Author: **Bharvad, Mukesh**

Title: ***Effect of Dried Blueberry on the Oxidative Stability of Plumpy Nut Toddler Food Fortified with Omega-3 Fatty Acid***

The accompanying research report is submitted to the **University of Wisconsin-Stout**, **Graduate School** in partial completion of the requirements for the

Graduate Degree/ Major: MS Food & Nutritional Sciences

Research Adviser: Hans Zoerb, Ph.D.

Submission Term/Year: Spring, 2013

Number of Pages: 44

Style Manual Used: American Psychological Association, 6th edition

☒ **I understand that this research report must be officially approved by the Graduate School and that an electronic copy of the approved version will be made available through the University Library website**

☒ **I attest that the research report is my original work (that any copyrightable materials have been used with the permission of the original authors), and as such, it is automatically protected by the laws, rules, and regulations of the U.S. Copyright Office.**

☒ **My research adviser has approved the content and quality of this paper.**

STUDENT:

NAME Mukesh Bharvad **DATE: 01/07/13**

ADVISER: (Committee Chair if MS Plan A or EdS Thesis or Field Project/Problem):

NAME Dr. Hans Zoerb **DATE: 01/07/13**

This section for MS Plan A Thesis or EdS Thesis/Field Project papers only
Committee members (other than your adviser who is listed in the section above)

1. **CMTE MEMBER'S NAME: DATE:**

2. **CMTE MEMBER'S NAME: DATE:**

3. **CMTE MEMBER'S NAME: DATE:**

This section to be completed by the Graduate School

This final research report has been approved by the Graduate School.

Director, Office of Graduate Studies: **DATE:**

Bharvad, M. ***Effect of Dried Blueberry on the Oxidative Stability of Plumpy Nut Toddler Food Fortified with Omega-3 Fatty Acid***

Abstract

The purpose of the study was to discover the effect of dried blueberry on the oxidative stability of Plumpy Nut toddler food fortified with omega-3 fatty acid**.** For sensory analysis, various attributes like peanut flavor, texture, off-flavor, and overall ranking were tested in three samples: a) Peanut butter with no addition of antioxidant (control sample); b) Peanut butter with only omega-3 fatty acids; and c) Peanut butter with omega-3 fatty acids and dried blueberry**.** Statistical analysis of the results indicated that there was a significant difference ($p<.0001$) in off-flavor of samples with omega-3 fatty acid from 0 to 8 days; while in control samples there was no statistically significant difference. Also, samples that contained dried blueberry and omega-3 fatty acid had more off-flavor intensity than control samples and less off-flavor intensity compared to samples containing omega-3 fatty acid. The texture of both the control samples and the samples with omega-3 fatty acid increased, while in samples with dried blueberry and omega-3 fatty acids, texture decreased. Peanut flavor intensity in the control samples increased, while in the other two samples it decreased. Finally, Plumpy Nut with no additives was highly preferred over the Plumpy Nut with omega-3 fatty acid and Plumpy Nut with dried blueberry and omega-3 fatty acid in terms of overall ranking.

Acknowledgments

I sincerely thank Dr. Hans Zoerb, my research advisor, for his professional advice, moral support, and limitless time. Without his help, it would have not been possible for me to complete my research study. His immense knowledge, experience, and encouragement was very helpful. I am greatly appreciative and indebted to Dr. Carolyn Barnhart for her great support, encouragement, and guidance during my entire graduate school process, and my research. I would also like to thank Dr. Carol Seaborn and Gladys Earl for all the timely help and support they provided during my memorable days at UW-Stout. I also wish to express my personal appreciation and gratitude to Connie Galep, who was of tremendous help in conducting my experiments. I also thank Vicki Weber for her support and help. Last but not least, I would also like to thank my family and friends for their unconditional love, advice, and support.

Table of Contents

List of Tables

List of Figures

Page

Chapter I: Introduction

Plumpy Nut, a food product that is based on peanut butter, was developed by the French company, Nutriset, to resolve severe malnutrition among the children of undeveloped countries such as Sudan, Darfur, and Niger (Thurow, 2005). Modeled after Nutella spread, Plumpy Nut was formulated by Andre Brined in 1999. The main ingredients in this product are peanut butter, vegetable oil, non-fat dried milk powder, sugar, vitamins, and minerals. Plumpy Nut contains a balance of lipids, carbohydrates, protein (macronutrients), minerals (calcium, phosphorus, potassium, magnesium, zinc, iron, iodine, sodium, and selenium) and vitamins (micronutrients) that help to promote normal growth in severely malnourished children. It is slightly sweeter than peanut butter (Hollis, 2010) and is recognized or categorized as Ready to Use Therapeutic Food (RUTF). Plumpy Nut is easy to use and easy to make product (Linneman et al., 2007). Water is not added during preparation of Plumpy Nut so it resists microbial growth and, therefore, it is stable at ambient temperatures for up to two years (Enserink, 2008a). Each prepared Plumpy Nut pack provides 500 kcal or 2.1 M J (Bhandari & Tiplady, 2005). A nutritionally inspired and designed product, Plumpy Nut has been adopted by "Doctors Without Borders" as part of their nutritional program in developing countries (*Peanut paste treats malnutrition*, 2008).

