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Utilizing Nutritive Sweeteners to Control Lipid Oxidation in Low Moisture Baked Goods

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**UTILIZING NUTRITIVE SWEETENERS TO CONTROL LIPID OXIDATION
IN LOW MOISTURE BAKED GOODS**

A Thesis Presented

by

SAMANTHA A. VIEIRA

Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

MASTER OF SCIENCE

SEPTEMBER 2016

Department of Food Science

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ABSTRACT

UTILIZING NUTRITIVE SWEETENERS TO CONTROL LIPID OXIDATION IN LOW MOISTURE BAKED GOODS

SEPTEMBER 2016

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In this study, we determined the effect of nutritive sweeteners at 0 to 0.50 moles/kg on lipid oxidation in a model cookie system. Confocal microscopy using Bodipy 493 as a fat soluble dye showed that the fat formed a continuous phase surrounding the starch granules regardless of sugar type. The impact of glucose concentration on lipid oxidation was monitored by lipid hydroperoxides and headspace hexanal during storage at 55°C. Low concentrations of glucose (<0.09) increased lipid oxidation rates while high concentrations (>0.09) were strong inhibitors. At equal molar concentrations, reducing sugars (glucose and fructose) inhibited lipid oxidation, greater than a two months increase in lag phase compared to the control. Sucrose inhibited lipid oxidation, but to a much lesser extent than reducing sugars. The inhibition of lipid oxidation is potentially due to sugar's ability to bind water. Additionally, reducing sugars may exhibit this effect due to their ability to act as a hydrogen donor which could inactivate free radicals or due to the production of Maillard reaction products (MRPs). For example, the I-values were lower and b-values were higher for cookies with non-reducing sugars compared to cookies with sucrose indicating that there were more MRPs. The addition of cysteine, sulfites, and ascorbic acid acted as a strong browning inhibitors however cysteine was showed to be antioxidative. When compared to synthetic antioxidants, glucose proved to be a strong natural alternative. These results could be utilized to develop effective means of controlling water activity and extending shelf-life of low moisture baked goods.

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CHAPTER 1

INTRODUCTION

Lipid oxidation can reduce shelf life, product nutrition, and can produce toxic lipid oxidation products. Methods of preventing lipid oxidation are necessary for economic and consumer health, specifically because it is recommended to use unsaturated fats in foods which are more prone to lipid oxidation than saturated fats.

The average American consumes 38 grams of saturated fat per day (HHS and USDA 2015). The 2015 U.S. Dietary Guidelines recommend that one should not consume more than 10% (22 grams) of their daily calories in saturated fat (HHS and USDA 2015). 18% of the American dietary intake of saturated fat derives from refined grain desserts such as cookies, cereals, and crackers (HHS and USDA 2015). Refined grain desserts are considered low moisture foods due to their water activity below 0.5 (Labuza et al, 1970). Therefore, the nutritional profile of low moisture foods can be increased by substituting the saturated fats in the product for unsaturated fats. However, increased consumer demand for elimination of synthetic additives has led researches to pursue other natural solutions to prevent lipid oxidation. Unfortunately, natural antioxidants are often not as effective as their synthetic counterparts. Therefore, there is a need for increased research in natural methods to prevent lipid oxidation. Specifically, controlling water activity of the system by utilizing the water binding properties of sugar, and hydrogen donating ability of reducing sugar, to prolong lag phase, and decrease the primary product (hydroperoxides) breakdown into secondary lipid oxidation products (hexanal). A literature review on the impact of lipid oxidation was also performed to emphasize the importance of preventing lipid oxidation.

The first study utilizes confocal microscopy to examine the microstructure of low moisture short bread cookies made with glucose or sucrose to examine their influence on the

location of the lipid and protein phase (Labuza et al. 1970). Samples with varying concentrations of glucose and sucrose were then subjected to 55°C storage studies. The results suggested that an increase in molar concentration of sugar also increases the inhibition of lipid oxidation. Next, the inhibition potential of sucrose, glucose, and fructose were compared in controlled and uncontrolled water activity environments. These results proposed that controlling water activity can prolong lag phase and that reducing sugars (glucose and fructose) provide stronger inhibition of lipid oxidation potentially due to their ability to produce maillard browning pigments, donate hydrogen, and scavenge free radicals.

The production of maillard browning pigments was measured by colorimetry readings and further inhibited by the addition of browning inhibitors which were then subjected to 55°C storage studies and were characterized by the measurement of primary lipid hydroperoxides, and secondary hexanal headspace analysis. Results suggest that maillard browning pigments do not influence lipid oxidation, therefore strengthening the potential of sugar's ability to bind water thus inhibiting lipid oxidation.

The lipid oxidation inhibition of glucose was compared to synthetic antioxidants and proved to potentially be a strong natural mechanisms of preventing lipid oxidation in low moisture foods. Finally, the impact of water activity versus lag phase was examined to determine if controlling water activity could prolong lag phase of the system.

CHAPTER 2

BIOLOGICAL IMPLICATIONS OF LIPID OXIDATION PRODUCTS

2.1. Introduction

Lipid oxidation causes deterioration in food quality due to the generation of off-flavors and degradation of colors and nutrients. Food lipids tend to have higher levels of lipid oxidation products than the biological lipids from which they originate due to the processes required for extraction and refining of the fats and oils. In addition, food processing operations such as cooking and frying increase lipid oxidation rates and any lipid containing food is likely to accumulate oxidation products during prolonged storage. The susceptibility of foods to lipid oxidation means that many diets would contain compounds such as lipid hydroperoxides, fatty acid decomposition products (e.g. aldehydes, ketones and epoxides) and oxidized sterols. These compounds have the potential to cause a variety of biological responses through their dietary consumption and through their production within the body. Fortunately, the complex antioxidant systems in humans (e.g. dietary antioxidants, metal-binding proteins and antioxidant enzymes) are typically adequate to prevent damage from dietary lipid oxidation products. However, in some cases, lipid oxidation products can lead to situations which cause biological abnormalities that would lead to disease. The aim of the present review is to summarize studies that demonstrate whether or not dietary lipid oxidation products pose a risk to human health.

2.2. Background

Lipid oxidation primarily occurs in foods containing unsaturated lipids such as meat, low moisture foods, emulsions, oils, and nuts. However, it can also occur with compounds such as

sterols during prolong storage or in highly processed products (e.g. spray dried). Lipid oxidation can cause repugnant aromas and flavors known as rancidity, a darkening of color, a change in texture, and the co-oxidation of nutrients and pigments such as vitamin A, vitamin E, vitamin C, and carotenoids. The oxidation of lipids also generates potentially cytotoxic fatty acid degradation products that can form genotoxic advanced lipid oxidation endproducts (ALEs) which are produced by conjugation of lipid oxidation carbonyls with proteins, cell membranes and nucleic acids. The stability of the lipid is dependent upon the degree and nature of the unsaturation of fatty acids, antioxidant content, pro-oxidants, food processing operations and storage conditions. Highly unsaturated fatty acids such as the omega-3s are the most susceptible to the effects of oxidation and potentially generate the most toxic degradation products.

In terms of food processing operations, deep fat frying can be particularly problematic due to the combination of high temperatures and water that accelerate triacylglycerol hydrolysis, fatty acid oxidation, double bond isomerization and polymerization of fatty acids and glycerol esters of fatty acids (Zhang et al. 2012). Meats are also susceptible to lipid oxidation because cooking releases protein-bound metals, inactivates antioxidant enzymes, and physically disrupts lipid membranes (Bou et al. 2010). Emulsions are another category of food susceptible to oxidation due to their high surface area that creates lipid-water interfaces where lipid and prooxidants can readily interact (Waraho et al. 2011).

Normally, foods that have undergone lipid oxidation are rejected by consumers because fatty acid degradation products that produce off-flavors are detectable at concentrations below 10 ppb (Kochhar 1996). However, flavors in food can mask the aroma of lipid oxidation products and processes such as deep fat frying, can distill out the volatile products that decreases off-flavors meaning that a food can have significant lipid oxidation that is difficult to detect by

sensory perception. However, even when lipid oxidation products are removed from foods by processes such as distillation, there are still lipid oxidation products esterified to the glycerol (core aldehydes) still remain and could be released by lipase during digestion. Given these facts, it's not surprising that consumers commonly ingest lipid oxidation products. For example, French fries from a fast food establishment contained 8.2% oxidized products (Frankel 1984). After 80 hours of use at 170F, oil containing 90% tallow and 10% cottonseed oil showed an increase in thiobarbituric acid reactive substances (TBARS) from 0.27 to 0.73ppm (Lake and Scholes 1997). The level of TBARS found in the frying oil were lower than TBARS found in the deep fried fish indicating that both the oil and the product fried in the oil contained lipid oxidation products. Yagi et al. (1986) measured peroxides in 30 kinds of commercially available foods and determined that the peroxide content was as high as 600 nmoles/g food. It has been estimated that the daily intake of lipid hydroperoxides (a primary product of lipid peroxidation) is around 1.4 mmol (Kanner 2007).

The above suggests that many diets contain oxidized lipids due to the consumption of fried, cooked (e.g. meats); and emulsified lipids as well as other lipid containing foods. The potential issue with consumption of oxidized lipids is further exacerbated because oxidatively susceptible vegetable oils has increased 130% in the United States between 1909 to 1999 (Gerrior and Bente 2002) and could be even more prevalent in the future as dietary recommendation continue to encourage greater consumption of unsaturated, liquid oils and omega-3 fatty acids (HHS and USDA 2015).

2.3. Evidence that oxidized lipid are absorbed from the diet

Lipid oxidation products have been associated with chronic diseases such as inflammation, cancer, cardiovascular disease (Halyna and Semchyshyn, 2014). Researches have

suggested that lipid oxidation products could produce toxicity if they are present in dietary fats or if oxidation of lipids occurs within the digestive tract. However, many of the studies implicating the negative health effects of lipid oxidation products have only been observed in cell culture and animal studies. The direct absorption of lipid oxidation product in humans is limited to only a few publications.

Strapans et al. (1994) found that diets high in oxidized corn oil increased conjugated dienes and TBARS in the chylomicron fraction of humans. No lipid oxidation products were found in LDL and HDL suggesting that these products were metabolized in the liver and not repackaged into other lipoproteins. Nuora et al., (2015) found an increase in a variety of lipid oxidation products in plasma of humans following consumption of cooked beef and that plasma inflammatory markers tended to increase with increasing levels of beef oxidation. Wilson et al., (2002) reported an increase in hydroxyl fatty acid (a decomposition product of lipid hydroperoxides) in women following ingestion of C-13 labeled hydroxyl or dihydroxy triacylglycerols. Consumption of a meal made with deep fried oils rich in lipid breakdown products reduced endothelium dependent flow-mediated dilation by a factor of seven, whereas no effect was found following consumption of foods fried in fresh cooking oil again suggesting that lipid oxidation products would be absorbed by humans (Williams et al. 1999). Diabetic patients were found to have greater levels of lipid oxidation products (conjugated dienes) in chylomicrons than healthy subjects after consumption of oxidized corn oil suggesting that overall health could also impact absorption of lipid oxidation products. However, others such as Ottestad et al. (2012) found no increased in lipid oxidation products in humans after consumption of oxidized fish oils. This could be due to the type of lipid oxidation products formed from different fat types as unsaturated carbonyls react more readily with proteins than saturated carbonyls.

2.4. Potentially Toxic Lipid Oxidation Products

Lipid oxidation is a complex process that produces lipid hydroperoxides (primary products) which can decompose into a variety of oxygenated and aliphatic fatty acid scission products. These decomposition products are found in foods and linoleic acid hydroperoxides have been reported to decompose to secondary lipid oxidation products in the gastric lumen (Kanazawa and Ashida, 1998). Oxidized dietary lipids can either directly enter the portal vein system and be transported to the liver, or they can enter circulation via the lymph as chylomicrons (Palay and Karlin 1959). Since lipid hydroperoxides do not seem to be absorbed into the plasma, research on the toxicity of lipid oxidation products has focused on secondary lipid oxidation products and in particular, aldehydes.

2.4.1. α,β -Unsaturated carbonyl compounds

One of the most reactive lipid oxidation products is α,β -unsaturated aldehydes (Figure 1). These compounds are electrophiles with nucleophiles attacking at the β -carbon (Figure 1). Nucleophile that can be modified by α,β -unsaturated aldehydes include nucleic acids, proteins and membranes as well as sulfhydryl containing compounds such as glutathione. These reaction products are referred to as advanced lipoxidation end products which have been postulated to be involved in diseases such as diabetes, kidney disease, cardiovascular disease and cancer (Halyna and Semchyshyn, 2014). Examples of α,β -unsaturated aldehydes in oxidized food lipids includes acrolein, crotonaldehyde and 4-hydroxyalkenals.

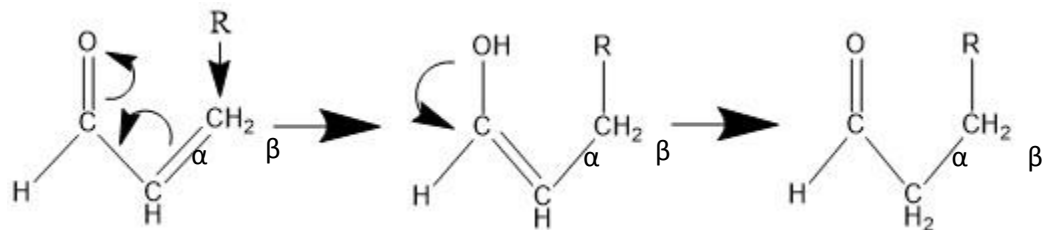


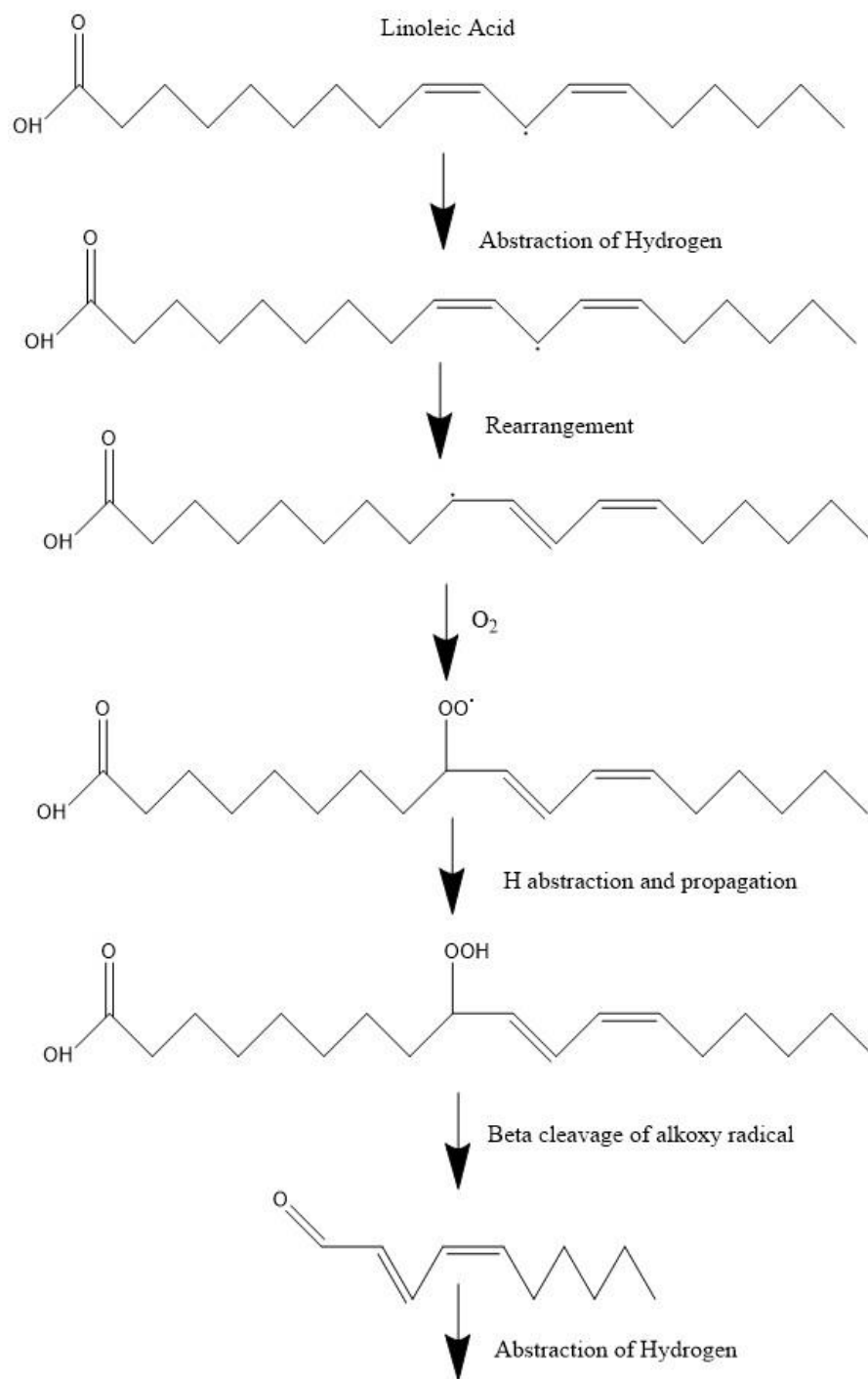
Figure 2.1: Nucleophilic Attack of α,β -unsaturated aldehyde

2.4.2. Acrolein

Acrolein (2-propenal) is formed by the oxidation of linoleic acid via the formation of lipid hydroperoxides on carbon 9, β -cleavage between carbon 8 and 9 to form an aldehyde, formation of a second hydroperoxide on carbon 12 of the original linoleic acid and β -cleavage between carbons 11 and 12 (Ewert et al., 2014; Figure 2). Acrolein is found in fried foods, alcoholic beverages, and charred meat. Researchers found that in vegetable oils heated to 180°C, acrolein was formed from PUFAs, especially linolenic acid (Endo et al. 2013). In olive oil used for frying, acrolein concentration ranged between 72.3 and 491.2 $\mu\text{g}/\text{kg}$ after 30 min and 123.2 and 427.1 $\mu\text{g}/\text{kg}$ after 60 min frying cycles containing gelatinized maize starch (Procida et al. 2009). According to the World Health Organization, the Tolerable Daily Intake of acrolein is 7.5 $\mu\text{g}/\text{kg}$ of body weight. It has been estimated that humans consume 5 mg/kg of acrolein in food. (Moghe et al., 2015). The Department of Homeland Security, Agency for Toxic Substances and Disease Registry, and Environmental Protection Agency consider acrolein an industrial chemical and biocide and list it as a high priority toxic chemical (ATSDR, 2007).

