Spread may have strong peroxide decomposers (other than heat) and also strong radical quenchers that convert intermediate alkoxyl radicals to lipid alcohols that retain conjugated diene structure (alcohols are not normally detected in lipid oxidation analyses).

c) That aldehydes parallel hydroperoxides (LOOH) formation and remain elevated after LOOH decomposition suggests that aldehydes do indeed derive from LOOH decomposition and that there is a constant bleed of alkoxyl radicals that undergo scission and escape into this pathway before contacting antioxidants.

d) That some inhibition of all lipid oxidation parameters by CRP begins to show up only after 60 days of incubation suggests that either endogenous TBHQ is becoming exhausted, that tocopherols have finally accumulated to sufficient levels in the Choice Spread or reached critical molecular targets to have distinguishable effects, or a combination of the two. These results also suggest that antioxidant CRP may in fact exert its greatest value in long term storage and at elevated temperatures.

5.3.3.   Sub-objective 3

The aim of this objective was to compare the effectiveness of CRP with tocopherols versus nitrogen flushing in inhibiting lipid oxidation in MRE Choice Spread to estimate practical feasibility in terms of functionality.

5.3.3.1.   40°C Storage Study

Change in color: There is negligible change in color of Choice Spread when stored at 40°C for 106 days: L^* values were essentially constant over time and packaging (Figure 25), b^* values decreased very slightly and slowly over time but showed no difference between types of packaging (Figure 26) and a^* values varied but showed no clear pattern of change with incubation time or packaging (Figure 27).
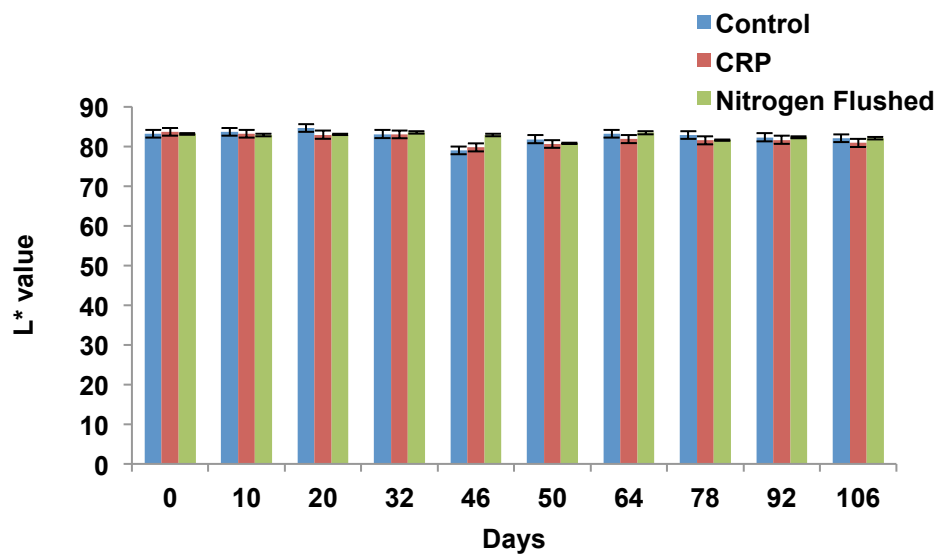
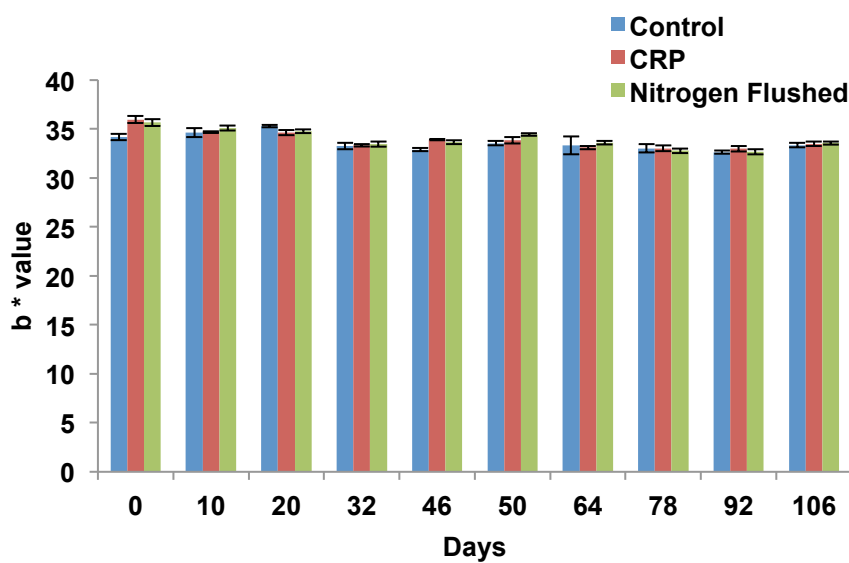Figure 25: Change in L* value of Choice Spread over 106 days at 40°C

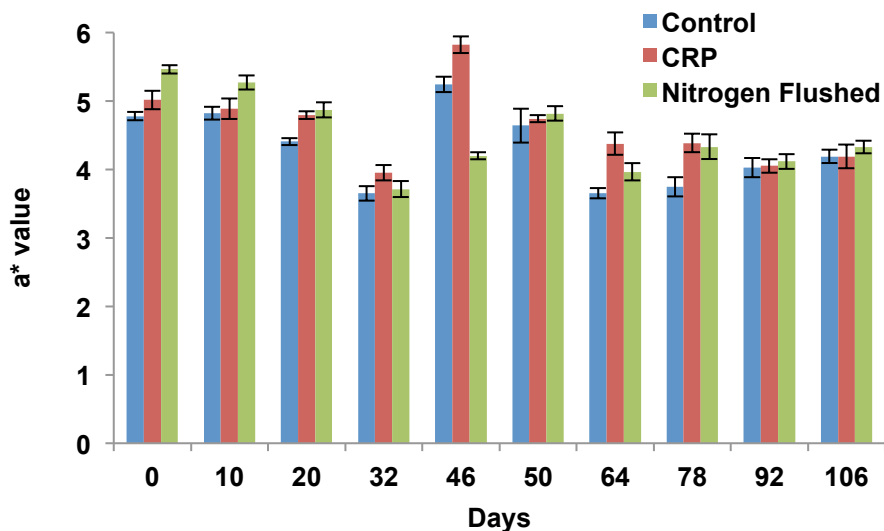Figure 26: Change in b* value of Choice Spread over 106 days at 40°C

