OXIDATIVE STABILITY OF PEANUT BUTTER BITES

By

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OXIDATIVE STABILITY OF PEANUT BUTTER BITES

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Title of Study: OXIDATIVE STABILITY OF PEANUT BUTTER BITES

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Abstract:

Peanut butter continues to be a mainstay in the American diet with more than a billion dollars in annual sales, but in its current form peanut butter lacks the convenience of other foods. A peanut butter bite snack food has been developed that is individually wrapped, high in protein, great tasting, and made mostly from peanut butter. The target market for the product is the active, health-conscious segment of the population that wants a high protein peanut butter snack that is easy to pack, carry, and eat.

The objective of the current study was to evaluate the shelf life of peanut butter bites under different storage and packaging conditions, and specifically to monitor oxidative stability of the samples over time.

In order to evaluate product shelf life, peanut butter bite samples were prepared with three different levels of added antioxidant (Vitamin E). Products were sealed in two different types of packaging (metallized polyethylene and plastic polyethylene), and were stored at two different temperatures to determine the rate of degradation of the product under various conditions. Oxidative stability was evaluated using two different analytical methods (peroxide value and TBARS testing) to evaluate primary and secondary oxidation products. All treatments were conducted in triplicate.

Results show that the both packaging materials provided oxidative stability for the peanut butter product. Also, higher levels of vitamin E resulted in greater stability. As expected, oxidation proceeded more quickly under higher temperature storage conditions.

A shelf stable individually-wrapped peanut butter snack product may be appealing to a large audience, and could result in an increase in the consumption of peanuts.

Keywords: peanut butter, oxidation, snack, high protein, Vitamin E

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CHAPTER I

INTRODUCTION

Peanut butter accounts for about 50% of the U.S. edible use of peanuts, which amounts to more than $850 million in retail sales annually (FAO, 2010). As peanut butter is both nutritious and economical, it is popularly used as a sandwich spread. Due to its richness in protein content, it can serve as a healthy snack option.

Since peanut butter lacks the convenience of other food products in its current form, there is a need to formulate individually-wrapped peanut butter products in different forms and shapes. A peanut butter snack food has been developed that is individually wrapped, high in protein, great tasting, and made mostly from peanut butter. The target market for this snack product is health-conscious millennials who want a high protein peanut butter snack that is easy to pack, carry and eat. Formulation of peanut butter chunks involves application of an enzyme technology which converts semi-solid peanut butter into solid chunks in the presence of additives.

Peanut butter is a high fat food product, so it is highly prone to oxidative rancidity. After a period of time, oxidation products are formed due to rancidity. Therefore, it is essential to study shelf life characteristics of peanut butter and peanut butter snacks. The present study focuses on the shelf life analysis of peanut butter bites under different temperature, antioxidant and packaging conditions.

Objectives

The overall goal of the research study was to evaluate the oxidative stability of peanut butter bites under different temperature conditions, antioxidant concentrations and packaging materials, and to choose the best formulation which can enhance the oxidative stability and delay the rancidity.

Specific objectives:

1. To quantify the primary and secondary oxidation products formed during 6 months of storage
2. To determine the effect of temperature on oxidative stability
3. To determine the effect of packaging material on oxidative stability
4. To determine the effect of antioxidant addition on oxidative stability

CHAPTER II

REVIEW OF LITERATURE

Peanuts

Peanuts (Arachis hypogaea) are a grain legume and oil crop widely grown in tropical and subtropical climates of Asia, Africa, Australia, and North and South America. Peanuts are similar in taste and nutritional profile to tree nuts such as walnuts and almonds but are not classified as nuts and are considered grain legumes. As per 2014-2015 annual report, world peanut production totals about 39.85 million metric tons annually (FAS USDA, 2016). The United States is the 4th largest peanut producer in the world, after China, Nigeria and India (USDA, 2016).

Peanuts are commercially grown in 13 states of the USA: Alabama, Arkansas, Florida, Georgia, Louisiana, Missouri, Mississippi, North Carolina, New Mexico, Oklahoma, South Carolina, Texas and Virginia. Out of these 13 states, 6 major peanut producing states grow nearly all of the US peanut crop. The United States is one of the world's leading peanut exporters, with average annual exports of between 200,000 and 250,000 metric tons (FAS USDA, 2016).

Peanuts are the 12th most valuable cash crop grown in the USA with a farm value of over one billion US dollars (ERS USDA, 2015). On average, an American consumer eats peanut products, worth more than $2 billion at the retail level (FAO, 2013). Value-added products such as peanut flour, peanut oil, roasted peanuts and peanut butter have been developed which have numerous applications in bakery and confectionery sectors. In the USA, most of the top-selling confectionery products either contain peanuts or peanut butter.

Peanut Butter

Peanut Butter is a food paste made primarily from ground dry roasted peanuts. It is included as an ingredient in many recipes, especially cookies and candies. Its flavor combines well with other flavors, such as chocolate, cured meats, savory sauces, and various types of breads and crackers. Peanut butter is high in plant protein, contains no cholesterol, and has many important vitamins and minerals, including niacin, vitamin E and other antioxidants, and natural folic acid (American Peanut Council, 2014).

Dr. John Harvey Kellogg invented a version of peanut butter in 1895. A St. Louis physician may have developed a version of peanut butter as a protein substitute for his older patients who had poor teeth and couldn't chew meat. Peanut butter was introduced at the St. Louis World's Fair in 1904. Peanut butter became an integral part of military rations in the 1st and 2nd World Wars (American Peanut Council, 2014).

There are many varieties of peanut butter available in the market today. Some of them are creamy, chunky, reduced fat, vitamin-fortified and omega-3fatty acid enriched peanut butters. Peanuts are roasted, blanched and sorted before grinding into a creamy consistency in the case of creamy peanut butter. Reduced fat peanut butter provides a fat reduction of at least 25% when compared to the regular creamy variety. Peanut pieces can be added to provide a crunchy texture in chunky peanut butter (National Nutrition Database, 2013).

In the USA, processed peanut butter should contain a minimum of 90 percent peanuts to be labeled as peanut butter. Otherwise, it is labeled as peanut spread. Other additives like sweeteners and salt are added to enhance flavor. Small amounts of stabilizers such as mono-di glycerides, hydrogenated palm oil etc., are added to prevent the oil separation from peanut butter.

Table 1. Peanut butter amino acid profile (Haytowitz, 2013).

Amino Acid	Amount (g per 100g PB)	1 cup (258 g)	2 Table spoons (32 g)
Tryptophan	0.227	0.586	0.073
Threonine	0.515	1.329	0.165
Isoleucine	0.605	1.561	0.194
Leucine	1.518	3.916	0.486
Lysine	0.669	1.726	0.214
Methionine	0.261	0.673	0.084
Cystine	0.225	0.580	0.072
Phenylalanine	1.180	3.044	0.378
Tyrosine	0.814	2.100	0.260
Valine	0.768	1.981	0.246
Arginine	2.719	7.015	0.870
Histidine	0.547	1.411	0.175
Alanine	0.899	2.319	0.288
Aspartic Acid	2.999	7.737	0.960
Glutamic Acid	5.001	12.903	1.600
Glycine	1.411	3.640	0.452
Proline	1.383	3.568	0.443
Serine	1.455	3.754	0.466

Table 1 gives the amino acid profile of peanut butter. Peanut butter is rich in the amino acids leucine, phenylalanine, arginine, aspartic acid and glutamic acid. Arginine is a precursor of

nitric oxide, a compound which expands blood vessels. It has been proven to help in decreasing blood pressure (Peanut Institute, 2016).

There are 7 steps involved in the manufacture of peanut butter as shown in Figure 1.

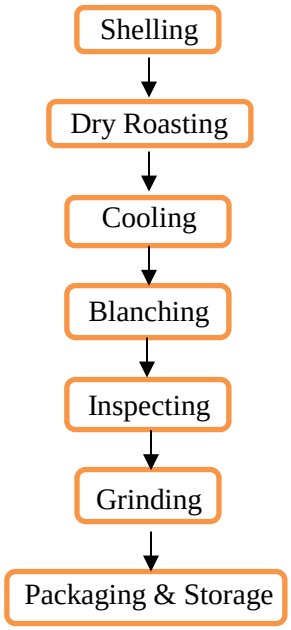


Figure 1. Steps involved in peanut butter manufacture.

1. Shelling

   The hull or shell of the peanuts is removed by machines. After peanuts are shelled, kernels are passed over separators to remove unshelled peanuts, contaminants and split kernels. From separators, shelled kernels are passed to a conveyor belt where defective kernels are removed. Then kernels are graded, sized and bagged for shipment to the roasting area.

2. Dry Roasting

   Roasting of shelled peanuts in a batch method requires heating at 320 F with a holding time of 40-60 min to obtain a satisfactory roast. Multi-stage roasters apply two or more

separate heating zones with different temperatures to produce more even roast by raising the temperature of the peanuts slowly to the roasting temperature. An optimum process requires approximately five separate heating zones to raise the temperature of the nuts slowly to the roasting temperature and roasting the peanuts at low temperatures for longer duration gives the best flavor and longer shelf life due to even roasting (Moss and Otten, 1989).

3. Cooling

To get a uniform product, after a definite roasting time, roasting should be stopped and peanuts are cooled quickly to avoid oil, color and flavor losses. Roasted peanuts are allowed to pass through a cooling chamber where a large amount of air is pulled through the mass of peanuts by suction fans.

4. Dry Blanching

Cooled peanuts are passed through a blancher and subjected to heat at 280 F for 25 min and abrasion between brushes to loosen, crack and remove skins and hearts. Blanching also cleans the kernels from dust and other impurities. Tannins from skins and hearts are removed during the blanching process and this results in a milder flavored product.

5. Inspecting

Blanched peanuts are screened and inspected to remove stones, poor quality peanuts and other undesirable material. Light peanuts are separated by blowers and metal by magnets.

6. Grinding

Grinding of peanuts is performed in 2 operations. Primary grinding reduces peanuts to a medium grind and secondary grinding reduces the medium grind particles to a smooth texture. Peanut butter is heated to about 170 F during the grinding process and pressure and temperature are constantly monitored to ensure that the product is not overheated. After stabilizer is added, peanut butter is rapidly cooled. Cooling crystallizes the stabilizer, causing trapping of the peanut oil released during the grinding step. To ensure the assimilation of all additives into the peanut butter, the final mixture is sent into a mixing pump where

homogenization takes place. Once butter is homogenized, it is filled into jars at a temperature less than 110 F.

7. Packaging & Storage

Before packaging, heat should be removed to ensure crystallization of fats and oils. Vacuum packaging is usually done to remove air which can cause oxidative rancidity. After filling, the jars are closed, labeled and palletized and sent to transportation and storage.

Nutritional Composition of Peanut Butter

Peanut butter, which is considered one of the America's favorite food products and a staple food much like bread, meat and milk, contains 22% protein and 51% fat. Peanut butter is considered a microbiologically stable and semi perishable food at ambient temperature due to low moisture content and low water activity content (Jay, 1996).

Table 2 summarizes the nutritional composition of creamy peanut butter. Peanut butter contains 25.9% of mono unsaturated fatty acids (MUFAs). MUFAs are known to reduce LDL cholesterol levels, thereby improving blood lipid profile and lowering the risk for cardiovascular diseases like heart attacks and atherosclerosis. Switching from a trans-fat diet to a MUFA rich diet results in weight loss. According to the American Diabetes Association (2000), diets with MUFAs could improve the loss of belly fat. Peanut butter contains 12.5% poly unsaturated fatty acids (PUFAs), which are omega-3 and DHA-containing omega-6 fatty acids. Peanut butter contains alpha-linolenic acid (ALA), an omega-3 fatty acid which may prevent hypertension, cardiovascular disease and excessive blood clotting and fibrocystic breast disease (Lista, Martinez, Miranda, et al, 2012).

Since peanut butter contains more than 51% of fat/lipids, over a period of time it is susceptible to oxidative rancidity. Hence, there is a need to study the effects of all the factors which can cause, accelerate and delay the rancidity.

Table 2. Nutritional composition of creamy peanut butter (National Nutrient Database, 2013)

Nutrient	Unit	Value per 100 g
Proximates		
Water	g	1.23
Energy	kcal	598
Protein	g	22.21
Total lipid (fat)	g	51.36
Carbohydrate	g	22.31
Fiber, total dietary	g	5
Sugars, total	g	10.49
Minerals		
Calcium, Ca	mg	49
Iron, Fe	mg	1.74
Magnesium, Mg	mg	168
Phosphorus, P	mg	335
Potassium, K	mg	558
Sodium, Na	mg	426
Zinc, Zn	mg	2.51
Vitamins		
Vitamin C	mg	0
Thiamin	mg	0.15
Riboflavin	mg	0.192

Niacin	mg	13.112
Vitamin B-6	mg	0.441
Folate, DFE	µg	87
Vitamin B-12	µg	0
Vitamin A, RAE	µg	0
Vitamin A, IU	IU	0
Vitamin E (alpha)	mg	9.1
Vitamin D	IU	0
Vitamin K	µg	0.3
Lipids		
Fatty acids, total saturated	g	10.325
Fatty acids, total monounsaturated	g	25.941
Fatty acids, total polyunsaturated	g	12.535
Fatty acids, total trans	g	0.075
Cholesterol	mg	0

Peanut Butter Snacks

Peanut butter slices were first formulated in 1941 by Dale and Weisgurt (Weisgurt and Van, 1941) using peanut butter and beeswax. In 1962, Ferguson proposed a patent on a shape-retaining spreadable peanut product consisting of oils, peanut butter, honey, salt and skimmed milk that was stable over a broad range of temperatures (Edgar, 1962). In 1999, researchers at Oklahoma State University's Food and Agricultural Products Center (FAPC) initiated a new sliced peanut butter project with funding from the Oklahoma Peanut Commission to continue the

development of the product (Cox, 2000). Peanut butter slices were developed using additives such as gums, starches, waxes, oil and peanut butter (Pareja Diaz, 2000). Gradually, various formulation and processing procedures were developed by combination of various binding agents, emulsifiers and texturizers. Many trial runs were conducted by incorporating different additives to the peanut butter to create different consistencies. Later, transglutaminase enzyme, a novel food ingredient, was used in the formulation of peanut butter slices replacing paraffin wax and gums (Nault, 2014). Peanut butter snacks are enriched protein snacks in a more convenient form than peanut butter (Hormel, 2016).

Ingredients Used in the Formulation

Enriched Protein: Different protein sources such as whey, egg, soy, pea, etc., are added to snack products to enhance the protein content. Proteins act as emulsifying agents and water-binding agents. Protein isolates of soy, pea, wheat, potato, dairy (whey), casein, pork are commonly used in the sweet and savory sectors (Hall, NW Foods). Mannoprotein extracted from yeast can also be used as an emulsifier in food and beverages (Kawahara et al. 2007).

Gellan Gum: Gellan gum is a high molecular weight extracellular polysaccharide produced by fermentation of pure culture of Sphingomonas elodea, by Kelco. It acts as a binding, texturizing and stabilizing agent in peanut butter bite preparation. Basically, gums are a group of complex hydrophilic carbohydrates containing several thousand monosaccharide units. Gellan gum associates with water molecules in such a way that the behavior of the water is modified, exhibiting special functional properties which alter the rheology of peanut butter. Gum binds with protein and other molecules and improves the firmness of the snack. This multi-functional hydrocolloid is used at low levels in formulating new products which require texturizing, stabilizing and structuring. In the formulation of confectionery snacks, 0.08% gellan gum was used (Kelco, 2012). Gellan gum is used as a thickening agent and binding agent in non-dairy

milks such as almond milk and soy milk (Blue Diamond, 2014). Along with emulsifiers such as carrageenan gum, it is used in the preparation of chocolate milks (Organic Valley, 2013).

Mono di glycerides: Acylgylcerols or mono di glycerides (commonly called fats) are natural emulsifiers which act as binding, trapping, emulsifying and gelling agents. MDG molecules trap peanut oil molecules within the matrix and maintain the integrity of the snack product. They are added during the peanut butter manufacture process. Further addition of these molecules during snack preparation is to enhance the textural properties of the snack. Partially hydrogenated vegetable oils, palm oil, and rapeseed oil can be substitutes of MDG. They help delay the oil leaking from peanut butter bites. However, upon heating the bites to a temperature above 50 C, oil leaking from the interior of the snack to the outer surface is observed. Addition of MDG will prevent oil leaking when the snack is stored at room temperature or cooler. Mono di glycerides are added during the manufacture of peanut butter to stabilize the emulsion mixture. Citric acid esters of mono di glycerides are added to infant formulas for emulsification purposes (Amara et al, 2014). Along with the blend of soy/palm oils; mono di glycerides are used for the formulation of cakes, icings, dough (Tiffany et al, 2015).

Transglutaminase: Transglutaminase is an enzyme present throughout nature (from microorganisms to mammals) and is commonly referred to as meat glue (ACTIVA TG, 2012). This enzyme catalyzes the acyl-transfer reactions between Y-carboxyamide groups of glutamine residues and amino acid groups of lysines in proteins, which leads to inter- and/or intra-molecular cross-linking (Koppelman, 2002). In a product containing protein, if it has sufficient levels of glutamine and lysine, it can form covalent bonds both internally (within same protein) and externally (between different proteins). This crosslinking modifies the structure and texture of the food products. Ajinomoto is one of the major producers of TG, which produces different varieties of TG such as TG-GS, TG-RM, TG-TI, TG-YG that have diverse applications in different food sectors. In the products which lack glutamine and lysine amino acids, transglutaminase along

with gelatin-rich water can help the binding properties of food products (ACTIVA TG, 2012). Wylie Dufresne at Harvard used transglutaminase to prepare noodles from peanut butter. TG can form bonds with proteins of peanut butter that allows it to be made into sheets and cut into noodles (Souza, 2011).

Lipid Oxidation

The spontaneous reaction of atmospheric oxygen or oxygen species compounds (super oxide, hydroxide, oxygen free radical, peroxide radical) with lipids is called lipid oxidation. Lipid oxidation is a very common process which leads to oxidative deterioration. Poly unsaturated fatty acids are usually decomposed by a lipid autoxidation process (Gordon, 2003). Figure 2 shows the pathways for different oxidation products formed due to oxidation of lipid food products.

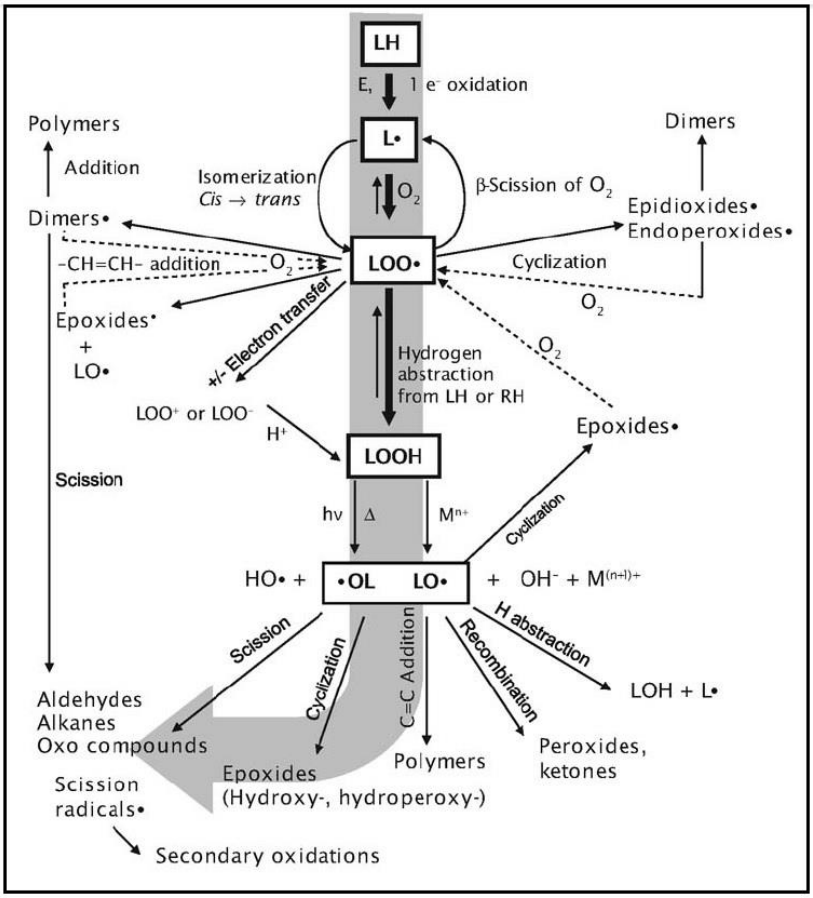


Figure 2. Lipid oxidation products (Gordon, 2003).

Fatty acids are converted to epoxides, peroxides, aldehydes, alkanes, oxo compounds, epi-dioxides, endo peroxides and ketones through chemical processes such as isomerization, scission, recombination and cyclization (Gordon, 2003). When light is present, oxygen activation to singlet oxygen may play a role in the initiation of oxidative deterioration, thus initiating lipid oxidation. Alternatively, metals like copper or iron, can also play a significant role in the process by which oxidation is initiated (Gordon, 2003).