Acrolein is a very strong electrophile which allows it to react with glutathione 150 times faster than 4-hydroxy-*trans*-2-nonenal (VanderJagt et al., 1997). Ismahil et al. (2011) directly exposed mice to 0.01 μM of acrolein which resulted in numerous adverse biological effects

including blood vessel dysfunction, increased myocardial oxidative stress and varying levels of myocardial protein adducts. Similarly, Burcham et al. (2012) exposed mouse hepatocytes to acrolein leading to protein carbonylation causing hepatocyte death. Furthermore, Uchida et al. (1998) demonstrated that acrolein-lysine adducts are involved in the modification of human low-density lipoproteins.



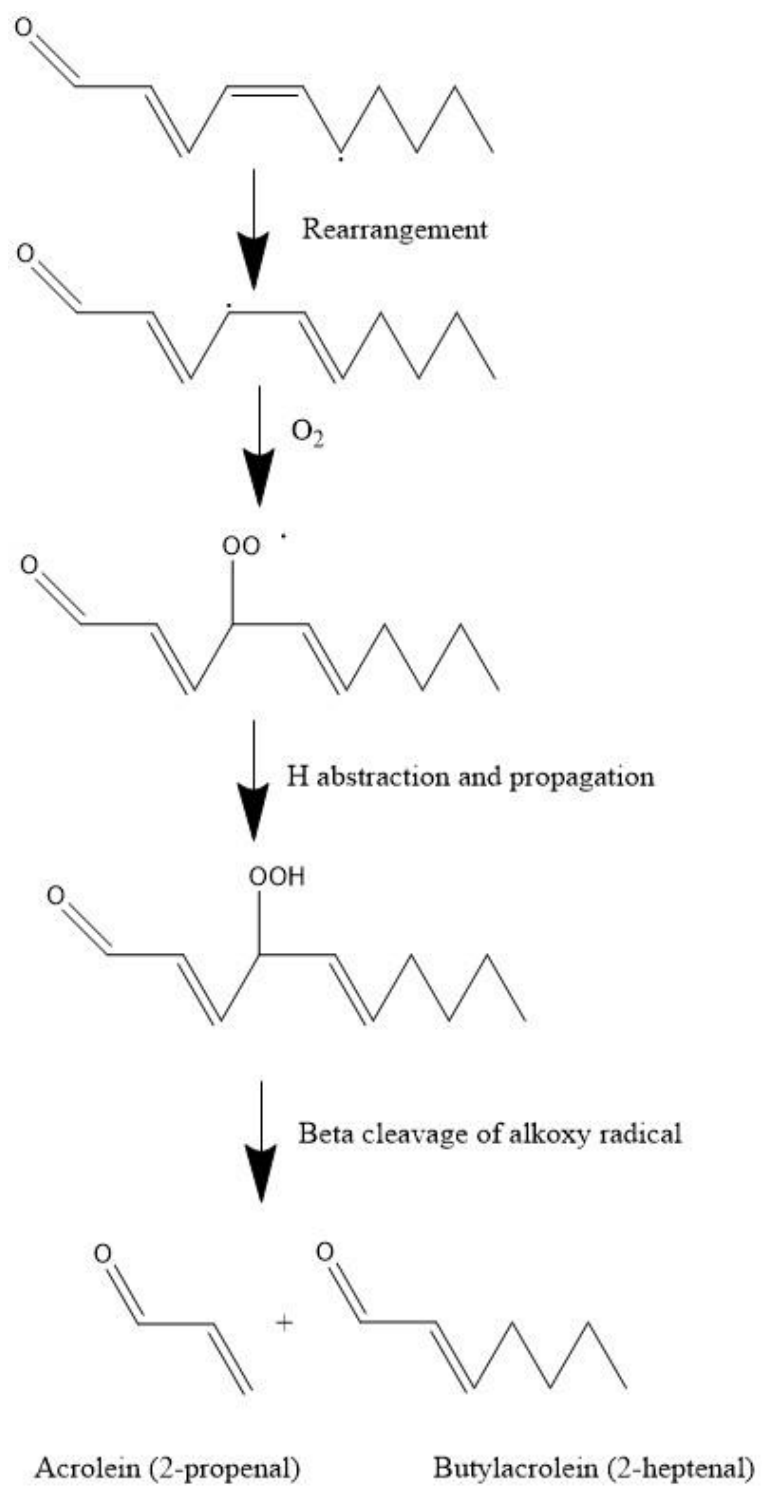


Figure 2.2: Formation of Acrolein

2.4.3. Crotonaldehyde

The exact formation crotonaldehyde (2-butenal) pathway is not well established but it has been shown to be formed from unsaturated fatty acids (Granvogel, 2014), ethanol and acetaldehyde (Early et al., 2015). Crotonaldehyde is found in wine (0.3-1.2 mg/liter), whiskey (30-210 µg/liter), vegetables and fruit (1-100 µg/kg), fish (71-1000 µg/kg), meat (10-270 µg/kg), heated canola oil (12.3 mg/kg after 24 h at 180°C), fried potato chips (12 to 25µg/kg) and donuts (8 to 19 µg/kg) (Eder and Budiawan 2001). In addition, crotonaldehyde is important in the synthesis of industrial chemicals such as 3-methylbutanol, tocopherol, and sorbic acid (Eder and Budiawan 2001).

As with other α,β -unsaturated aldehydes, crotonaldehyde has a strong reactive electrophilic carbonyl group, which allows it to conjugate with glutathione thus reducing glutathione within the body (VangerJagt et al. 1997). The electrophilic nature of crotonaldehyde means that it can form propanodeoxyguanosine adducts in DNA resulting in mutagenic, genotoxic and carcinogenic activity and thus it can produce hepatic tumors in rodents (Chung et al., 1986). Eder and Budiawan (2001) fed 1 and 10 mg/kg of crotonaldehyde to rats in order to simulate the propanodeoxyguanosine adduct levels similar to levels of crotonaldehyde in food. Adduct levels of crotonaldehyde were within the range of 3 adducts/10⁹ nucleotides. This concentration of 1,N²-propanodeoxyguanosine adducts is an indication of a possible cause of cancer (Chung et al. 1986). Chung et al. (1986) noted that 2 weeks after feeding, 19% of crotonaldehyde given to the subjects remained within the propanodeoxyguanosine adducts.

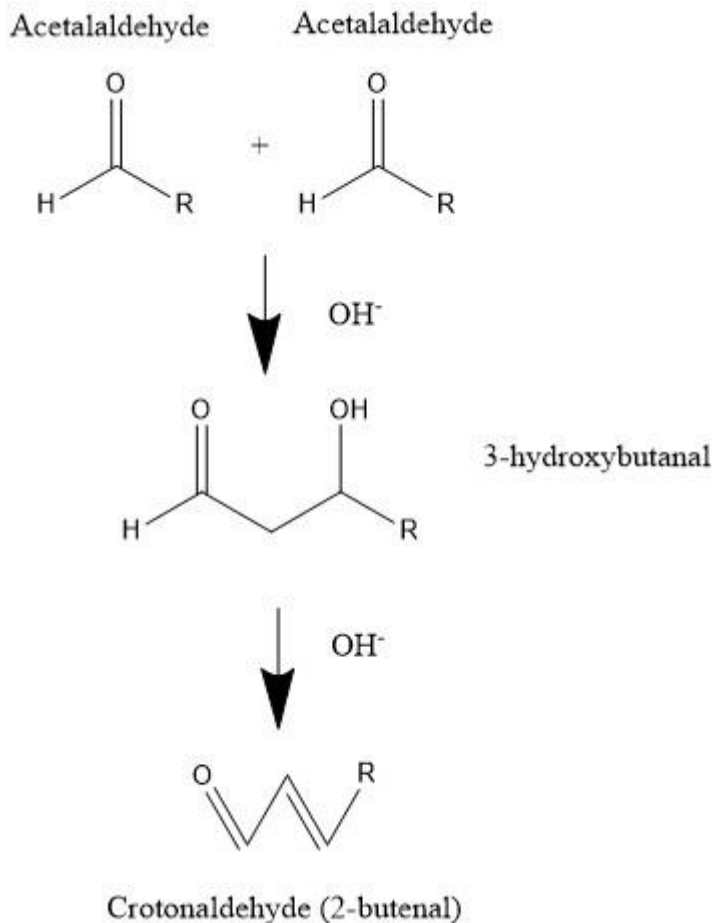


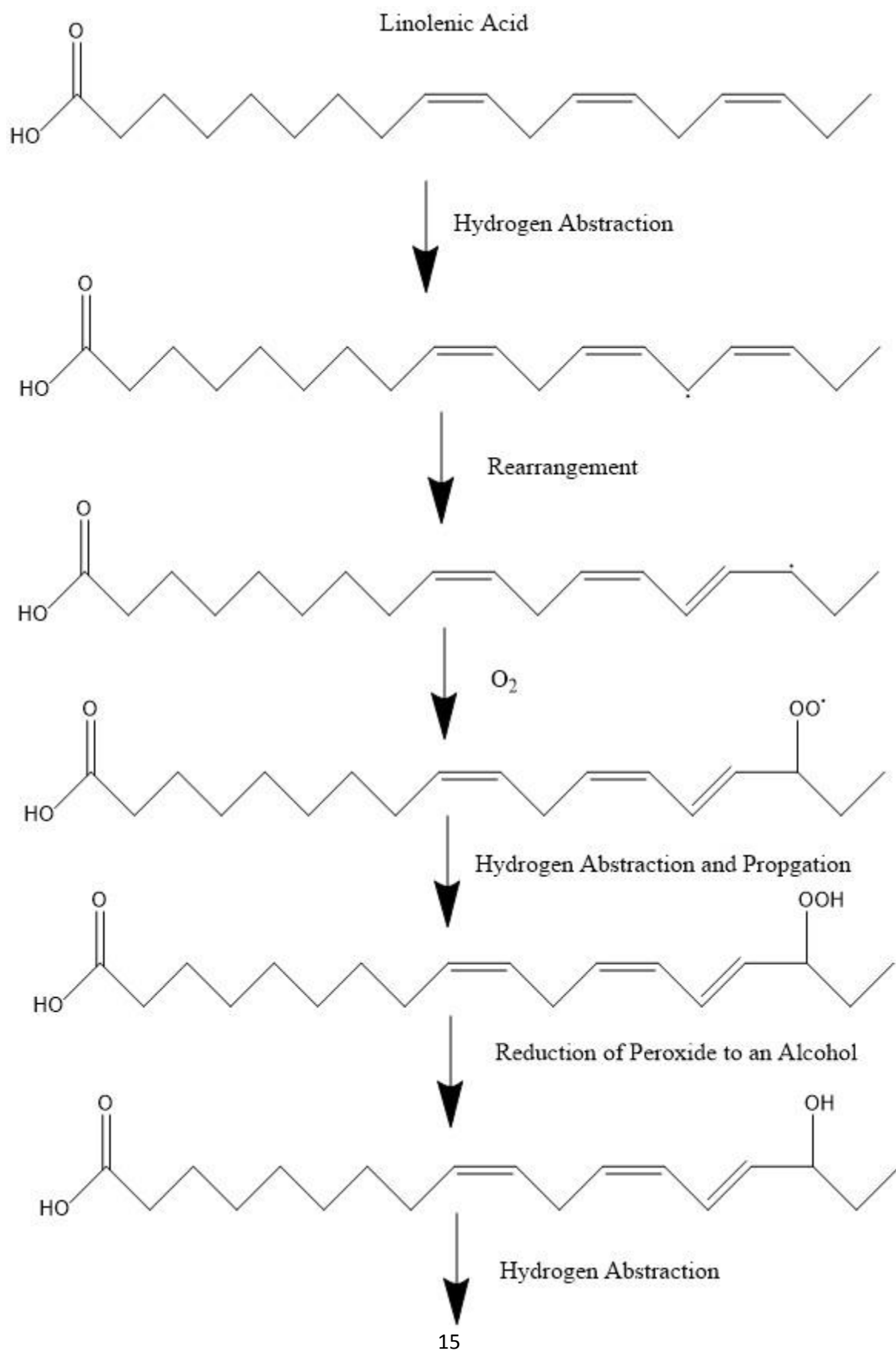
Figure 2.3: Crotonaldehyde

2.4.4. 4-Hydroxy-*trans*-2-nonenal and 4-Hydroxy-*trans*-2-hexenal

Two additional α,β -unsaturated aldehydes that are found in food and are potentially toxic are 4-hydroxy-*trans*-2-nonenal and 4-hydroxy-*trans*-2-hexenal which originate from ω -6 and ω -3 fatty acids, respectively (Esterbauer et al., 1991; Long and Picklo, 2010). Both of these aldehydes are produced by double oxidation of the fatty acid with the first step being the decomposition of a hydroperoxide at the double bond on the methyl end of the fatty acids (e.g. the ω -3 or 6 double bond) to an alcohol followed by formation of a second hydroperoxide on the ω -6 or 9 carbon which then undergoes β -scission to fragment the fatty acids into 6 or 9

carbon aldehydes (Figure 3) with an alcohol group. Both 4-hydroxy-*trans*-2-nonenal and 4-hydroxy-*trans*-2-hexenal form Michael adducts with proteins, membranes, DNA and glutathione which are responsible for their cytopathological effects (Esterbauer et al., 1991; Long and Picklo, 2010). There are differences in where these adducts are formed, with 4-hydroxy-*trans*-2-hexenal adducts forming in high ω -3 fatty acid tissues such as brain and eye. These α,β -unsaturated aldehydes can be cytotoxic when present in the diet of lab animals. For example, mice fed a 4-hydroxy-*trans*-2-nonenal rich fraction of oxidized linoleic acid had severe lymphocyte necrosis in the thymus 24 hours after exposure (Esterbauer 1993). These α,β -unsaturated aldehydes can also be detected in human tissue using antibodies to the aldehyde-protein adducts. For example, Oberly et al. (1999, 2000) found high levels of 4-hydroxy-*trans*-2-nonenal modified proteins in metastatic prostate tissue and in the nucleus and mitochondria in human renal cancer. Similarly, there is an increase in 4-hydroxy-*trans*-2-nonenal modified proteins in human colon cancer cells (Kondo et al. 1999).

4-Hydroxy-*trans*-2-nonenal has been reported in French fries at 59.7 +/- 11.9mg per 100 g oil (Seppanen and Csallany 2004). In addition, both 4-hydroxy-*trans*-2-nonenal and 4-hydroxy-*trans*-2-hexenal have been detected in infant formula and baby foods (Surh et al., 2007) as well as fresh and oxidized vegetable and fish oils (Viau et al., 2016) and meat and fish products (Tirosh et al., 2015).