Figure 27: Change in a* value of Choice Spread over 106 days at 40°C

Change in concentration of lipid oxidation products: As was noted for the control-CRP comparison above, oxidation at 40°C remained low throughout the storage period for all parameters and differences between types of packaging were not notable (Figures 28-30). However, there was a trend for nitrogen flushed samples to have slightly higher oxidation than the control or CRP packaging. If this effect can be reproduced in replicate studies, it may be explained by inadequate nitrogen flushing, extra exposure and handling during flushing, or residual oxygen in the nitrogen gas.
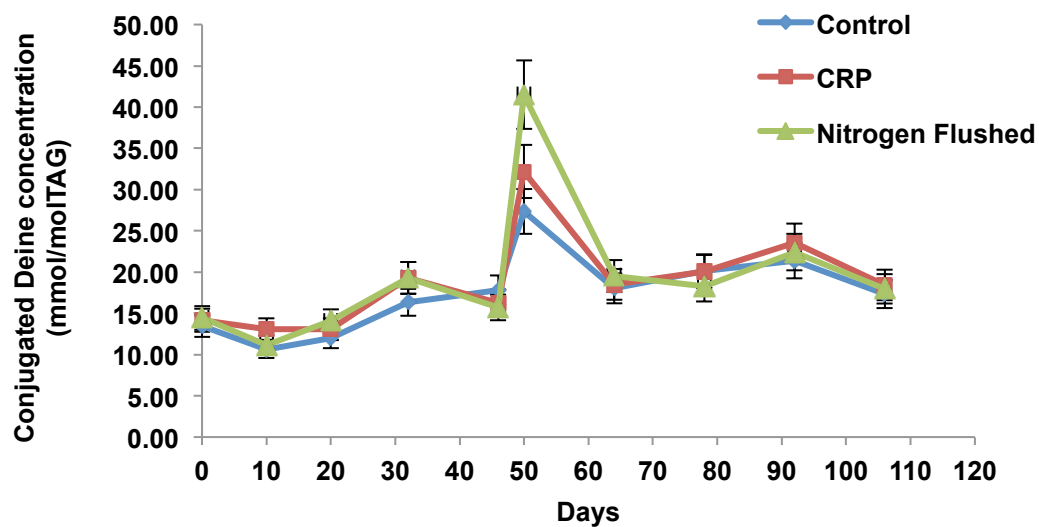
Figure 28: Changes in conjugated dienes concentrations over 106 days at 40°C
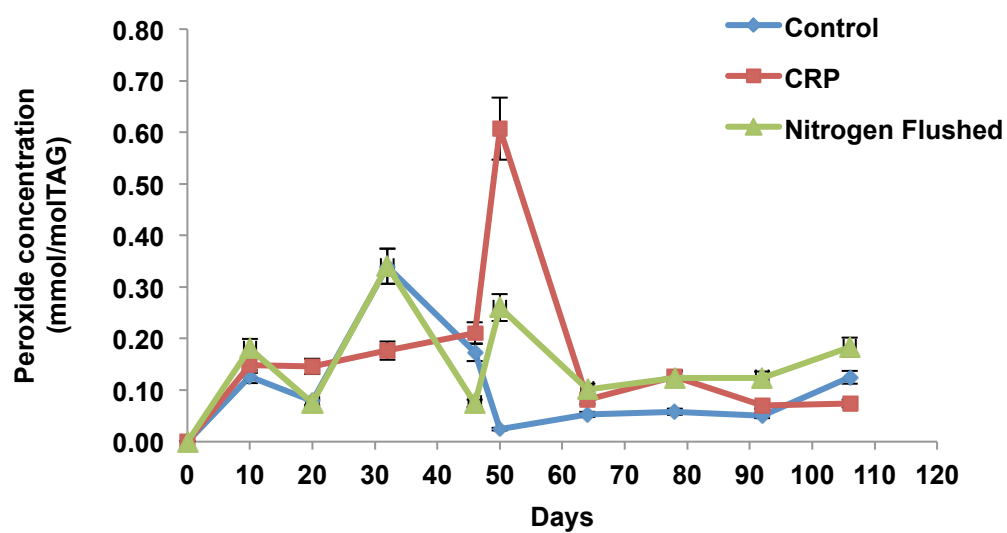
Figure 29: Changes in peroxide concentration over 106 days at 40°C
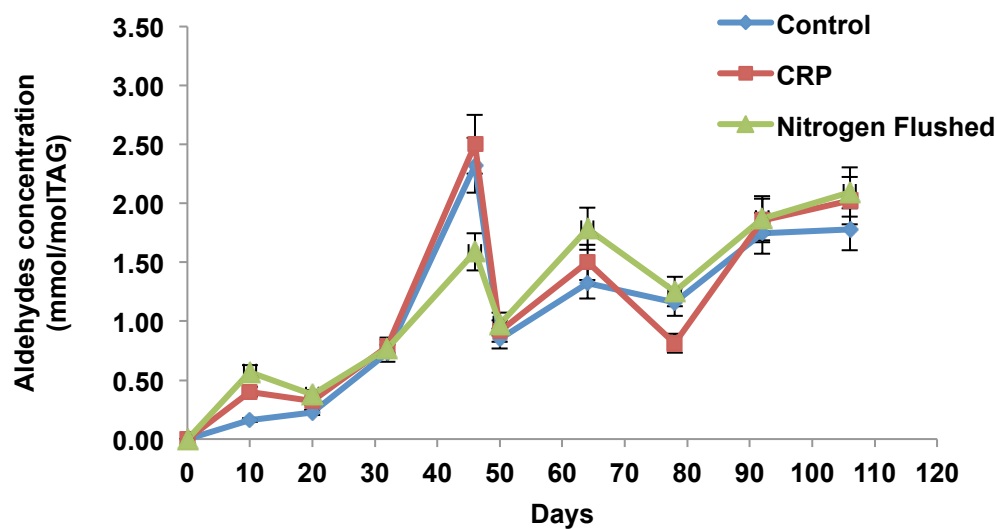
Figure 30: Changes in aldehydes concentration over 106 days at 40°C