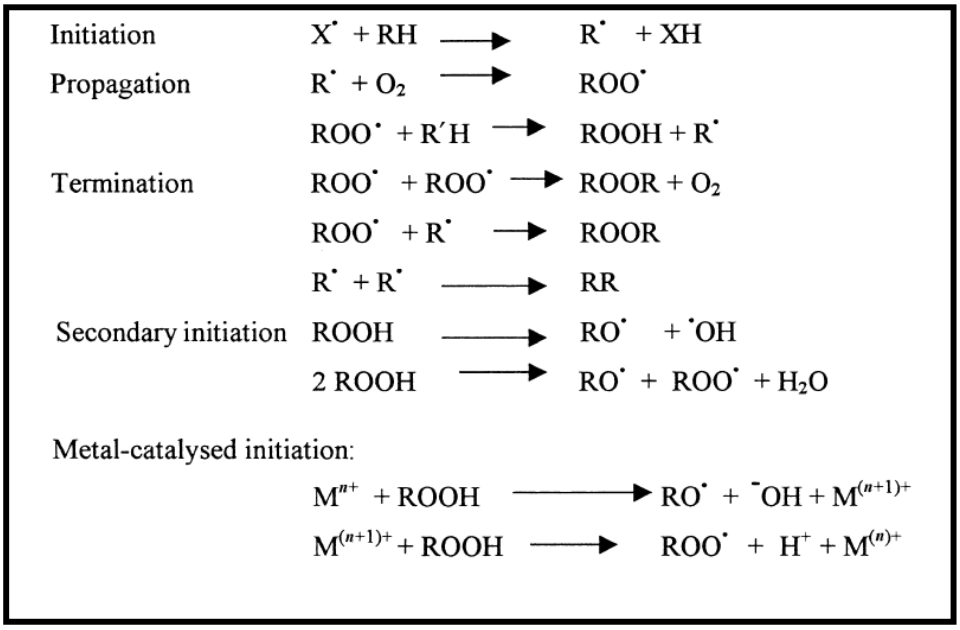


Figure 3. Mechanism of lipid auto-oxidation (Gordon, 2003)

As shown in the Figure 3, lipid auto-oxidation is a 3 step free-radical reaction. The first step is initiation, in which lipid radicals are formed from lipid molecules. Removal of a hydrogen atom by a reactive species such as a hydroxyl radical may lead to initiation of lipid oxidation. Secondary initiation by homolytic cleavage of hydroperoxides is mainly observed (main initiation reaction in oils) in edible oils. Oils have traces of hydroperoxides, formed by the action of lipoxygenase action. Metal-catalyzed initiation is catalyzed by metal ions. The second step is propagation, where one lipid radical is converted into a different lipid radical. These reactions

involve the removal of hydrogen atom from lipid molecules or addition of oxygen to an alkyl radical. The propagation step occurs very rapidly when compared to the initiation step, as the enthalpy of reaction is relatively low for the propagation reaction compared to the initiation reaction. Termination reactions involve the formation of compounds from free radicals which usually are different from the alkyl group compounds that started the initiation reaction in the presence of free radicals.

Oxidation Products

The components formed during the early stages of the lipid oxidation process are hydroperoxides. Figure 4 shows the mechanism of formation of 1,3 hydroperoxide, a primary oxidation product from linoleic acid. These peroxide products are also formed during the enzyme-induced lipid oxidation and during lipoxygenase-catalyzed oxidation (Gordon, 2003).

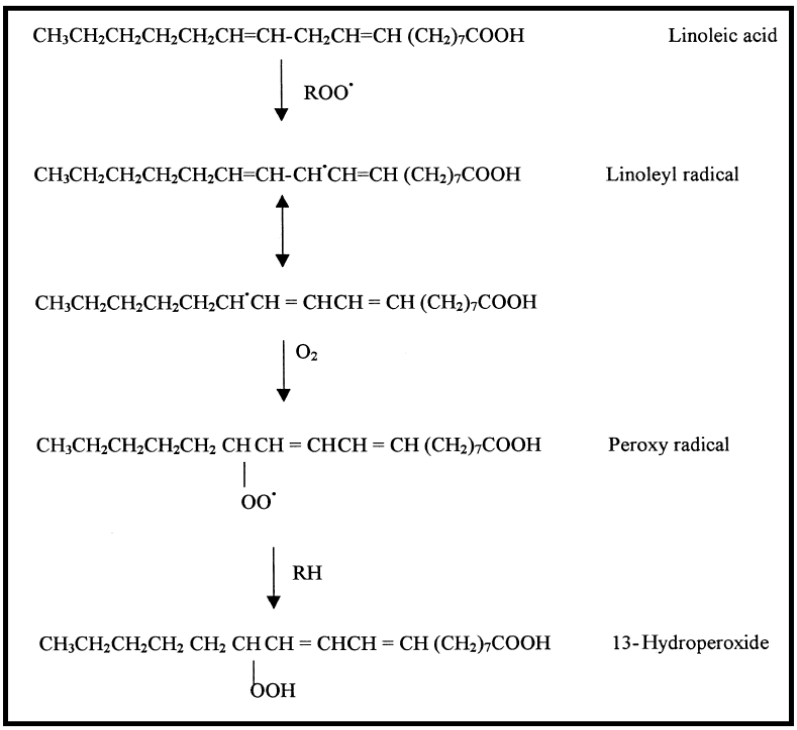


Figure 4. 1,3 Hydroperoxide formation from linoleic acid (Gordon, 2003)

Hydroperoxides are involatile and odorless, unstable compounds. As shown in Figure 5, hydroperoxides decompose to form volatile aroma compounds such as aldehydes and ketones, which give out off-flavors. The nature of the off-flavor mainly depends on the fatty acid composition of the food product and the extent to which the food product is oxidized. Off-flavors developed during lipid oxidation normally act as a warning that a food product is no longer edible. Exceptions are capsulated polyunsaturated lipid supplements like omega 3 capsules (Gordon, 2003).

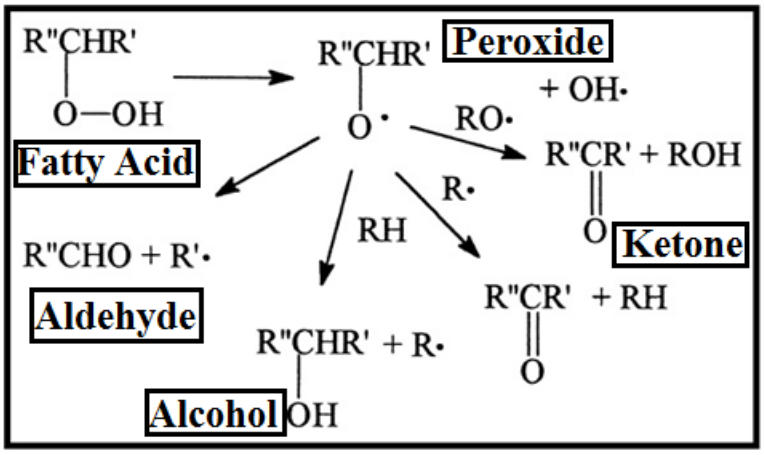


Figure 5. Primary and secondary oxidation compounds (Gordon, 2003)

Alkoxy radicals formed by hydro peroxide decomposition decompose to release volatile hydrocarbons like aldehydes, ketones, and alcohols which are no longer bound to the glycerol backbone. In normal form, fatty acids are bound to glycerol in triglyceride form. Volatile aldehydes are the main contributors to the aroma of oxidized oils and fats. In some cases, primary oxidation compounds such as hydro peroxides will not decompose to secondary oxidation products. Hydroperoxides formed from polyunsaturated fatty acids undergo further oxidation reactions to form dihydroperoxides and molecules which have oxygen-containing rings including hydroperoxy epidioxides and bicycloendoperoxides.

Oxidative Rancidity

Perception of objectionable flavors and odors caused by oxidation of the unsaturated fatty acid chains of lipids is called oxidative rancidity. Rancidity can be detected by chemical assays and reduced and/or delayed by addition of antioxidants and packaging.

Oxidative rancidity is studied by comparing the susceptibility of the original lipids (control) and oxidized products and investigating the effect of other chemicals like anti-oxidants and oxidation conditions like light, temperature and packaging material (Kamal, Eldin, 2010). The products of lipid oxidation are hydroperoxides, alcohols, aldehydes and ketones. These are studied and quantified using classical analytical methods, volumetric, titrimetric and colorimetric methods. Some of them include Peroxide Value, Anisidine Value, Epoxide Value and TBARS Assay. Carbonyl value, Permanganate assay, total polar compounds (Fritsch, 1981). These methods are simple and are reasonably sensitive, reliable and reproducible when carried out under standardized conditions (Kamal, Eldin, 2010).

Out of all the methods to detect primary oxidation products, peroxide value gives important characteristic of lipid quality. It is relatively simple and requires less chemical reagents than other tests. As per official AOCS method (American Oil Chemists Society, 1990), hydroperoxides are reacted with iodide ions to liberate iodine and PV is determined by titration of liberated iodine with sodium thiosulfate using starch as an indicator.

To detect the secondary oxidation products, chromatographic methods or spectroscopic methods are most reliable. Spectroscopic methods analyze the samples based on the specific functional group present in the oxidized product. They require no or minimal sample treatment and samples can be run in a short duration of time. Chromatographic methods are faster than spectroscopic methods but they consume time during protocol standardization and sample preparation. The TBARS assay is a well-known spectroscopic method for screening and

monitoring of lipid oxidation. In this assay, malondialdehyde (MDA), is formed as an oxidation product. Thiobarbituric acid at low pH and elevated temperatures by nucleophilic addition, forms a complex TBA-MDA-TBA adduct which shows pinkish red color at 531 nm wavelength (Janero, 1990).

In order to evaluate the oxidation products and to study the process of oxidation in a relatively short time, shelf life studies are done in accelerated conditions which exponentially increase the oxidation. Any method which is capable of evaluating product stability, based on the data that are obtained in a significantly shorter period than the actual shelf life of the product under elevated physical conditions can be called accelerated shelf life testing.

Antioxidants

Oxidation can be counteracted by 2 methods: physical and chemical means. The physical method involves prevention of contact between the oxidizable substrate such as unsaturated food products and oxidant species such as free radicals, atmospheric O2 and transition metals. This can be done through modified atmospheric packaging or physiochemical barriers. The chemical method is to incorporate antioxidant molecules which can prevent or delay the natural process of oxidation of unsaturated fatty acids.

An antioxidant should be localized at the site (substrate meets oxidant species) where oxidation occurs. It should be non-toxic and should not cause any side-effects when consumed. Antioxidants can inhibit the rate and extent of lipid oxidation-induced changes.

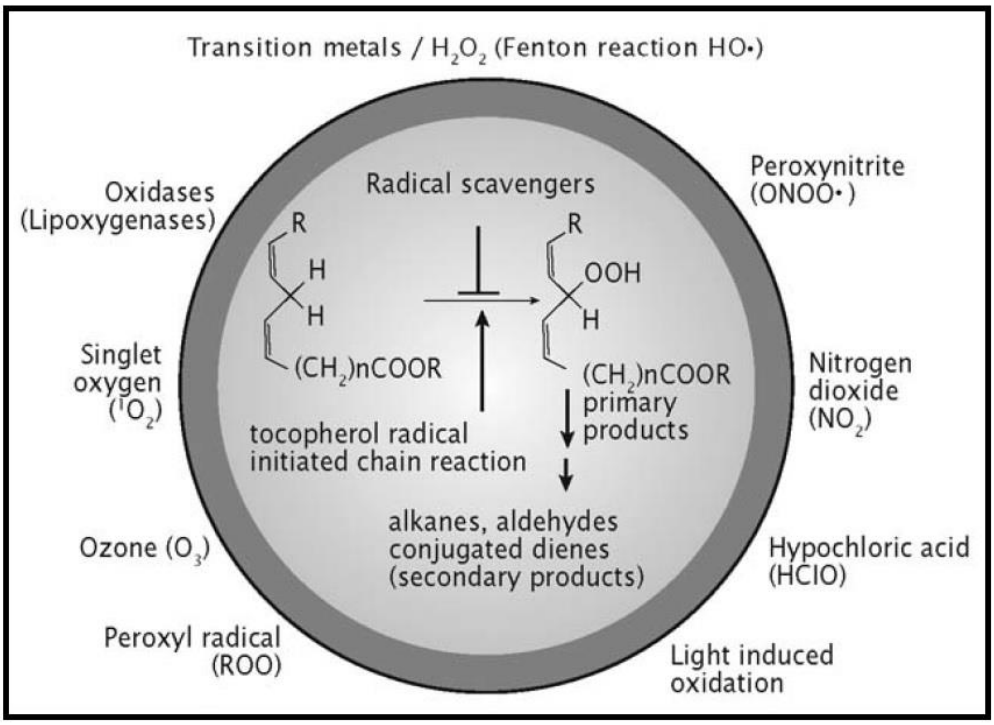


Figure 6. Oxidation products (inner circle) and oxidants (outermost space) (Wong, 2013)

Figure 6 shows various oxidants and oxidation products. Oxidants such as enzymes, light, a wide range of reactive oxygen species, and a combination of transition metal ions and peroxide molecules will cause oxidation. Antioxidants prevent lipid oxidation by preventing the fatty acid molecules from transforming into radicals in chain reactions. Radical chain breakers work within the lipid phase to stop the reaction (Huang, Wong, 2013).

Vitamin E as an Antioxidant

Vitamin E is a natural antioxidant so it was used in the formulation and oxidative studies involving peanut butter bites. Vitamin E acts as a radical scavenger which can quench free radicals. Vitamin E is a common term for tocopherols and tocotrienols that co-exist with lipids in biological systems. One significant trend observed in recent years is to substitute synthetic food ingredients like BHA, BHT, TBHQ with natural antioxidants like vitamins E & C.

The functional groups susceptible to oxidation are bis-allylic carbons in linoleate (18:2, n-6), linolenate (18:3, n-3) and longer chain polyunsaturated fatty acids having the structure ($-CH_2-(CH = CH-CH2)_n-CH_2-$) (Huang, Wong, 2013).

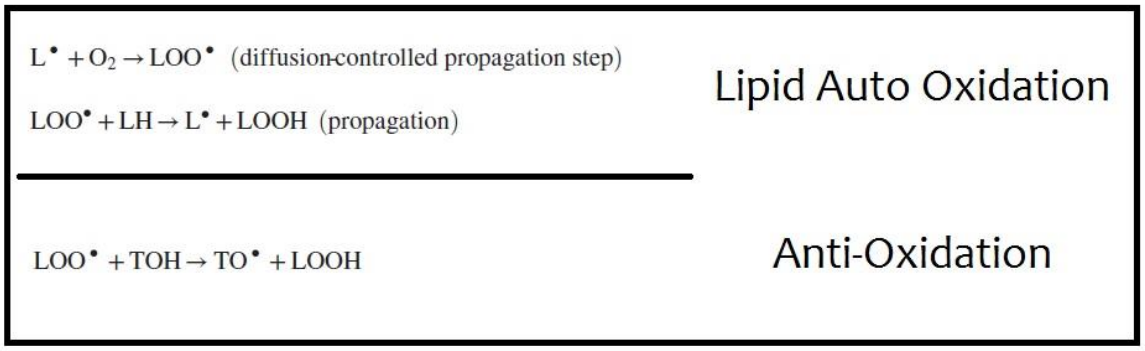


Figure 7. Auto-oxidation vs antioxidant mechanism.

The mechanism of antioxidants in preventing the lipid oxidation is almost similar to the mechanism of lipid auto-oxidation discussed in the 'Lipid Oxidation' section. As shown in Figure 7, the difference lies in the chain propagation step where the tocopherol molecule quenches the free radical and becomes a tocopherol radical and continues the reaction until the chain termination step, thus preventing the unsaturated fatty acid molecules from undergoing lipid oxidation.

Packaging

Food Packaging is defined as a coordinated system of preparing food products for transport, warehousing, logistics, sale, and end use. Apart from providing physical & barrier protection, convenience, information transmission and marketing information, packaging plays a significant role in shelf life and product stability.

Packaging is one of the primary processes which can impact the quality of a lipid food product as it can delay the lipid oxidation by preventing the contact of the oxidizable substrate (lipid) with the active oxidant species (oxygen, metal ions).

Plastics are the most commonly used packaging material for preserving food products. Plastics are special group of polymers with characteristics that differentiate them from fibers, rubbers, adhesives and other polymeric materials. Plastics provide chemical and heat resistance; low, medium, and high gas permeability; water vapor transmission rate, thermal and mechanical behavior (Siracusa, 2012). More than 100 million pounds of plastics, such as polyethylene, polypropylene, polyvinyl chloride, polystyrene were produced in 2011, which are recognized as indirect food additives (American Chemistry Council, 2012).

Oxygen and water permeability, material density, degree of polymerization, glass transition temperature, melting temperature, and chemical resistance are the properties to be considered while selecting packaging material for food products.

For oxidative stability of peanut butter slices, 4 different packaging materials: saran wrap, HB1 (high barrier material), DK11 (low barrier material) and Printpack (cheese packing material) were tested based on a wide range of moisture and oxygen permeability values of materials and most widely available polymers (Pareja Diaz, 2000). DK11 exhibited minimum adherence to the peanut butter slices, followed by Printpack material, HB1 and Saran.

CHAPTER III

MATERIALS AND METHODS

Peanut Butter Bite Preparation

Peanut butter bites were formulated using Jif™ Peanut Butter. Each bite weighed about 12.5 grams and contained peanut butter as the major ingredient along with binding, sweetening, and emulsifying agents. Preparation of peanut butter bites involved melting creamy peanut butter (Jif) at 50° C using a water bath (Precision, Reciprocal Shaking Bath Model 50). Pea protein (Growing Naturals), peanut flour (Protein Plus), cacao butter (Navitas) and Alphadim PBK mono-di-glycerides (Corbion) were added to the peanut butter and mixed. This mixture was kept at 50° C. Brown sugar (Great Value) and Kelcogel gellan gum (Modernist Pantry) were added to water in a glass beaker and heated to 90° C using a water bath (Thermo, Microprocessor-controlled Bath 280 Series) and mixed thoroughly.

The gel solution was added to the peanut butter mixture and mixed rigorously. The specific composition of the bites is shown in Table 3. After cooling the mixture to 45° C, the ACTIVA® Transglutaminase GS enzyme (Ajinomoto) was added to the mixture and mixed. The mixture was immediately scraped into saran-covered molds to form peanut butter bites. After cooling for 30-45 min, the wrapped peanut butter bites were packed tightly in saran wrap as shown in Figure 8.

Figure 8. Peanut butter bite samples wrapped in saran wrap.

For shelf-life studies, 3 different formulations of peanut butter bites were prepared. They differed in the level of antioxidant added. Formula 1 (control) was the common formulation used for the development of peanut butter bites. From the 50g formula weight, 4 bites were prepared.

Table 3. Formulation of Peanut Butter Bites with Different Levels of Vitamin E.

Ingredient	**Formula 1 (g)**	**Formula 2 (g)**	**Formula 3 (g)**
Peanut Butter	39.50	39.50	39.50
Peanut Flour	2.00	2.00	2.00
Pea Protein	2.00	2.00	2.00
Cacao Butter	1.00	1.00	1.00
Mono-di-glycerides	1.00	1.00	1.00
Gellan Gum	0.04	0.04	0.04
Brown Sugar	3.00	3.00	3.00
Water	0.96	0.96	0.96
Enzyme TG-GS	0.50	0.50	0.50
Vitamin E	0.00 (0 ppm)	0.01 (200 ppm)	0.025 (500 ppm)
Total	**50 g**	**50.01 g**	**50.025 g**

Each saran-wrapped snack bite was packaged in 2 different packaging materials: 2 mil transparent polyethylene (Uline, IL, S-947) and 2.5 mil metallized polyester and polyethylene bonded film (Uline, IL, S-11661) shown in Figure 9. Each package was sealed using an impulse heat sealer (Pack Secure, OH, MP-12C). Oxygen and moisture permeability properties of the two packages are shown in Table 4.

Table 4. Characteristic Properties of Packaging Materials (Uline, 2016)

Packaging	**Level**	**Material**	**Moisture Permeability ($10^{\wedge(-5)}$ g/in^2-hr)**	**Oxygen Permeability ($10^{\wedge(-5)}$ cc/in^2-hr)**
Saran Wrap	Primary	Polyvinyl chloride	8528.45553	3.53828
Metallized Pouch	Secondary	Polyester and poly ethylene bonded film	4.1875	0.33332
Plastic Pouch	Secondary	Polyethylene	776.671	0.4927536

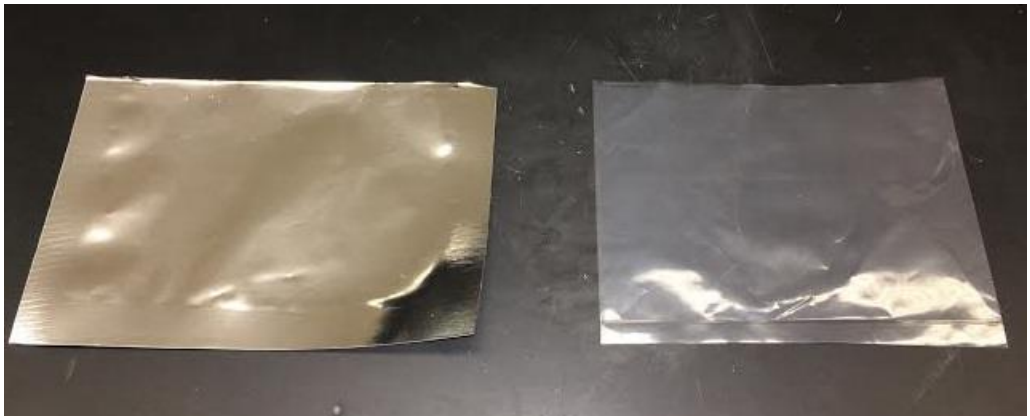
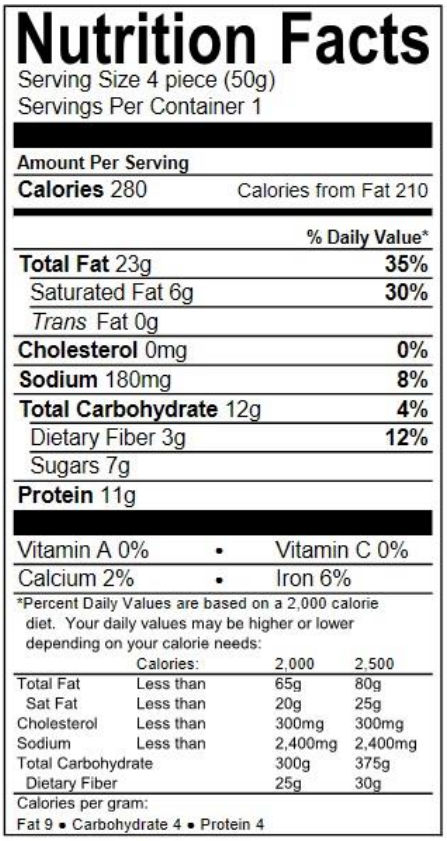


Figure 9. Metallized (left) and plastic (right) packaging materials

Nutrition Label for Peanut Butter Bites

Figure 10 shows the nutritional label for peanut butter bites generated by Genesis nutritional labeling software (Genesis, Version 11.0.137, Salem, OR, ESHA). The nutritional content is similar to that of peanut butter, since the major ingredient in the snack product is peanut butter itself. Added protein, sugar and fat alter the proximates to some extent. A major food component of the bites is fat, which is susceptible to oxidative rancidity. This research study focuses on delaying the rancidity by the addition of the antioxidant vitamin E.