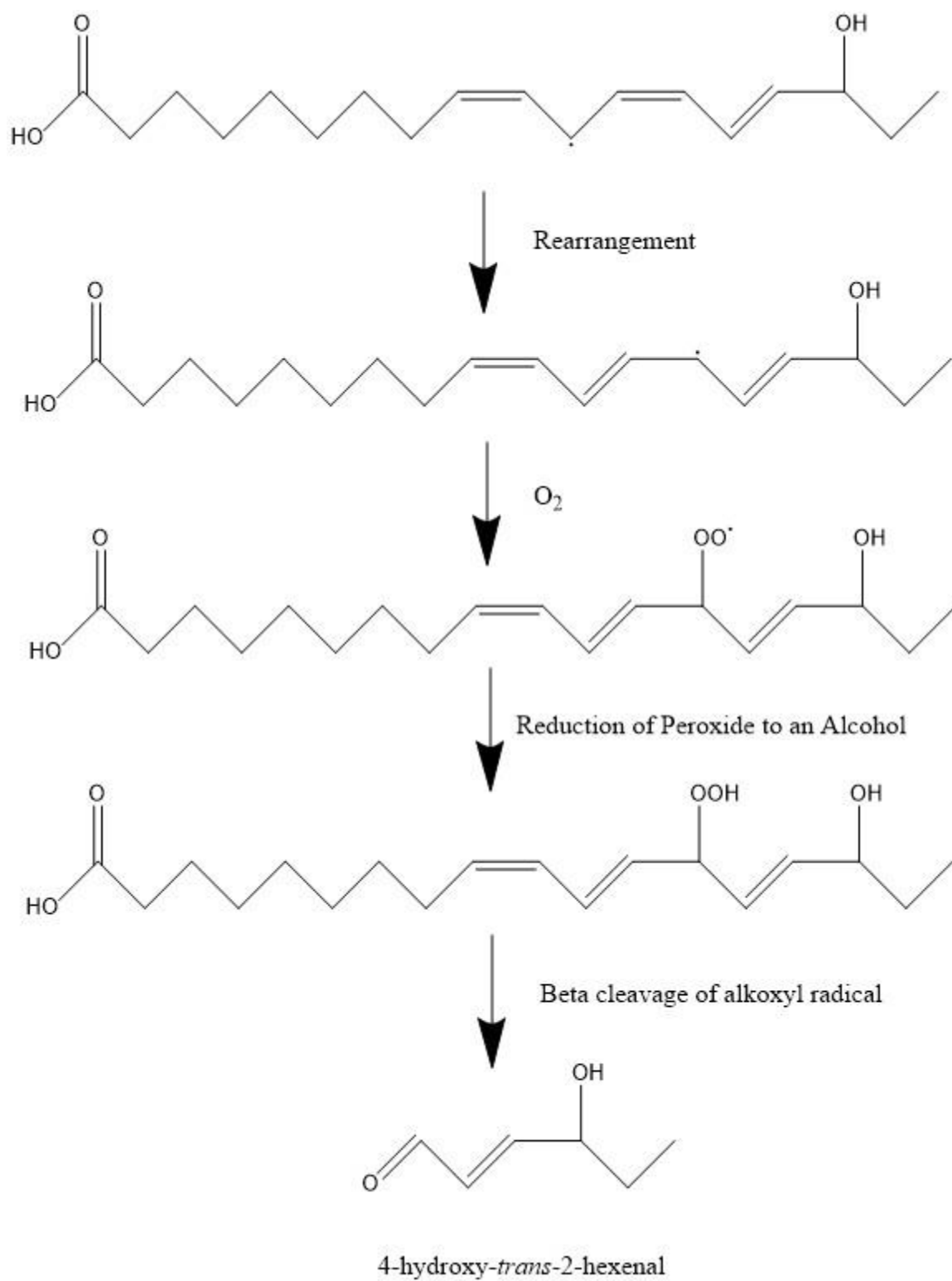
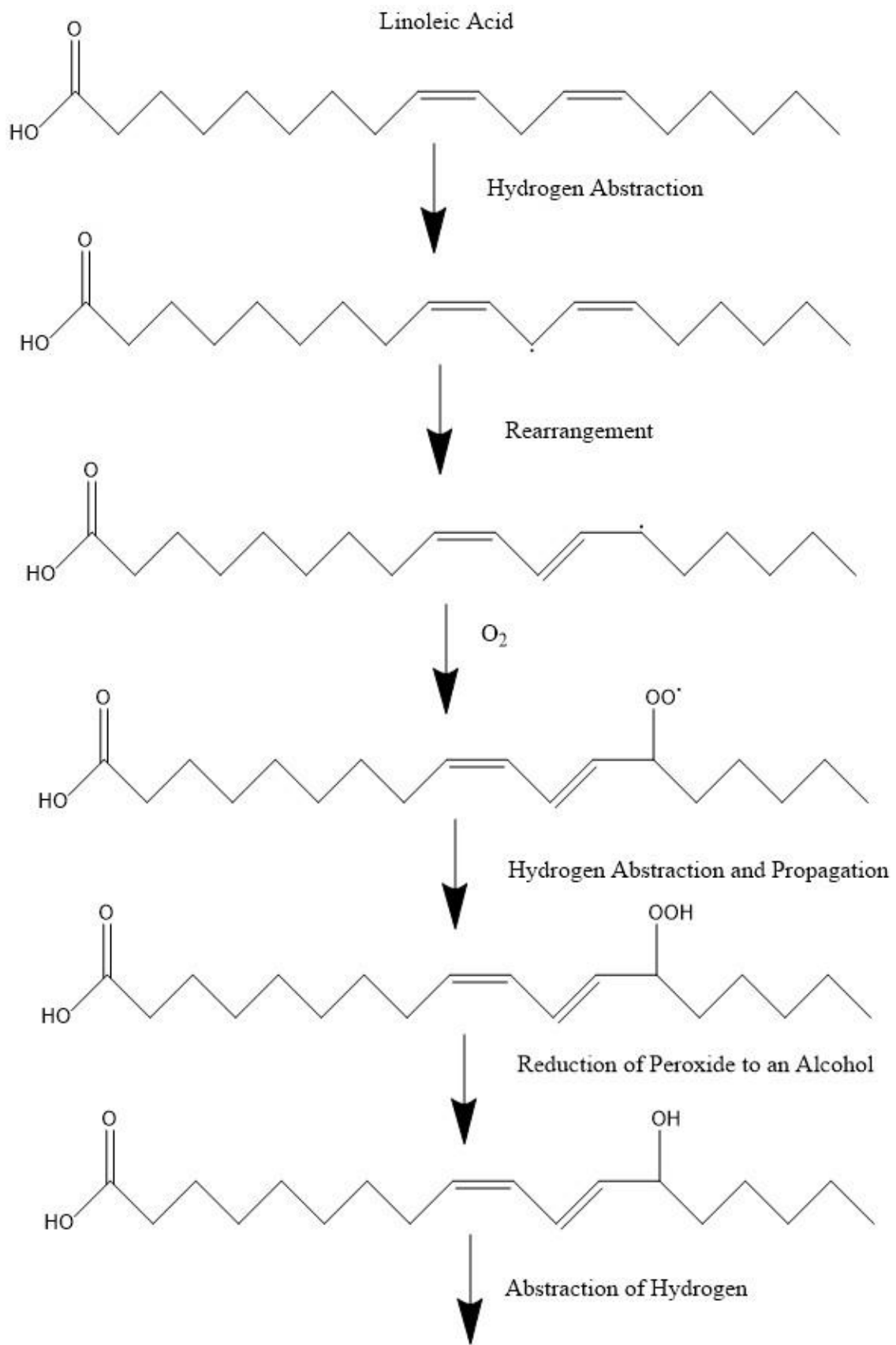


Figure 2.4: 4-hydroxy-*trans*-2-hexenal



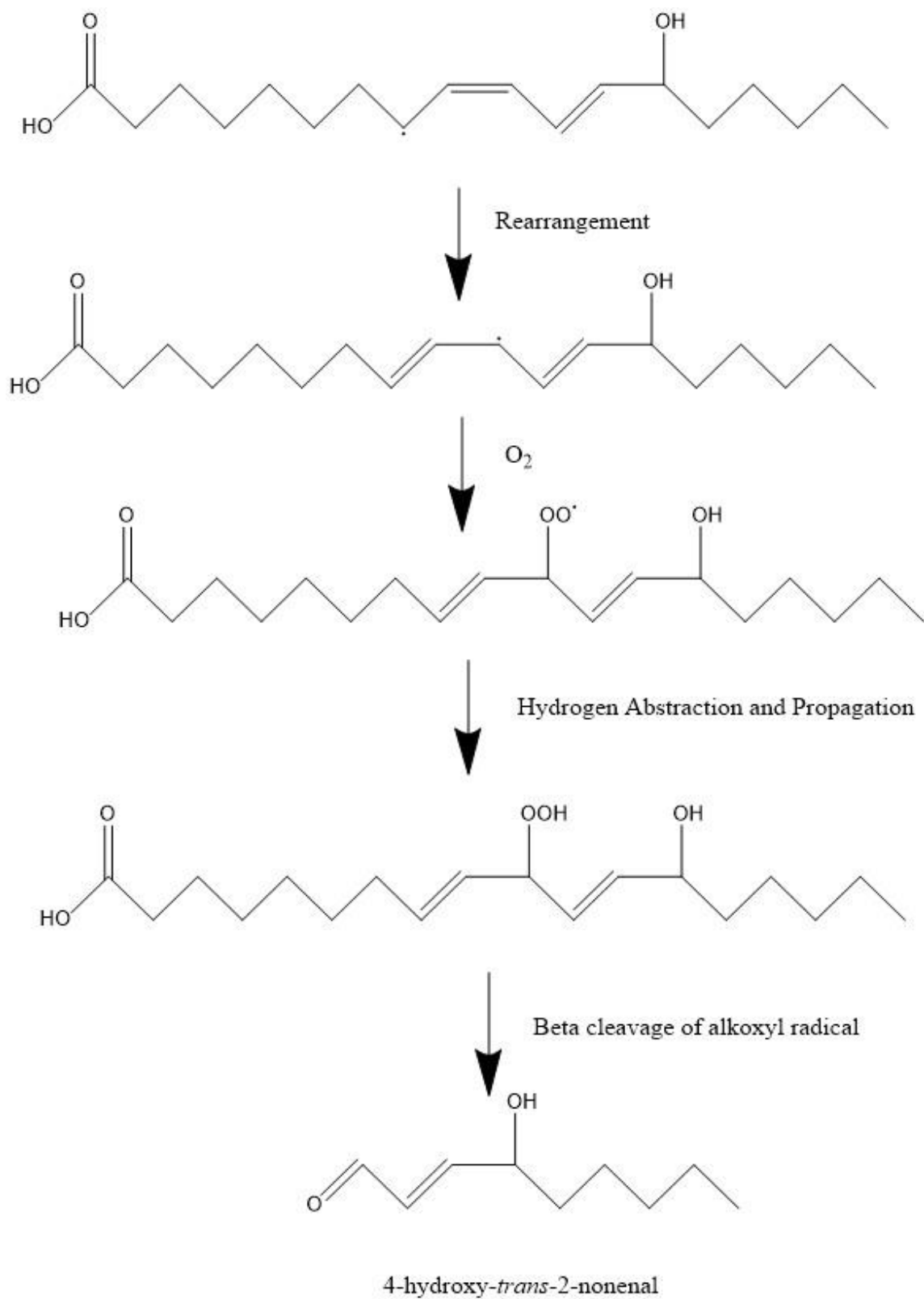


Figure 2.5: 4-hydroxy-trans-2-nonenal

2.4.5. Malonaldehyde

The second most studied lipid oxidation product that can form from ALE's is malonaldehyde (MDA). MDA is a crosslinking agent that reacts with amino groups of enzymes, proteins, and DNA. MDA appears to be more mutagenic whereas a 4-hydroxy-*trans*-2-nonenal tends to be more toxic (Esterbauer 1993). Fatty acids with at least 3 double bonds are required for the formation of MDA (Decker and McClements, 2008). MDA is one of the more abundant fatty acid decomposition aldehydes in foods (Kanner 2007). For example, 4-hydroxy-*trans*-2-nonenal, has been shown to reach 120 μM whereas MDA has been shown to reach more than 300 μM in oxidized meat and fish products (Tirosch et al., 2015).

MDA has been found bound to lysine residues of proteins in food, which can be released during digestion and is absorbed from the gut into the organs (Ayala et al., 2014). Ando et al. (1995) found higher levels of MDA (free and bound forms) in old human red blood cells compared to young red blood cells indicating an increase of oxidative damage as red blood cells age. Isolated kidney and liver nuclei treated with MDA showed significant formation of DNA-protein crosslinking (Voitkun and Zhitkovich 1998). MDA-DNA adducts have also been reported in human breast and liver tissues (Sun et al., 2004).

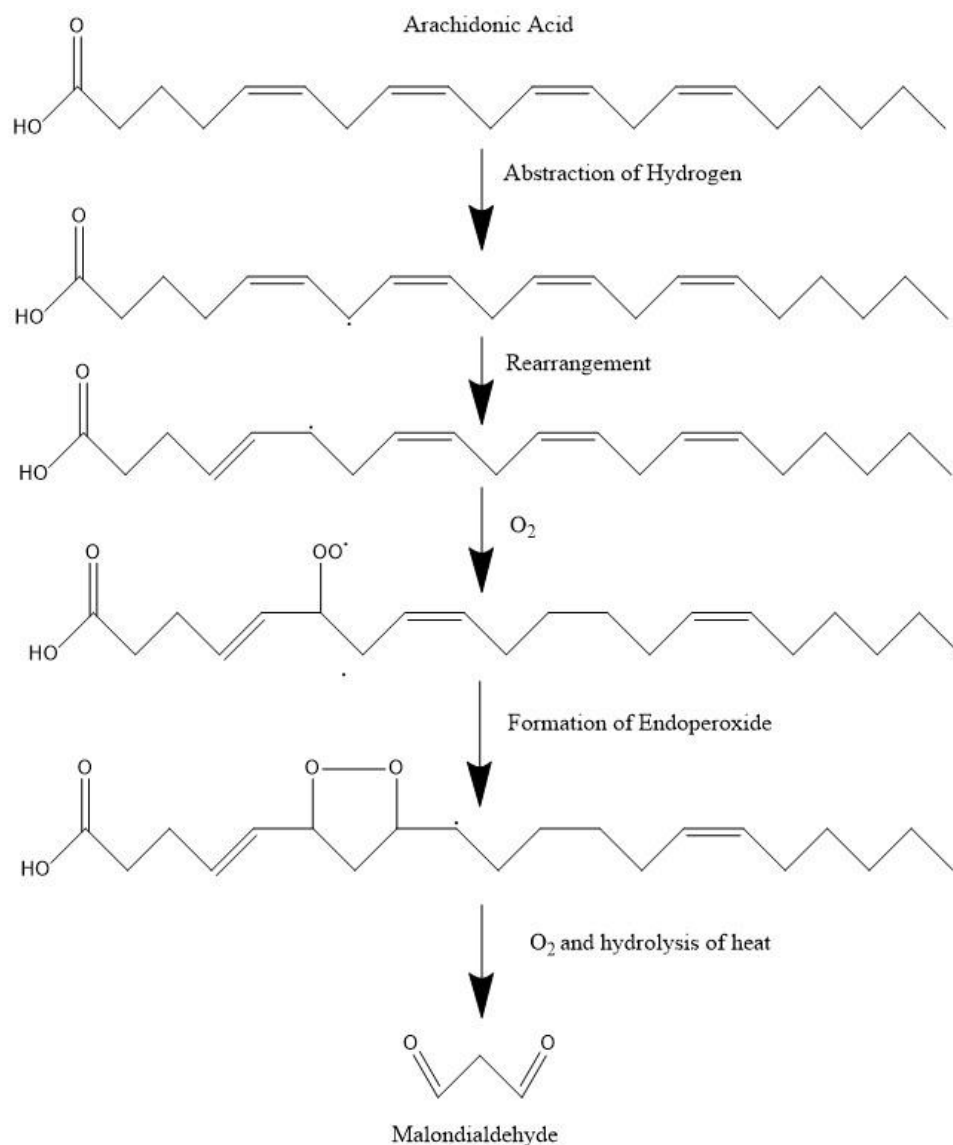


Figure 2.6: Malondialdehyde

2.4.6. Cholesterol

Another commonly studied group of oxidation products that impact health are those that arise from cholesterol. The oxidation of cholesterol results in the formation of many byproducts called oxysterols such as 7- α and 7- β -hydroxy-cholesterol, 7-ketocholesterol and 5- α 6- α epoxy-cholesterol (Figure 4; Vejux et al., 2011). Oxidized cholesterol products are

found in cholesterol-rich processed foods such as dried egg, milk powders, heated butter-ghee, and can reach a level of 10 to 100 μM (Guardiola et al., 2002). Oxidized cholesterols have been shown to have proatherogenic biological activities. 7-Oxygenated species of oxysterol are cytotoxic toward endothelial cells (Smith 1980). Oxysterols also effect the gene expression that impacts cytotoxicity, atherogenicity, mutagenicity, and lipid metabolism (Smith 1980). Mice fed a combination of oxidized cholesterol and salmon oil showed an expression of 50 genes compared to the 7 genes when fed oxidized cholesterol and coconut oil (Ringseis and Eder 2005) suggesting that the composition of the diet could impact their toxicity. The transcriptionally affected genes were those that involved in xenobiotic metabolism and stress response (Ringseis and Eder 2005). Strapans et al. (2005) found that when human subjects were fed alpha-epoxy cholesterol, it was taken up by chylomicrons and incorporated to endogenous VLDL and HDL in the liver. The ability of oxidized cholesterol to be transferred from the liver to VLDL and HDL is unlike lipid oxidation aldehydes which are primarily found in the chylomicrons (Strapans et al., 1994).

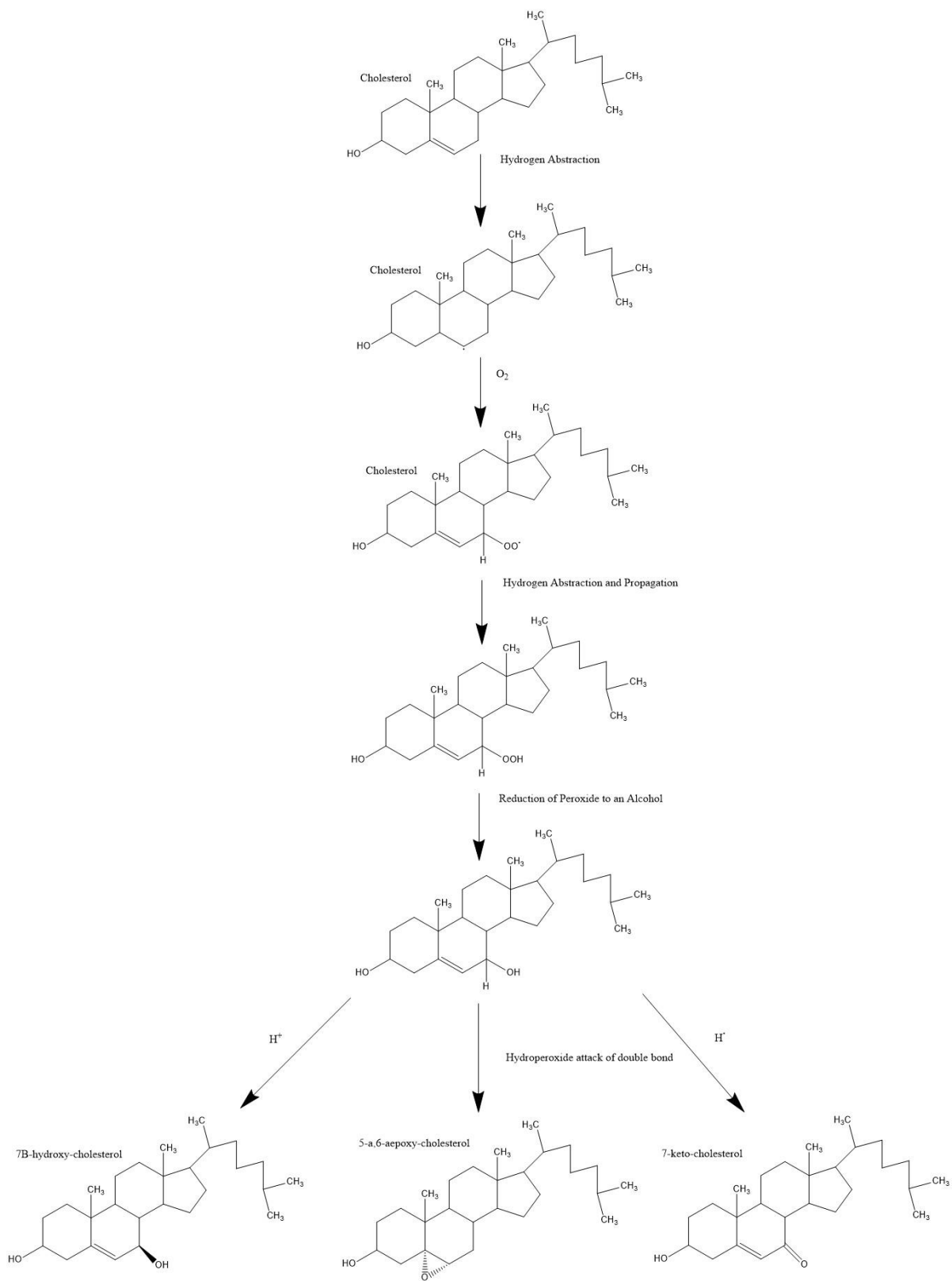


Figure 2.7: Cholesterol and Oxysterol Derivatives

2.5. *In Vivo* Production of Lipid Oxidation Products

Extensive lipid oxidation in healthy individuals is very unusual as biological systems have very complex methods to control prooxidants, scavenge free radicals, regenerate antioxidants and repair and/or replace oxidized compounds (e.g. membranes, proteins and DNA) (Aw et al. 1991, Pietta 2000). Oxidation can be associated with diseases however; it is difficult to identify whether lipid oxidation caused the disease or if the disease was the result of tissue malfunction that damaged inherent oxidation control leading to lipid oxidation (Baynes 2007). In addition, human behavior such as smoking and extreme exercise (e.g. marathon runners) can result in increased oxidative reactions (Ryu et al. 2016).