5.3.3.2. 60°C Storage Study

Change in color: CRP with tocopherol did show some beneficial effect in color retention in comparison to nitrogen flushed samples during storage at 60°C (Figures 32-34). Color retention was most notable in a^* values (red tones, Figure 34) and b^* values (yellow tones, Figure 33), with less difference in overall lightness (L^*, Figure 32). Lightness and yellow decreased during storage, while red tones increased in parallel with, but not totally attributable to, browning.
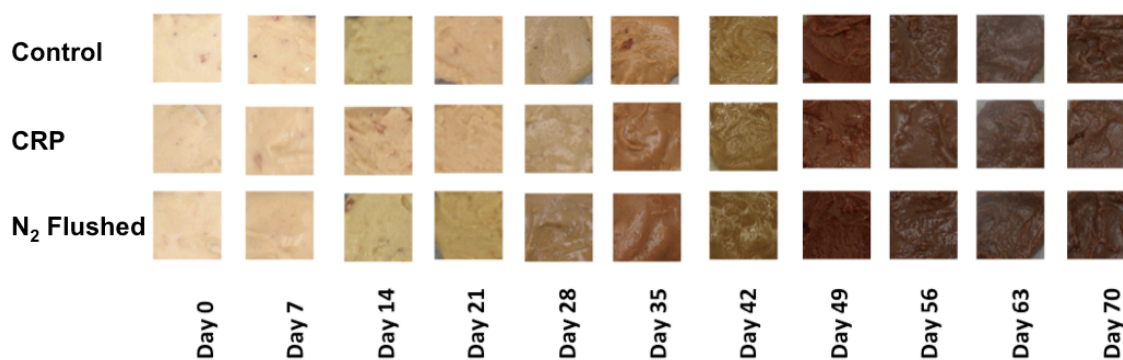
Figure 31: Browning at 60°C
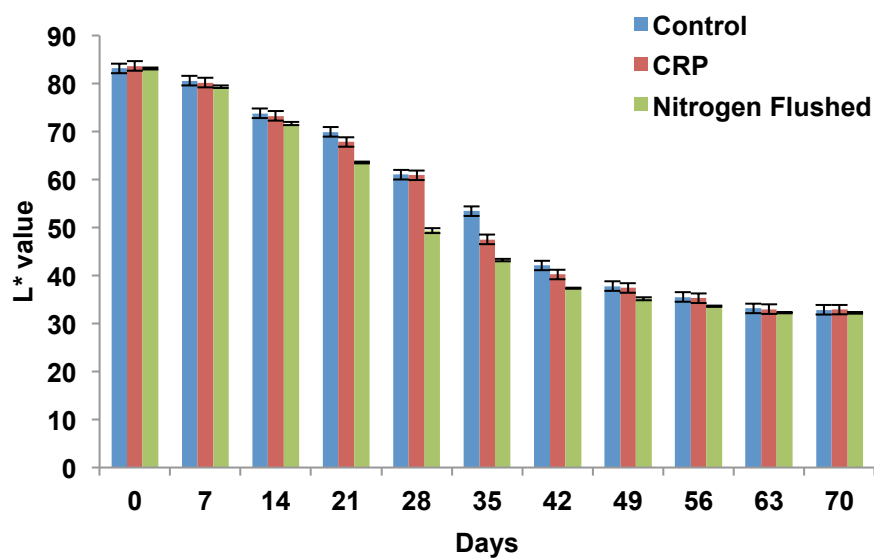
Figure 32: Change in L^* value of Choice Spread over 70 days at 60°C
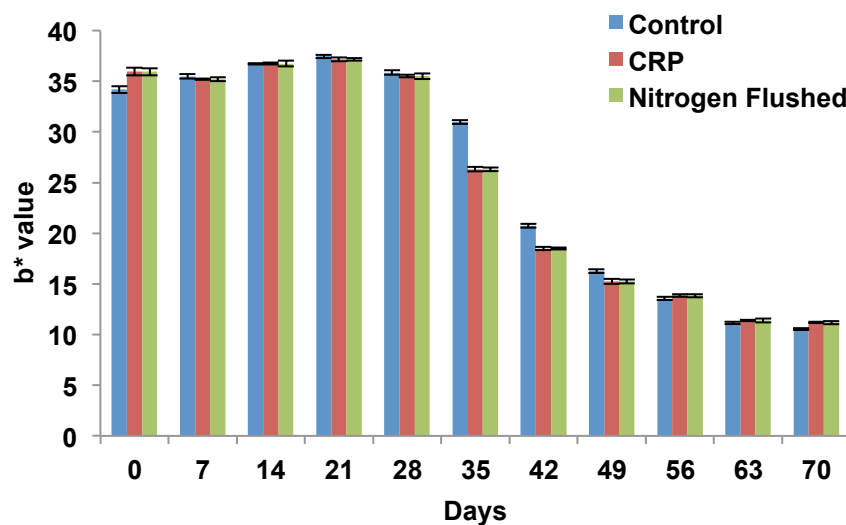
Figure 33: Change in b* value of Choice Spread over 70 days at 60°C
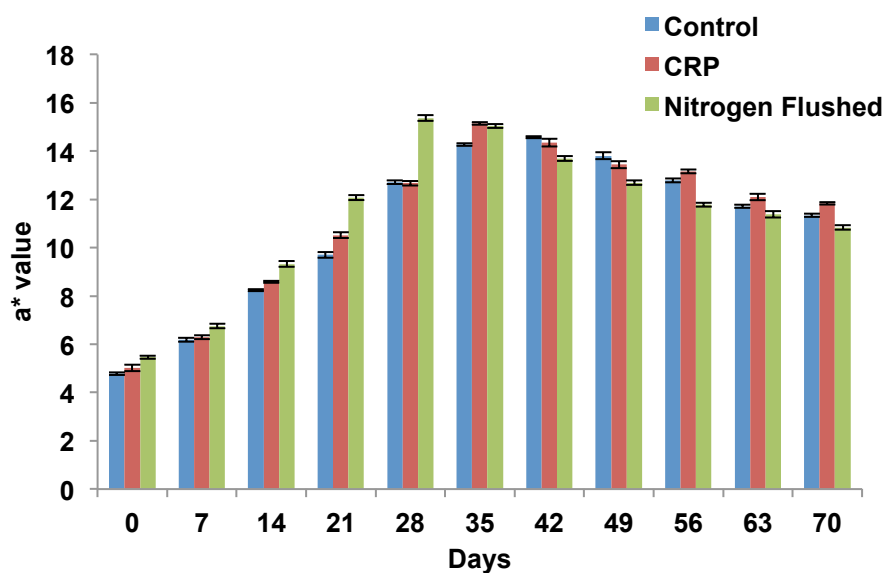
Figure 34: Change in a* value of Choice Spread over 70 days at 60°C