Nutrition Facts

Serving Size 4 piece (50g)
Servings Per Container 1

Amount Per Serving

Calories 280	Calories from Fat 210
	% Daily Value*
Total Fat 23g	**35%**
Saturated Fat 6g	**30%**
Trans Fat 0g	
Cholesterol 0mg	**0%**
Sodium 180mg	**8%**
Total Carbohydrate 12g	**4%**
Dietary Fiber 3g	**12%**
Sugars 7g	
Protein 11g	

Vitamin A 0%	•	Vitamin C 0%	
Calcium 2%	•	Iron 6%	

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs:

	Calories:	2,000	2,500
Total Fat	Less than	65g	80g
Sat Fat	Less than	20g	25g
Cholesterol	Less than	300mg	300mg
Sodium	Less than	2,400mg	2,400mg
Total Carbohydrate		300g	375g
Dietary Fiber		25g	30g

Calories per gram:
Fat 9 • Carbohydrate 4 • Protein 4

Figure 10. Nutritional label for peanut butter bites.

Experimental Design

The design of the experiment was based on evaluation of oxidative rancidity (primary and secondary oxidation) of the peanut butter bites under different combinations of temperature conditions, antioxidant levels and packaging materials. The oven temperature (50° C) was chosen based on the most probable upper temperature limit during transportation and storage. The packaging materials were selected based on common materials used for packing food products, specifically to pack oxidation-sensitive food products. Experiments were carried out over 6 months of storage time, with 10 observations in total. The first measurements were taken on the formulation day, the next 5 observations were taken on a weekly basis, and the last 4 observations were taken on a monthly basis.

The following storage conditions were chosen:

- Room temperature and Humidity : 25°C ± 5°C, 45% RH ± 10%
- Oven Temperature : 50°C ± 1°C

The following antioxidant concentration levels were chosen:

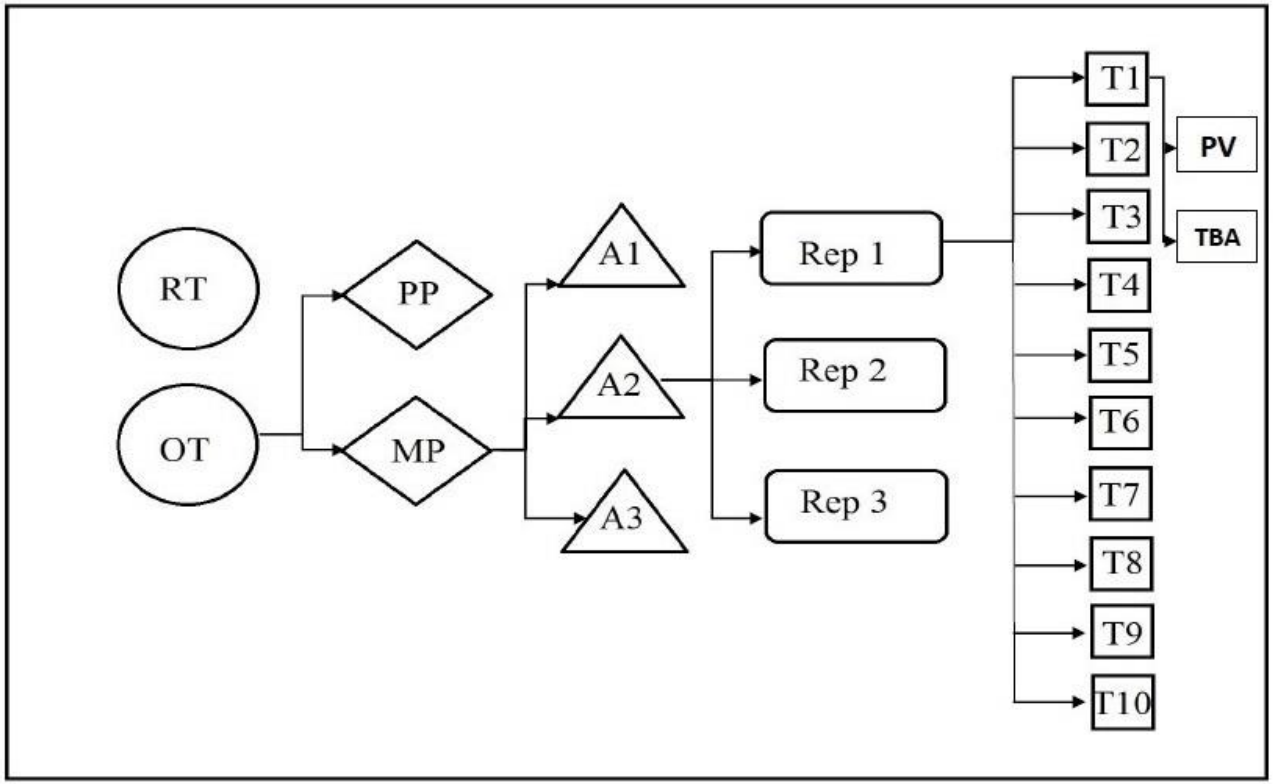
- 0 ppm of vitamin E (0 mg for 12.5 g product)
- 200 ppm of vitamin E (2.5 mg for 12.5 g product)
- 500 ppm of vitamin E (6.25 mg for 12.5 g product)

The following packaging materials were chosen:

- Transparent flat polyethylene packaging
- Metallized polyester & polyethylene bonded film packaging

The basis for selection of these materials was to the study how the rancidity is affected by the oxygen and moisture permeability characteristics of packaging materials. The reason for using

saran plastic wrap as a primary packing material was to hold the integral structure of the bite and metallized and/or transparent polyethylene packaging bags as a secondary packing material was to observe how rancidity gets affected with the 2 commonly available food bags which have different permeability characteristics. The experiments were carried out in 3 replications. The experimental design is shown in Figure 11.



Temperature: RT – Room Temperature (25° C) OT – Oven Temperature (50° C)

Packaging: PP – Plastic Packaging MP – Metallized Packaging

Antioxidant: A1 – 0 ppm Vitamin E A2 – 200 ppm Vitamin E A3 – 500 ppm Vitamin E

Time Interval: T1-Week0 T2-Week1 T3-Week2 T4-Week3 T5-Week4 T6-Week5 T7-Week11 T8-Week15 T9-Week19 T10-Week23

Replications: Rep 1 Rep 2 Rep 3

Figure 11. Experimental design for peanut butter bite oxidative stability tests.

Statistical Methods

Samples were stored at 2 different temperature conditions and 2 different packaging materials were used. Samples with 3 different levels of antioxidant (Vitamin E) concentration were chosen to study the oxidative rancidity. There were 3 replications of each unique sample. In total, for every observation day, either for Peroxide test or TBA test, there were 2 X 2 X 3 X 3 = 36 samples. Testing was done 10 times, so there were 360 samples tested for peroxide value assay and 360 samples for TBARS assay. All the samples were given a unique number immediately after the formulation.

Statistically, this was an experimental project with a 2 X 2 X 3 X 10 factorial treatment structure in a Completely Randomized Design (CRD) with 3 replications at each combination of storage, antioxidant level and packaging material tested at 10 time periods for both peroxide value and TBARS assays. The oxidation data was analyzed using SAS Institute (Version 9.4, Cary, NC). The statistical analysis methods used were ANOVA, F-test, Least Square Means and Difference in Least Square Means.

Peroxide Value Assay

When lipids are oxidized, hydro-peroxides are formed which are unstable in nature. These are primary oxidation compounds formed during the early stages of rancidity. The most commonly used quantitative assay for hydro-peroxide detection is the peroxide value assay. It measures the amount of peroxide formed and is expressed in units of milli-equivalents of oxygen per kilogram of fat, which is a direct measure of the amount of fat that has been oxidized. For the peroxide value assay (American Oil Chemists Society, 1990), 5g of peanut butter bite sample was homogenized in 30 ml 3:2 glacial acetic acid and chloroform mixture. To this mixture, 0.5 ml of saturated potassium iodide solution was added. Peroxides formed in the peanut butter react with liberated iodine, which is chemically titrated against sodium-thio sulfate solution using starch as

an indicator. The titration value gives an estimate of peroxide value. The PV assay protocol is summarized in Figure 12.

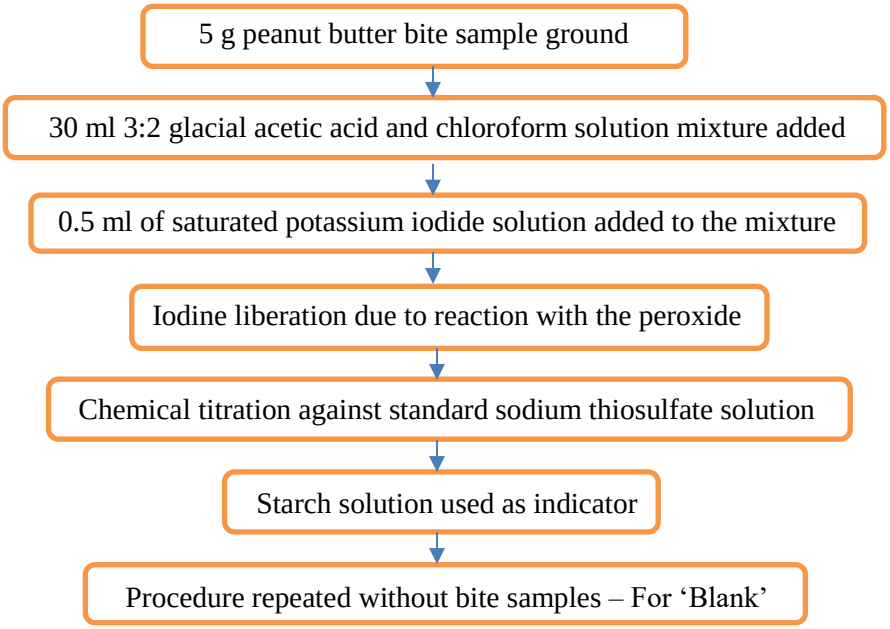


Figure 12. Peroxide value assay protocol.

The peroxide value was calculated as follows:

PV = (S-B)*N*1000/(sample weight) where

S = Titration value of the sample (ml)

B = Titration value of the blank sample (ml)

N = Normality of Sodium thio sulfate solution (0.1 N)

Sample weight = 5 grams

Following are some precautions that should be taken into account during the peroxide value assay:

- Sodium thiosulfate should be accurately standardized. It should be made up from concentrated solution and care must be taken to avoid contaminating or diluting the standard solution.
- Potassium iodide solution must be saturated i.e., there must be undissolved crystals of iodide in the beaker or container.
- Potassium iodide should be stored in dark and tested to ensure that it contains no iodine prior to usage for peanut butter oxidation, else peroxide value will be altered.
- Solutions should be made with recently boiled distilled water to eliminate the possibility of oxidation of iodide by dissolved oxygen.
- Amount of iodine liberated in the test is sensitive to the conditions used such as the time the peroxides are allowed to react with iodide, reaction temperature and pH. Precise conditions of the test must be followed.
- Liberation of iodine from potassium iodide by oxygen in the sample can lead to a higher peroxide value, hence care should be taken to ensure that right amount of potassium iodide is added to the sample mixture.

TBARS Assay

The Thio Barbituric Acid Reactive Substances (TBARS) assay is an analytical method which quantifies rancidity caused by secondary oxidation compounds in high lipid food systems. This method gives a measure of the aldehydes present in a food product. After a period of time, unstable primary oxidation compounds (hydro-peroxides) break down and aldehydes or ketones are formed. At a wavelength of 531 nm, Malondialdehyde (MDA) reacts with Thio-Barbituric Acid (TBA) and Tri Chloro-acetic Acid (TCA) to form a pinkish-red colored complex. The absorbance of this oxidized chromogen is measured at 531 nm using a spectrophotometer. This absorbance is a direct measure of concentration of the secondary oxidation product, MDA, produced in peanut butter bites during the oxidation process. The 531 nm wavelength is

specifically chosen because this aldehyde exhibits a maximum absorption peak at this particular wavelength.

The TBARS assay protocol is summarized in Figure 13. For the TBARS assay (American Oil Chemists Society, 2004), 5g of peanut butter bite sample is dissolved in 15 ml of distilled water and homogenized thoroughly. To prepare TCA solution, 20 g of Tri Chloro-acetic acid (TCA) is dissolved in 100 ml distilled water and mixed thoroughly. This solution is kept in a closed glass bottle and stored under a fume hood. To prepare the TCA-TBA reagent, 0.6 g of Thio barbituric acid (TBA) is dissolved in 120 ml of TCA solution and mixed thoroughly. This solution is kept in a cool place in a closed glass bottle. Two ml of the TCA-TBA reagent is added to 1 ml of peanut butter homogenate and 50 ul of Butylated Hydroxy Anisole is added immediately to avoid further oxidation during the sample analysis.

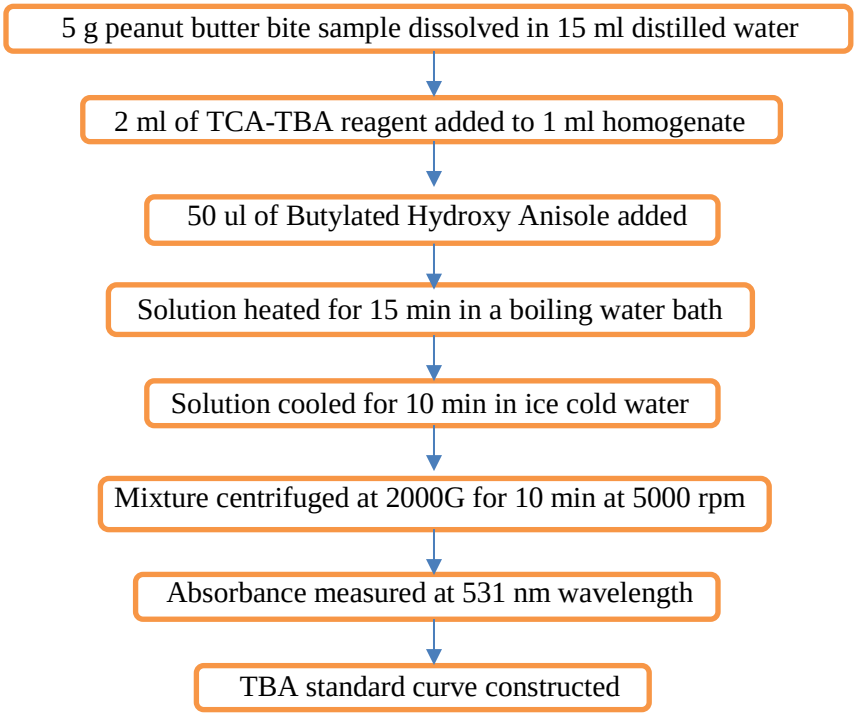


Figure 13. TBARS assay protocol.

The solution is heated for 15 minutes in a boiling water bath and then cooled for 10 minutes in ice cold water. The mixture is centrifuged at 2000G for 10 min at 5000 rpm. Absorbance is measured using a spectrophotometer at 531 wavelength. A TBA standard curve is developed using TBA standards to calculate the concentration of MDA from absorbance values.

Following are some precautions that should be taken into account during the TBARS assay:

- Acids used while working on TBARS assay protocol are corrosive and harmful so care must be taken while using them. They should be used under a fume hood. Gloves are mandatory.
- While removing samples from the boiling water bath, care should be exercised.
- BHA should be added immediately to avoid oxidation reaction occurring during the boiling duration or the whole TBARS process.
- It is mandatory to centrifuge the supernatant multiple times if there is cloudiness in the supernatant. Cloudiness can affect the spectrophotometric observations.

CHAPTER IV

RESULTS AND DISCUSSION

This chapter includes the results from two oxidative rancidity assays: the peroxide value assay and the Thio-Barbituric Acid Reactive Substances (TBARS) assay. The data includes oxidation results from peanut butter bites stored at different temperatures, under different packaging conditions, and with 3 antioxidant concentration levels during 6 months of storage.

Peroxide Value Assay

Peroxide value gives a measure of primary oxidation and is expressed in meq/kg units. The results obtained from the peroxide value assay are shown in Figures 14 and 15 for plastic and metallized packaging materials, respectively. It can be observed that during the 6 month storage period, the peroxide values ranged from 2.6 – 31.51 meq/kg. The peroxide value (P.V.) of freshly made samples was about 2.57 meq/kg and the maximum P.V. observed in peanut butter bite samples during the 6 months of storage was 31.51 meq/kg. The peroxides produced during initial sub-intervals will be decomposed into stable compounds such as aldehydes and ketones and the peroxide values should eventually decrease. However, as the time proceeds, more fatty acids in the peanut butter bites are converted to peroxides, thus resulting in an increase in the concentration of peroxides from one interval to another during this 6 month storage period. This phenomenon should continue until all the fatty acids get converted to peroxides and other primary oxidation products. After complete conversion, followed by prolonged storage, the peroxides decompose to stable secondary oxidation products – aldehydes and ketones. This results in a

permanent decrease in the peroxide value, which is in tune with the normal trend of peroxide formation during the oxidation of fatty acids.

From Figure 14, it can be observed that all the curves follow a similar trend with peroxide values increasing from the lowermost curve to the uppermost curve. The three lower curves show the peroxide value results for peanut butter bite samples stored at room temperature (25° C) and the three upper curves show the peroxide values for peanut butter bite samples stored at oven temperature (50° C). Samples stored at oven temperature had consistently higher peroxide values than the samples stored at room temperature.

Samples with different levels of antioxidant concentration (200 ppm and 500 ppm) exhibited lower peroxide values than the control samples (0 ppm), and the 500 ppm samples showed the lowest peroxide values, indicating the least amount of oxidation among different treatment levels. Peroxide values show an increasing trend from week 0 to week 23 in all the curves, indicating that oxidation increases during the storage period. The samples with 0 ppm vitamin E stored at oven temperature (50° C) exhibited the highest peroxide values among the six treatments whereas samples with 500 ppm vitamin E stored at room temperature (25° C) exhibited the lowest peroxide values. Both temperature and antioxidant level had a significant impact on the primary oxidation in peanut butter bites stored in plastic packaging material.

It can also be observed that the curves in Figures 14 and 15 are very similar.

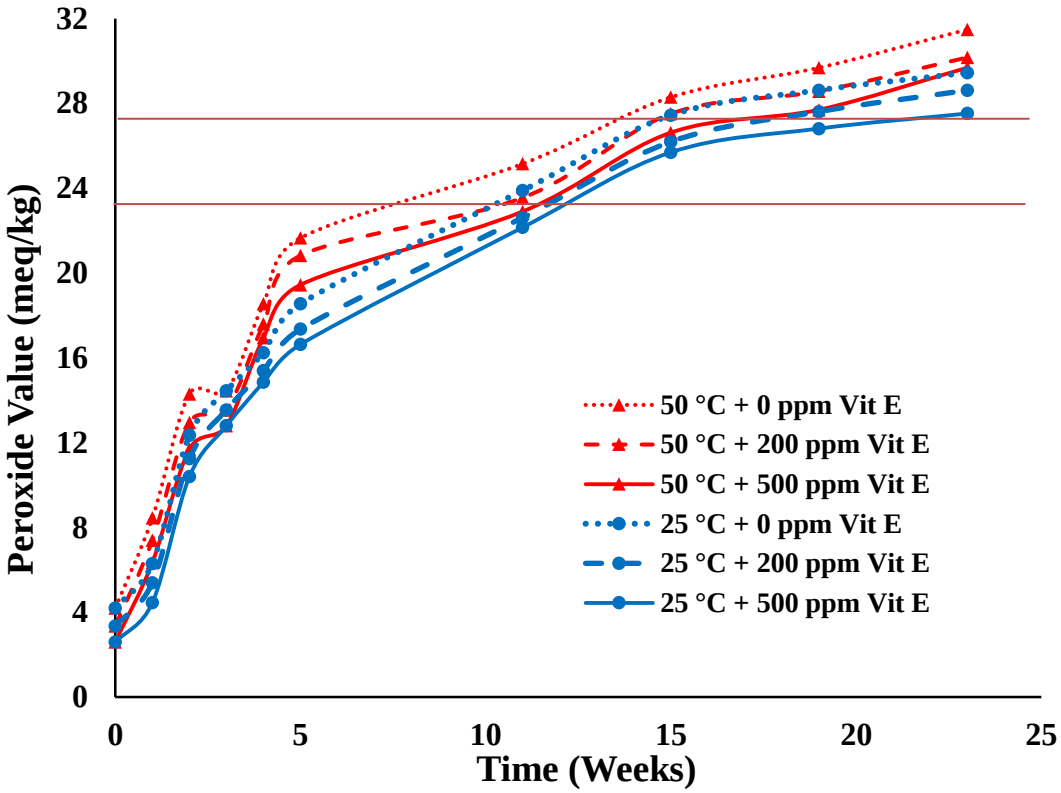


Figure 14. Peroxide values for peanut butter bite samples in plastic packaging material. Values shown are the average of three replicates. Red lines indicate typical range of values for onset of rancidity.