It was originally suggested that the main risk of lipid oxidation products to human health would be consumption of oxidation products that were produced during food processing and subsequent storage. More recently, interest has increased in the possibility that lipid oxidation products could be formed during consumption of foods. Lactoperoxidase is a natural peroxidase enzyme found in the saliva, tears, gastric juices, and is naturally present in fresh raw milk. Salvolini et al. (2000) studied human whole saliva in 169 healthy subjects and found that with increasing age there is a decrease in peroxidase activity and a subsequent increase in saliva lipid hydroperoxides levels. In meats, iron and myoglobin are strong prooxidants which have been shown to increase lipid hydroperoxides in the consumed meat up to 6 fold (1200 μ M after 180 min) in the acid environment of the stomach (Kanner and Lippidot, 2001).

Formation of lipid hydroperoxides would not be that problematic since, as discussed above, they are not readily absorbed into blood. However, lipid hydroperoxides can decompose into free radicals that can oxidize other unsaturated fatty acids and the decomposition of hydroperoxides will then form aldehydes that are potentially toxic. Kanazawa and Ashida (1998)

found that linoleic hydroperoxides were broken down into hydroxides, epoxides, ketones, and aldehydes in the rat stomach. Lipid hydroperoxides in meat were decomposed by metmyoglobin within 15mins (Gobert et al. 2014). Ninety min after ingestion of turkey, MDA, and lipid hydroperoxides decomposition products were found to increase significantly in human plasma. Oxidation of lipid under gastrointestinal conditions has also been reported in fish oil (Kristinova et al., 2013) and oil-in-water emulsions (Lorrain et al., 2012).

While these studies on the oxidation of lipids during digestion are interesting, it is unclear how oxygen concentrations would impact these reactions. For example, MDA, 4-hydroxy-*trans*-2-nonenal, and 4-hydroxy-*trans*-2-hexenal formation from cod liver oil were found to only increase slightly under gastric condition but increased more substantially during duodenal digestion (Tullberg et al., 2016). It is clear that most foods contain oxygen and the process of chewing could increase oxygen concentrations. However, it is less clear what happens to oxygen concentrations once the food moves through the digestive tract. Tullberg et al (2016) used nitrogen flushing in the initial digestion steps but did not measure exact oxygen concentrations throughout the study. It is likely that the stomach would contain oxygen immediately after swallowing but if oxidation is rapid, oxygen concentrations could decrease and slow reaction chemistry. As the food moves into the lower GI tract, the microbiome would also consume oxygen until an anaerobic environment was created. Thus while it is interesting that *in vitro* studies have shown that oxidation can occur under simulated conditions, more work is needed to monitor oxygen levels during digestion and match these oxygen levels to those that exist *in vivo*.

2.6. Protection against Toxicity

Although there is ample evidence that lipid oxidation products could be toxic to the human body because they can react with proteins, membranes and DNA that are essential to health; there is still skepticism as to whether or not these products are actually toxic. This is due to the body's natural defensive mechanisms and natural antioxidants found in food that can inhibit lipid oxidation or counteract the side effects of lipid oxidation products. However, it is also possible that lipid oxidation products do not directly cause chronic diseases but could increase the body's susceptibility to diseases if an individual has a pre-existing health condition. There are several studies conducted on the impact of lipid oxidation products in individuals with pre-existing conditions. For example, advanced lipid oxidation end products increased inflammatory biomarkers in diabetic patients with pre-existing renal and/or vascular complications (Baynes 2007).

Free radical scavenging antioxidants break the autooxidation chain by interacting with the (peroxyl radicals (LOO^\bullet) to form stable antioxidant radicals (A^\bullet), which are less reactive or transform to nonradical products. The body has a number of natural protective mechanisms found in nucleated cells in the body and in the intestinal epithelia which assist in detoxification; these antioxidant systems include glutathione peroxidase, catalase, superoxide dismutase, free radical scavenging antioxidants (e.g. ubiquinone) and metal chelators (e.g. transferrin and ferritin) (Chan and Decker, 1994). In addition, the body's antioxidant defense is supplemented by dietary antioxidants including tocopherols and ascorbic acid. This multi-component antioxidant system is extremely effective at inhibiting lipid oxidation in healthy individuals.

In vivo antioxidants are also found in the gastrointestinal tract. Glutathione is found in the mucosal cells of the GI tract and can originate from foods as humans consume around 100-

150 mg/day of glutathione (Aw et al., 1991). Glutathione is important in the GI tract since it's able to reduce hydroperoxides and decrease absorption of hydroperoxides from the gut into the lymph in both rats and humans (Lomaestro and Malone 1995). Tanel and Averill (2007) also found that N-acetylcysteine (a precursor of glutathione) increased intracellular glutathione levels by 30% in Chinese hamster ovary cells protecting the cells against acrolein-induced apoptosis. They proposed that N-acetylcysteine inhibited activation of the mitochondrial death pathway and could be useful as a protective agent. However, lipid oxidation products can negatively impact the endogenous antioxidant pathways as 4-hydroxy-*trans*-2-nonenal, acrolein, crotonaldehyde and MDA have been shown to inactivate glutathione reductase, which maintains cellular glutathione levels that is essential for the activity of glutathione peroxidases (VanderJagt et al. 1997).

In addition to the body's defensive mechanisms, nature provides a widespread variety of antioxidants, which include phenols, phenolic acids, tannins, and carotenoids. Humans' dietary intake of total flavonoids ranges between 50-800 mg/day (Zhu et al., 2004). Red wine and tea have been reported to contain approximately 200 mg flavonoids/serving (Pandey and Rizvi 2009). A high fruit, low fat diet has been proven to reduce the effects of toxic lipid products as well as reduce the chance of cardiovascular disease. For example, polyphenolic metabolites found in red wine, cider, and tea contain strong antioxidants (Gobert et al. 2014). Flavonoids also suppress reactive oxygen species formation by upregulating and protecting the body's natural antioxidant defenses, inhibiting enzymes, and chelating transition metals that aid in lipid oxidation (Pietta 2000).

Red wine polyphenols have been reported to prevent the formation of the MDA in plasma and decrease the oxidation of postprandial LDL by 34% compared to a 50% increase in MDA without wine (Gorelik et al. 2008). Dietary antioxidants can also be important in the GI

tract. Kanner and Lapidot (2001) found that red-wine polyphenols were able to inhibit metmyoglobin-catalyzed oxidation under stomach conditions in a dose-dependent manner with higher concentrations of polyphenols leading to no accumulation of hydroperoxides. Gobert et al. 2014 found that 240 min after the consumption of beef meal, minipigs had a 5-fold increase in TBARS compared to 2-fold increase when fruit and vegetables or polyphenols were added to a beef meal. Even if lipids oxidize during digestion, lipid oxidation products could react with food proteins limiting their digestion and absorption thus providing another level of protection (Baynes, 2007). However, not all dietary antioxidants are effective in the GI tract. For example, ascorbic acid was prooxidative during the gastric digestion of beef (Van Hecke et al., 2016) which is likely due to the ability of ascorbic acid to reduce iron to its more prooxidative state (Decker, 1997).

2.7. Conclusion

The western diet involves a high dietary consumption of unsaturated fats that can potentially produce high amounts lipid oxidation products. These lipid oxidation products can enter the body through the diet, be formed in gastrointestinal tract or be produced in the body, especially in unhealthy individuals. Lipid oxidation aldehydes are potentially toxic especially α , β -unsaturated aldehydes that are highly reactive with proteins, cell membranes and nucleic acids. Lipid hydroperoxides do not seem to be readily absorbed into plasma from the diet but other oxidation products such as aldehydes, alcohols and oxidized cholesterol are incorporated into chylomicrons thus can be transported to tissues. Of these products, only oxidized cholesterol seems to be incorporated into VLDL and HDL in the liver and thus further incorporated into tissues. Many lipid oxidation products might not be absorbed into the plasma but still might have health implications by promoting gut inflammation and possibly altering the

microbiome. While this field of toxic lipid oxidation products is still relatively young, it's obvious that consumption of oxidized lipids could cause health problems and thus it is critical that food oils are protected from oxidation and that diets include antioxidants that could decrease lipid oxidation *in vivo*.

CHAPTER 3
UTILIZING NUTRITIVE SWEETENERS TO CONTROL LIPID OXIDATION
IN LOW MOISTURE BAKED GOODS

3.1. INTRODUCTION

The western diet involves a high dietary consumption of saturated fats from low moisture snack foods (National Cancer Institute, 2010). Therefore, companies have begun replacing chemically stable saturated fat with healthier, more chemically unstable unsaturated fat. Unsaturated fats are prone to producing high amounts of potentially toxic lipid oxidation products such as ketones, epoxides, and aldehydes which can lead to various biological consequences (Halyna and Semchyshyn, 2014). Consequently, the incorporation of unsaturated fats requires additional strategies to maximizing the chemical stability of the low moisture food. The food industry is focused on formulating consumer friendly, clean-labels leading to increased research in natural antioxidants to prevent lipid oxidation of unsaturated fats. However, natural antioxidants are often needed in high concentrations which can alter the texture or flavor of a food. Therefore, it is more applicable to maximize the stability of foods without adding additional ingredients. This requires in depth knowledge of the mechanisms of lipid oxidation so prooxidative and antioxidative factors can be identified and controlled.

In order to maximize the stability of unsaturated fats, one mechanism researchers have focused on is controlling the water activity of the system. In low water activity foods, the motility of prooxidants is decreased due to the water binding of the system and a decrease in the availability of water molecules for chemical reactions (Eichner and Karel. 1972, Labuza et al. 1970). To control this important quality parameter, the food industry has focused its research on packaging to prevent moisture transfer within the system. However, once the product

reaches the consumer and the packaging is opened, then the water activity of the product changes. For example, Patriganani et al. (2014) found that the water activity of biscuits increased over 124 days and was the main limiting factor of the biscuit shelf-life.

Sugar has the potential to impact lipid oxidation because it can alter viscosity, change water activity, scavenger free radical via its hydroxyl groups and donate hydrogen if it is a reducing sugar. For instance, sugar in emulsions can retard lipid oxidation by increasing the viscosity of the system (Hisieh et al. 1987, Sims et al. 1979, Joslyn and Supplee 1949).

Ponginebbi et al. (1999) found that sucrose significantly retarded lipid oxidation in linoleic acid emulsions and more inhibition occurred when the sucrose concentration increased from 0 to 14% (wt./vol). Between 22-31% there was no additional effect on oxidation therefore they assumed that the inhibitory effect had reached a maximum at 14%. However, at low sugar concentrations, sugars accelerated lipid oxidation in a Triton X-100 stabilized methyl linoleate emulsion (Yamauchi et al., 1982). These researchers also reported that reducing sugars could accelerate the decomposition of lipid hydroperoxides. EDTA inhibited this reaction suggesting that reducing sugars were converting transition metals to their more prooxidative reduced state which in turn rapidly decomposed hydroperoxides (Yamauchi et al., 1983). These studies suggest that sugars can be both prooxidative and antioxidative depending on their concentrations. The role of sugar on lipid oxidation reactions in low moisture foods remains unstudied.

The term “low moisture foods” in this study refers to prepared foods such as cookies, crackers, cereal etc. Our laboratory has previously studied lipid oxidation in a cracker model system which was lower in fat (8%), did not contain added sugar and was kneaded to develop gluten cross linking. In this study, we utilized short-bread cookies, another low moisture food with a water activity below 0.5 (Labuza et al., 1970), as a model system to study the effect of

sugars on lipid oxidation. Shortbread cookies are higher in both fat (30-40%) and sugar ($\approx 14\%$) than the cracker model and are a typical drop cookie that has minimal mixing and kneading to minimize gluten cross linking. How sugars impact physical structure was examined by confocal microscopy. The impact of sugar on lipid oxidation reactions was determined by varying sugar concentration and type and by studying the relationship between how sugar type influenced browning reactions, water activity and lipid oxidation. This information was then used to evaluate how cookie formulations could be utilized to increase the shelf-life of high fat cookies.

3.2. Materials and Methods

All-purpose flour (Gold Medal, original) was purchased from local grocery stores. Interesterified soybean oil #762420 was kindly donated by Archer Daniels Midland Company (Decatur, IL) and selected for its recommended use in baking applications. According to the supplier's literature, the interesterified soybean oil contained 11% palmitic, 31% stearic, 14% oleic, 35% linoleic and 4% linolenic. Since the interesterified soybean oil contained substantial levels of the omega-6 fatty acid, linoleic acid, hexanal would be an appropriate secondary lipid oxidation product to monitor lipid oxidation (Table 1). Dry goods were stored in closeable freezer bags; interesterified soybean oil was kept frozen (-20°C) until use. The non-polar dye Bodipy 493/503 ("Bodipy") was purchased from Invitrogen (Carlsbad, CA). Rhodamine B, ammonium thiocyanate, barium chloride, iron (II) sulfate heptahydrate, hydrochloric acid, propyl gallate (PG), tert-butylhydroquinone (TBHQ), butylhydroxy toluene (BHT) and cumene hydroperoxide, were purchased from Sigma-Aldrich (St. Louis, MO). Methanol, hexanal, ethanol, and chloroform were purchased from Fisher Scientific (Waltham, MA). All solvents and reagents were of analytical grade or higher. Double distilled water was used to prepare all solutions.

3.2.1. Sample Preparation

The composition of the cookie formulation is shown in Table 1. Cookies were prepared by creaming interesterified soybean oil with the sugar with a rubber spatula until a homogenous mixture was obtained. After creaming, water was added followed by flour. In experiments with varying sugar concentrations, flour was used to replace sugar. Dough was rolled into balls (27.0g) by hand and placed onto an ungreased baking sheet where they were compressed to approximately 3 cm. The cookies were baked at 190°C for 18 min in a General Electric oven (GE model JB350DFWW; Fairfield, CT). After baking and cooling for 20 min, the cookies were coarsely crumbled using a mortar and pestle and 0.5 g was placed into acid-washed, 10-ml glass GC vials (Supelco Analytical; Bellefonte, PA) and closed with aluminum caps containing PTFE/silicone septa (Supelco Analytical; Bellefonte, PA). Samples were stored at 55°C in the dark or in desiccator containing saturated magnesium chloride at 55°C in the dark. When stored in a desiccator, samples were left open for two days until an Acurite humidity meter reached 30% humidity.

Table 3.1: Ingredient Composition for Model System

| Ingredient | Industrial Cookie Percentage (w/w) | Control Percentage (w/w) |
|-----------------------------|---|---------------------------------|
| Interesterified Soybean Oil | 30.0 | 30.0 |
| Sugar | 14.0 | 0 |
| Flour | 53.0 | 67.0 |
| Water | 3.0 | 3.0 |
| Total | 100.0 | 100.0 |

3.2.1.1. Addition of confocal dyes

Bodipy and Rhodamine B were added at concentrations based on the work of McClements and Matalanis (2012). Bodipy (1.0 mg) was dissolved in 1 mL ethanol, and fully mixed with the lipid phase until even distribution of colored dye was visually detected before incorporating the sugar. Rhodamine B (0.3 mg) was dissolved in double distilled water and added directly to the lipid phase prior to sugar addition.

3.2.1.2. Addition of Browning Inhibitors

Sulfite, Cysteine and Ascorbic Acid were added to the cookies on an equal molar basis according 21 C.F.R. § 130.9 2015. upper limit for sulfites in final food products. Browning inhibitors were added directly to the flour before being added to the lipid phase.

3.2.1.3. Addition of Antioxidants

Synthetic antioxidants [propyl gallate, tert-butylhydroquinone, and butylhydroxy toluene] were dissolved in ethanol and mixed with the interesterified soybean oil for 1 min before sugar was added according to 21 C.F.R. § 172.115 2015

3.2.1.4. Characterization of the Cookies

Cookie pH was determined by crushing cookies from each treatment and forming a 5:1 (wt./wt.) slurry in double distilled water by vortexing. The sample was then vacuum filtrated and the pH of the supernatant was taken. Water activity was determined at 23-28°C using a Decagon Devices AquaLab Series 3 water activity meter (Pullman, WA). Cookies (2.0g) were crushed to form a consistent layer across the bottom of the sampling cup. Colorimetric readings of intact cookies were analyzed using Hunter Lab Colorimeter ColorFlex EZ SOP (Restion, VA, USA) on a white backdrop.