Change in concentration of lipid oxidation products: The production of peroxides, conjugated dienes and aldehydes was comparable in all types of packaging (Figures 35-

37). Base levels of conjugated dienes, peroxides and aldehydes were higher than at 40°C, consistent with accelerated oxidation.
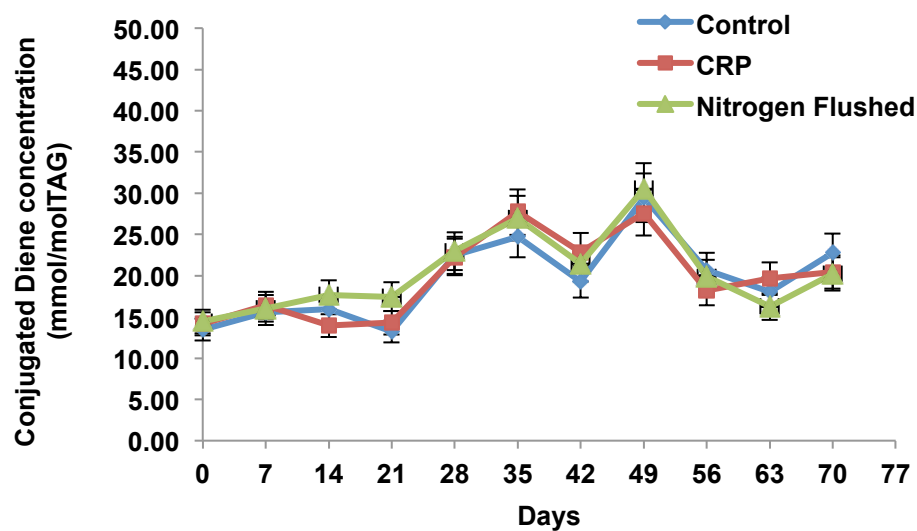
Figure 35: Changes in conjugated dienes concentration over 70 days at 60°C
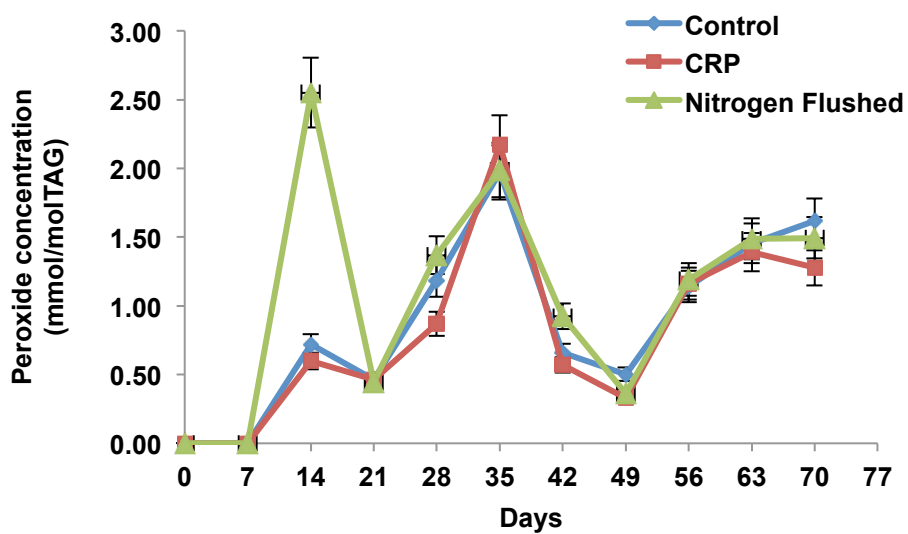
Figure 36: Change in peroxide concentration over 70 days at 60°C
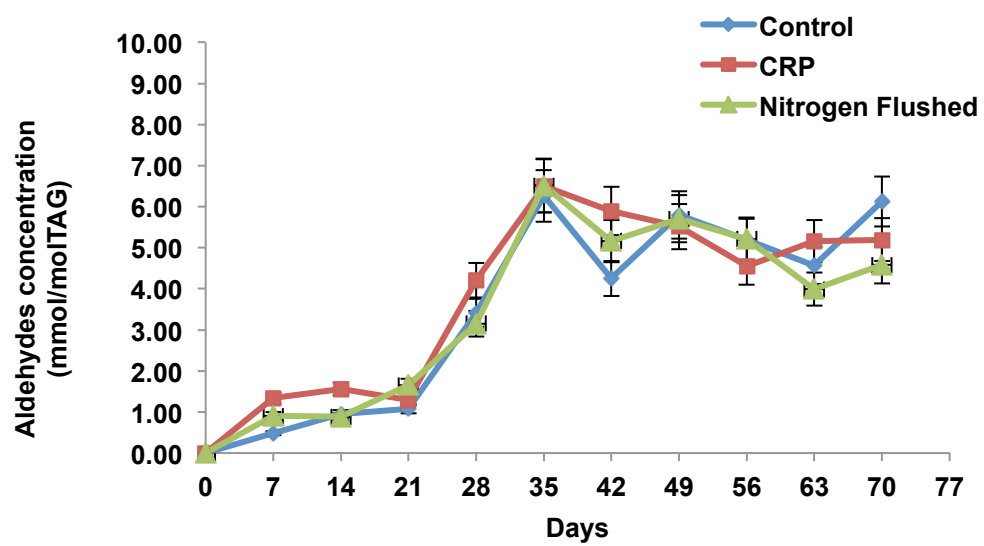
Figure 37: Change in aldehydes concentration over 70 days at 60°C