From Figure 15, it can be observed that all the curves follow a similar trend with peroxide values increasing from the lowermost curve to the uppermost curve. The three lower curves show the peroxide value results for peanut butter bite samples stored at room temperature (25° C) and the three upper curves show the peroxide values for peanut butter bite samples stored at oven temperature (50° C). Samples stored at oven temperature had consistently higher peroxide values than the samples stored at room temperature.

Samples with no antioxidant (0 ppm vitamin E) exhibited the highest peroxide values among the six treatments and samples with 500 ppm vitamin E exhibited the lowest peroxide values, indicating the least amount of oxidation among the different treatments. Peroxide values

show an increasing trend from week 0 to week 23 in all the curves, indicating that oxidation increases during the storage period.

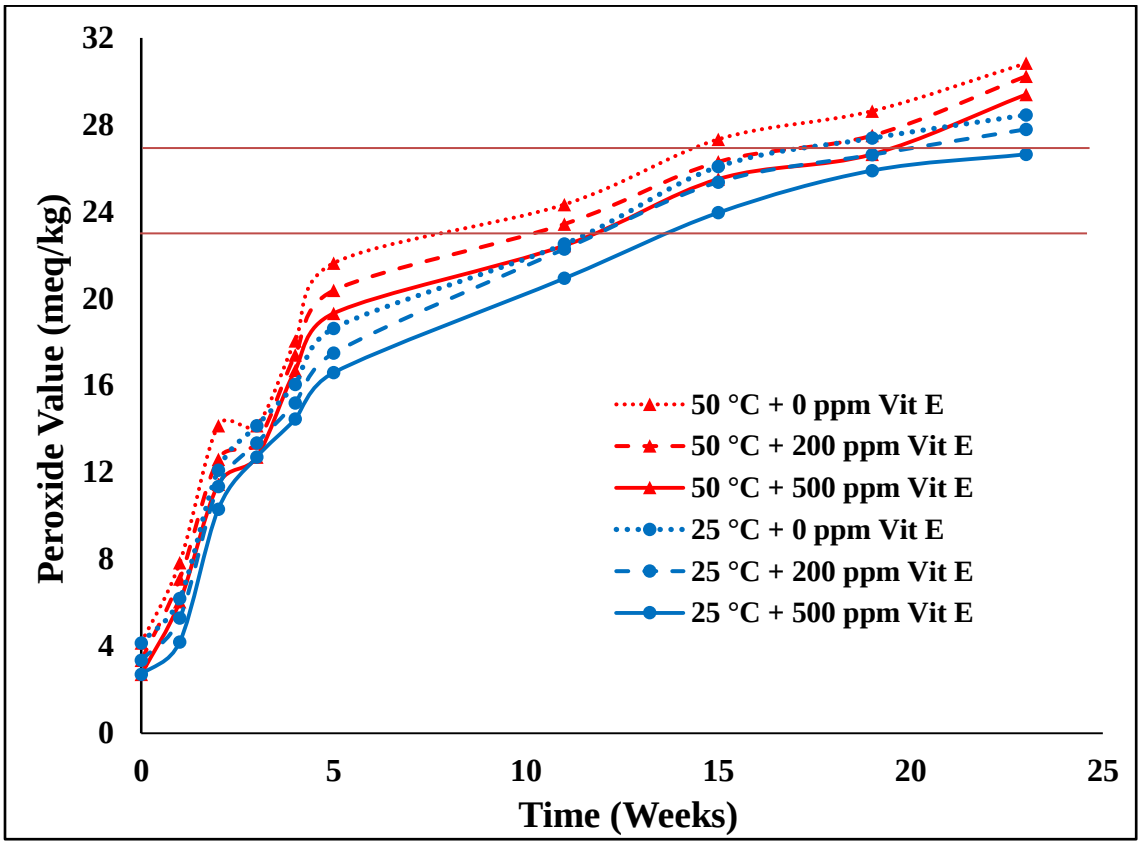


Figure 15. Peroxide values for peanut butter bite samples in metallized packaging material. Values shown are the average of three replicates. Red lines indicate typical range of values for onset of rancidity.

Overall, the samples with 0 ppm vitamin E stored at oven temperature (50° C) exhibited the highest peroxide values among the six treatments whereas the samples with 500 ppm vitamin E stored at room temperature (25° C) exhibited the lowest peroxide values. Both temperature and antioxidant level had a significant impact on the primary oxidation in peanut butter bites stored in metallized packaging material.

TBARS Assay

The TBARS assay gives a measure of secondary oxidation. Malondialdehyde (MDA) is a secondary oxidation product whose concentration is expressed in ng/ml units. The results obtained from the TBARS assay are shown in Figures 16 and 17 for plastic and metallized packaging materials, respectively. TBARS values ranged from 0.02 to 1.16 ng/ml depending on the packaging material and storage conditions. In general, TBARS values seemed to increase steadily from the initial value and continued to increase during the entire time of storage.

From Figure 16, it can be observed that all the curves follow a similar trend with TBARS values increasing from the lowermost curve to the uppermost curve. The three lower curves show the TBARS values for peanut butter bite samples stored at room temperature (25° C) and the three upper curves show the TBARS value results of peanut butter bite samples stored at oven temperature (50° C). Samples stored at oven temperature had significantly higher TBARS values than the samples stored at room temperature.

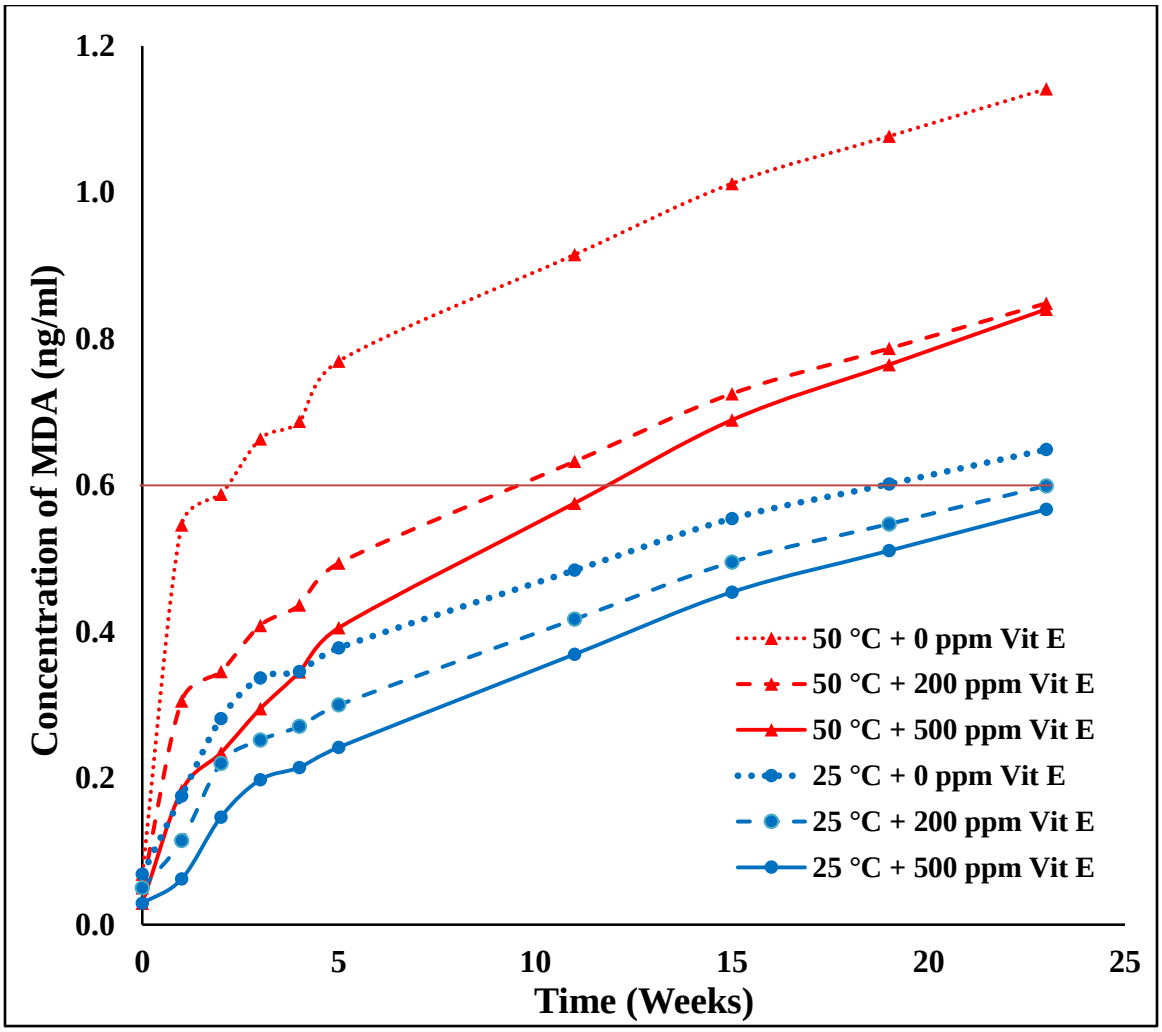


Figure 16. TBARS values for peanut butter bite samples in plastic packaging material. Values shown are the average of three replicates. The red line indicates the typical value for onset of rancidity.

Samples with different levels of antioxidant concentration (200 ppm and 500 ppm) exhibited lower TBARS values than the control samples (0 ppm). The samples with 500 ppm vitamin E showed the lowest TBARS values, indicating the least amount of oxidation among the different treatment levels. TBARS values show an increasing trend from week 0 to week 23 in all the curves, indicating that oxidation increased during the storage period. The samples with 0 ppm vitamin E stored at oven temperature (50° C) exhibited the highest TBARS values among the 6

curves whereas the samples with 500 ppm vitamin E stored at room temperature (25° C) exhibited the lowest TBARS values. Both temperature and antioxidant level had a significant impact on the secondary oxidation in peanut butter bites stored in plastic packaging material. It can also be observed that the curves in Figures 16 and 17 are similar.

From Figure 17, it can be observed that all the curves follow a similar trend with TBARS values increasing from the lowermost curve to the uppermost curve. The three lower curves show the TBARS values for peanut butter bite samples stored at room temperature (25° C) and the three upper curves show the TBARS values for peanut butter bite samples stored at oven temperature (50° C). Samples stored at oven temperature had significantly higher TBARS values than the samples stored at room temperature.

Samples with no antioxidant (0 ppm vitamin E) exhibited the highest TBARS values among the six treatments and samples with 500 ppm vitamin E exhibited the lowest TBARS values, indicating the least amount of oxidation among the different treatments. TBARS values show an increasing trend from week 0 to week 23 in all the curves indicating that oxidation increased during the storage period. Samples with 0 ppm vitamin E stored at oven temperature (50° C) exhibited the highest TBARS values among the six curves whereas samples with 500 ppm vitamin E stored at room temperature (25° C) exhibited the lowest TBARS values. Both temperature and antioxidant level significantly impact the secondary oxidation in peanut butter bites stored in metallized packaging material.

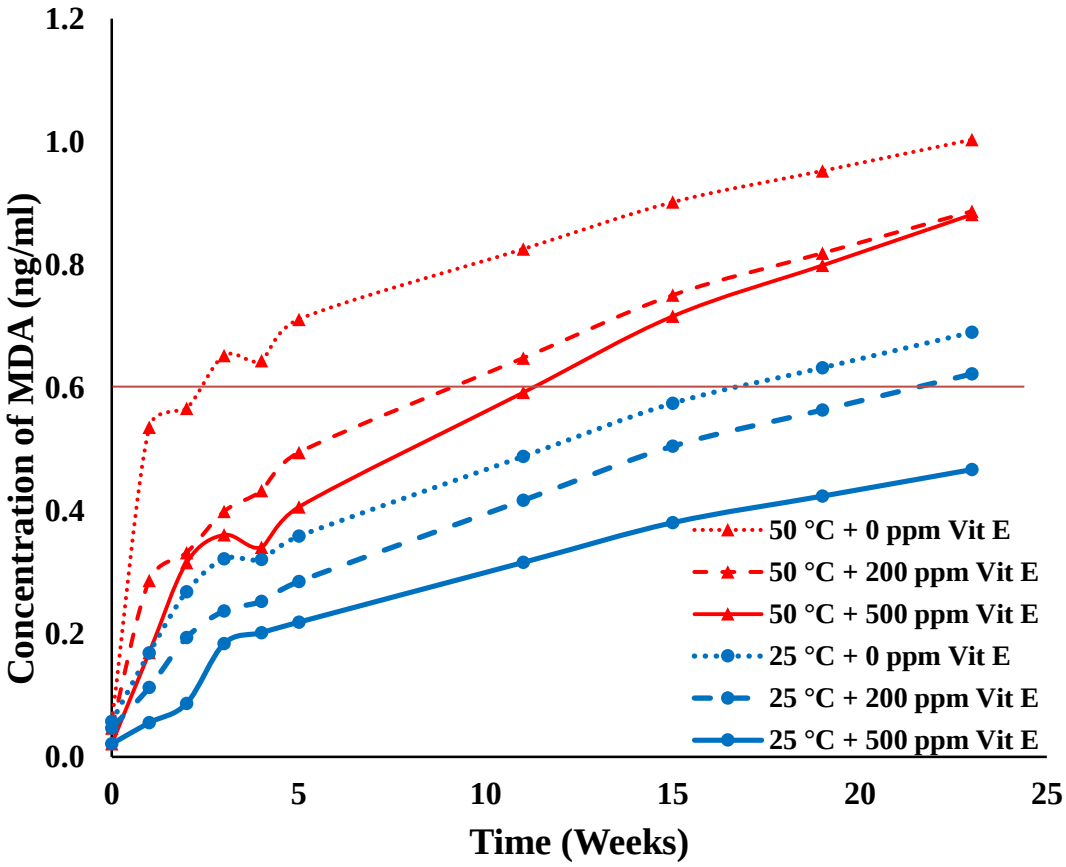


Figure 17. TBARS values for peanut butter bite samples in metallized packaging material. Values shown are the average of three replicates. The red line indicates the typical value for onset of rancidity.

As the TBARS value gives an indirect measure of the oxidation products, especially aldehydes, formed during lipid oxidation, this trend in graphical curves can be explained by the fact that as the duration of storage increases, TBARS values increase within the oxidized food product. Some aldehydes are secondary or tertiary by-products of the fatty acids in the food products. Other aldehydes are by-products of other non-fat constituents.

Comparison at Specific Time Points

In order to more easily compare the twelve different treatments, snapshots of the oxidation values at five different time points (week 2, week 5, week 11, week 15 and week 23) are shown in Figures 18-27.

Figure 18 shows peroxide values for all treatments at week 2. It can be observed from the figure that temperature had the biggest effect on peroxide values, antioxidant concentration had the second biggest effect, and packaging had the smallest effect on the peroxide values. Most treatments were significantly different from each other, with the exception of a few treatments where packaging was not significantly different. Peroxide values for plastic and metallized packaging materials ranged from 10.4 to 12.4 meq/kg for samples stored at room temperature. Samples stored at oven temperature (50° C) exhibited peroxide values between 11.4 and 14.3 meq/kg for both packaging materials. The 500 ppm samples at room temperature had the lowest peroxide values, but the two different packaging materials were not significantly different at that point. The samples with 0 ppm vitamin E had significantly different oxidation values with respect to temperature and packaging. The samples with 500 ppm vitamin E were significantly different with respect to temperature, but the oxidation values for samples at oven temperature and at room temperature did not show significant differences between the plastic and metallized packaging materials. Again, temperature and antioxidant concentration were significantly different in every case, but packaging was not significantly different for several treatments.

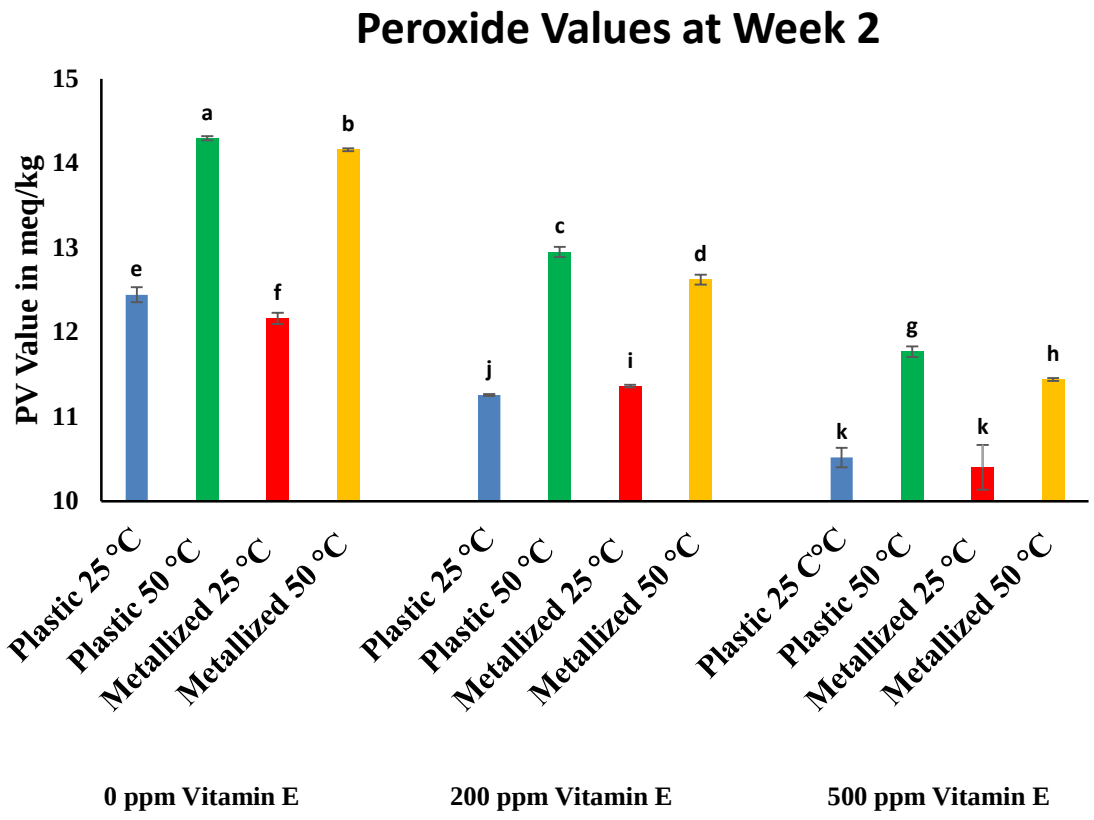


Figure 18. Comparison of peroxide values at week 2. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 19 shows peroxide values for all treatments at week 5. It can be observed from Figure 19 that peroxide values for plastic and metallized packaging materials ranged from 16.5 to 18.8 meq/kg for samples stored at room temperature. Samples stored at oven temperature (50° C) exhibited peroxide values between 19.2 and 21.9 meq/kg for both packaging materials.

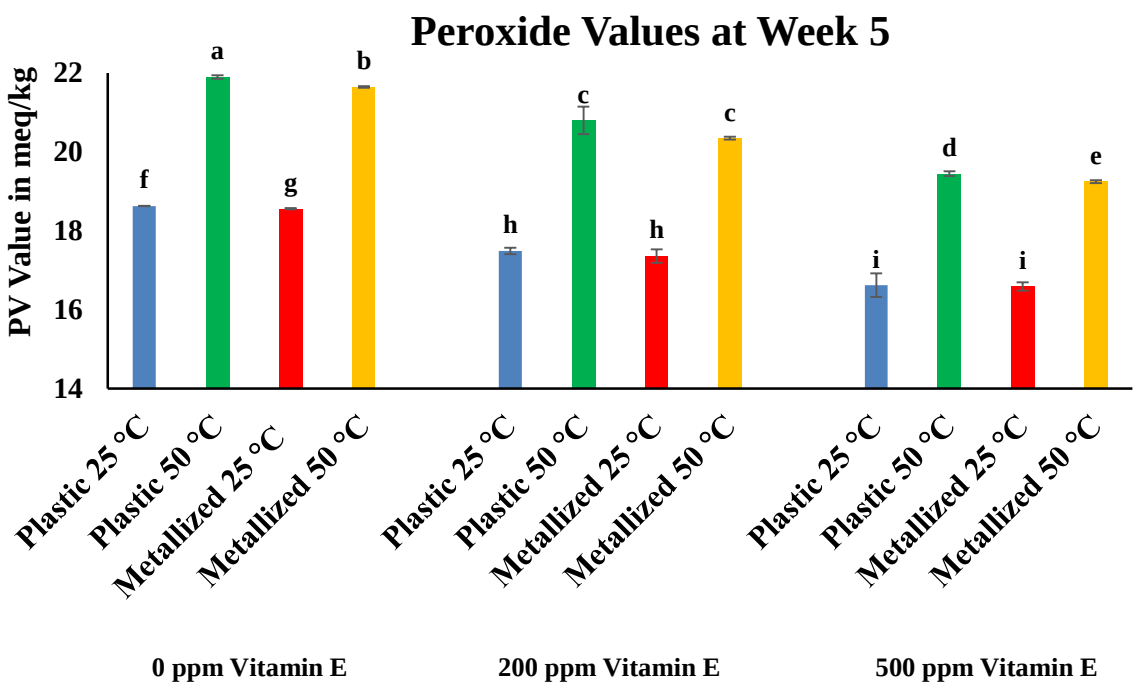


Figure 19. Comparison of peroxide values at week 5. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

From Figure 19 it is apparent that the 500 ppm samples at room temperature had the lowest peroxide values, but the two different packaging materials were not significantly different at that point. The samples with 0 ppm vitamin E had significantly different oxidation values with respect to temperature and packaging. The samples with 200 ppm vitamin E were significantly different with respect to temperature, but the oxidation values for samples at oven temperature and at room temperature did not show significant differences between the plastic and metallized packaging materials. Again, temperature and antioxidant concentration were significantly different in every case, but packaging was not significantly different for several treatments.