3.3. Oxidation Measurements

3.3.1. Hydroperoxide Analysis

Lipid hydroperoxides, a primary oxidation product, were measured using a modified version of the International Dairy Federation method as described by Shantha and Decker (1994). Cookie samples (0.1 g) were crushed using a mortar and pestle and added to a mixture of chloroform and methanol (5 mL; 2:1 v/v). The samples were vortexed briefly, and centrifuged (2500g) for 10 min to extract the hydroperoxide fraction. The top layer (200 µl) was then mixed with 16.7 µl of a 1:1 solution (ferrous sulfate dissolved in double distilled water + barium chloride dissolved in 0.4 N hydrochloric acid) and 16.7 µl of ammonium thiocyanate dissolved in double distilled water. This final mixture was then vortexed, covered to prevent evaporation, and incubated for 20 min to allow color development. Absorbance was measured at 500 nm on a Genesys 20 spectrophotometer (ThermoSpectronic; Waltham, 61 MA). The final concentration of hydroperoxides was calculated using a cumene hydroperoxide standard curve.

3.3.2. Hexanal Headspace Analysis

Headspace hexanal, a secondary oxidation product, was measured with a GC-2014 Shimadzu gas chromatograph equipped with an AOC-5000 autosampler (Shimadzu; Kyoto, Japan) based on the method of Panya et al. (2012). A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/Carboxen/PDMS) stable flex solid phase microextraction (SPME) fiber (Supelco, Bellefonte, PA) was inserted through the vial septum and exposed to the sample headspace for 10 min at 55°C. The volatiles on the SPME fiber were desorbed at 250°C for 3 min in the GC detector at a split ratio of 1:7. The chromatographic separation of volatile aldehydes was performed on a fused-silica capillary column (30 m \times 0.32 mm i.d. \times 1 μm) coated with 100% poly(dimethylsiloxane) (Equity-1, Supelco). The temperatures of the oven, injector, and flame ionization detector were 65, 250, and 250°C, respectively. Sample run time was 6 min. Peak integration was calculated using Shimadzu EZstart (version 7.4). Concentrations were calculated using a standard curve made from crushed cookie spiked with known hexanal concentrations.

3.3.3. Imaging by microscopy

A Nikon Confocal Microscope (C1 Digital Eclipse, Tokyo, Japan) with a PL FLUOTAR ELWD 20.0x0.45 objective lens was used to capture confocal images. Cookies containing both Bodipy (lipid soluble) and Rhodamine B (protein specific) were imaged consecutively, and the final images were overlaid. Bodipy was excited at 488 nm, and emission spectra were collected at 515 \pm 30 nm. Rhodamine B was excited at 543 nm and emission spectra were collected at 605 \pm 75 nm. Detector pinhole size was 150 μm and final images consisted of 512 \times 512 pixels, with a pixel size of 1243.2 nm, and a pixel dwell time of 1.68 μs . Confocal microscopy images were

evaluated using EZ-CS1 (version 3.8) software (Nikon; Melville, NY), while optical images taken on the same microscope were analyzed using NIS-Elements (version 3.0) software (Nikon, Melville, NY).

3.4. Statistical analysis

All experiments were conducted in triplicate. Oxidation lag phases were defined as the first data point that was consistently significantly greater than the time-zero value. Statistical analysis was carried out by analysis of variance (ANOVA) and Turkey's Honestly Significant Difference test using SPSS software (SPSS., Armonock, NY, USA). A significance level of $p < 0.05$ was defined as being statistically different.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Composition and Microstructure

The pH of the model cookie was 6.3 and did not vary significantly with the addition of confocal dyes, different sugars, or the change in sugar concentration. Water Activity of the uncooked dough ranged from 0.66-.74. Post baking the water activity of cookies ranged between 0.14-.25 depending on the system sugar type.

Table 4.1: Water Activity of samples pre and post baking

| | Before | After |
|-----------------|-----------|-----------|
| | Aw | Aw |
| Sucrose | 0.73±0.01 | 0.14±0.01 |
| Glucose | 0.71±0.01 | 0.25±0.02 |
| Fructose | 0.72±0.01 | 0.17±0.01 |
| Control | 0.71±.01 | 0.15±0.00 |

Bodipy and Rhodamine B dyes were added to visualize the lipid and protein portions, respectively, with confocal microscopy. Air bubbles and starch granules were determined based on their absence of dye stain and size (Barden et al., 2015). Lipid formed a continuous phase surrounding the starch granules in cookies made with either sucrose or glucose (Figure 1,2). This structure is similar to a cracker model system made with lower levels of interesterified soybean oil (8%). Protein levels were less in the model cookie than cracker which is likely due to the lower level of flour in the cookie due to the higher levels of sugar and fat.

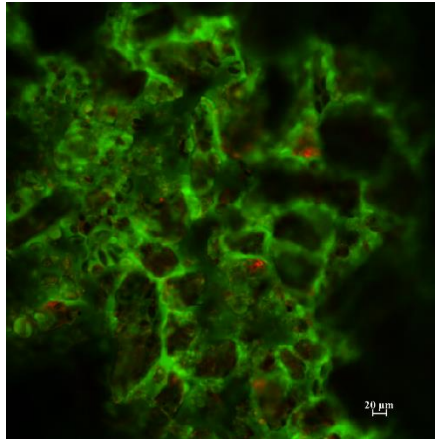


Figure 4.1 Sucrose

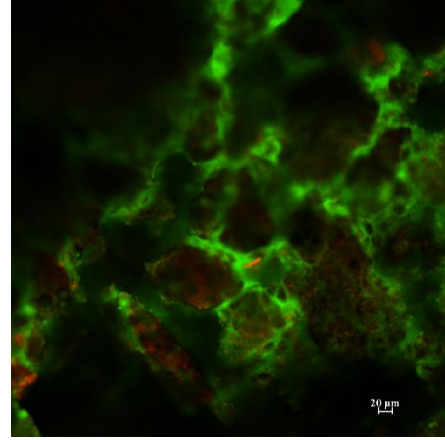


Figure 4.2 Glucose

4.2. Lipid Oxidation of Cookies

4.2.1. Lipid Oxidation of Cookies at High Molar Concentrations of Sucrose or Glucose

A typical shortbread cookie contains approximately 14% sucrose. Therefore, cookies were prepared with 14% sucrose as well as lower concentrations (0.17-.50 moles sugar/kg cookie) to determine the impact of sugar on lipid oxidation. Glucose was also tested as it is a reducing sugar and thus could have different impact on lipid oxidation kinetics. Lipid hydroperoxide lag phase for the control was 18 days. All sucrose concentrations had a hydroperoxides lag phase of 38 days. For hexanal formation, the control had a lag phase of 18 days and all sucrose concentrations had a lag phase of 41 days. Glucose (0.17 moles/kg) had a slight increase in lipid hydroperoxides and headspace hexanal after 63 days. The two higher glucose concentrations (0.33, and 0.50 moles glucose/kg cookie) never exited the lag phase after 112 days of storage at 55°C for either lipid hydroperoxides or headspace hexanal.

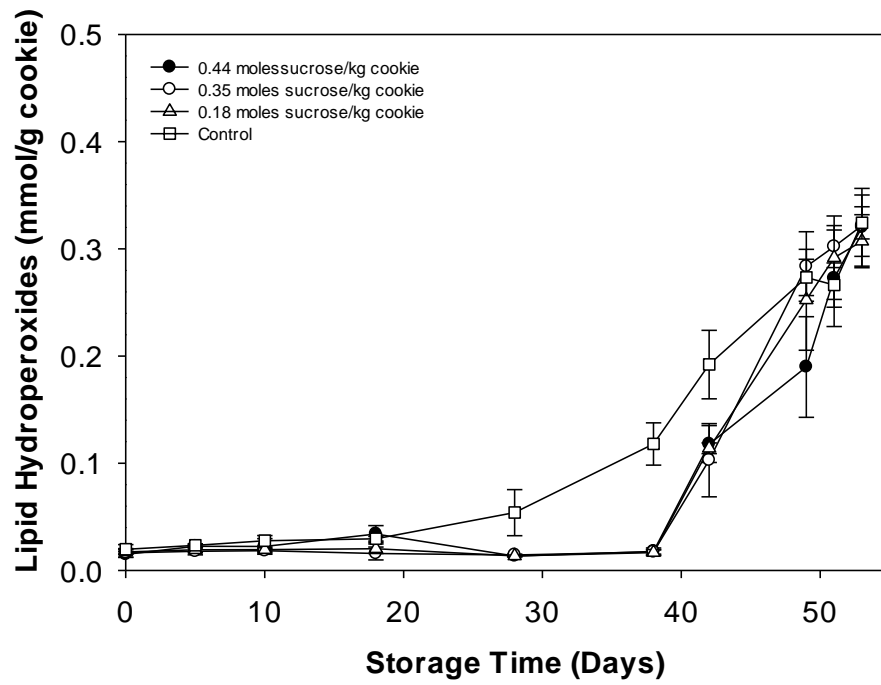


Figure 4.3: Lipid Hydroperoxides of Sucrose at High Molar Concentrations

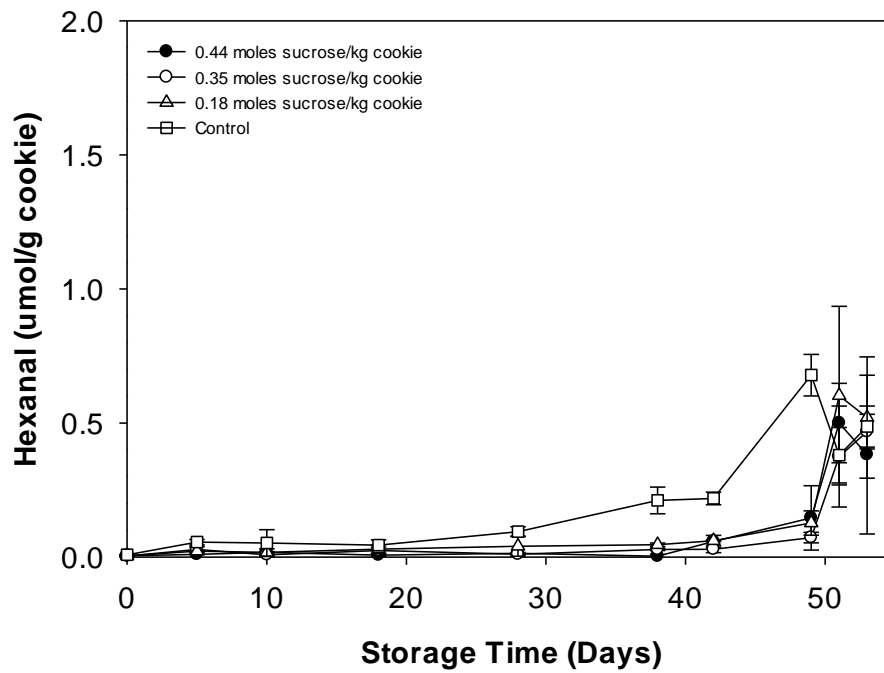


Figure 4.4: Hexanal of Sucrose at High Molar Concentrations

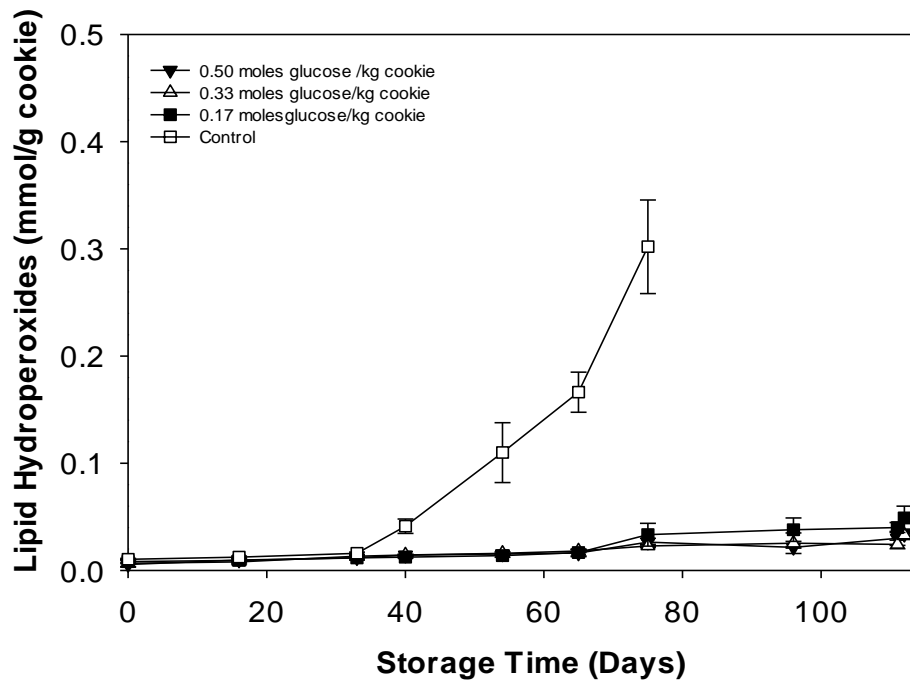


Figure 4.5: Lipid Hydroperoxides of Glucose at High Molar Concentrations

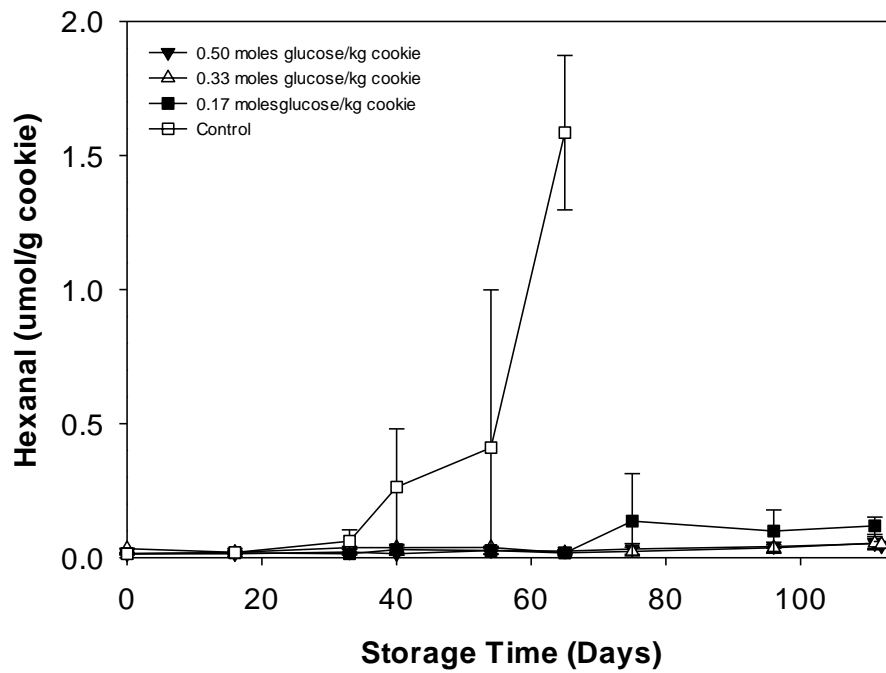


Figure 4.6: Hexanal of Glucose at High Molar Concentrations

4.2.2. Lipid Oxidation of Cookies at Low Molar Concentrations of Sucrose or Glucose

Since glucose was strongly antioxidative and samples did not exit the lag phase, lower sugar concentrations were tested. Control samples had a lag phase of 28 days for hydroperoxides and 31 days for headspace hexanal. Hydroperoxides lag phases were 31, 35 and 38 days for 0.09, 0.03 and 0.03 moles sucrose/kg cookie, respectively. Hexanal lag phases were 34,53, 41 days for 0.09, 0.03 and 0.03 moles sucrose/kg cookie, respectively. For glucose, samples made with lower molar concentrations, the samples had a hydroperoxide lag phases of 21 and 34 days and a hexanal lag phase of 34 and 41 days for concentrations 0.03 and 0.003 moles glucose/ kg cookie, respectively. The sample with 0.09 moles glucose/ kg cookie did not exit hydroperoxide or hexanal lag phase.