6. DISCUSSION

It has been established that lipid oxidation in MRE Choice Spread is limiting the launch of this product for the US Armed forces. Oxidation leads to browning and flavor changes, affecting the sensory attributes that are important to a consumer. The preliminary two-week study suggested that there is potential for Controlled Release Packaging impregnated with mixed tocopherols to extend the shelf life of MRE Choice Spread by limiting browning and production of off-flavors. This motivated the development of a more systematic study to investigate the full effect of CRP with mixed tocopherols in stabilizing lipid oxidation in MRE Choice Spread by quantification of color and lipid oxidation products at 40°C and 60°C. This technology was also compared with nitrogen flushing that is used commercially to retard oxidation.

Surprisingly, results of the shelf life study did not meet the expectations predicted from previous model system studies and the preliminary study in any of the objectives. The key questions are:

1) Were there really no differences in oxidation protection by the three types of packaging?

2) Were differences present but in characteristics not measured? or

3) Were any potential differences obscured by other factors such as levels of endogenous antioxidants or processing of the test films?

We agree that superficial viewing of the data suggests any differences between effects of the three types of packaging were marginal at best, as expressed in question 1. However,

questions 2 and 3 look beyond this to understand what may actually be happening in the Choice Spread.

It is likely that excessively high endogenous levels of TBHQ played a major role in aberrant results of this study. After 70 days at 60°C, peroxide values from all three types of packaging were still less than 2mmol/mol TAG and aldehydes were still low, indicating very low oxidation. In equivalent time at ambient temperature, this corresponds to 2.6 years stabilized by the TBHQ alone. Under such conditions, it is unlikely that low levels of tocopherols delivered slowly can make a dramatic impact on primary properties, but tocopherols released from packaging may exert important selective effects, as will be discussed later.

Color changes in Choice Spread were comparable in control, CRP and nitrogen flushed packages, in contrast to the color protection observed previously for both processed cheese and Choice Spread. There was more browning at 60°C because Maillard reactions are catalyzed by heat and ingredients such as lactose present in Non-Fat Dry Milk (NFDM) [4]. Aldehydes from oxidizing lipids can also participate in Maillard reactions. The change in color at 40°C was negligible for all samples over the storage period because thermal driving forces were minimal.

So why were there no differences in color between types of packaging? The inhibition of browning seen previously in processed cheese spread was attributed in part to two effects: rapid conversion of alkoxyl radicals to alcohols so that scission to reactive carbonyl products was limited and tocopherol interactions with protein amine groups. Hence, tocopherols blocked both the carbonyl and the amine functions required for Maillard

browning (Schaich, K.M. and Yam, K., unpublished data). In the current study, the Choice Spread unexpectedly contained 200 ppm TBHQ as an endogenous or instant addition antioxidant. This is a high level compared to the tocopherols slowly migrating in and we speculate that until TBHQ is consumed it dominates effects on lipid oxidation kinetics and course in Choice Spread and in addition blocks access of tocopherols to both lipid and protein sites. TBHQ reacts with protein thiol groups but not amine groups, so it does not block browning reactions.

Beyond browning, there remains the small protection of a^* values by CRP with tocopherols. We speculate that tocopherols released from CRP may inhibit oxidation of the Red40 food dye added to the artificial bacon bits in the Choice Spread or pigments in the cheese powders. However, it should be emphasized that although these differences in a^* are measurable instrumentally, they are not distinguishable visually so do not compromise sensory qualities during the storage periods of this study.

Patterns of lipid oxidation products observed were rather complicated, being similar in all types of packaging but not straightforward in the oxidation processes they portrayed. After an initial lag period of about two weeks, conjugated dienes increased and remained elevated between 20 and 30 mmols/mol TAG for the entire test period. In contrast, hydroperoxides and aldehydes increased and decreased when measured at intervals of 7 to 14 days during the storage period in cyclic manner. Peroxides reached levels less than 10% of the conjugated dienes and aldehydes accumulated to slightly higher levels. Similar cycling has been observed in chocolate [92], methyl linoleate, peanut butter, tortilla chips and other food systems (K.M. Schaich, unpublished data) and even processed cheese [93]. This can be explained by the continuous nature of lipid oxidation

where formation and simultaneous degradation of products is an ongoing process, especially at high storage temperatures. It is highly unlikely that lipid oxidation occurs homogeneously throughout a food, all at the same time and rate. Rather, oxidation in complex food matrices such as Choice Spread probably occurs in localized regions or perhaps layers where critical concentrations of catalysts and oxygen come together; oxidation flares and hydroperoxides increase until counteracted by decomposition forces (high peroxide concentrations or O-O scission catalysts), then hydroperoxides decrease temporarily until oxidation begins in another region. The result is cycles of oxidation that reach beyond just hydroperoxides.