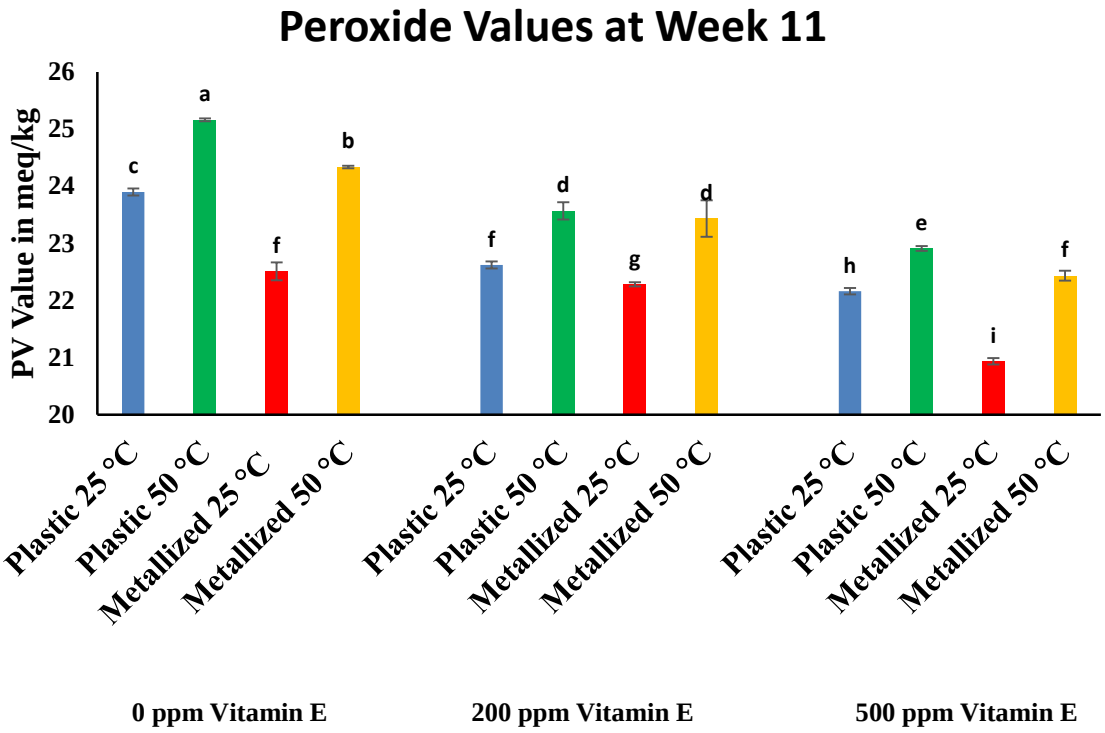


Figure 20. Comparison of peroxide values at week 11. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 20 shows peroxide values for all treatments at week 11. It is clear from the figure 20 that temperature had the biggest effect on oxidative rancidity. All treatments were significantly different from each other, with the exception of two pairs (d, f). The oxidation values for samples with 200 ppm vitamin E at oven temperature were not significantly different between the plastic and metallized packaging materials (d). However, at room temperature, the oxidation values were significantly different (f, g). The oxidation values for samples with 0 ppm and 200 ppm vitamin E at room temperature were not significantly different between a pair of treatments (f).

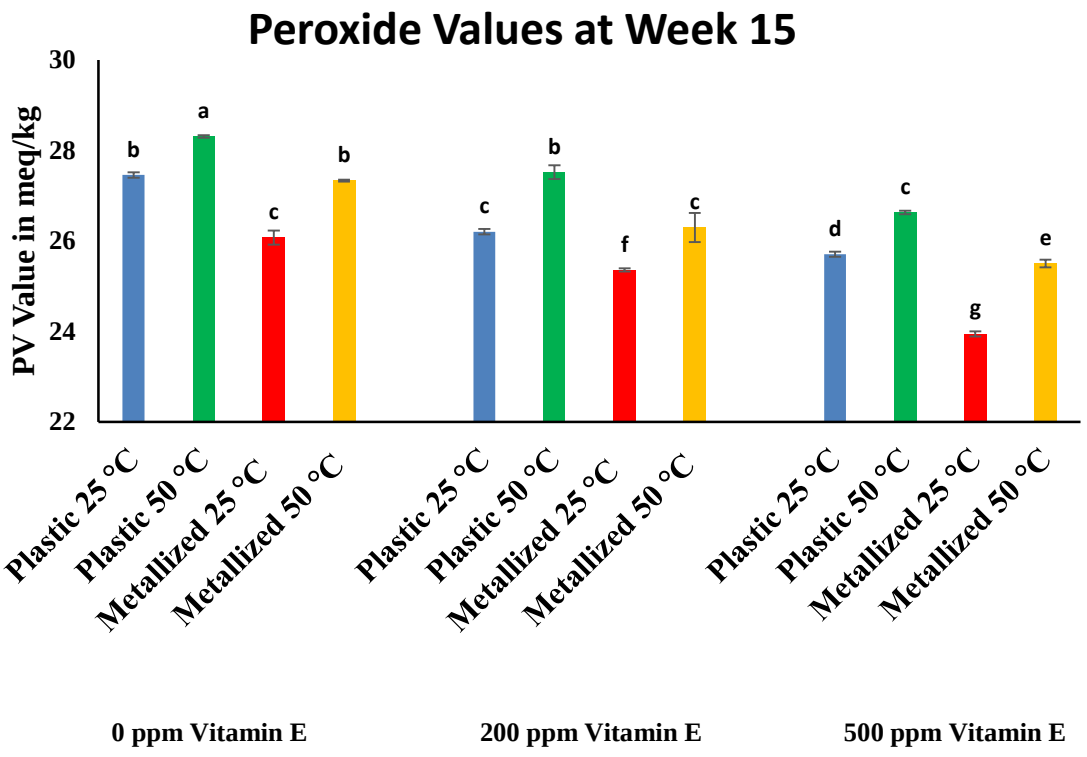


Figure 21. Comparison of peroxide values at week 15. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 21 shows peroxide values for all treatments at week 15. Again, it is clear from the figure that temperature had the biggest effect on oxidative rancidity. The 500 ppm samples stored in metallized packaging at room temperature had the lowest peroxide values. Temperature, packaging and antioxidant concentration were not significantly different for several treatments.

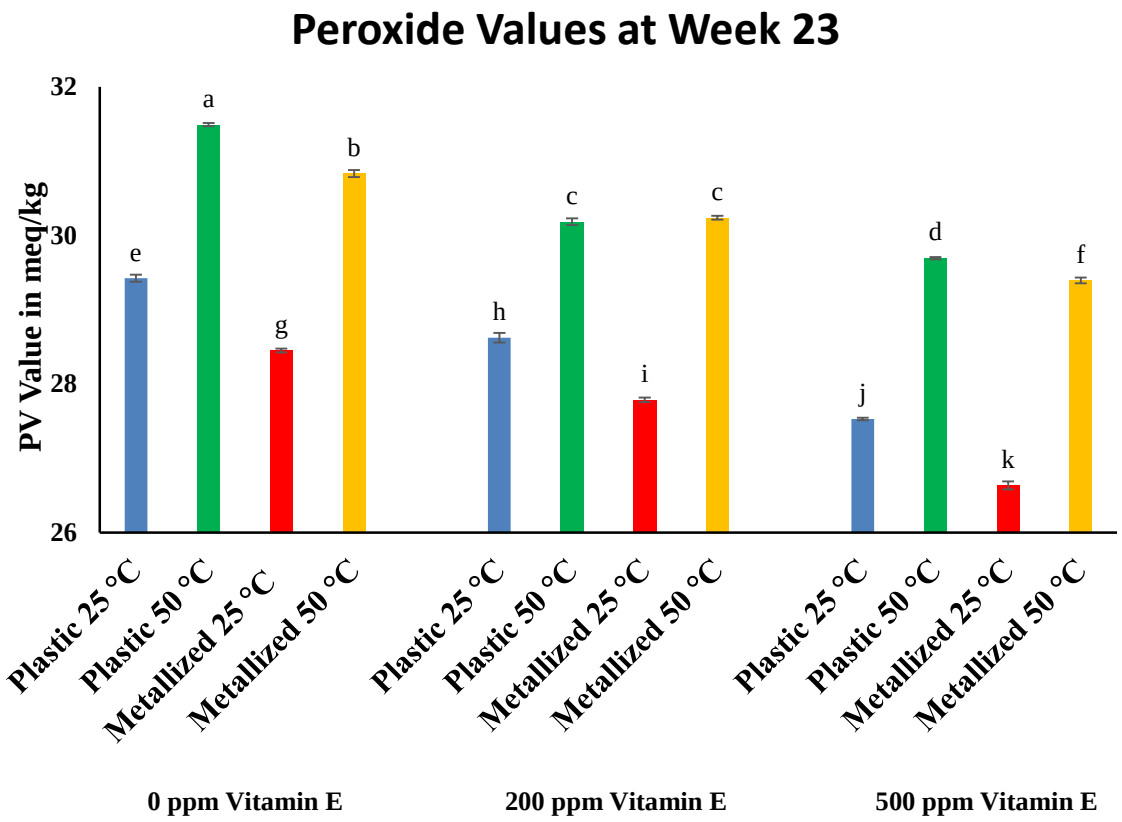


Figure 22. Comparison of peroxide values at week 23. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 22 shows peroxide values for all treatments at week 23. Again, it is clear from the figure that temperature had the biggest effect on oxidative rancidity.

All treatments were significantly different from each other, with the exception of one pair. The oxidation values for samples with 200 ppm vitamin E at oven temperature were not significantly different between the plastic and metallized packaging materials. However, at room temperature, the oxidation values were significantly different.

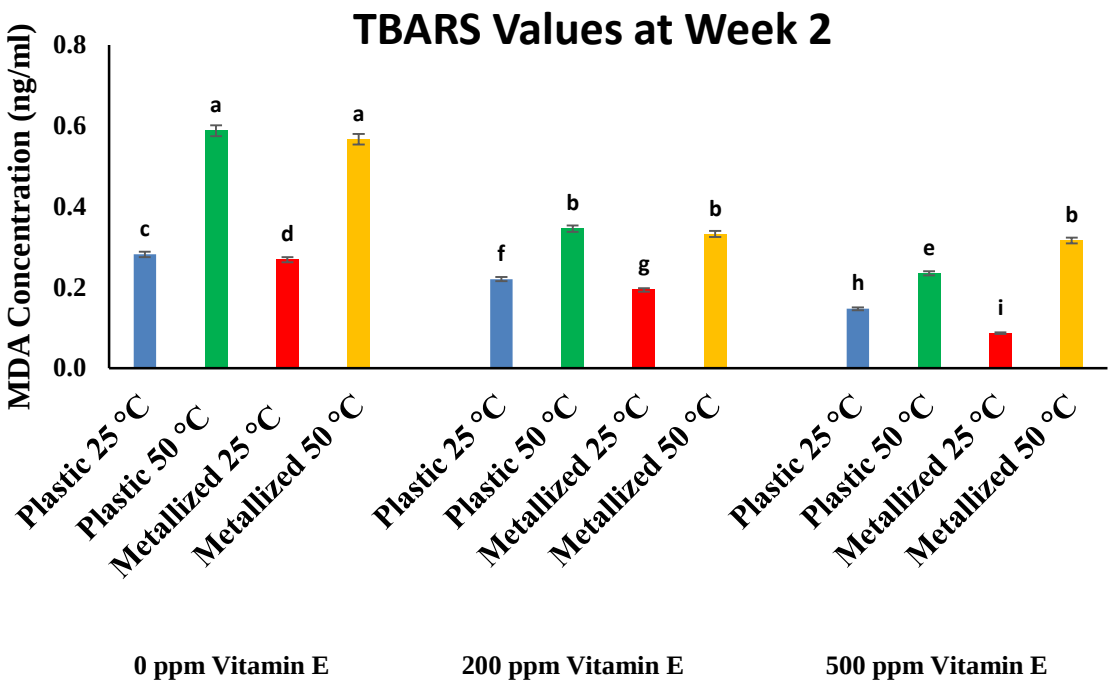


Figure 23. Comparison of TBARS values at week 2. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 23 shows TBARS values for all treatments at week 2. It can be observed from the figure that temperature had the biggest effect on MDA values, antioxidant concentration had the second biggest effect, and packaging had the smallest effect on the oxidation values. Most treatments were significantly different from each other, with the exception of three treatments, out of which packaging was not significantly different in two treatments (a, b) and antioxidant concentration in one treatment (b). Room temperature stored samples had rancidity values ranging from 0.09 to 0.28 ng/ml Malondialdehyde concentration with 0.09 ng/ml for 500 ppm samples and 0.28 ng/ml for control samples. Rancidity values in oven temperature stored samples varied from 0.23 ng/ml to 0.59 ng/ml with 0.23 ng/ml for 500 ppm samples. The samples with 0ppm vitamin E had significantly different oxidation values with respect to temperature but not

with respect to packaging. Samples stored at oven temperature with 200 and 500 ppm vitamin E concentrations showed no significant differences between the plastic and metallized packaging materials. Samples with 500 ppm vitamin E at room temperature had the lowest oxidation values.

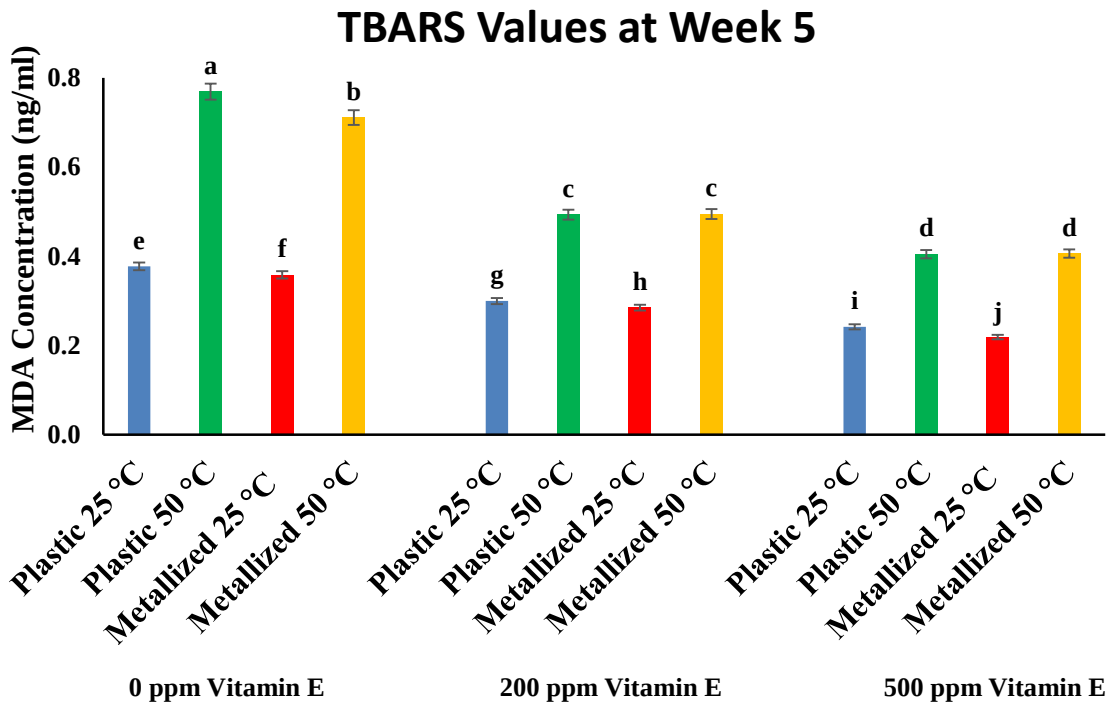


Figure 24. Comparison of TBARS values at week 5. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 24 shows TBARS values for all treatments at week 5. It can be observed from the figure that temperature had the biggest effect on MDA values, antioxidant concentration had the second biggest effect, and packaging had the smallest effect on the oxidation values. Most treatments were significantly different from each other, with the exception of two treatments where packaging was not significantly different. Room temperature stored samples had rancidity values ranging from 0.22 to 0.38 ng/ml Malondialdehyde concentration with 0.22 ng/ml for 500 ppm samples and 0.38 ng/ml for control samples. Rancidity values in oven temperature stored samples varied from 0.41 ng/ml to 0.77 ng/ml with 0.41 ng/ml for 500 ppm samples. The samples

with 0ppm vitamin E had significantly different oxidation values with respect to temperature and packaging. Samples stored at oven temperature with 200 and 500 ppm vitamin E concentrations showed no significant differences between the plastic and metallized packaging materials. Samples with 500 ppm vitamin E at room temperature had the lowest oxidation values.

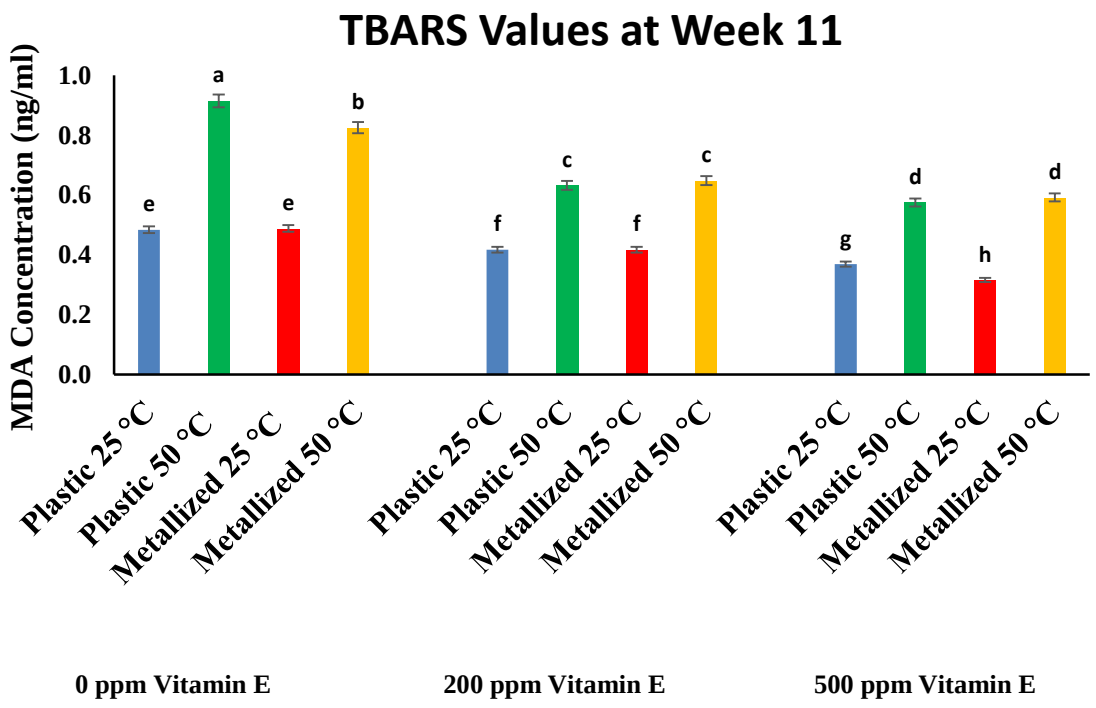


Figure 25. Comparison of TBARS values at week 11. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 25 shows TBARS values for all treatments at week 11. It can be observed from the figure that temperature had the biggest effect on MDA values, antioxidant concentration had the second biggest effect, and packaging had the smallest effect on the oxidation values. Most treatments were significantly different from each other, with the exception of four pairs where packaging was not significantly different at various temperature and antioxidant concentration levels (c, d, e and f).

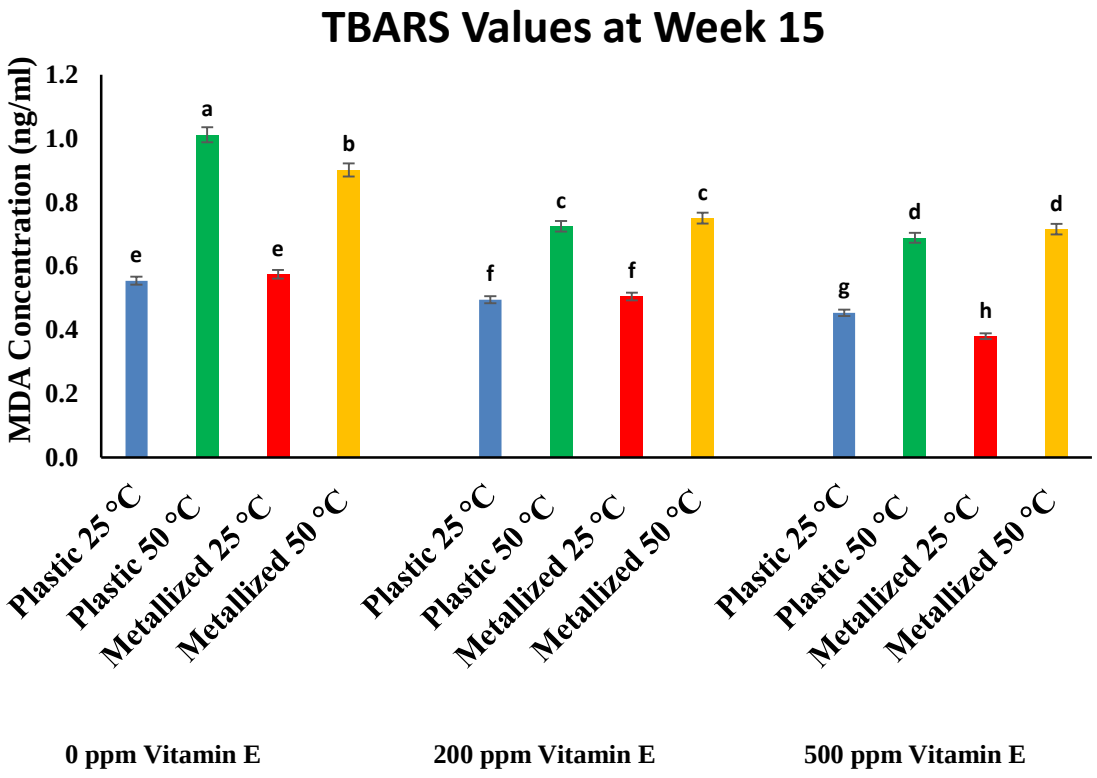


Figure 26. Comparison of TBARS values at week 15. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 26 shows TBARS values for all treatments at week 15. From the figure, again temperature had the biggest effect on MDA values, antioxidant concentration had the second biggest effect, and packaging had the smallest effect on the oxidation values. Most treatments were significantly different from each other, with the exception of four pairs where packaging was not significantly different at various temperature and antioxidant concentration levels (c, d, e and f), similar to that of Figure 25.