Breast milk is a healthy food or diet for newborn babies. It provides all the nutrients and energy that are required during the first six months of life to regulate basal metabolism and fulfill energy demands for growth and development (Krebs, 2007). Alone, breast milk fulfills the nutritional demands during the beginning of active growth when a newborn can double its weight in three months. However, weaning or complementary foods are required to meet toddlers' nutritional demands during the post infant phase of growth (Onyango, Receveur, & Esrey, 2002).

The term ‘toddler’ is specifically used for two- to three year-old children. These children begin to walk, blend with other children at or in pre-school and become more independent. This period is crucial, not just for the toddler’s health, but is also the phase of continuity that can affect growth and development later in life. It is also a time of nervousness, sleeplessness, teething, tantrums, and accidents. During this period, nutritional requirements begin to change because toddlers start to consume a different variety of foods, develop different tastes, and build a cultural as well as sensory connection with the food they eat (Weaver, More, & Harris, 2008).

Toddlers’ Nutritional Requirements

Toddlers’ nutritional requirements are distinct from those of adults and older children. Toddlers grow rapidly compared to adults, so they need more calories and nutrients (w/w) to sustain their growth. A toddler’s energy requirement is approximately 95kcal/kg bodyweight, about one-third higher than that of an adult (approximately 30-35 kcal/kg bodyweight). Conversely, a toddler’s gastric capacity is about one-third lower than that of an adult. To meet their energy and nutrient requirements, toddlers must consume more calories per kilogram of body weight than adults in order to balance their needs.

To meet their nutritional requirements, toddlers must consume three balanced meals and two to three energy-rich snacks (Weaver et al., 2008). Recommendations for toddlers are based on blending of some ‘low energy’ and some ‘high energy’ foods (Fox, Pac, Devaney, & Jankowski, 2004). Toddlers’ diets should not be restricted to predetermined serving sizes. Instead they should be allowed to eat based on their appetites. The proportions they eat will differ from toddler to toddler, meal to meal, and day to day. Toddlers get hungry based on their energy needs satiety, so their bodies can manage their appetites to meet their nutritional needs. (Briefel, Reidy, Karwe, Jankowski, & Hendricks, 2004).

Battling Malnutrition

In Africa, food security is critical issue, and in West Africa child malnutrition is the highest in the world. Children suffer from malnutrition because of increased cost of food on the global market and because of shortages from local crop production (Simms, 2010). Food commodities are often inconsistent in many developing countries. One health volunteer in Aouloumat, a rural region of Niger, reported that malnutrition is caused not by a lack of diversity in food or vitamin deficiencies, rather availability and reliability of basic food stuffs. If cereals such as millet and rice are in short supply and demand is high, the cost increases and people eat less expensive or more available alternatives. Poorly balanced meals using adulteration or non-nutritive fillers in foods is often the result. Basic grain supplies are limited or unavailable during the dry season or pre-harvest period. Most of the vitamin and protein rich foods are unavailable. Even when inexpensive and readily available food is present, general consumption of grains, proteins and fruits or vegetables can be limited (Bruegel, 2011). In addition, while adults can digest and survive for short periods on diets that are deficient in certain nutritional aspects, toddlers and children of developmental age are very susceptible to dietary deficiencies. Nutritional deficits leading to retarded physical and neural development at a young age can have lifelong consequences.

Importing food to address food shortages is not a long term solution to malnutrition, and many non-governmental organizations are involved in attempts to alleviate malnutrition in developing countries by developing consistent, secure sources of local food. Plumpy Nut is an example. People in rural areas obtain supplements like Plumpy Nut from clinics and specific distribution sites, and they use it as a medicine (Dyer, 2010). Local production and distribution of similar foods as a staple rather than a "treatment" would be far better option. In Niger, one out of five children dies before the age of five due to malnutrition (Ghosh, 2009; Ghosh, 2009). However, a new study revealed that malnutrition rates among children in Niger have significantly improved as a result of intervention by non-governmental agencies (Hampshire, Casiday, Kilpatrick, & Panter-Brick, 2009).

UNICEF and its co-partners have treated an estimated 382,400 malnourished children since 2006, with thousands of lives saved by Plumpy Nut in the past decade (Hampshire, Casiday, Kilpatrick, & Panter-Brick, 2009). Plumpy Nut and similar supplements are effective because they contain high levels of easily digested protein, fat and carbohydrates. Because it uses peanut butter as a base into which sugar and protein powders are dispersed, it contains little water and is stable at ambient temperatures for long periods of time. “Plumpy Nut is known as the magical product,” says Regional Humanitarian Advisor Jan Eijkenaar of the European Commission’s Humanitarian Aid Department (ECHO). “It helps children recover very quickly when they suffer from malnourishment (POLGREEN, 2008).