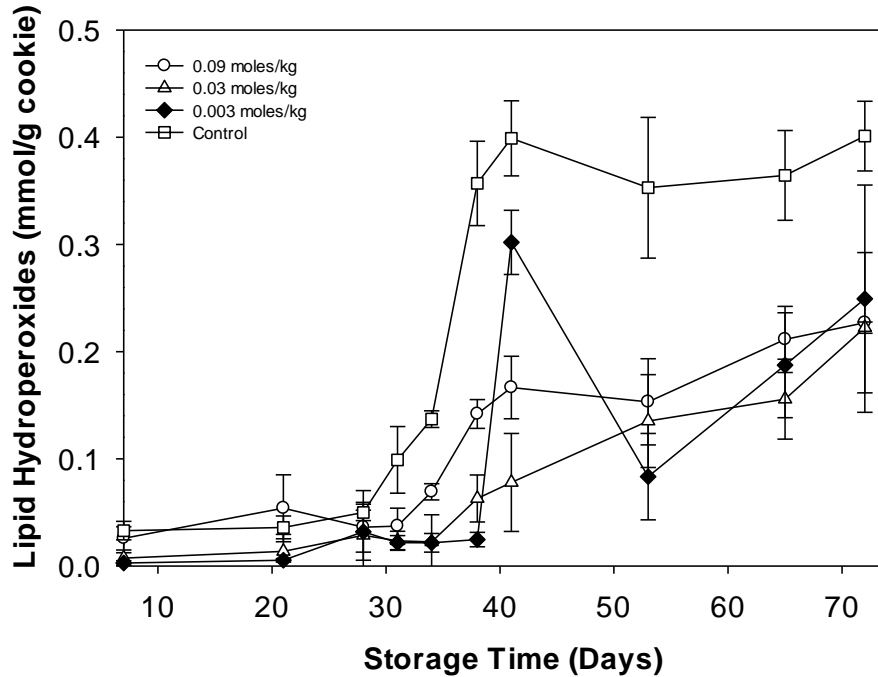


Figure 4.7: Lipid Hydroperoxides of Sucrose at Low Molar Concentrations

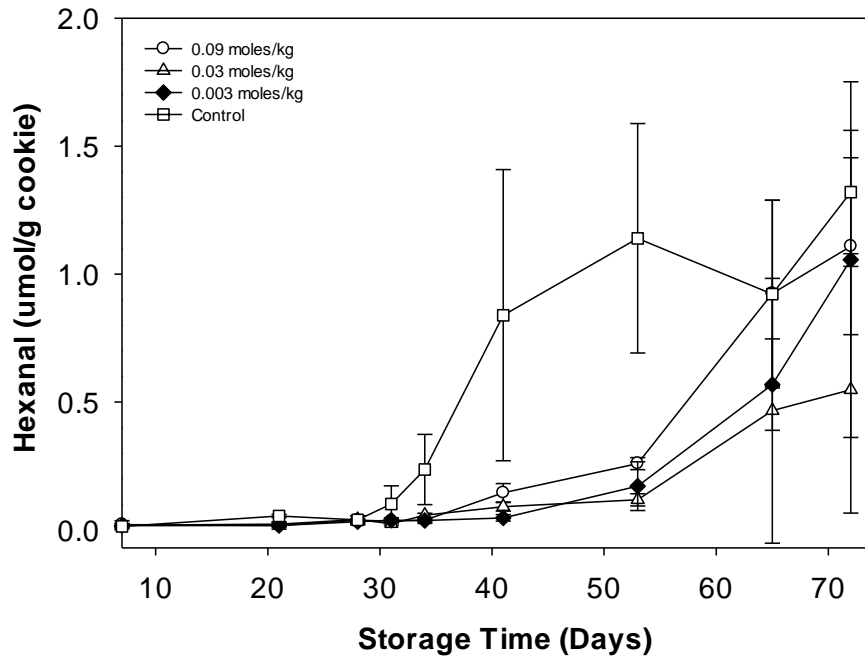


Figure 4.8: Hexanal of Sucrose at Low Molar Concentrations

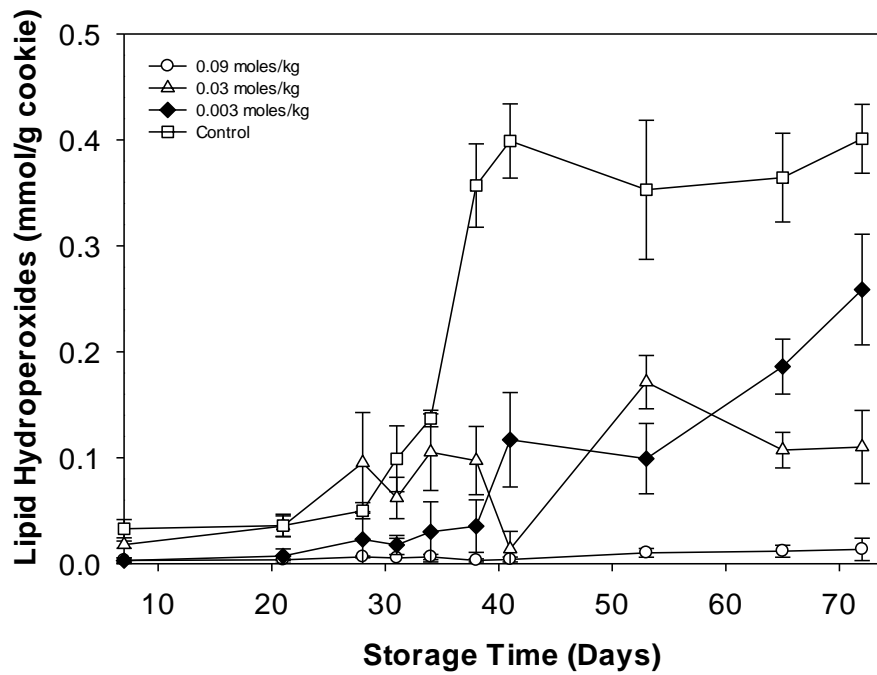


Figure 4.9: Lipid Hydroperoxides of Glucose at Low Molar Concentrations

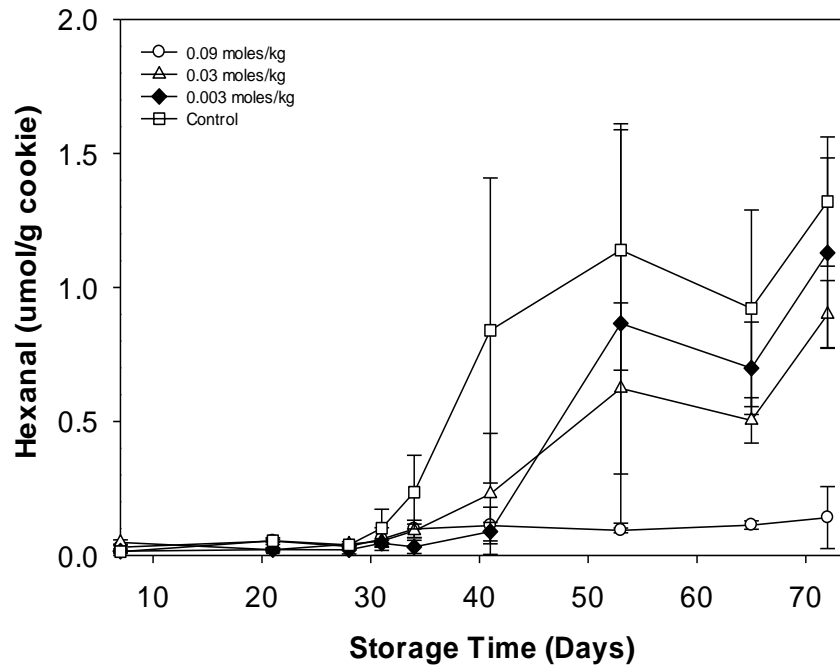


Figure 4.10: Hexanal of Glucose at Low Molar Concentrations

4.2.3. Lipid Oxidation of Cookies made with Various Sugars

Overall, an increase in either sucrose or glucose concentration lead to a stronger antioxidant effect. In terms of the short-bread cookies it was proven that glucose was more effective than sucrose in extending the lag phase of both hydroperoxide and hexanal formation. This effect has also been shown to occur in oil-in-water emulsions in which increasing sucrose (0 to 14%) increased inhibition of lipid oxidation (Ponginebbi et al. 1999). Hydroperoxide and hexanal formation was also compared in cookie samples containing sucrose, glucose, and fructose at 0.09 moles sugar/kg cookie. Hydroperoxide lag phase ranged from Control < Fructose < Sucrose < Glucose while Hexanal lag phase was in the order of Control < Sucrose < Glucose < Fructose. Again we see that reducing sugars, glucose and fructose,

had a greater difference in lag phases than the non-reducing sugar, sucrose. It has been noted that reducing sugars like glucose can inhibit lipid oxidation by donating hydrogen to free radicals (Yamauchi et al., 1982). However, sugars could potentially also inhibit lipid oxidation by binding water and altering water activity.

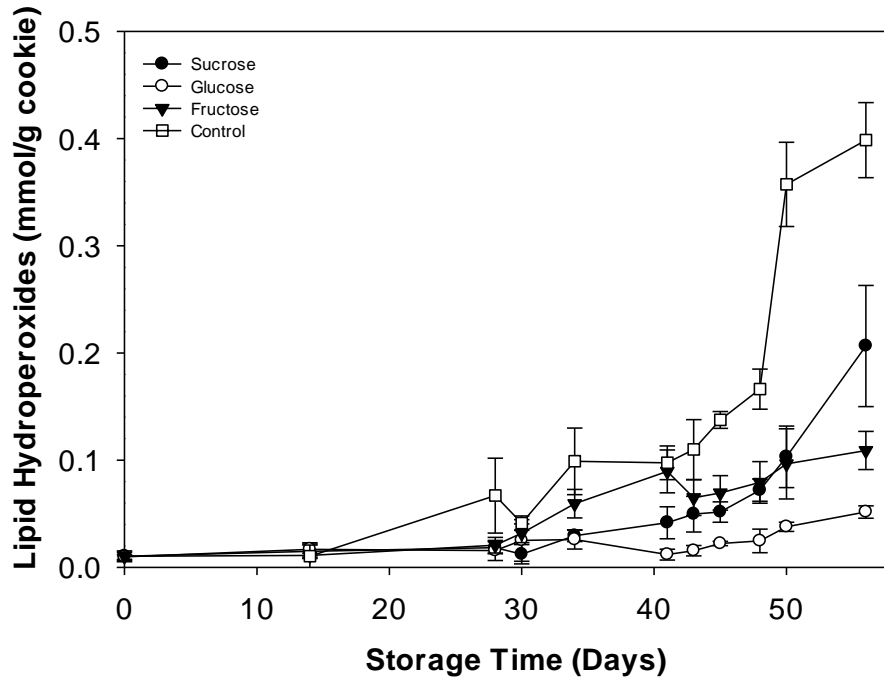


Figure 4.11: Lipid Hydroperoxides of Various Sugars

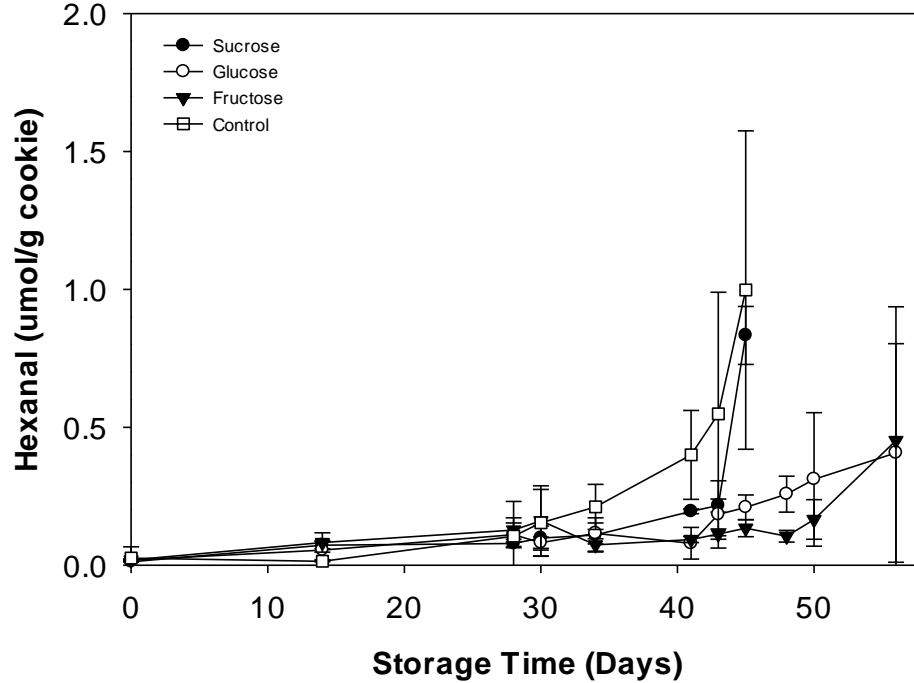


Figure 4.12: Hexanal of Various Sugars

4.3. Effect of Controlled Water Activity

In the above experiments, cookies with sucrose, glucose and fructose had average water activities of 0.14, 0.25 and 0.17, respectively. Since water activity is well known to influence lipid oxidation kinetics, hydroperoxide and hexanal formation were determined for the different sugars (0.09 moles sugar/kg cookie) in a controlled humidity environment which produced a similar water activity in all samples ranging from 0.20 to 0.23. Hydroperoxide lag phases under controlled water activity were in the order of Control<Sucrose=Fructose=Glucose. All samples containing sugar had the same hexanal lag phases 36 days.

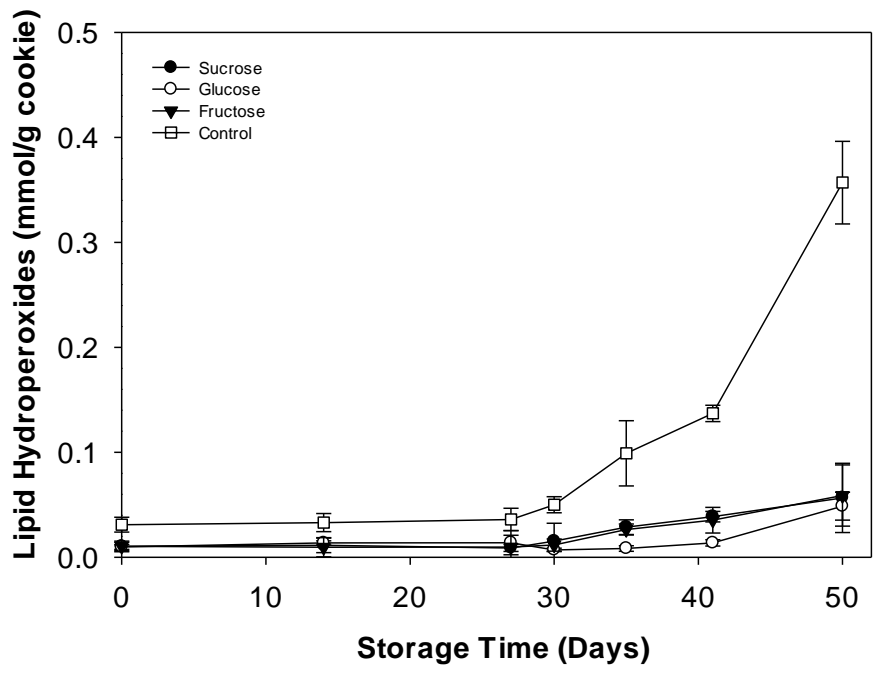


Figure 4.13: Lipid Hydroperoxides of Various Sugars in Controlled Water Activity Environment

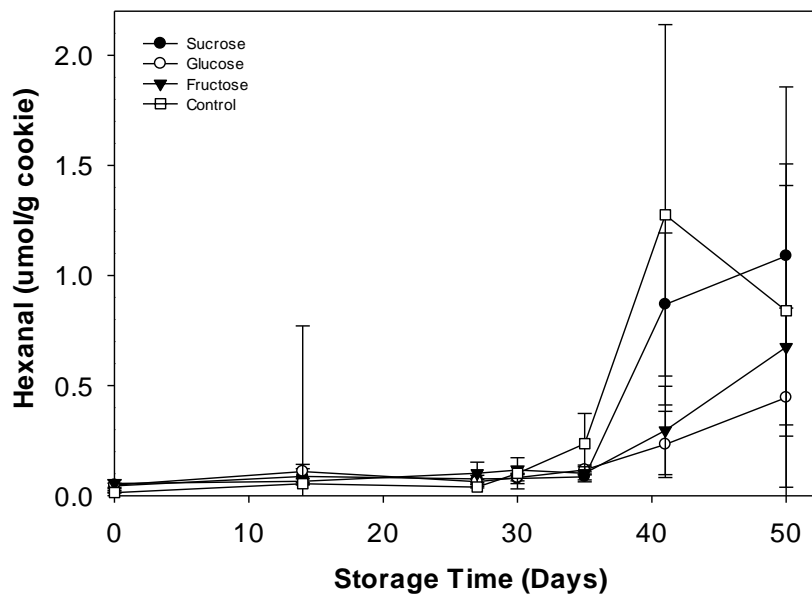


Figure 4.14: Hexanal of Various Sugars in Controlled Water Activity Environment

4.4. Impact of Maillard Browning Pigments

4.4.1. Colorimetry of Samples

Sugar type also impacted the color of the cookies as they participated differently in browning reactions. Color in terms of L values was in the order of control \cong sucrose > glucose > fructose; in terms of a values they were sucrose < control < glucose < fructose; and in terms of b values they were sucrose < control \cong glucose < fructose. As expected the cookies containing the reducing sugars had more nonenzymatic browning than the control and sucrose containing cookies. Maillard browning products have been reported to inhibit lipid oxidation in many food systems due to their ability to decompose hydroperoxides (Tanka et al. 1992), scavenge free radicals (Marales and Jimenez-Perez, 2001; Yen and Hsieh, 1995) and chelate metals (Wijewickereme et al. 1997). The addition of glucose and glycine to bakery products was shown to inhibit lipid oxidation (Griffth and Johnson, 1957). However, in the model cookie system used in this study, fructose created the most browning products as can be seen from its lower L value, but was not any more oxidatively stable than cookies with glucose or sucrose when water activity was controlled. However, fructose did make the cookies more stable than glucose and sucrose when water activity was not controlled.