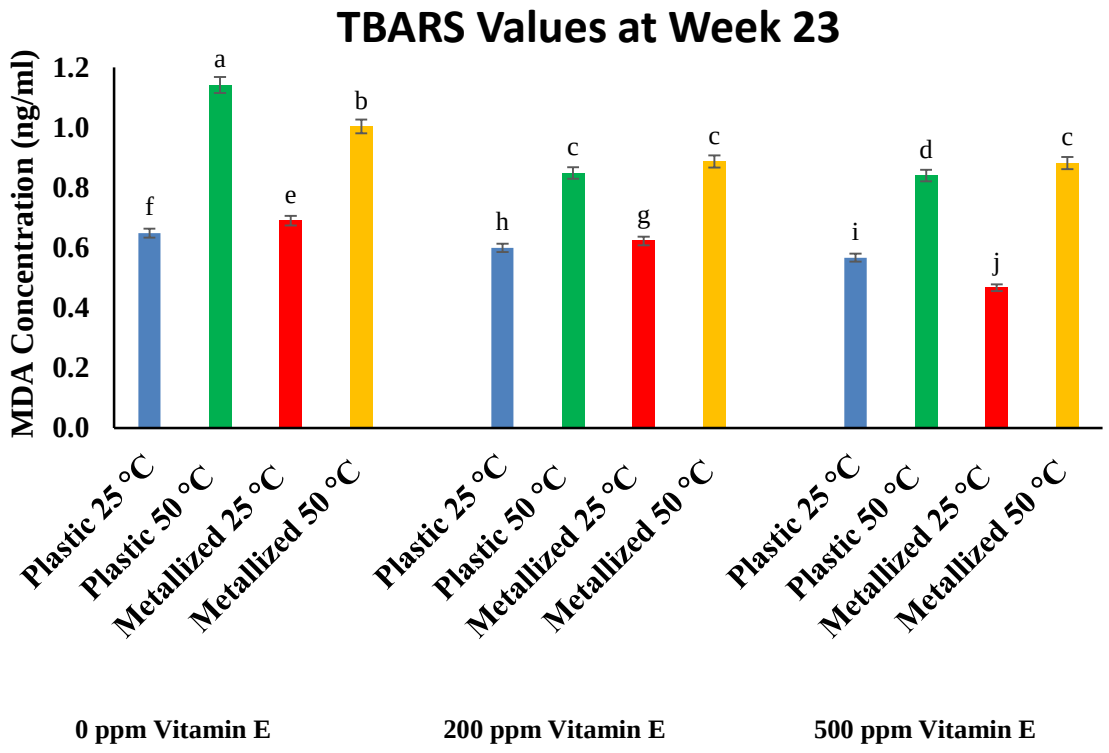


Figure 27. Comparison of TBARS values at week 23. Values shown are the average of three replicates. Error bars represent standard deviation. Values marked with the same letter are not significantly different (α=.05).

Figure 27 shows TBARS values for all treatments at week 23. Again, temperature had the greatest effect on oxidative rancidity at week 23. Room temperature stored samples had rancidity values ranging from 0.47 to 0.65 ng/ml Malondialdehyde with 0.47 ng/ml for 500 ppm vitamin E samples and 0.65 ng/ml for control samples. Rancidity values in oven temperature stored samples varied from 0.88 to 1.14 ng/ml for vitamin E samples. The samples with 0ppm vitamin E had significantly different oxidation values for all four treatments involving temperature and packaging (a, b, e, f). The samples with 200 ppm and 500 ppm vitamin E also had significantly different oxidation values with respect to temperature (g, h, i, j). However, the oxidation values of 200 ppm vitamin E samples for both plastic and metallized packaging materials and of 500 ppm vitamin E samples for metallized packaging material were not significantly different.

Temperature Effects on Rancidity

Storage temperature (25° C and 50° C) had a significant effect on the development of oxidative rancidity. It is commonly accepted that peroxide values ranging from 23 – 27 meq/kg represent the early stages of rancidity and peroxide values greater than 30 indicate complete rancidity (Gray, 1978). TBARS values greater than 0.6 ng/ml typically indicate the early stages of rancidity (Gray, 1978). From figures 14 and 15, where red lines represent onset of rancidity, the time to onset of rancidity from peroxide value data was 15 weeks at room temperature and 11 weeks at oven temperature. From figures 16 and 17, where red lines represent onset of rancidity, the time to onset of rancidity from TBARS data was 19 weeks at room temperature and 11 weeks at oven temperature. The 'estimated rancidity onset time' as a function of storage temperature and packaging are shown in Table 5.

Table 5. Table Showing Estimated Rancidity Onset Time vs Temperature for Both Assays.

Assays	Storage Temperature (°C)	Time to Onset of Rancidity (weeks)	
		Metallized Packaging	Plastic Packaging
Peroxide Value Assay	25	15	15
	50	11	11
TBARS Assay	25	19	19
	50	11	11

From the peroxide value assay at week 2, for 0ppm vitamin E samples stored in plastic packaging, the average peroxide value was 12.3% higher at oven temperature than at room temperature. For 0ppm vitamin E samples stored in metallized packaging, the average peroxide value was 14.1% higher at oven temperature than at room temperature. For 200ppm vitamin E samples stored in plastic packaging, the average peroxide value was 13.07% higher at oven

temperature than at room temperature. In most cases, oven temperature samples were 9-10% higher than room temperature samples.

From the TBARS assay at week2, both of the 0 ppm samples were about 52%. Most of the others ranged from 36-42%.

It can be clearly observed that the peanut butter bites stored at room temperature take a longer time duration to show signs of rancidity for both peroxide and TBARS values. Higher temperatures increase formation of free radicals which accelerate the lipid oxidation process. Hence, higher temperature stored samples exhibited higher rancidity values than the room temperature stored ones. It can be concluded that temperature effects on rancidity development are significant.

Other studies have also shown that temperature is a critical factor in rancidity development. Refined sunflower oil samples were stored in different packaging materials at ambient temperature (18-32 °C) and 37 °C and tested over a period of time to record the progress of secondary oxidation with respect to storage variation and time. Samples stored at 37 °C deteriorated faster than the ones stored ambient temperature (Nasirullah and Nagaraja, 1989). The effect of carnosic acid (an antioxidant extracted from rosemary) on oxidative stability of sunflower oil was studied during accelerated storage. At 60 °C, different oxidation assays were performed and the higher temperature yielded higher oxidation values than the control (Zhang and Liu, 2010).

Temperature also played a vital role in accelerating rancidity of peanut butter slices. For smaller storage periods, rancidity in terms of peroxide value and aldehyde generation increased rapidly with rise in temperature (Adhikary, 2001). It can be concluded that temperature is a significant factor in rancidity development in this product.

Effect of Packaging Material on Oxidative Rancidity

Statistical analysis showed that overall, packaging had a significant effect on oxidation for both peroxide and TBARS assays, and the metallized packaging material performed better than the plastic packaging (shown in Appendix table). However, at specific time intervals, packaging was not significantly different for some of the treatments. Rancidity values for both types of packaging were fairly close to each other for most treatments, and the differences between packages were the smallest at high temperature storage. Saran wrap was used as an initial packaging material to maintain the shape of the peanut butter bites, and the metallized and plastic packaging were used as a second layer for rancidity studies.

From the peroxide value assay at week 2, for 0ppm vitamin E samples, average peroxide value was 2.2% higher in plastic packaging than metallized packaging at room temperature and was 0.9% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature. For 200ppm vitamin E samples, average peroxide value was 0.9% higher in metallized packaging than plastic packaging at room temperature and was 2.5% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature. For 500ppm vitamin E samples, average peroxide value was 1.1% higher in plastic packaging than metallized packaging at room temperature and average peroxide value was 2.8% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature.

From the TBARS assay at week 2, for 0ppm vitamin E samples, average TBARS value was 4.66% higher in plastic packaging than metallized packaging at room temperature and average TBARS value was 3.60% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature. For 200ppm vitamin E samples, average TBARS value was 12.03% higher in metallized packaging than plastic packaging at room

temperature and average TBARS value was 3.91% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature. For 500ppm vitamin E samples, average TBARS value was 40.87% higher in plastic packaging than metallized packaging at room temperature and average TBARS value was 25.72% higher for samples stored in plastic packaging than those stored in metallized packaging at oven temperature.

Oxygen and moisture permeability values are critical parameters which determine the suitability of particular material as a good packaging material. Permeability values for saran wrap, metallized and plastic packaging materials are shown in Table 4. In determination of oxidative stability in high lipid food systems like peanut butter, oxygen permeability of packaging material plays a more significant role than the moisture permeability. From the results, peanut butter bite samples stored in metallized packaging in combination with saran wrap were less oxidized than the samples stored in plastic packaging in combination with saran wrap. These results can be expected due to the lower oxygen permeability in the metallized packaging material compared to that of the plastic material. Overall, peanut butter bites wrapped in saran-metallized packaging materials exhibited better shelf-life properties than saran-plastic materials. Also, the use of the saran wrap as a primary packing could have minimized differences between the two types of packaging.

After 4 months of storage, there was a little oil seepage in the packaging materials stored at room temperature, but there was a lot of visible oil seepage through all packaging materials in samples stored at 50° C. The oil separation likely contributed to the development of oxidative rancidity.

Other studies have found that metallized packaging worked best for preventing oxidation in other products. The effects of different packaging materials including five layers of compound film, modified polypropylene and metallized plastic film, and packaging atmosphere on the

quality of roasted pistachio nuts were investigated. Results showed that packaging pistachio nuts in metallized film kept the quality of pistachio nuts better than the other packaging options and lengthened oxidative shelf life (Raei and Pourazarang, 2009).

The effect of four different packaging materials (low barrier material DK11, high barrier material HB1, cheese packaging material print pack and saran wrap) on oxidative rancidity of peanut butter slices was also studied. Out of the 4 packaging materials, the cheese packaging print pack material and the HB1 seemed to perform the best (Adhikary, 2001).

Effect of Antioxidant Concentration on Oxidative Rancidity

To delay the oxidative rancidity, different natural antioxidants were incorporated into the formulation and lipid oxidation was analyzed during a preliminary experiment. Out of 3 antioxidants tested, including vitamin A, beta-carotene and vitamin E, vitamin E was found to be the most effective in delaying the lipid oxidation of peanut butter bites. Hence vitamin E was chosen for use in this research study. Oxidative rancidity was studied using 3 different concentrations of vitamin E: 0 ppm, 200 ppm and 500 ppm.

From the peroxide value assay, different levels of added vitamin E had a significant effect on oxidation at each time interval. From week 0 to week 23, there was an overall significant effect of antioxidant concentration on the oxidative rancidity, with higher rancidity values for every succeeding time interval. Vitamin E is utilized for its antioxidant properties, and as time proceeds, it gets depleted. Depletion of vitamin E in the snacks could be one reason for higher rancidity values over time. In samples with the highest level of vitamin E (500 ppm), the onset of rancidity occurs during week 15 for room temperature stored samples, where the rancidity values range from 23-27 meq/kg PV. For oven temperature stored samples, the onset of rancidity occurred at week 11, where rancidity values range from 22-26 meq/kg PV. It can be

concluded that the highest level of antioxidant concentration in combination with room temperature storage resulted in the lowest peroxide value as a result of delayed oxidation.

From the TBARS value assay, antioxidant concentration had an overall significant effect on oxidative stability at all time intervals, similar to the peroxide value assay. Samples with 500 ppm vitamin E resulted in much less oxidation than the control samples and the 200 ppm samples. The onset of rancidity occurs during week 19 for room temperature stored samples and during week 11 for oven temperature stored samples, where the MDA concentration values exceed 0.6 ng/ml in both cases.

From the peroxide value assay at week 2, average peroxide values for 0ppm and 200ppm vitamin E samples were 15.5% and 6.6% higher, respectively, than that of 500 ppm vitamin E samples stored in plastic packaging material at room temperature. Average peroxide values for 0ppm and 200ppm vitamin E samples were 17.6% and 9.1% higher, respectively, than that of 500 ppm vitamin E samples stored in plastic packaging material at oven temperature. Average peroxide values for 0ppm and 200ppm vitamin E samples were 14.5% and 7.9% higher, respectively, than that of 500 ppm vitamin E samples stored in metallized packaging material at room temperature. Average peroxide values for 0ppm and 200ppm vitamin E samples were 19.2% and 9.4% higher, respectively, than that of 500 ppm vitamin E samples respectively stored in metallized packaging material at oven temperature.

From the TBARS assay at week 2, average TBARS values for 0ppm and 200ppm vitamin E samples were 47-60% and 5-30% higher, respectively, than that of 500 ppm vitamin E samples stored at both temperatures and in both packaging materials.

One other observation was that the samples with vitamin E exhibited a more oily appearance and oily texture than the control samples. Samples formulated with 500 ppm of vitamin E had the oiliest appearance compared to other treatments.

Vitamin E has been used as an antioxidant in many different applications. Alpha-Tocopherol (Vitamin E) and synthetic antioxidants (BHA, BHT) were added to pasteurized butter samples at two concentrations: 50 and 100 ppm (Ozturk and Cakmakci, 2006). Peroxide and TBARS values showed that both natural and synthetic antioxidants used were capable of protecting the butter samples against oxidation. Results indicated that alpha-tocopherol exhibited a very strong antioxidant activity almost equal to that of the synthetic antioxidants. Another study showed that tocopherols were effective in decelerating lipid oxidation in fish oil-enriched energy bars when added in smaller concentrations (Jacobsen et al, 2008) and fish fillets (Ana and Mancini-Filho, 2000). Studies have also shown that tocopherols can be very effective in butterfat containing food products (Dougherty, 1993). Tocopherols also appear to be a useful antioxidant when added directly in both raw and cooked meat (McCarthy et al, 2001).

Oxidation Rate

Oxidation rate was calculated for three different time intervals: 0-2 weeks, 2-5 weeks and 5-23 weeks. Table 6 shows the oxidation rates based on peroxide values for the 12 treatments. Table 7 shows the oxidation rates based on TBARS values for the 12 treatments. Similar trends in oxidation rate were observed for both assays.

From the peroxide value results, the slope of oxidation with respect to time is highest during the first 2 weeks of storage, ranging from 3.80 to 5.05 meq/kg-week as shown in Table 6. Gradually, the slope decreases from week 2 to week 5 with a range of 1.73 to 2.62 meq/kg-week. This decline in slope indicates a decrease in oxidation rate during the intermediate storage period compared to the initial rate. During week 5 to week 23, the oxidation rate was at a minimum, ranging from 0.51 to 0.61 meq/kg-week.

Table 6. Rate of Oxidation in meq/kg-week for Peroxide Values

Anti-oxidant	Storage Combination	Oxidation Rate 0-2 weeks	Oxidation Rate 2-5 weeks	Oxidation Rate 5-23 weeks
0 ppm Vit E	Plastic 25 °C	4.08	2.15	0.59
	Plastic 50 °C	5.05	2.53	0.53
	Metallized 25 °C	3.98	2.17	0.54
	Metallized 50 °C	5.00	2.50	0.51
200 ppm Vit E	Plastic 25 °C	3.95	1.95	0.55
	Plastic 50 °C	4.80	2.62	0.52
	Metallized 25 °C	4.00	1.73	0.57
	Metallized 50 °C	4.63	2.58	0.57
500 ppm Vit E	Plastic 25 °C	3.90	2.08	0.60
	Plastic 50 °C	4.55	2.58	0.56
	Metallized 25 °C	3.80	2.08	0.58
	Metallized 50 °C	4.38	2.60	0.61
Range		**3.80 - 5.05**	**1.73 - 2.62**	**0.51 - 0.61**

The oxidation rate is similar for samples stored at room temperature in both plastic and metallized packaging materials. Similarly, oxidation rate is similar for samples stored at oven temperature in both plastic and metallized packaging materials. Samples stored at oven temperature exhibited higher oxidation rates when compared to those stored at room temperature. Antioxidant level had a big effect on oxidation rate. Control samples exhibited higher oxidation rates than samples formulated with different levels of vitamin E. Samples with 0 ppm vitamin E and oven temperature (50 °C) had the highest oxidation rate whereas the samples with 500 ppm vitamin E and room temperature had the lowest oxidation rate.

Table 7. Rate of Oxidation in ng/ml-week for TBARS Values

Anti-oxidant	Storage Combination	Oxidation rate during 0-2 weeks	Oxidation rate during 2-5 weeks	Oxidation rate during 5-23 weeks
0 ppm Vit E	**Plastic 25 °C**	0.106	0.032	0.015
	Plastic 50 °C	0.259	0.061	0.021
	Metallized 25 °C	0.105	0.030	0.018
	Metallized 50 °C	0.254	0.048	0.016
200 ppm Vit E	**Plastic 25 °C**	0.085	0.027	0.017
	Plastic 50 °C	0.148	0.049	0.020
	Metallized 25 °C	0.073	0.030	0.019
	Metallized 50 °C	0.143	0.054	0.022
500 ppm Vit E	**Plastic 25 °C**	0.059	0.032	0.018
	Plastic 50 °C	0.103	0.057	0.024
	Metallized 25 °C	0.063	0.024	0.014
	Metallized 50 °C	0.147	0.030	0.026
Range		**0.103 - 0.259**	**0.027 - 0.061**	**0.015 - 0.026**

From the TBARS assay results, the slope of oxidation with respect to time is highest during the first 2 weeks of storage, ranging from 0.103 to 0.259 ng/ml-week as shown in Table 7. Gradually, the slope decreases from week 2 to week 5 with a range of 0.027 - 0.061 ng/ml-week. This decline in slope indicates a decrease in oxidation rate during the intermediate storage period compared to the initial rate. During week 5 to week 23, the oxidation rate was at a minimum, ranging from 0.015 - 0.026 ng/ml-week.

At week 0, based on the obtained oxidation values, it is apparent that oxidation already had begun. It could be due to utilization of older ingredients such as monodiglycerides, peanut butter in formulation. Usage of fresh ingredients could result in negligible oxidation values at week 0.

Statistical Analysis

For identification of significant differences due to the effect of temperature, packaging, antioxidant concentration and different time intervals on the oxidation of peanut butter bites, statistical analysis was performed using SAS Institute (Version 9.4, Cary, NC). The statistical model involved 2 temperatures, 2 packaging materials, 3 antioxidant concentration levels, 3 replications and 10 time intervals. The treatment structure was a 2 X 2 X 3 X 10 factorial and the design structure was a Completely Randomized Design (CRD).

There were 4 main source factors: temperature, packaging, antioxidant concentration and time interval. As there were 4 sources, 6 two-way interactions and 4 three-way interactions and 1 four-way interaction were observed. Totally, there were 4 + 6 + 4 + 1 = 15 source-interaction combinations and 1 residual error as shown in Table 8.

Appendix tables A.2.1. to A.2.7. show the ANOVA data and least square means data for peroxide value assay. Appendix tables A.3.1. to A.3.7. show the ANOVA data and least square means data for TBARS assay. All estimations were done at 95% confidence intervals (Type 3 Tests of Fixed Effects). From the 'Type 3 Tests of Fixed Effects' table, four source effects and all possible interactions were tested using the F-test at 95 % confidence interval.

From the SAS output as shown in appendix tables A.2.3. and A.3.3., it was observed that for both PV and TBARS assays, overall source effects and all possible interactions were significant ($p<0.05$). It is apparent that there are significant interactions between temperature, packaging, antioxidant concentration and time interval. Individual significant source effects were

observed from the figures A.2.1. to A.2.4. and A.3.1. to A.3.4. Some interactions had a non-significant effect on primary and secondary lipid oxidation.

Table 8: Statistical Table of Inputs Used in Analysis for PV and TBARS Assays

Sl No.	Source	Degrees of Freedom
1	Temperature	1
2	Packaging	1
3	Temperature * Packaging	1
4	Antioxidant	2
5	Temperature * Antioxidant	2
6	Packaging * Antioxidant	2
7	Temperature * Packaging * Antioxidant	2
8	Time	9
9	Temperature * Time	9
10	Packaging * Time	9
11	Temperature * Packaging * Time	9
12	Antioxidant * Time	18
13	Temperature * Antioxidant * Time	18
14	Packaging * Antioxidant * Time	18
15	Temperature * Packaging * Antioxidant * Time	18
16	Residual Error	288

Assumptions Made in the Experiment

Throughout the experiment, it was assumed that:

- Peanut butter bite samples stored at room temperature had negligible temperature and humidity variations.
- Oven temperature fluctuations were negligible.
- Sealing of packaging materials was adequately efficient at the same temperature setting in the impulse sealer.
- Oxidative rancidity caused by exposure to light and metals was not taken into account.
- Peanut butter bites were equally susceptible to oxidation.
- Antioxidant added was equally distributed into the snack samples.
- Oxidation during the sample testing was not significant.

CHAPTER V

CONCLUSIONS

This research study provides some insights on oxidative stability of peanut butter bites and the effects of storage conditions such as storage temperature, packaging material and concentration of antioxidant in the snack.