In terms of oxidation products themselves, let us examine each one separately and then see how the patterns fit together as a whole. As a general rule, conjugated dienes are the first change occurring in lipids after initiation and in the presence of non-limiting oxygen, hydroperoxides form almost immediately after this. Thus, conjugated dienes and hydroperoxides should accumulate in parallel and at the same concentration since they derive from the same process. However, in this study hydroperoxide concentrations lagged behind conjugated dienes and most of the time remained at levels less than 10% of conjugated dienes on a molar basis. Thus, both products presented aberrant behavior.

The elevated levels of conjugated dienes maintained even as hydroperoxides were cyclically decomposing and reforming suggests that a) there was some initiating reaction not affected by antioxidants or lipid decompositions (e.g. metals) that maintained a constant supply of new radical chains and/or b) hydroperoxides decomposed to products that retained the conjugated diene structure. It is reasonable to expect both processes to be active.

The most obvious explanation for the lower hydroperoxides is that the LOOH were being constantly decomposed by the heat and other factors during the incubations to form alkoxyl radicals plus and hydroxyl radicals (heat, UV light) or hydroxide ions (metals).

$$LOOH \rightarrow LO^{\bullet} + {^{\bullet}OH} \text{ or } OH^{-}$$

The fate of the radicals formed in this process, alkoxyl radicals in particular, controls downstream oxidation. Both $LO^{\bullet}$ and ${^{\bullet}OH}$ radicals are very active radical propagators. If they react faster with other lipid chains than with TBHQ or tocopherols, their reactions could in fact contribute in a major way to creating new conjugated dienes. Unstopped, this process would lead to ever-increasing conjugated diene levels, but dienes accumulation could also be slowed by competitive hydrogen abstraction from TBHQ (antioxidant action) or from other molecules in the system, particularly proteins (co-oxidation reactions). Since overall, the oxidation level remains low in Choice Spread, it would appear that alkoxyl radical reactions with antioxidants and proteins are faster than with lipids.

An interesting and important consequence when alkoxyl radicals abstract hydrogens from any molecular target is that the products are alcohols that retain the conjugated diene structure. In this way, conjugated dienes can remain elevated without formation of new radical chains. At the same time, lipid alcohols are stable and they don't smell or mediate other reactions, so they just accumulate innocuously in the system. It is surprising that although lipid alcohols are the second stable product always drawn into radical chain reactions (hydroperoxides are the first), these products are seldom if ever measured. Adding lipid alcohols to the products analyzed would provide complementary

information that makes the critical link between alternate pathways in lipid oxidation.

A second competitive fate of alkoxyl radicals is scission to generate aldehydes, shown here for the C13 alkoxyl of linoleic acid:

$$CH_3(CH_2)_4\overset{b}{C}H\overset{O}{|}\overset{a}{C}H=CH-CH=CH-R \;\rightarrow\; pentane \;+ OCH-CH=CH-CH=CH-R \quad (b)$$

$$hexanal \;+\; {}^{\bullet}CH=CH-CH=CH-R \qquad (a)$$
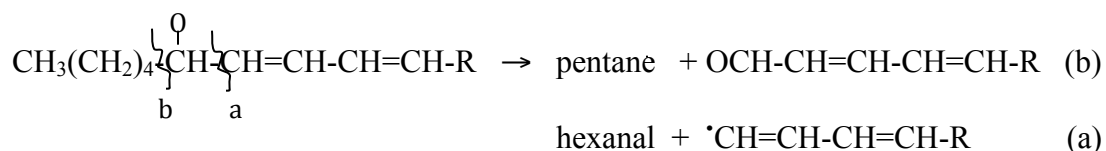
Accumulation of secondary products such as aldehydes may parallel hydroperoxide decomposition if the dominant oxidation pathway in a given food is alkoxyl radical scission. However, scission occurs in competition at least with alkoxyl radical hydrogen abstraction to form alcohols and internal rearrangement to form epoxides. Complicating the picture still further, all these products react rapidly with proteins, which removes them from detection in assays. Hence, low aldehyde levels do not necessarily mean low levels of lipid oxidation, only that alternate pathways must be investigated before drawing conclusions.

Comments also need to be made about several other observations from the study. First, although replacing oxygen in the package headspace with nitrogen should have delayed oxidation effects, it showed no beneficial effects. This can be attributed to improper handling during the gas flushing stages, e.g. use of brass regulators for flushing can introduce metal contaminants that accelerate oxidation, even pre-purified nitrogen contains about 2% oxygen which is also incorporated into the package and extra handling exposes samples longer before sealing, all of which counteract the benefit of flushing with inert gases.

Another important observation was the texture of Choice Spread during the storage study. This attribute was not identified to be critical to the shelf life prior to designing the study and was not systematically quantified. However, casual observations during the storage study noted that control and nitrogen flushed samples became firmer and more gritty in nature while products in CRP packages retained better texture (softer, more cohesive), flavor and had lower levels of grittiness. This was also confirmed by visually inspecting samples incubated for 14 weeks at 60°C (Figure 12, Table 4), where CRP retained the smoothness and moisture, unlike control samples. Texture stiffening can be explained by attack of lipid radicals and oxidation products on proteins, leading to protein cross-linking, polymerization and co-oxidation that markedly alter consistency.