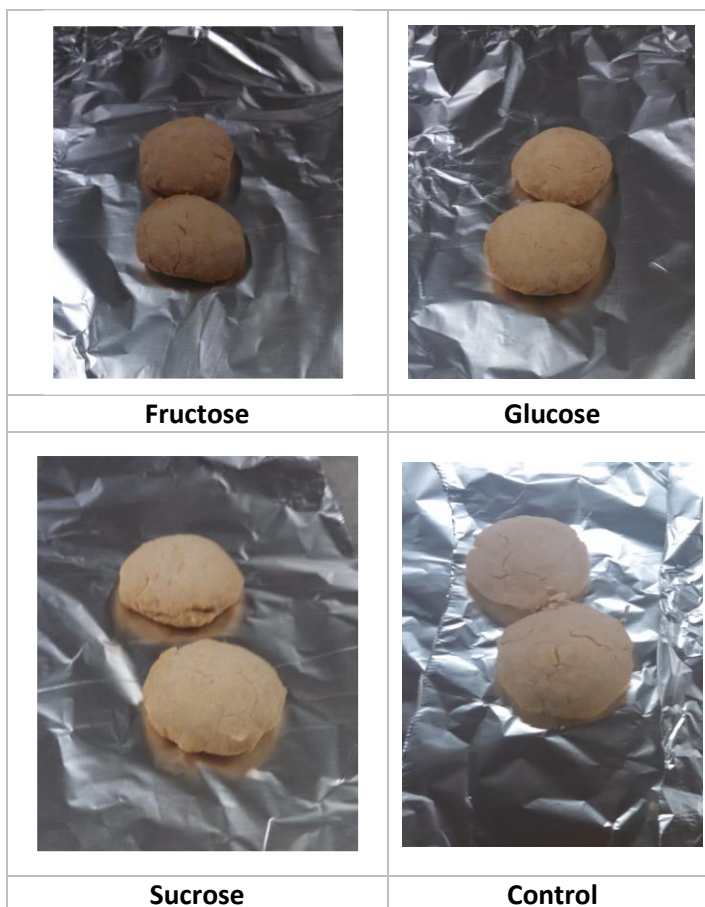


Figure 4.15: Images of Samples

Table 4.2: Colorimetric Values

| | L | a | B |
|-----------------|-----------|----------|----------|
| Control | 69.2±0.4 | 5.9±0.1 | 27.1±0.3 |
| Sucrose | 68.7±2.44 | 3.6±0.3 | 22.1±0.7 |
| Glucose | 65.7±0.8 | 6.9±0.3 | 28.7±0.1 |
| Fructose | 59.5±0.4 | 11.9±0.1 | 30.9±0.0 |

4.4.2. Addition of Browning Inhibitors

In an attempt to verify whether Maillard browning pigments were contributing to the overall oxidative stability of the cookies, cysteine, sulfite and ascorbic acid were added to cookies made with glucose to inhibit browning. Ascorbic acid, sulfite and cysteine had L values of 69.1, 67.2, and 74.6, respectively, compared to 65.7 for the glucose control cookies indicating

that all three decrease in non-enzymatic browning. The addition of ascorbic acid and sulfite caused an increase in lipid oxidation. For ascorbic acid, hydroperoxide and hexanal lag phases decreased to 0 and 37 days, respectively. For sulfite, hydroperoxide and hexanal lag phases decreased to 0 and 37 days, respectively. Cysteine had a strong prevention of lipid oxidation and did not exit the lag phase of both hydroperoxides and hexanal formation during the entire study.

The addition of ascorbic acid may have been prooxidative due to ascorbic acid's ability to reduce transition metal ions such as ferric ion to ferrous ion (Yamauchi et al. 1984). Therefore, the addition of ascorbic acid can accelerate the lipid hydroperoxide degradation and generating aldehydes such as hexanal. Sulfite could be prooxidative for the same reason but it is unclear why cysteine would be antioxidative as it is also able to reduce metals.

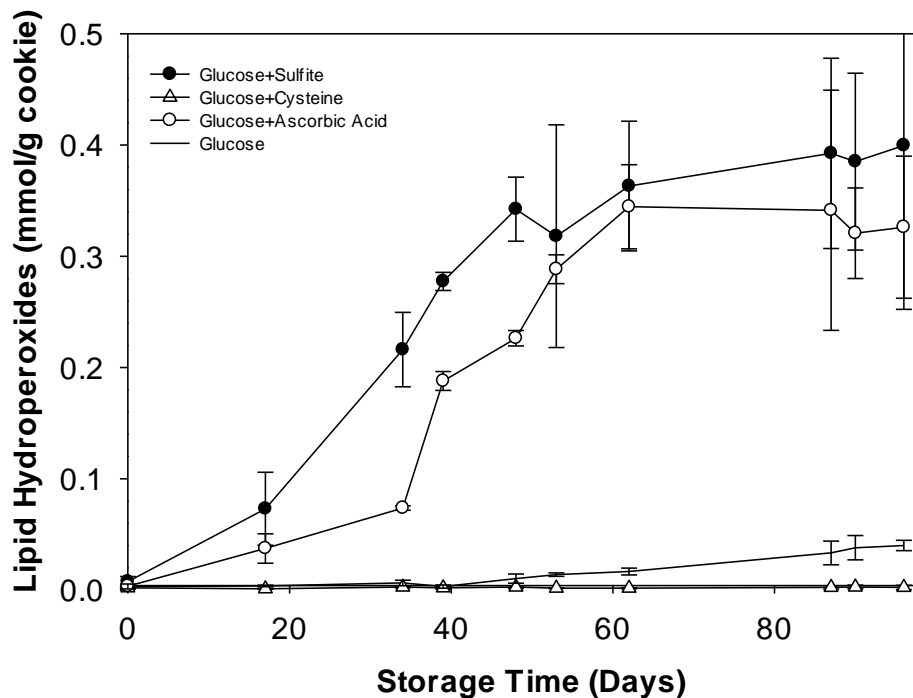


Figure 4.16: Lipid Hydroperoxide of Samples with Inhibitors

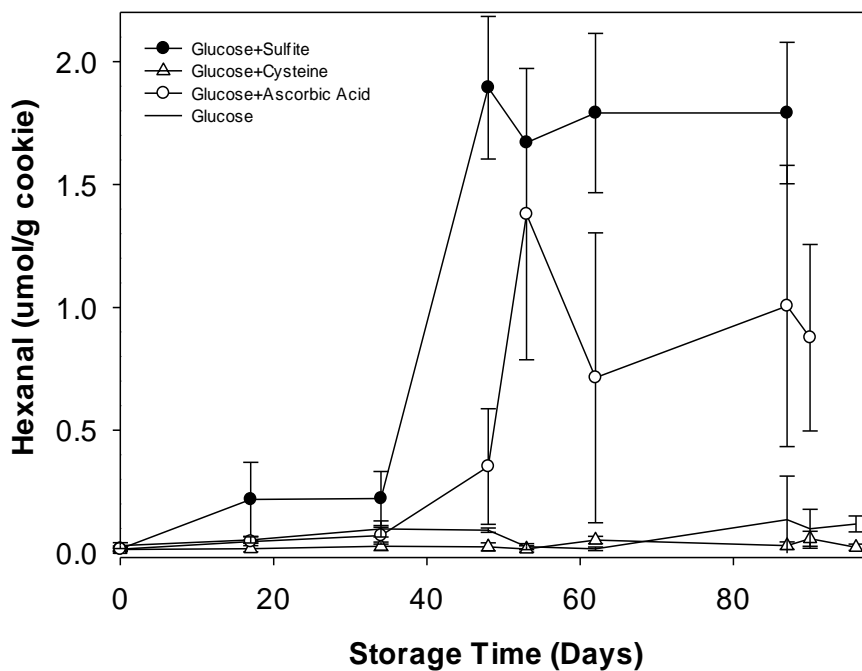


Figure 4.17: Hexanal of Samples with Inhibitors

Table 4.3: Comparison of colorimetric values of samples containing non-enzymatic browning inhibitors used in oxidation studies

| | L | a | b |
|-----------------------|----------|----------|----------|
| Control | 69.2±0.4 | 5.9±0.1 | 27.1±0.3 |
| Glucose | 65.7±0.8 | 6.9±0.3 | 28.7±0.1 |
| Glucose+Sulfite | 67.2±1.4 | 3.4±0.7 | 26.2±0.6 |
| Glucose+Ascorbic Acid | 69.1±0.7 | 3.8±0.3 | 25.6±0.1 |
| Glucose+Cysteine | 74.6±0.3 | 2.11±0.3 | 24.5±0.1 |

4.5. Addition of Various Antioxidants

Due to the ability of glucose to greatly inhibit lipid oxidation, the next step was to determine how the combination of glucose and synthetic antioxidants impacted oxidation. TBHQ, PG, and BHT all increased lipid hydroperoxide formation which would be due to their ability to donate a hydrogen to a peroxy radical thus increase the formation of hydroperoxides. In terms of hexanal BHT (50 day lag phase) and PG (30 day lag phase) increased off-flavor

development compared to glucose alone (64 day lag phase). Samples with TBHQ did not exit the hexanal lag phase during the entire storage study. These results demonstrate that glucose is a strong natural alternative to the use of synthetic antioxidants.

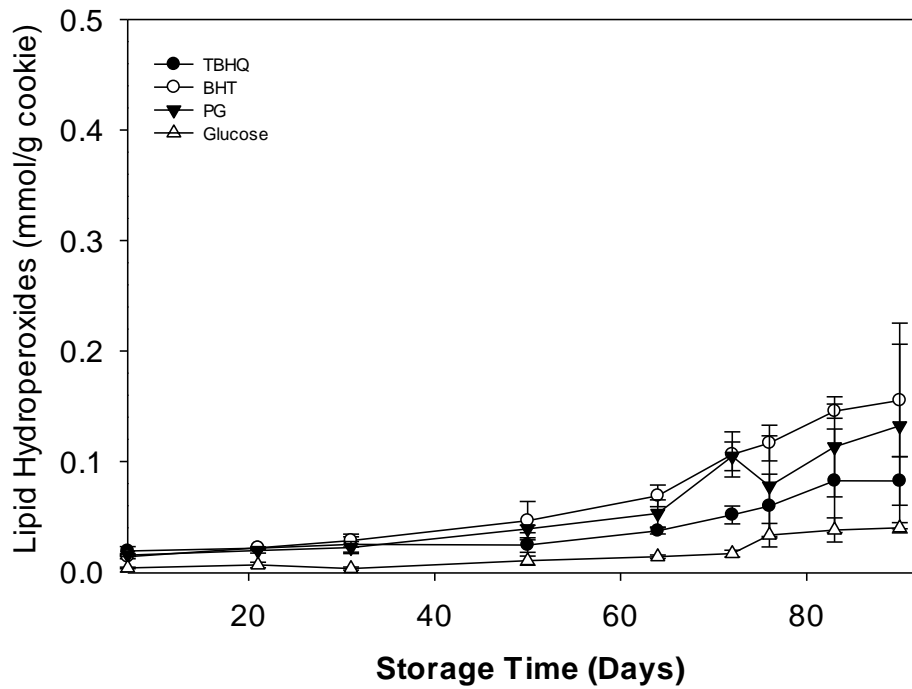


Figure 4.18: Lipid Hydroperoxides of Glucose and Synthetic Antioxidants

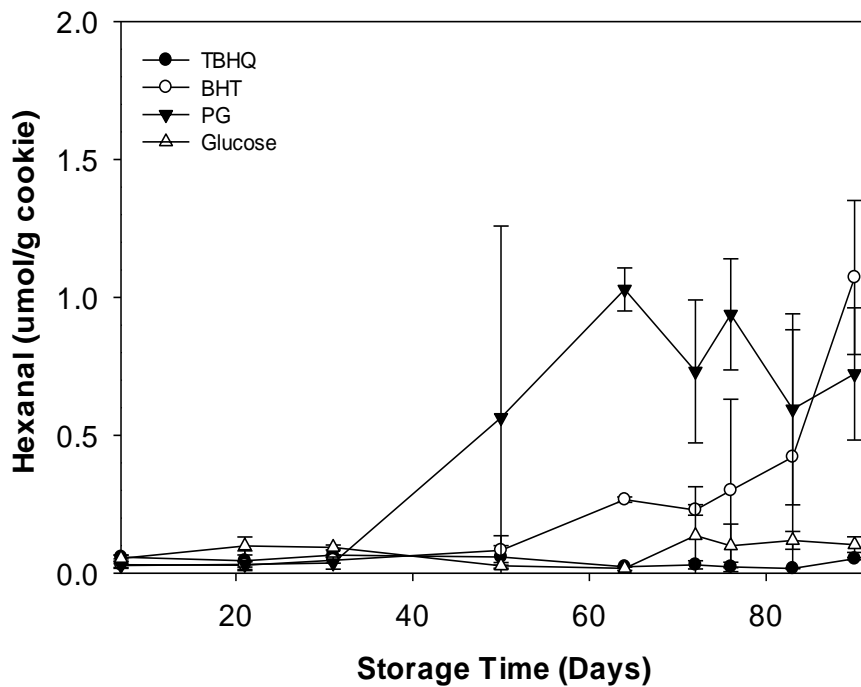


Figure 4.18: Hexanal Glucose and Synthetic Antioxidants

4.6. Correlation between lag phase and water activity

When the lag phase of various samples was plotted against their water activity, it was observed that there was no correlation between lag phase and water activity having an R^2 value of 0.0195. This concludes that controlling water activity is only one factor needed to take into consideration when preventing lag phase

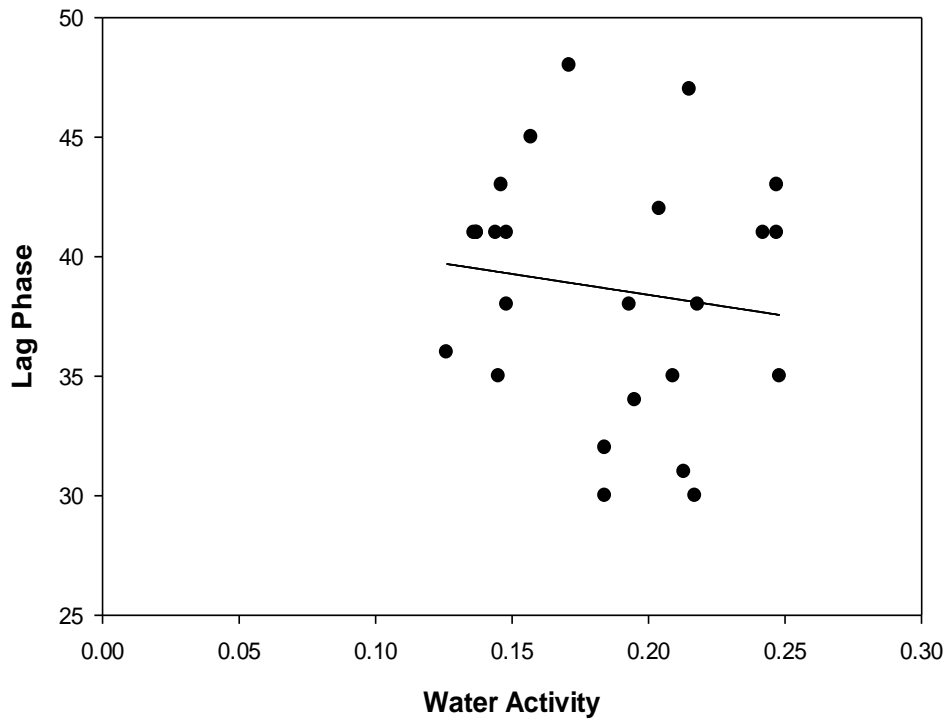


Figure 4.20: Correlation between Water Activity and Lag Phase

CHAPTER 5

CONCLUSION

Sugar more specifically reducing sugars, show great potential in inhibiting lipid oxidation in low moisture foods. Sugar has the ability to bind water while reducing sugars can also potentially donate hydrogen and scavenge free radicals. Our study first demonstrated that the addition of sucrose or glucose maintain the same lipid and protein microstructure.

Additionally, we found that higher concentrations of sugar have greater inhibition of lipid oxidation. Sucrose inhibited lipid oxidation at concentrations above 0.17 moles sucrose/kg cookie. Glucose inhibited lipid oxidation at concentrations above 0.09 moles glucose/kg cookie. Different concentrations of sugar may have positive or negative impacts on the systems susceptibility to lipid oxidation. These findings suggest that sugar may be a suitable natural alternative to currently used synthetic antioxidants.

Our study also demonstrated that browning pigments may not inhibit lipid oxidation. Glucose and fructose out performed sucrose by extending lag phase two months greater than the control. Fructose produced more maillard browning pigments based on colorimetry L values, than glucose however, in a controlled water activity environment it did not show greater inhibition of lipid oxidation compared to glucose or sucrose.

Lastly, water activity influences the rate of lipid oxidation and it must be considered when preventing lipid oxidation. However, we have shown that the water activity of a sample does not correlate with a change in lag phase therefore, other factors must be taken into consideration when preventing lipid oxidation. Further studies should be conducted to identify other factors that will enhance the inhibition properties of sugar for increased oxidative stability while maintaining physical stability.

BIBLIOGRAPHY

Agency for Toxic Substances and Disease Registry (Accessed Nov. 2015). Toxicological Profile For Acrolein. <http://www.atsdr.cdc.gov/toxprofiles/tp124.pdf>.

Alexander JC (1986) Heated and oxidized fats. *Prog Clin Biol Re* 222 :185-209.

Allen JC, Wrieden WL (1982) Influence of Milk-Proteins on Lipid Oxidation in Aqueous Emulsion .2. Lactoperoxidase, Lactoferrin, Superoxide-Dismutase and Xanthine-Oxidase. *J Dairy Res* 49.2: 249-63.

Aando K, Beppu M, Kikugawa K (1995) Evidence for Accumulation of Lipid Hydroperoxides during the Aging of Human Red-Blood-Cells in the Circulation. *Biol Pharm Bull* 18.5: 659-63.

Aw TY, Wierzbicka G, Jones DP (1991) Oral Glutathione Increases Tissue Glutathione in Vivo. *Chem- Biol Interact* 80.1: 89-97.

Antonio A, Muñoz MF, Sandro A (2014) Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxid Med Cell Longevity* 2014: 31.

Barden L, Vollmer D, Johnson D, Decker E (2015) Impact of Iron, Chelators, and Free Fatty Acids on Lipid Oxidation in Low-Moisture Crackers. *J. Agric Food Chem* 63: 1812-1818.

Baynes JW (2007) Dietary ALEs are a Risk to Human Health – NOT! *Mol Nutr Food Res* 51.9: 1102-6.

Blankenhorn DH (1990) Diet and New Lesions of the Coronary-Arterie. *Jama* 264.10:1252

Blasbalg TL, Hibbeln JR, Ramsden CE, Majchrzak SF, Rawlings RR (2011) Changes in Consumption of Omega-3 and Omega-6 Fatty Acids in the United States during the 20th Century. *Am J Clin Nutr* 93.5: 950-62.