The major conclusions drawn from this research study are as follows:

1. Storage temperature had a significant effect on oxidative rancidity of peanut butter bites. The 50^{o}C storage temperature resulted in oxidation values that were nearly 100% higher than those observed at 25^{o}C storage.
2. Addition of the antioxidant Vitamin-E was found to be effective in delaying the oxidative rancidity of peanut butter bites, and antioxidant level had a significant effect on both peroxide and TBARS oxidation numbers.
3. Overall, the effects of packaging on oxidative rancidity were significant, with the metallized package resulting in the best oxidative barrier. However, differences between the two types of packaging were not significant for several treatments at specific time points.
4. Time to onset of rancidity varied from 11 weeks to 15 weeks, depending on the treatment. The shortest times occurred at high temperature storage.

5. Overall, temperature, packaging, antioxidant concentration and time interval had a significant effect on oxidation.
6. Treatments used with a combination of higher antioxidant concentration and room temperature were found to be shelf stable for 15 weeks, after which there was a sign of oxidative rancidity.
7. The slowest oxidation rates occurred in samples prepared with 500 ppm vitamin E, in the metallized packaging material, and stored at room temperature.

CHAPTER VI

RECOMMENDATIONS FOR FUTURE STUDY

1. Future studies could involve in the texture analysis of peanut butter bites. How the textural properties such as adhesiveness, cohesiveness, gumminess, springiness, mouthfeel, grittiness, etc., vary with respect to temperature, time and packaging material should be studied.
2. Different emulsifiers such as lecithin, SBK, transcendin etc., could be added to the formulation and the textural parameters should be compared to find the best emulsifier for the snack.
3. Apart from metallized and plastic packaging materials, different packaging materials could be used for the oxidative stability research.
4. Pilot plant studies could be conducted to study the oxidative stability and textural parameter variations on a large scale.
5. Use of other nut butters could be evaluated as potential line extensions to the product.

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APPENDICES

A.1 SAS Code

A.1.1 SAS Code for Peroxide Value Assay

```
ODS listing;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';
Options Pageno=1 LS=100;
DATA Oxidation;
INPUT temp pack anti time oxid @@;
DATALINES;

/* Comment: temp (1 - room temperature, 2 - oven temperature); pack (1 - plastic packaging, 2 - metallized packaging); anti (1 - 0ppmVitE, 2 - 200ppmVitE, 3 - 500ppmVitE), time (0, 1, 2, 3, 4, 5, 11, 15, 19, 23 - weeks); oxid (peroxide value in meq/kg) */

1 1 1 0 4.200 1 1 1 1 6.300 1 1 1 2 12.350 1 1 1 3 14.300 1 1 1 4 16.250 1 1 1 5 18.800 1 1 1 11
23.950 1 1 1 15 27.380 1 1 1 19 28.760 1 1 1 23 29.450 1 1 1 0 4.100 1 1 1 1 6.200 1 1 1 2 12.450
1 1 1 3 14.210 1 1 1 4 16.340 1 1 1 5 18.300 1 1 1 11 23.830 1 1 1 15 27.430 1 1 1 19 28.580 1 1
1 23 29.370 1 1 1 0 4.200 1 1 1 1 6.500 1 1 1 2 12.530 1 1 1 3 14.370 1 1 1 4 16.180 1 1 1 5
18.600 1 1 1 11 23.920 1 1 1 15 27.560 1 1 1 19 28.540 1 1 1 23 29.450 1 1 2 0 3.350 1 1 2 1
5.400 1 1 2 2 11.250 1 1 2 3 13.330 1 1 2 4 15.400 1 1 2 5 17.100 1 1 2 11 22.550 1 1 2 15 26.180
1 1 2 19 27.620 1 1 2 23 28.620 1 1 2 0 3.410 1 1 2 1 5.300 1 1 2 2 11.270 1 1 2 3 13.420 1 1 2 4
15.420 1 1 2 5 17.900 1 1 2 11 22.640 1 1 2 15 26.250 1 1 2 19 27.580 1 1 2 23 28.690 1 1 2 0
3.360 1 1 2 1 5.420 1 1 2 2 11.250 1 1 2 3 13.350 1 1 2 4 15.380 1 1 2 5 17.100 1 1 2 11 22.670 1
1 2 15 26.180 1 1 2 19 27.670 1 1 2 23 28.560 1 1 3 0 2.600 1 1 3 1 4.450 1 1 3 2 10.400 1 1 3 3
12.630 1 1 3 4 14.850 1 1 3 5 16.650 1 1 3 11 22.100 1 1 3 15 25.730 1 1 3 19 26.840 1 1 3 23
27.540 1 1 3 0 2.600 1 1 3 1 4.510 1 1 3 2 10.520 1 1 3 3 12.590 1 1 3 4 14.780 1 1 3 5 16.620 1 1
3 11 22.210 1 1 3 15 25.670 1 1 3 19 26.760 1 1 3 23 27.510 1 1 3 0 2.490 1 1 3 1 4.560 1 1 3 2
10.630 1 1 3 3 12.670 1 1 3 4 14.850 1 1 3 5 16.650 1 1 3 11 22.170 1 1 3 15 25.710 1 1 3 19
26.870 1 1 3 23 27.540 1 2 1 0 4.150 1 2 1 1 6.200 1 2 1 2 12.100 1 2 1 3 14.080 1 2 1 4 16.050 1
2 1 5 18.600 1 2 1 11 22.650 1 2 1 15 26.110 1 2 1 19 27.350 1 2 1 23 28.440 1 2 1 0 4.120 1 2 1
1 6.130 1 2 1 2 12.230 1 2 1 3 14.130 1 2 1 4 16.110 1 2 1 5 18.800 1 2 1 11 22.540 1 2 1 15
26.060 1 2 1 19 27.390 1 2 1 23 28.480 1 2 1 0 4.170 1 2 1 1 6.170 1 2 1 2 12.160 1 2 1 3 14.090
1 2 1 4 16.080 1 2 1 5 18.500 1 2 1 11 22.340 1 2 1 15 26.050 1 2 1 19 27.410 1 2 1 23 28.430 1 2
2 0 3.350 1 2 2 1 5.300 1 2 2 2 11.350 1 2 2 3 13.280 1 2 2 4 15.200 1 2 2 5 17.480 1 2 2 11
22.250 1 2 2 15 25.350 1 2 2 19 26.580 1 2 2 23 27.820 1 2 2 0 3.420 1 2 2 1 5.200 1 2 2 2 11.380
1 2 2 3 13.320 1 2 2 4 15.250 1 2 2 5 17.520 1 2 2 11 22.280 1 2 2 15 25.340 1 2 2 19 26.620 1 2
```

2 23 27.780 1 2 2 0 3.360 1 2 2 1 5.270 1 2 2 2 11.360 1 2 2 3 13.270 1 2 2 4 15.220 1 2 2 5 17.490 1 2 2 11 22.320 1 2 2 15 25.380 1 2 2 19 26.650 1 2 2 23 27.760 1 2 3 0 2.700 1 2 3 1 4.200 1 2 3 2 10.300 1 2 3 3 12.380 1 2 3 4 14.450 1 2 3 5 16.550 1 2 3 11 20.950 1 2 3 15 23.880 1 2 3 19 25.900 1 2 3 23 26.690 1 2 3 2 10.700 1 2 3 3 12.450 1 2 3 4 14.530 1 2 3 5 16.520 1 2 3 11 20.870 1 2 3 15 24.010 1 2 3 19 25.830 1 2 3 23 26.580 1 2 3 0 2.680 1 2 3 1 4.180 1 2 3 2 10.200 1 2 3 3 12.410 1 2 3 4 14.450 1 2 3 5 16.710 1 2 3 11 20.980 1 2 3 15 23.950 1 2 3 19 25.940 1 2 3 23 26.640 2 1 1 0 4.200 2 1 1 1 8.450 2 1 1 2 14.300 2 1 1 3 16.330 2 1 1 4 18.350 2 1 1 5 21.900 2 1 1 11 25.150 2 1 1 15 28.280 2 1 1 19 29.670 2 1 1 23 31.470 2 1 1 0 4.100 2 1 1 1 8.420 2 1 1 2 14.270 2 1 1 3 16.370 2 1 1 4 18.410 2 1 1 5 21.400 2 1 1 11 25.190 2 1 1 15 28.310 2 1 1 19 29.720 2 1 1 23 31.490 2 1 1 0 4.200 2 1 1 1 8.470 2 1 1 2 14.320 2 1 1 3 16.420 2 1 1 4 18.370 2 1 1 5 21.700 2 1 1 11 25.140 2 1 1 15 28.330 2 1 1 19 29.670 2 1 1 23 31.510 2 1 2 0 3.350 2 1 2 1 7.400 2 1 2 2 12.950 2 1 2 3 15.280 2 1 2 4 17.600 2 1 2 5 20.800 2 1 2 11 23.600 2 1 2 15 27.540 2 1 2 19 28.610 2 1 2 23 30.180 2 1 2 0 3.410 2 1 2 1 7.320 2 1 2 2 13.010 2 1 2 3 15.350 2 1 2 4 17.800 2 1 2 5 20.400 2 1 2 11 23.700 2 1 2 15 27.510 2 1 2 19 28.490 2 1 2 23 30.140 2 1 2 0 3.360 2 1 2 1 7.460 2 1 2 2 12.890 2 1 2 3 15.290 2 1 2 4 17.950 2 1 2 5 21.300 2 1 2 11 23.400 2 1 2 15 27.510 2 1 2 19 28.640 2 1 2 23 30.230 2 1 3 0 2.600 2 1 3 1 6.350 2 1 3 2 11.700 2 1 3 3 14.330 2 1 3 4 16.950 2 1 3 5 19.450 2 1 3 11 22.950 2 1 3 15 26.680 2 1 3 19 27.750 2 1 3 23 29.590 2 1 3 0 2.600 2 1 3 1 6.320 2 1 3 2 11.790 2 1 3 3 14.420 2 1 3 4 16.920 2 1 3 5 19.390 2 1 3 11 22.870 2 1 3 15 26.620 2 1 3 19 27.720 2 1 3 23 29.620 2 1 3 0 2.490 2 1 3 1 6.360 2 1 3 2 11.820 2 1 3 3 14.280 2 1 3 4 16.980 2 1 3 5 19.520 2 1 3 11 22.910 2 1 3 15 26.590 2 1 3 19 27.680 2 1 3 23 29.870 2 2 1 0 4.150 2 2 1 1 7.850 2 2 1 2 14.150 2 2 1 3 16.100 2 2 1 4 18.050 2 2 1 5 21.650 2 2 1 11 24.350 2 2 1 15 27.410 2 2 1 19 28.620 2 2 1 23 30.850 2 2 1 0 4.120 2 2 1 1 7.830 2 2 1 2 14.180 2 2 1 3 16.400 2 2 1 4 18.080 2 2 1 5 21.670 2 2 1 11 24.310 2 2 1 15 27.320 2 2 1 19 28.630 2 2 1 23 30.780 2 2 1 0 4.170 2 2 1 1 7.850 2 2 1 2 14.150 2 2 1 3 16.400 2 2 1 4 18.050 2 2 1 5 21.580 2 2 1 11 24.350 2 2 1 15 27.270 2 2 1 19 28.650 2 2 1 23 30.870 2 2 2 0 3.350 2 2 2 1 7.100 2 2 2 2 12.600 2 2 2 3 15.000 2 2 2 4 17.400 2 2 2 5 20.350 2 2 2 11 23.300 2 2 2 15 26.330 2 2 2 19 27.520 2 2 2 23 30.240 2 2 2 0 3.420 2 2 2 1 7.320 2 2 2 2 12.690 2 2 2 3 15.230 2 2 2 4 17.330 2 2 2 5 20.410 2 2 2 11 23.800 2 2 2 15 26.290 2 2 2 19 27.480 2 2 2 23 30.210 2 2 2 0 3.360 2 2 2 1 7.250 2 2 2 2 12.580 2 2 2 3 15.110 2 2 2 4 17.390 2 2 2 5 20.370 2 2 2 11 23.200 2 2 2 15 26.270 2 2 2 19 27.490 2 2 2 23 30.260 2 2 3 0 2.700 2 2 3 1 6.050 2 2 3 2 11.450 2 2 3 3 14.080 2 2 3 4 16.700 2 2 3 5 19.250 2 2 3 11 22.400 2 2 3 15 25.380 2 2 3 19 26.730 2 2 3 23 29.420 2 2 3 0 2.820 2 2 3 1 6.030 2 2 3 2 11.420 2 2 3 3 14.050 2 2 3 4 16.750 2 2 3 5 19.320 2 2 3 11 22.370 2 2 3 15 25.400 2 2 3 19 26.620 2 2 3 23 29.350 2 2 3 0 2.680 2 2 3 1 6.070 2 2 3 2 11.450 2 2 3 3 14.110 2 2 3 4 16.680 2 2 3 5 19.410 2 2 3 11 22.530 2 2 3 15 25.720 2 2 3 19 26.590 2 2 3 23 29.410

;

```
PROC GLM DATA=Oxidation;
TITLE 'Peroxide Value Result for Peanut Butter Bites';
CLASS temp pack anti time;
MODEL oxid = temp | pack | anti | time;
LSMEANS all / PDIFF ADJUST=TUKEY;
RUN;
```

A.1.2 SAS Code for TBARS Assay

```
ODS listing;
DM 'LOG; CLEAR; OUTPUT; CLEAR;';
Options Pageno=1 LS=100;
DATA Oxidation;
INPUT temp pack anti time oxid @@;
DATALINES;

/* Comment: temp (1 - Room temperature, 2 - Oven temperature); pack (1 – Plastic, 2 – Metallized packaging); anti (1 - 0ppmVitE, 2 - 200ppmVitE, 3 - 500ppmVitE), time (0, 1, 2, 3, 4, 5, 11, 15, 19, 23 - weeks); oxid (TBARS value or MDA concentration in ng/ml) */

1 1 1 0 0.0672 1 1 1 1 0.1716 1 1 1 2 0.2749 1 1 1 3 0.3291 1 1 1 4 0.3375 1 1 1 0 0.0688 1 1 1 1
0.1756 1 1 1 2 0.2814 1 1 1 3 0.3368 1 1 1 4 0.3454 1 1 1 0 0.0704 1 1 1 1 0.1796 1 1 1 2 0.2879
1 1 1 3 0.3445 1 1 1 4 0.3533 1 1 1 5 0.3691 1 1 1 11 0.4727 1 1 1 15 0.5416 1 1 1 19 0.5877 1 1
1 23 0.6337 1 1 1 5 0.3778 1 1 1 11 0.4838 1 1 1 15 0.5544 1 1 1 19 0.6015 1 1 1 23 0.6486 1 1 1
5 0.3865 1 1 1 11 0.4949 1 1 1 15 0.5672 1 1 1 19 0.6153 1 1 1 23 0.6635 1 1 2 0 0.0488 1 1 2 1
0.1121 1 1 2 2 0.2151 1 1 2 3 0.2461 1 1 2 4 0.2643 1 1 2 0 0.0499 1 1 2 1 0.1147 1 1 2 2 0.2202
1 1 2 3 0.2519 1 1 2 4 0.2705 1 1 2 0 0.0510 1 1 2 1 0.1173 1 1 2 2 0.2253 1 1 2 3 0.2577 1 1 2 4
0.2767 1 1 2 5 0.2929 1 1 2 11 0.4073 1 1 2 15 0.4836 1 1 2 19 0.5344 1 1 2 23 0.5852 1 1 2 5
0.2998 1 1 2 11 0.4169 1 1 2 15 0.4950 1 1 2 19 0.5470 1 1 2 23 0.5990 1 1 2 5 0.3067 1 1 2 11
0.4265 1 1 2 15 0.5064 1 1 2 19 0.5596 1 1 2 23 0.6128 1 1 3 0 0.0282 1 1 3 1 0.0607 1 1 3 2
0.1434 1 1 3 3 0.1932 1 1 3 4 0.2096 1 1 3 0 0.0289 1 1 3 1 0.0621 1 1 3 2 0.1468 1 1 3 3 0.1977
1 1 3 4 0.2145 1 1 3 0 0.0296 1 1 3 1 0.0635 1 1 3 2 0.1502 1 1 3 3 0.2022 1 1 3 4 0.2194 1 1 3 5
0.2364 1 1 3 11 0.3606 1 1 3 15 0.4435 1 1 3 19 0.4987 1 1 3 23 0.5539 1 1 3 5 0.2420 1 1 3 11
0.3691 1 1 3 15 0.4539 1 1 3 19 0.5104 1 1 3 23 0.5669 1 1 3 5 0.2476 1 1 3 11 0.3776 1 1 3 15
0.4643 1 1 3 19 0.5221 1 1 3 23 0.5799 1 2 1 0 0.0561 1 2 1 1 0.1650 1 2 1 2 0.2621 1 2 1 3
0.3143 1 2 1 4 0.3136 1 2 1 0 0.0574 1 2 1 1 0.1689 1 2 1 2 0.2683 1 2 1 3 0.3217 1 2 1 4 0.3210
1 2 1 0 0.0587 1 2 1 1 0.1728 1 2 1 2 0.2745 1 2 1 3 0.3291 1 2 1 4 0.3284 1 2 1 5 0.3503 1 2 1 11
0.4771 1 2 1 15 0.5615 1 2 1 19 0.6179 1 2 1 23 0.6741 1 2 1 5 0.3585 1 2 1 11 0.4883 1 2 1 15
0.5747 1 2 1 19 0.6324 1 2 1 23 0.6900 1 2 1 5 0.3667 1 2 1 11 0.4995 1 2 1 15 0.5879 1 2 1 19
0.6469 1 2 1 23 0.7059 1 2 2 0 0.0457 1 2 2 1 0.1100 1 2 2 2 0.1892 1 2 2 3 0.2317 1 2 2 4 0.2467
1 2 2 0 0.0468 1 2 2 1 0.1126 1 2 2 2 0.1937 1 2 2 3 0.2372 1 2 2 4 0.2525 1 2 2 0 0.0479 1 2 2 1
0.1152 1 2 2 2 0.1982 1 2 2 3 0.2427 1 2 2 4 0.2583 1 2 2 5 0.2782 1 2 2 11 0.4073 1 2 2 15
0.4934 1 2 2 19 0.5507 1 2 2 23 0.6081 1 2 2 5 0.2848 1 2 2 11 0.4169 1 2 2 15 0.5050 1 2 2 19
0.5637 1 2 2 23 0.6224 1 2 2 5 0.2914 1 2 2 11 0.4265 1 2 2 15 0.5166 1 2 2 19 0.5767 1 2 2 23
0.6367 1 2 3 0 0.0205 1 2 3 1 0.0541 1 2 3 2 0.0848 1 2 3 3 0.1797 1 2 3 4 0.1971 1 2 3 0 0.0210
1 2 3 1 0.0554 1 2 3 2 0.0868 1 2 3 3 0.1839 1 2 3 4 0.2017 1 2 3 0 0.0215 1 2 3 1 0.0567 1 2 3 2
0.0888 1 2 3 3 0.1881 1 2 3 4 0.2063 1 2 3 5 0.2139 1 2 3 11 0.3086 1 2 3 15 0.3718 1 2 3 19
0.4140 1 2 3 23 0.4561 1 2 3 5 0.2189 1 2 3 11 0.3159 1 2 3 15 0.3806 1 2 3 19 0.4237 1 2 3 23
0.4668 1 2 3 5 0.2239 1 2 3 11 0.3232 1 2 3 15 0.3894 1 2 3 19 0.4334 1 2 3 23 0.4775 2 1 1 0
0.0672 2 1 1 1 0.5331 2 1 1 2 0.5739 2 1 1 3 0.6480 2 1 1 4 0.6713 2 1 1 0 0.0688 2 1 1 1 0.5456
2 1 1 2 0.5874 2 1 1 3 0.6633 2 1 1 4 0.6871 2 1 1 0 0.0704 2 1 1 1 0.5581 2 1 1 2 0.6009 2 1 1 3
0.6786 2 1 1 4 0.7029 2 1 1 5 0.7517 2 1 1 11 0.8939 2 1 1 15 0.9886 2 1 1 19 1.0517 2 1 1 23
1.1150 2 1 1 5 0.7694 2 1 1 11 0.9149 2 1 1 15 1.0119 2 1 1 19 1.0765 2 1 1 23 1.1412 2 1 1 5
0.7871 2 1 1 11 0.9359 2 1 1 15 1.0352 2 1 1 19 1.1013 2 1 1 23 1.1674 2 1 2 0 0.0488 2 1 2 1
0.2983 2 1 2 2 0.3376 2 1 2 3 0.3990 2 1 2 4 0.4262 2 1 2 0 0.0499 2 1 2 1 0.3053 2 1 2 2 0.3455
2 1 2 3 0.4084 2 1 2 4 0.4362 2 1 2 0 0.0510 2 1 2 1 0.3123 2 1 2 2 0.3534 2 1 2 3 0.4178 2 1 2 4
0.4462 2 1 2 5 0.4821 2 1 2 11 0.6179 2 1 2 15 0.7082 2 1 2 19 0.7686 2 1 2 23 0.8289 2 1 2 5
```