Similarly in informal observations, packaging delivering controlled release of tocopherols showed better retention of original flavor and less generation of stale or off-flavors in Choice Spread than nitrogen flushing or controls. Since lipid oxidation products were not depressed by the CRP packaging impregnated with tocopherols, we speculate that the tocopherols were instead interfering in reactions not measured, e.g. Strecker degradation reactions that generate flavors.

Results of this study were unexpectedly contrary to what has been observed in model systems during development of CRP in this research program, as well as in previous test foods. Detailed explanations for this disparity will have to be the subjects of future studies, but some plausible explanations and potential areas for investigation may be offered here.

1) Antioxidant Overloading: Choice Spread, a commercial MRE product manufactured for the Armed Forces, is stabilized by adding the antioxidant- TBHQ at 200 ppm of the formulation. Since this product was reported to have significant shelf-life problems, CRP looked attractive although the TBHQ levels were sufficient to keep the product stable for close to 3 years at room temperature, according to predictive calculations based on peroxide levels. However, this level of antioxidant protection prevents deterioration from occurring during the much shorter time period of this study, despite exposure to elevated temperatures. Potentially even more problematic is that both TBHQ and tocopherol levels will be highest during this initial stage of storage and the two antioxidants together may well be causing an antioxidant overload within the system. Results of this study suggest that the two antioxidants may not behave synergistically to provide stabilization against oxidation. This effect of endogenous antioxidants should be considered while developing a Controlled Released Packaging system and can be included in the Target Release Rate concept being developed in this research program [94, 95]. According to this concept, there exists an ideal rate for the release of active compound from package to attain a desired shelf life based on marketing and logistic considerations as well as stability of the food product. This ideal rate depends on the polymers used, its processing conditions, structure and release properties as well as food variables such as composition, contact with package and storage conditions. The presence of endogenous antioxidants (concentration and type) will also affect the diffusion of active compounds impregnated in the films into the food and its activity and thus the target release rate should be modified to suit these new conditions for long term shelf life stabilization. Perhaps, using

antioxidants that behave synergistically such as ascorbic acid in food with tocopherol in the package can provide better stabilization against oxidative degradation.

2) Migration Issues of Tocopherol: A model system such as linoleic acid is an extremely simplified version of real foods where the effect of CRP is magnified as it is in liquid phase, composed of lipid molecules only and the tocopherols are homogenously distributed by constant mixing (Figure 38).
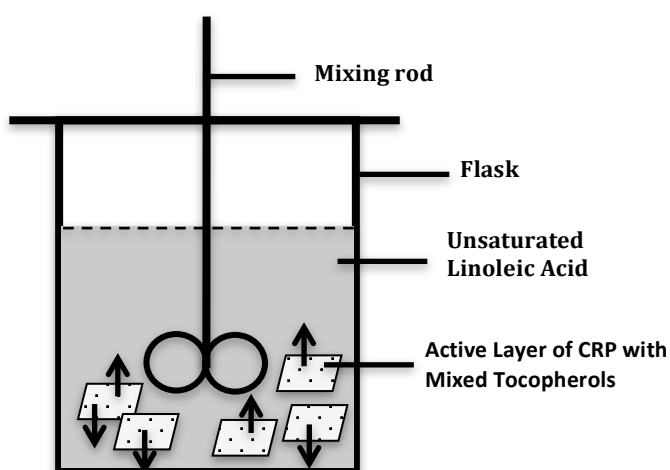
Figure 38: Tocopherol migration in a model food system with CRP films

In contrast, Choice Spread is a semisolid matrix composed of ingredients that go beyond lipids, emulsifiers and proteins, making it a complex system with multiple ingredient interactions, unlike model systems. Also, Choice Spread is an emulsion with interfacial phenomena that are not present in a model system and the behavior of antioxidants in emulsions is very different from bulk oils that could lead to to over-estimation of the antioxidant efficacy [32]. The tocopherol distribution in Choice Spread, instead of being homogenous like in linoleic acid, is heavily laden at the food-package contact layer and has uncertain diffusion into and within the food matrix (Figure 39). The complex matrix

may inhibit both migration of tocopherols into the food and accessibility of tocopherols to active sites of oxidation within the food or the matrix could even completely inhibit tocopherols transfer.
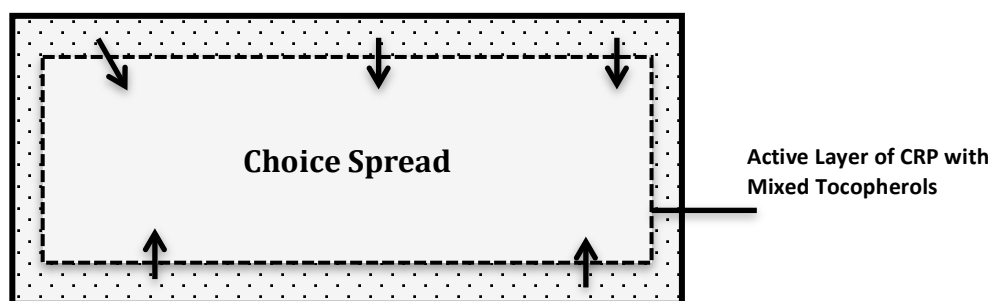
Figure 39: Tocopherol migration from packaging films into real food systems

3) Limited Analytical Endpoints: It is difficult to predict all the reactions that occur in complex reaction systems of real foods; it is even more difficult to predict how tocopherols will intervene in these reactions. Moreover, the deterioration is not limited to browning and lipid oxidation, but effects can be broadcasted to carbohydrates, proteins, pigments and other molecules through co-oxidation. Thus, when analyses of foods in storage are limited to the traditional lipid oxidation measures (usually only conjugated dienes and hydroperoxides, perhaps volatile carbonyls) plus physical color, a very incomplete picture of the system chemistry is generated, the presence of multiple competing reactions is not detected and product transformations cannot be tracked. Under such circumstances, results can easily be misinterpreted and incorrect conclusions drawn.