Bou R, Hanquet N, Codony R, Guardiola F, Decker EA (2010) Effect of heating oxyhemoglobin and methemoglobin on microsomes oxidation. *Meat Sci* 85:47-53.

Buege JA, Aust SD (1976) Lactoperoxidase-Catalyzed Lipid Peroxidation of Microsomal and Artificial Membranes. *Biochim Biophys Acta Gen Subj* 444.1: 192-201.

Burcham PC, Raso A, Kaminskis LM (2012) Chaperone Heat Shock Protein 90 Mobilization and Hydralazine Cytoprotection Against Acrolein-Induced Carbonyl Stress. *Mol Pharmacol* 82.5: 876-86.

Chan KM, Decker EA (1994) Endogenous Skeletal Muscle Antioxidants. *Food Sci Nutr* 34: 403-426.

Chung FL, Tanaka T, Hecht SS (1986) Induction of Liver Tumors in F344 Rats by Crotonaldehyde. *Canc Res* 46.3: 1285-9

Cordain L, Watkins BA, Florant GL, Kelher M, Rogers L, Li Y (2002) Fatty Acid Analysis of Wild Ruminant Tissues: Evolutionary Implications for Reducing Diet-Related Chronic Disease. *Eur J Clin Nutr* 56.3: 181-91

David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Delvin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ (2014) Diet Rapidly and Reproducibly Alters the Human Gut Microbiome. *Nature* 505.7484: 559.

Earley JH, Bourne RA, Watson MJ, Poliakoff M (2015) Continuous catalytic upgrading of ethanol to n-butanol and >C-4 products over Cu/CeO₂ catalysts in supercritical CO₂. *Green Chem.* 17: 3018-3025

Eder E, Budiawan SD (2001) Cancer Risk Assessment for the Environmental Mutagen and Carcinogen Crotonaldehyde on the Basis of TD50 and Comparison with 1,N-2-Propanodeoxyguanosine Adduct Levels. *Cancer Epidemiol Biomarkers Prev* 10.8: 883-8

Endo Y, Hayashi C, Yamanaka T, Takayose K, Yamaoka M, Tsuno T, Nakajima S (2013) Linolenic Acid as the Main Source of Acrolein Formed during Heating of Vegetable Oils. *J Am Oil Chem Soc* 90.7: 959-64

Esterbauer H, Schaur RJ, Zollner H (1991) Chemistry and Biochemistry of 4-Hydroxynonenal, Malonaldehyde and Related Aldehydes. *Free Radic Biol Med* 11.1: 81-128

Esterbauer H. (1993) Cytotoxicity and Genotoxicity of Lipid-Oxidation Products. *Am J Clin Nutr* 57.5: 779-86

Ewert A, Granvogel M, Schieberle P (2014) Isotope-Labeling Studies on the Formation Pathway of Acrolein during Heat Processing of Oils. *J Agri Food Chem* 62: 8524-8529

Frankel EN, Smith LM, Hamblin CL, Creveling RK (1984) Occurrence of Cyclic Fatty Acid Monomers in Frying Oils used for Fast Foods. *J Am Oil Chem Soc* 61.1: 87-90

Frankel EN (1984) Lipid Oxidation - Mechanisms, Products and Biological Significance. *J Am Oil Chem. Soc* 61.12: 1908-17

Gerrior SA, Bente L (2001) The Interactive Food Supply: An on-Line Connection to Food and Nutrient Information. *Faseb J* 15.5: A746

Ghosh S, Molcan E, DeCoffe D, Dai C, Gibson DL (2013) Diets Rich in n-6 PUFA Induce Intestinal Microbial Dysbiosis in Aged Mice. *Br J Nutr* 110.3: 515-23

Gobert M, Remond D, Loonis M, Buffiere C, Dante-Lhoutellier V, Dufour C (2014) Fruits, Vegetables and their Polyphenols Protect Dietary Lipids from Oxidation during Gastric Digestion. *Food Funct* 5.9: 2166-74

Gorelik S, Ligumswky M, Kohen R, Kanner J (2008) A Novel Function of Red Wine Polyphenols in Humans: Prevention of Absorption of Cytotoxic Lipid Peroxidation Products. *Faseb* 22.1: 41-6

Granvogl M (2014) Development of Three Stable Isotope Dilution Assays for the Quantitation of (E)-2-Butenal (Crotonaldehyde) in Heat-Processed Edible Fats and Oils as Well as in Food. *J Agri Food Chem* 62: 1272-1282

Griffith T, Johnson JA (1957) Relation of the Browning Reaction to Storage of Sugar Cookies. *Cereal Chem* 34: 159-169

Guardiola F, Dutta PC, Codony R, Savage GP (2002) Cholesterol and phytosterol oxidation products: analysis, occurrence, and biological effects. AOCS Press, Champaign

Ismahil MA, Hamid T, Haberzettl P, Gu T, Chandrasekar B, Srivastava S, Bhatnagar A, Prabhu SD (2011) Chronic Oral Exposure to the Aldehyde Pollutant Acrolein Induces Dilated Cardiomyopathy. *Am J Physiol Heart Circ Physiol* 301.5: H2050-60

Kanazawa K, Ashida H (1998) Dietary Hydroperoxides of Linoleic Acid Decompose to Aldehydes in Stomach before being Absorbed into the Body. *Biochim Biophys Acta* 1393.2-3: 349-61

Kanner J, Lapidot T (2001) The Stomach as a Bioreactor: Dietary Lipid Peroxidation in the Gastric Fluid and the Effects of Plant-Derived Antioxidants. *Free Radic Biol Med* 31.11: 1388-95

Kanner J (2007) Dietary Advanced Lipid Oxidation Endproducts are Risk Factors to Human Health. *Mol Nutr Food Res* 51.9: 1094-101

Kenmogne-Domguia HB, Meynier A, Boulanger C, Genot C (2012) Lipid Oxidation in Food Emulsions Under Gastrointestinal-Simulated Conditions: The Key Role of Endogenous Tocopherols and Initiator. *Food Dig* 3.1: 46-52

Kochhar, SP., and Henry, CJ. (2009) Oxidative Stability and Shelf-Life Evaluation of Selected Culinary Oils. *Int J Food Sci Nutr* 60: 289-96

Kondo S, Toyokuni S, Iwasa Y, Tanaka T, Onodera H, Hiai H, Imamura M (1999) Persistent Oxidative Stress in Human Colorectal Carcinoma, but Not in Adenoma. *Free Radic Biol Med* 27(3-4):401-10

Kristinova V, Storro I, Rustad T (2013) Influence of human gastric juice on oxidation of marine lipids - in vitro study. *Food Chem* 141: 3859-3871

Lake RJ, Scholes P (1997) Quality and Consumption of Oxidized Lipids from Deep-Frying Fats and Oils in New Zealand. *J Am Oil Chem* 74.9: 1065-8

Lomaestro BM, Malone M (1995) Glutathione in Health and Disease: Pharmacotherapeutic Issues. *Ann Pharmacother* 29.12: 1263-73

- Long EK, Picklo MJ SR (2010) Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: Make some room HNE ... *Free Radic Biol Med* 49: 1-8
- Lorrain B, Dangles O, Loonis M, Armand M, Dufour C (2012) Dietary Iron-Initiated Lipid Oxidation and Its Inhibition by Polyphenols in Gastric Conditions. *J Agric Food Chem* 60: 9074-9081
- Matalanis, A. and McClements, D. J. (2012). Impact of encapsulation within hydrogel microspheres on lipid digestion: An in vitro study. *Food Biophys* 7: 145-154
- McClements DJ, Decker EA (2008) Lipids, In Fennema's Food Chemistry. Ed. Damodarin, S., Parkin, K., Fennema, O.R. CRC Press, Boca Raton, FL
- Moghe A, Ghare S, Lamoreau B, Mohammad M, Barve S, McClain C, Joshi-Barve S (2015) Molecular mechanisms of acrolein toxicity: relevance to human disease. *Toxicol Sci* 143 (2):242-55
- Morales and Jimenez-Perez. (2001). Free Radical Scavenging Capacity of Maillard reaction products as related to colour and fluorescence. *Food Chem* 72 (1): 119- 125
- Nuora A, Chiang VS, Milan AM, Tarvainen M, Pundir S, Quek S, Smith GC, Markworth JF, Ahotupa M, Cameron-Smith D, Linderborg KM (2015) The impact of beef steak thermal processing on lipid oxidation and postprandial inflammation related responses. *Food Chem* 184: 57-64
- Oberley TD, Toyokuni S, Szweda LI (1999) Localization of Hydroxynonenal Protein Adducts in Normal Human Kidney and Selected Human Kidney Cancers. *Free Radic Biol Med* 27.5-6: 695-703
- Oberley TC, Zhong W, Szweda LI, Oberley LW (2000) Localization of Antioxidant Enzymes and Oxidative Damage Products in Normal and Malignant Prostate Epithelium. *The Prostate* 44.2: 144-55
- Ottestad I, Vogt G, Retterstol K, Myhrstad MC, Haugen J, Nilsson A, Ravn-Haren G, Nordvi B, Bronner KW, Andersen LF, Holven KB, Ulven SM (2012) Oxidised fish oil does not influence established markers of oxidative stress in healthy human subjects: a randomised controlled trial. *Br J Nut.* 108: 315-326
- Palay SL, Karlin LJ (1959) An Electron Microscopic Study of the Intestinal Villus .2. the Pathway of Fat Absorption. *J Biophys Biochem Cytol* 5.3: 373.
- Panya, A., Laguerre, M., Bayrasy, C., Lecomte, J., Villeneuve, P., McClements, D. J., and Decker, E. A. (2012). An investigation of the versatile antioxidant mechanisms of action of rosmarinate alkyl esters in oil-in-water emulsions. *J Agric Food Chem* 60: 2692-2700
- Pandey KB, Rizvi SI (2009) Plant Polyphenols as Dietary Antioxidants in Human Health and Disease. *Oxid Med Cell Longevity* 2.5: 270-8
- Pietta PG (2000) Flavonoids as Antioxidants. *J Nat Prod* 63.7: 1035-42

- Ponginebbi L, Nawar WW, Chinachoti P (1999) Oxidation of linoleic acid in emulsions: Effect of substrate, emulsifier, and sugar concentration. *J Am Oil Chem* 76:131
- Procida G, Cichelli A, Compagnone D, Maggio RM, Cerretani L, Carlo MD (2009) Influence of Chemical Composition of Olive Oil on the Development of Volatile Compounds during Frying. *Eur Food Res Technol* 230.2: 217-29
- Reaven P, Parthasarathy S, Grasse BJ, Miller E, Almazan F, Mattson FH, Khoo JC, Steinberg D, Witztum JL (1991) Feasibility of using an Oleate-Rich Diet to Reduce the Susceptibility of Low-Density-Lipoprotein to Oxidative Modification in Humans. *Am J Clin Nutr* 54.4: 701-6
- Ringseis R, Eder K (2005) Effects of Dietary Fat and Oxidized Cholesterol on Gene Expression in Rat Liver as Assessed by cDNA Expression Array Analysis. *Eur J Nutr* 44.4: 231-41
- Ryu JH, Paik IY, Woo JH, Shin KO, Cho SY, Roh HT (2016) Impact of different running distances on muscle and lymphocyte DNA damage in amateur marathon runners. *J Phys Ther Sci* 28(2): 450–455
- Salvolini E, Martarelli D, Giorgio RD, Mazzanti L, Procaccini M, Curatola G. (2000) Age-Related Modifications in Human Unstimulated Whole Saliva: A Biochemical Study. *Aging Clin Exp Res* 12.6: 445-8
- Semchyshyn HM (2014) Reactive Carbonyl Species In Vivo: Generation and Dual Biological Effects. *Sci World J* 2014: 10
- Seppanen CM, Csallany AS (2004) Incorporation of the Toxic Aldehyde 4-Hydroxy-2-Trans-Nonenal into Food Fried in Thermally Oxidized Soybean Oil. *J Am Oil Chem* 81.12: 1137-41
- Shantha, N. C. and Decker, E. A. (1994). Rapid, sensitive, iron-based spectrophotometric methods for determination of peroxide values of food lipids. *J Am Oil Chem Int* 77: 421-424
- Smith LL (1980) Autoxidation in Food and Biological System
- Staprans I, Pan XM, Rapp JH, Reingold KR (2005) Development of Atherosclerosis *Mol Nutr Food Res* 49.11: 1075-82
- Staprans I, Rapp JH, Pan XM, Kim KY, Feingold KR (1994) Oxidized Lipids in the Diet are a Source of Oxidized Lipid in Chylomicrons of Human Serum. *Arterioscler Thromb* 14.12: 1900-5
- Sulfites in standardized food, 21 C.F.R. § 130.9 20115
- Sun X, Nair J, Bartsch H (2004) A modified immuno-enriched P-32-postlabeling method for analyzing the malondialdehyde-deoxyguanosine adduct, 3-(2-deoxy-beta-D-erythro-pentofuranosyl)-pyrimido [1,2-alpha]purin-10(3H)one in human tissue samples. *Chem Res Toxicol* 17: 268-272

Suomela JP, Ahotupa M, Kallio H (2005) Triacylglycerol Oxidation in Pig Lipoproteins After a Diet Rich in Oxidized Sunflower Seed Oil. *Lipids* 40.5: 437-44

Surh JL, Kwon SH (2007) 4-Hydroxy-2-alkenals in polyunsaturated fatty acids-fortified infant formulas and other commercial food products. *Food Addit Contam* 24: 1209-1218

Tanka M, Sugita S, Chiu WK, Nagashima Y, Taguchi T (1990) Influence of Water Activity on the Development of Antioxidative Effect during the Maillard Reaction between Histidine and Glucose. *Agric. Sci Technol* 56: 525-530

Tanel A, Averill-Bates DA (2007) Inhibition of Acrolein-Induced Apoptosis by the Antioxidant N-Acetylcysteine. *J Pharm Exp Therap* 321.1: 73-83

Tirosh O, Shpaizer A, Kanner J (2015) Lipid Peroxidation in a Stomach Medium is Affected by Dietary Oils (Olive/Fish) and Antioxidants: The Mediterranean Versus Western Diet. *J Agri Food Chem* 63.31: 7016-23

Tullberg C, Larsson K, Carlsson N, Comi I, Scheers N, Vegarud G, Undeland I (2016) Formation of reactive aldehydes (MDA, HHE, HNE) during the digestion of cod liver oil: comparison of human and porcine in vitro digestion models. *Food Funct* 7: 1401-1412

Uchida K, Kanematsu M, Sakai K, Matsuda T, Hattori N, Mizuno Y, Suzuki D, Miyata T, Noguchi N, Niki E, Osawa T (1998) Protein-Bound Acrolein: Potential Markers for Oxidative Stress. *Proceedings of the National Academy of Sciences of the United States of America*. *J Neurochem* 95.9: 4882-7

U.S. Department of Health and Human Services and U.S. Department of Agriculture (Accessed May 2016) 2015 – 2020 Dietary Guidelines for Americans. <http://health.gov/dietaryguidelines/2015/guidelines/>.

Vander Jagt DL, Hunsaker LA, Vander Jagt TJ, Gomez MS, Gonzales DM, Deck LM, Royer RE (1997) Inactivation of Glutathione Reductase by 4-Hydroxynonenal and Other Endogenous Aldehydes. *Biochem Pharma* 53.8: 1133-40

Vejux A, Samadi M, Lizard G (2011) Contribution of Cholesterol and Oxysterols in the Physiopathology of Cataract: Implication for the Development of Pharmacological Treatments. *J Ophthalmol* 2011: 471947

Viau M, Genot C, Ribourg L, Meynier A (2016) Amounts of the reactive aldehydes, malonaldehyde, 4-hydroxy-2-hexenal, and 4-hydroxy-2-nonenal in fresh and oxidized edible oils do not necessary reflect their peroxide and anisidine values. *Eur J Lipid Sci Technol* 118: 435–444

Voitkun V, Zhitkovich A (1999) Analysis of DNA-Protein Crosslinking Activity of Malondialdehyde in Vitro. *Mutat Res Fund Mol Mech Mut* 424.1-2: 97-106

Waraho T, McClements DJ, Decker EA (2011) Mechanisms of lipid oxidation in food dispersions. *Trends Food Sci Technol* 22: 3-13

Williams MJ, Sutherland WH, McCormic MP, de Jong SA, Walker RJ, Wilkins GT (1999) Impaired Endothelial Function Following a Meal Rich in used Cooking Fat. *J Am Coll Cardiol* 33.4: 1050-5

Wilson R, Lyall K, Smyth L, Fernie C, Riemersma R (2002) Dietary hydroxy fatty acids are absorbed in humans: Implications for the measurement of 'oxidative stress' in vivo. *Free Radic Biol Med* 32: 162-168

Wijewickereme AN, Kitts DD (1997) Influence of Reaction Conditions on the Oxidative Behavior of Model Maillard Reaction Products. *J Agri Food Chem* 45: 4571-4576

Yagi K, Kiuchi K, Saito Y, Mike A, Kayahara N, Tatano T, Ohishi N (1986) Use of a New Methylene Blue Derivative for Determination of Lipid Peroxides in Foods. *Biochem Intern* 12.2: 367-71

Yamauchi R, Aoki Y, Sugiura T, Kato K, Ueno Y (1982) Effect of Sugars and Sugar Analogs on Autoxidation of Methyl Linoleate and Safflower Oil. *Agri Bio Chem* 46.12: 2997-3002

Yamauchi R, Goto Y, Kato K, Ueno Y (1984) Prooxidant Effect of Dihydroxyacetone and Reducing Sugars on the Autoxidation of Methyl Linoleate in Emulsions. *Agri Bio Chem* 48.4: 843-848

Yen GC, Hsieh P (1995) Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction products. *J Sci Food Agri* 67:415-420

Zhu M, Rajamani S, Kaylor J, Han S, Zhou F, Fink AL (2004) The Flavonoid Baicalein Inhibits Fibrillation of Alpha-Synuclein and Disaggregates Existing Fibrils. *J Bio Chem* 279.26: 26846-57