0.4935 2 1 2 11 0.6324 2 1 2 15 0.7249 2 1 2 19 0.7867 2 1 2 23 0.8484 2 1 2 5 0.5049 2 1 2 11 0.6469 2 1 2 15 0.7416 2 1 2 19 0.8048 2 1 2 23 0.8679 2 1 3 0 0.0282 2 1 3 1 0.1785 2 1 3 2 0.2291 2 1 3 3 0.2878 2 1 3 4 0.3371 2 1 3 0 0.0289 2 1 3 1 0.1827 2 1 3 2 0.2345 2 1 3 3 0.2946 2 1 3 4 0.3450 2 1 3 0 0.0296 2 1 3 1 0.1869 2 1 3 2 0.2399 2 1 3 3 0.3014 2 1 3 4 0.3529 2 1 3 5 0.3959 2 1 3 11 0.5622 2 1 3 15 0.6731 2 1 3 19 0.7470 2 1 3 23 0.8209 2 1 3 5 0.4052 2 1 3 11 0.5754 2 1 3 15 0.6889 2 1 3 19 0.7646 2 1 3 23 0.8402 2 1 3 5 0.4145 2 1 3 11 0.5886 2 1 3 15 0.7047 2 1 3 19 0.7822 2 1 3 23 0.8595 2 2 1 0 0.0561 2 2 1 1 0.5233 2 2 1 2 0.5532 2 2 1 3 0.6372 2 2 1 4 0.6291 2 2 1 0 0.0574 2 2 1 1 0.5356 2 2 1 2 0.5662 2 2 1 3 0.6522 2 2 1 4 0.6439 2 2 1 0 0.0587 2 2 1 1 0.5479 2 2 1 2 0.5792 2 2 1 3 0.6672 2 2 1 4 0.6587 2 2 1 5 0.6947 2 2 1 11 0.8064 2 2 1 15 0.8810 2 2 1 19 0.9306 2 2 1 23 0.9802 2 2 1 5 0.7111 2 2 1 11 0.8254 2 2 1 15 0.9017 2 2 1 19 0.9525 2 2 1 23 1.0033 2 2 1 5 0.7275 2 2 1 11 0.8444 2 2 1 15 0.9224 2 2 1 19 0.9744 2 2 1 23 1.0264 2 2 2 0 0.0457 2 2 2 1 0.2800 2 2 2 2 0.3244 2 2 2 3 0.3896 2 2 2 4 0.4227 2 2 2 0 0.0468 2 2 2 1 0.2866 2 2 2 2 0.3320 2 2 2 3 0.3988 2 2 2 4 0.4326 2 2 2 0 0.0479 2 2 2 1 0.2932 2 2 2 2 0.3396 2 2 2 3 0.4080 2 2 2 4 0.4425 2 2 2 5 0.4834 2 2 2 11 0.6333 2 2 2 15 0.7332 2 2 2 19 0.7999 2 2 2 23 0.8665 2 2 2 5 0.4948 2 2 2 11 0.6482 2 2 2 15 0.7505 2 2 2 19 0.8187 2 2 2 23 0.8869 2 2 2 5 0.5062 2 2 2 11 0.6631 2 2 2 15 0.7678 2 2 2 19 0.8375 2 2 2 23 0.9073 2 2 3 0 0.0205 2 2 3 1 0.1654 2 2 3 2 0.3084 2 2 3 3 0.3524 2 2 3 4 0.3328 2 2 3 0 0.0210 2 2 3 1 0.1693 2 2 3 2 0.3157 2 2 3 3 0.3607 2 2 3 4 0.3406 2 2 3 0 0.0215 2 2 3 1 0.1732 2 2 3 2 0.3230 2 2 3 3 0.3690 2 2 3 4 0.3484 2 2 3 5 0.3968 2 2 3 11 0.5785 2 2 3 15 0.6996 2 2 3 19 0.7804 2 2 3 23 0.8612 2 2 3 5 0.4061 2 2 3 11 0.5921 2 2 3 15 0.7161 2 2 3 19 0.7988 2 2 3 23 0.8815 2 2 3 5 0.4154 2 2 3 11 0.6057 2 2 3 15 0.7326 2 2 3 19 0.8172 2 2 3 23 0.9018

;

```
PROC GLM DATA=Oxidation;
TITLE 'TBARS Result for Peanut Butter Bites';
CLASS temp pack anti time;
MODEL oxid = temp | pack | anti | time;
LSMEANS all / PDIFF ADJUST=TUKEY;
RUN;
```

A.2 Statistical Output – Peroxide value result

Table A.2.1. Class Distribution of Oxidation Parameters for Peroxide Value Result.

Class Level Information		
Class	Levels	Values
temp	2	1 2
pack	2	1 2
anti	3	1 2 3
time	10	0 1 2 3 4 5 11 15 19 23

Note: In temperature class (temp), 1 and 2 indicate room temperature and oven temperature respectively. In packaging class (pack), 1 and 2 indicate plastic and metallized packaging respectively. In antioxidant concentration class (anti), 1, 2 and 3 indicate 0ppm, 200ppm and 500ppm concentrations of vitamin E. In time period class (time), 0 to 23 indicate sample testing time periods in weeks.

Table A.2.2. ANOVA Table for Peroxide Value Result.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	119	26302.58924	221.03016	21741.9	<.0001
Error	240	2.43987	0.01017		
Corrected Total	359	26305.02911			

R-Square	Coeff Var	Root MSE	oxid Mean
0.999907	0.567098	0.100827	17.77950

Table A.2.3. 'Type 3 Tests of Fixed Effects' Table for Peroxide Value Result.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	1	232.38827	232.38827	22859.1	<.0001
pack	1	18.59587	18.59587	1829.20	<.0001
temp*pack	1	0.08962	0.08962	8.82	0.0033
anti	2	203.24218	101.62109	9996.06	<.0001
temp*anti	2	0.47965	0.23983	23.59	<.0001
pack*anti	2	0.46712	0.23356	22.97	<.0001
temp*pack*anti	2	0.23587	0.11794	11.60	<.0001
time	9	25767.01985	2863.00221	281622	<.0001
temp*time	9	55.53121	6.17013	606.93	<.0001
pack*time	9	15.05095	1.67233	164.50	<.0001
temp*pack*time	9	1.69432	0.18826	18.52	<.0001
anti*time	18	3.30622	0.18368	18.07	<.0001
temp*anti*time	18	2.06415	0.11468	11.28	<.0001
pack*anti*time	18	1.55100	0.08617	8.48	<.0001
temp*pack*anti*time	18	0.87294	0.04850	4.77	<.0001

The GLM Procedure
Least Squares Means
Adjustment for Multiple Comparisons: Tukey

Table A.2.4. Temperature (temp) LSMEANS Table for Peroxide Value Result.

		H0:LSMean1=LSMean2
temp	oxid LSMEAN	Pr > |t|
1	16.9760556	<.0001
2	18.5829444	

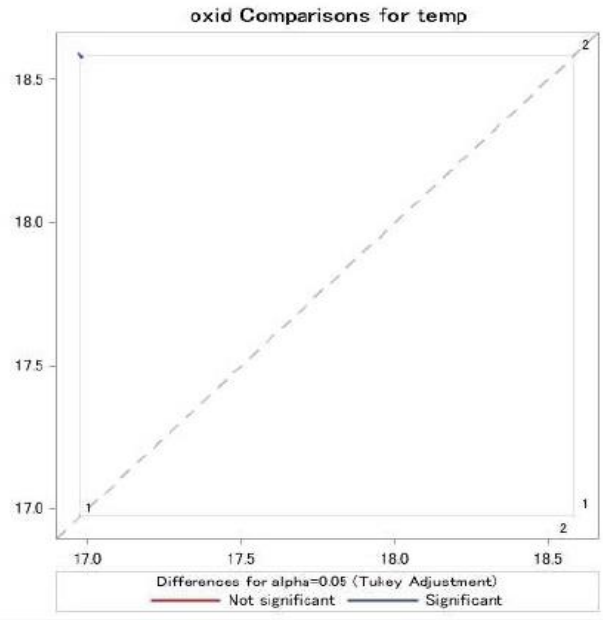

Figure A.2.1. Graph comparing mean peroxide values for temperature.

Table A.2.5. Packaging (pack) LSMEANS Table for Peroxide Value Result.

pack	oxid LSMEAN	H0:LSMean1=LSMean2 Pr > |t|
1	18.0067778	<.0001
2	17.5522222	

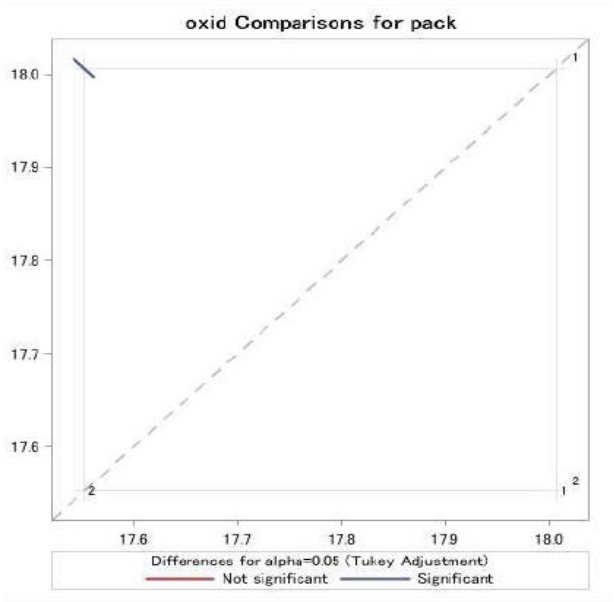

Figure A.2.2. Graph comparing mean peroxide values for packaging.

Table A.2.6. Antioxidant Concentration (anti) LSMEANS Table for Peroxide Value Result.

anti	oxid LSMEAN	LSMEAN Number
1	18.7105833	1
2	17.7574167	2
3	16.8705000	3

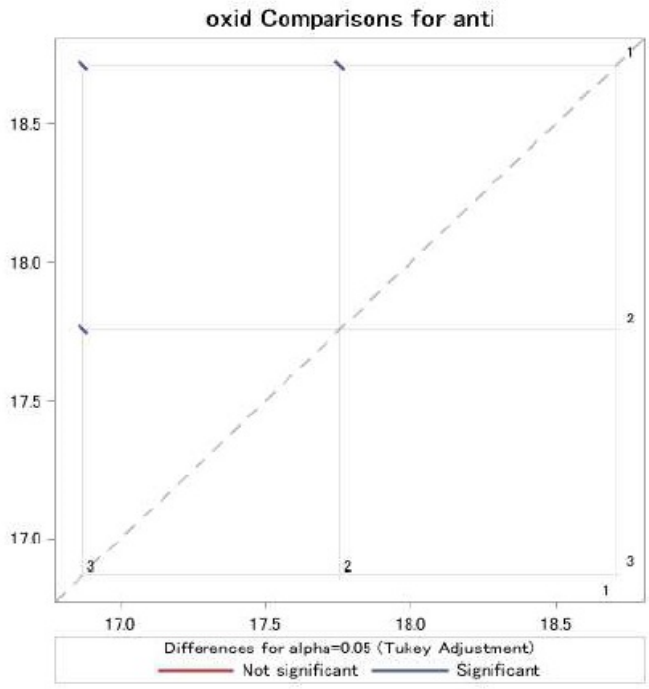

Figure A.2.3. Graph comparing mean peroxide values for antioxidant concentration.

Table A.2.7. Time Interval (time) LSMEANS Table for Peroxide Value Result.

time	oxid LSMEAN	LSMEAN Number
0	3.3933333	1
1	6.2622222	2
2	12.1152778	3
3	14.3008333	4
4	16.4597222	5
5	19.0488889	6
11	23.0205556	7
15	26.3605556	8
19	27.6436111	9
23	29.1900000	10

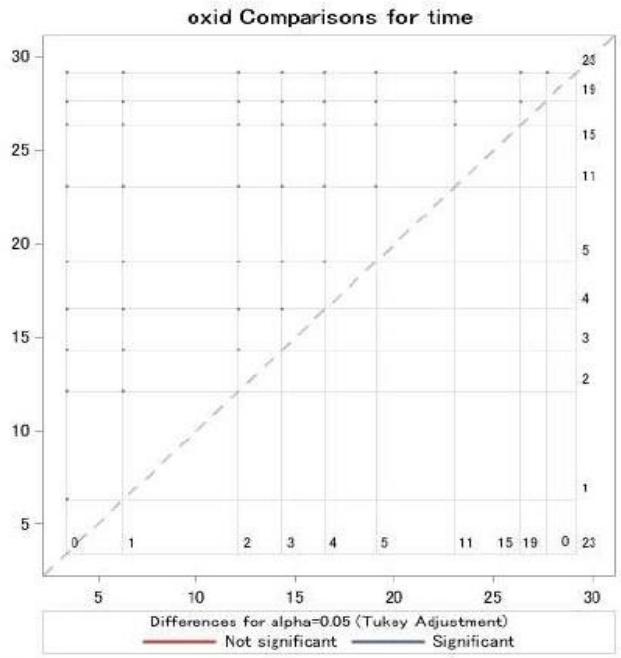

Figure A.2.4. Graph comparing mean peroxide values for time interval.

A.3 Statistical Output – TBARS result

TBARS Result for Peanut Butter Bites

The GLM Procedure

Table A.3.1. Class Distribution of Oxidation Parameters for TBARS Result.

Class Level Information		
Class	Levels	Values
temp	2	1 2
pack	2	1 2
anti	3	1 2 3
time	10	0 1 2 3 4 5 11 15 19 23

Note: In temperature class (temp), 1 and 2 indicate room temperature and oven temperature respectively. In packaging class (pack), 1 and 2 indicate plastic and metallized packaging respectively. In antioxidant concentration class (anti), 1, 2 and 3 indicate 0ppm, 200ppm and 500ppm concentrations of vitamin E. In time period class (time), 0 to 23 indicate sample testing time periods in weeks.

Table A.3.2. ANOVA Table for TBARS Result.

Dependent Variable: oxid

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	119	25.80464972	0.21684580	1545.69	<.0001
Error	240	0.03366980	0.00014029		
Corrected Total	359	25.83831952			

R-Square	Coeff Var	Root MSE	oxid Mean
0.998697	2.691552	0.011844	0.440060

Table A.3.3. 'Type 3 Tests of Fixed Effects' Table for TBARS Result.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	1	4.90112006	4.90112006	34935.4	<.0001
pack	1	0.01482250	0.01482250	105.66	<.0001
temp*pack	1	0.00055950	0.00055950	3.99	0.0470
anti	2	2.48403616	1.24201808	8853.17	<.0001
temp*anti	2	0.40442505	0.20221253	1441.38	<.0001
pack*anti	2	0.01602393	0.00801197	57.11	<.0001
temp*pack*anti	2	0.06392606	0.03196303	227.83	<.0001
time	9	16.78782944	1.86531438	13296.1	<.0001
temp*time	9	0.74258566	0.08250952	588.13	<.0001
pack*time	9	0.00310667	0.00034519	2.46	0.0107
temp*pack*time	9	0.00722481	0.00080276	5.72	<.0001
anti*time	18	0.21033384	0.01168521	83.29	<.0001
temp*anti*time	18	0.09855046	0.00547503	39.03	<.0001
pack*anti*time	18	0.01995268	0.00110848	7.90	<.0001
temp*pack*anti*time	18	0.05015289	0.00278627	19.86	<.0001

Table A.3.4. Temperature (temp) LSMEANS Table for TBARS Result.

temp	oxid LSMEAN	H0:LSMean1=LSMean2 Pr > |t|
1	0.32338000	<.0001
2	0.55674000	

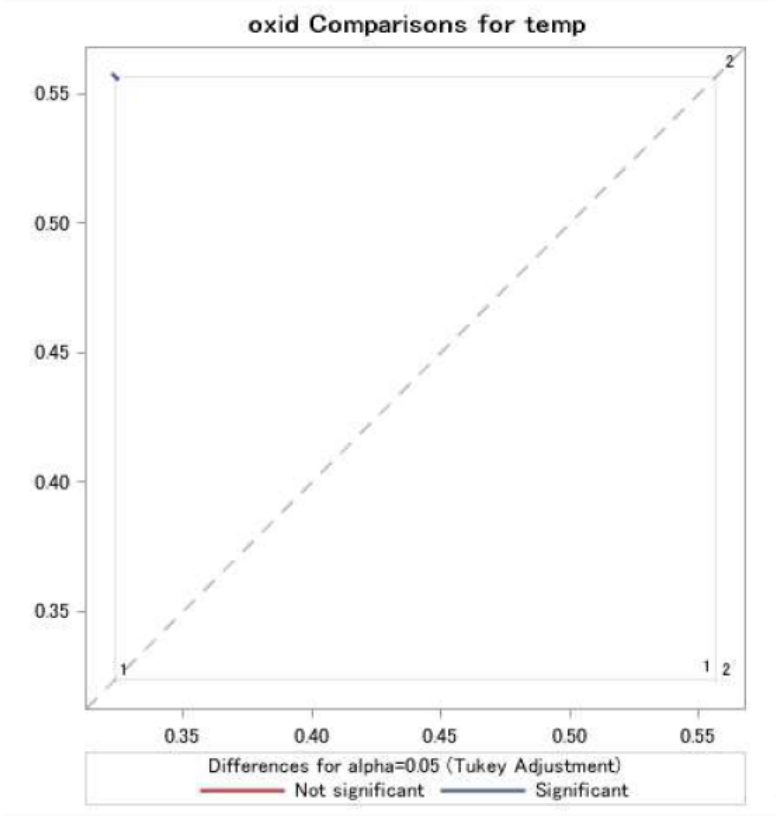

Figure A.3.1. Graph comparing mean TBARS values for temperature.

Table A.3.5. Packaging (pack) LSMEANS Table for TBARS Result.

pack	oxid LSMEAN	H0:LSMean1=LSMean2 Pr > |t|
1	0.44647667	<.0001
2	0.43364333	

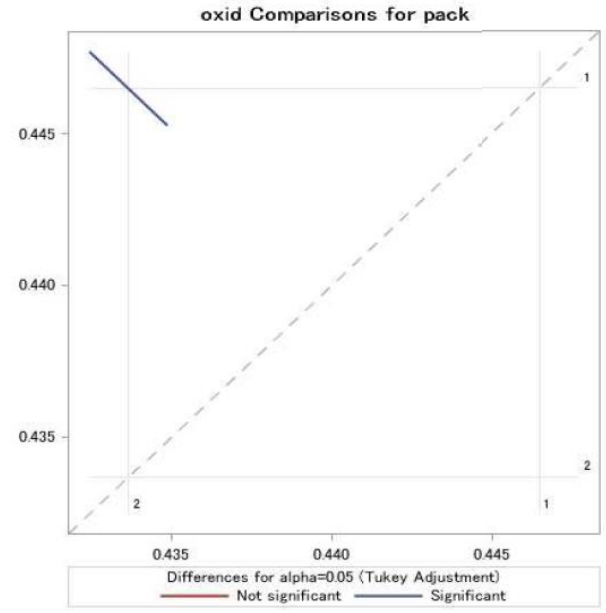

Figure A.3.2. Graph comparing mean TBARS values for packaging.

Table A.3.6 Antioxidant Concentration (anti) LSMEANS Table for TBARS Result.

anti	oxid LSMEAN	LSMEAN Number
1	0.55176750	1
2	0.41569000	2
3	0.35272250	3

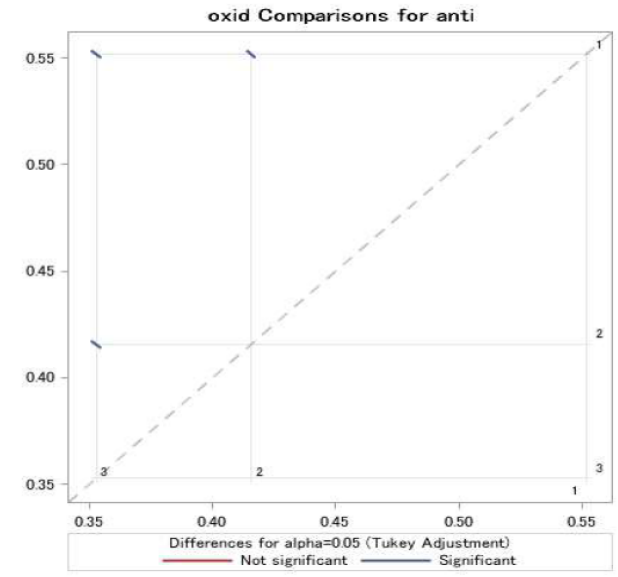

Figure A.3.3. Graph comparing mean TBARS values for antioxidant concentration.

Table A.3.7. Time Interval (time) LSMEANS Table for TBARS Result.

time	oxid LSMEAN	LSMEAN Number
0	0.04546667	1
1	0.22620000	2
2	0.29820833	3
3	0.35893333	4
4	0.37425000	5
5	0.42182500	6
11	0.55660833	7
15	0.64646667	8
19	0.70637500	9
23	0.76626667	10

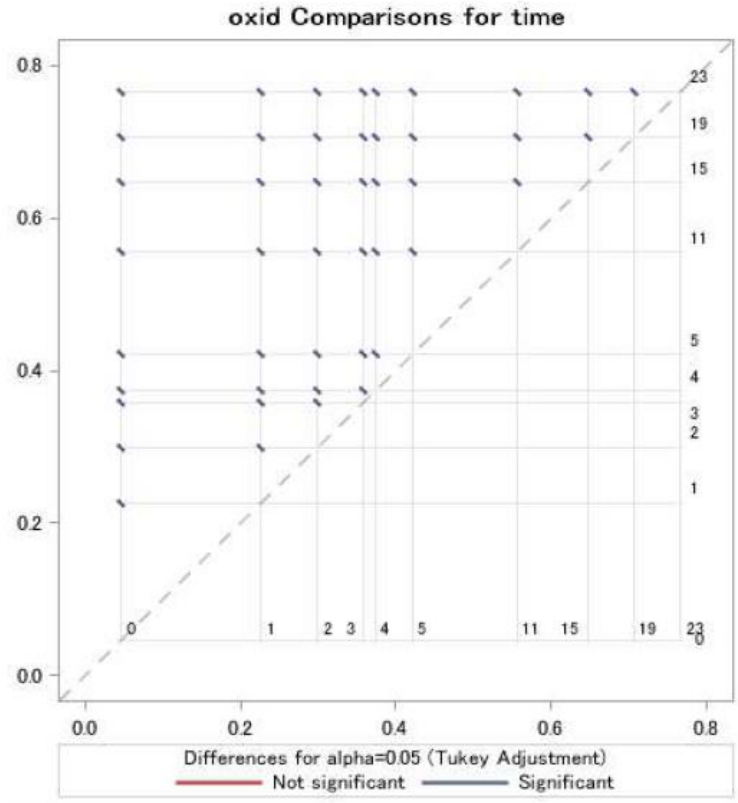

Figure A.3.4. Graph comparing mean TBARS values for time interval.

VITA

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