4) Antioxidant action on Maillard browning: It is well known that Maillard browning pigments inhibit lipid oxidation [96]. Browning set in early during the incubations and may well be the failure mode of this product at high temperatures, rather than lipid

oxidation. Especially at 60°C, accumulation of oxidation products began to slow at about the same time that browning was becoming quite pronounced (~35 days). Thus, side reactions in the food product itself, unrelated to the packaging, affect the major reactions of lipid oxidation and obscure any protection potentially provided by CRP during short-term shelf life. Even if CRP effects are most active and important with longer storage times, the protection may be irrelevant in this product because the high heat basically burns other components and makes product inedible.

The bottom line is that, although this study was designed to move applications of CRP out of the laboratory and model system studies to show that CRP can effectively extend the shelf life and protect sensory qualities of more complex real foods, perhaps the most important lessons learned were that there is more knowledge to be gathered about optimizing CRP and matching it to individual foods and also that effects of CRP on foods should be measured by a battery of analyses perhaps as complex as the degradation reactions themselves. Matching the release rates of antioxidants to the rate of major oxidation reactions in a food system to maintain appropriate levels of the antioxidant in the food system, under specific package and storage conditions is difficult but imperative [67]. The concept of Target Release Rate can be applied to future CRP package developments to allow for more customized package design for each type of food, storage conditions and required shelf life.

7. SUMMARY AND CONCLUSION

This research study was an attempt to move CRP technology from laboratory scale model system studies to plant level commercial applications to solve shelf life problems associated with a commercially processed real food system- MRE Choice Spread. The effectiveness of CRP impregnated with mixed tocopherols in delaying browning (sub-objective 1) and stabilizing oxidative degradation (sub-objective 2) was compared to regular MRE laminate packaging with and without nitrogen flushing (sub-objective 3), a common practice used in industry to inhibit oxidation. The results obtained are as follows:

Sub-objective 1: Within the 106 days of the study CRP had no effect on browning at 40°C. At 60°C, browning due to high temperatures was the failure mode, showing notable browning by 35 days. CRP impregnated with tocopherols did not prevent Maillard browning but did provide some protection to red pigments (a^* values).

Sub-objective 2: CRP packages did not improve stabilization against oxidative degradation as measured by conjugated dienes, peroxides and aldehydes over 106 and 70 days of storage at either 40°C or 60°C respectively.

Sub-objective 3: Choice Spread showed comparable color degradation and lipid oxidation in all three types of packaging at 40°C and 60°C, indicating that neither treatment provided protection in this food system under conditions of the study.

Although physical and chemical analyses indicated no effects of CRP or nitrogen flushing on browning or lipid oxidation, informal sensory evaluations revealed that CRP-tocopherol samples showed better texture retention and lower levels of grittiniess than

control or nitrogen flushed samples. This indicates that CRP exerts effects on the system beyond lipid oxidation and this needs to be investigated further.

Altogether, these results on the surface seem to suggest that neither CRP nor nitrogen flushing are able to supplement TBHQ stabilization of Choice Spread. However, the same results teach two important lessons for CRP:

1)   Customization of CRP films using the Target Release Rate concept described previously is required to suit a specific food product. When antioxidant levels in the food formulation are high, CRP can probably not provide additional protection for short term storage and may even push antioxidant concentrations into the prooxidant range. For long term stabilization under these conditions, the target release rate will be different, as the delivery of additional antioxidant from packaging needs to be slow and prolonged. Alternatives that should be considered include reducing formula levels of antioxidants or using antioxidants in the formula that act by different mechanisms or specificities than the antioxidant being delivered in the packaging.

2)   At least in initial studies, endpoint testing for oxidation should be broad, encompassing as many alternate and connected reactions as possible in order to accurately document how tocopherols delivered from CRP alters overall degradation. Limiting oxidation analyses to conjugated dienes, hydroperoxides and aldehydes provides an incomplete picture of what is really happening in the Choice Spread and may lead to erroneous conclusions regarding activity of CRP in this system. Not measuring protein effects misses oxidation chains that have been transferred away from lipids (so are no

longer detected as lipid oxidation) but still degrade sensory and nutritional properties and are subject to intervention by tocopherols.

Overall, results of this study show clearly the complications in translating antioxidant behavior in CRP model systems to real foods, highlighting the need for more real food system studies to be conducted and also that a "one size fits all" approach cannot be used when applying CRP technology. Formulation and packaging must be coordinated to work together rather than in opposition. Each food system is unique in its formulation, phase properties, endogenous catalysts, types of degradation reactions and degradation rates. All these factors must be considered in determining which antioxidants or other stabilizers to add initially to the formula, which to add to packaging and how fast the latter must be delivered to the food. These issues should be added to the concept of Target Release Rate being developed in this research program in order to design model systems that more accurately predict CRP behavior with a variety of real foods.

8. FUTURE WORK

Research that is still needed to improve effectiveness of CRP in extending shelf life for real food systems such as MRE Choice Spread includes the following.

1) A similar storage study should be conducted on Choice Spread without added antioxidants to observe CRP effects without interferences.

2) Other types of endogenous antioxidants should be considered, particularly metals chelators, in place of or in addition to TBHQ. Inclusion of citric or fumaric acid would provide a double advantage of metal complexing plus browning inhibition.

3) Additional or repeat storage studies should include detailed measures of texture changes, co-oxidation of proteins and more lipid oxidation products in order to identify the most active degradation pathways and determine which are amenable to intervention by CRP.

4) Because analysis of tocopherols release from CRP films into food was difficult to measure, more complex model systems need to be developed to determine effects of food system composition and structure on antioxidant release rates. Such systems should include at least proteins, carbohydrates and lipids to more accurately mimic real food matrices and to be able to draw useful conclusions before carrying out pilot and industrial scale studies.

5) Effects of endogenous antioxidants should be tested using simple syringe pump experiments to build into the Target Release Rate model.

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