Statement of the Problem

Plumpy Nut is a peanut-based product that is used as part of a strategy to minimize or erradicate malnutrition in toddlers in developing countries. One improvement to Plumpy Nut-type products is addition of omega-3 fatty acids to contibute to neural development in toddlers aged two to five years old. However, Omega-3 fatty acids are highly susceptible to oxidation because of the polyunsaturated fatty acids EPA and DHA. Lipid oxidation results in offensive flavors and as a result highly unsaturated fats like omega-3 fatty acids cannot be added to foods with extended shelf life without protection from antioxidants (Min, 1998). Natural antioxidants such as those found in dried berries might retard lipid oxidation in omega-3 fortified products. “The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Antioxidant compounds in like phenolic acids, polyphenols and flavonoids would inhibit the oxidative mechanisms that lead to degenerative diseases" (Prakash, Rigelhof & Miller, n.d., p. 207). Blueberries can be used as a model to determine if natural antioxidants can prevent oxidation of omega-3 fatty acid.in peanut butter based suppliments.

Purpose of the Study

The objective of this research is to improve stability of omega-3 fatty acid in fortified peanut butter based supplements through the addition of dried blueberry paste. The goals of the project are:

1. To evaluate the stability of added omega-3 fatty acid in a Plumpy-Nut like product.
2. To determine if the addition of natural antioxidants in the form of dried blueberries can retard the rate of oxidation of omega-3 fatty acids.

Limitations of the Study

The limitation of the study was based on the subjective analysis of untrained panelists. Therefore the results cannot be a global representation of a population. Because the age categories of the test population fell within the age range of 18-25 years old, this has also limited the results of certain populations.

Chapter II: Review of Literature

Omega-3 Polyunsaturated Fatty Acids (PUFA)

The term omega-3 fatty acid has its origins in the Greek word *Omega* referring to the end; in this case the end of the fatty acid chain. *Omega* identifies the methyl terminus of the chain (as opposed to the carboxylic acid terminus). In the *omega* nomenclature, the methyl group is number 1 and the double bond closest to the methyl end is numbered by the number of carbons from that end. Thus, for omega-3 fatty acids, the first unsaturated carbon bond occurs at the third carbon from the methyl end. Likewise, for omega -6 and -9 fatty acids, the first unsaturated carbon bond occurs at the sixth and ninth carbon, respectively, from the methyl end (Enserink, 2008b). There are three nutritionally important omega-3 fatty acids: alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Weers & Gulati, 1997). Salmon, flax seeds and walnuts are excellent sources of these omega-3 fatty acids.

Alpha-linolenic acid and linoleic acid are classified as "essential" because the human body cannot synthesize them in sufficient quantities. These fatty acids play a significant role in many physiological functions related to inflammatory response. As a result, humans require sufficient amount sufficient amounts of both alpha-linolenic acid and linoleic acid in their diets (Valentine, 2010).

Alpha-linolenic acid can be found in flaxseeds, walnuts, hemp seeds, soybeans and some dark green, leafy vegetables. Dietary sources of linoleic acid include corn oil, safflower oil, sunflower oil, and canola oil. Most people eat higher amounts of linoleic acid than alpha-linolenic acid (Kreiter, 2008).

The human body converts alpha-linolenic acid, via desaturation and elongation, into two important omega-3 fatty acids: eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA)

(Curtis et al., 2004; Arkhipenko & Sazontova, 1995). These fatty acids can be found in cold-water fish including salmon, tuna, halibut and herring. Eicosapentaenoic acid plays an important role in preventing cardiovascular disease, while DHA aids in proper brain and nerve development.

Important Role of Omega-3 Fatty Acid in Health Benefits

Cardiovascular benefits. The advantage to consuming omega-3 polyunsaturated fatty acids (omega-3 PUFAs) as a mitigator of cardiovascular disease was described by Stark, Crawford and Reifen (2008). The relationship between omega-3 fatty acids and cardiovascular disease was supported by the inverse correlation between the quantity of omega-3 fatty acids in in the diet and the blood with the development of coronary heart disease (Abeywardena & Patten, 2011). Numerous trials in animals, humans, and in tissue culture connect dietary omega-3 fatty acids with the prevention of heart disease by restricting combination of cytokines and mitogens that increase the inflammation and plaque formation (Patel, Tracey, Hughes & Lip, 2010; Gogus & Smith, 2010). Moreover, cytokins and mitogens activate nitric oxide, which assist endothelial repair, and lower plasma lipids (mainly triacylglycerols) and VLDL (very low density lipid) (Leaf, 2006).

Benefits for the brain and eyes. Omega-3 PUFAs play an essential role in development and function of the brain and nervous system (Abeywardena & Patten, 2011). Brain tissue is rich in DHA, suggesting a correlation between dietary omega-3 fatty acids and their deposition in neural membranes. Docosahexaenoic acid participates in the development of brain and formation of the retina during pregnancy (Zivkovic, Telis, German, & Hammock, 2011). Therefore, it is important that pregnant mothers consume diets rich in omega-3 fatty acids (Cole, Qiu-Lan, & MaFrautschy, 2010). The importance of DHA accumulation and brain development remain throughout the first two years of life (Cole, Qiu-Lan, & MaFrautschy, 2010; Dunstan, 2007). The brain of a typical child is comprised of more than 20 g of DHA (DeMar, Ma, Bell, & Rapoport, 2004). Docosahexanoic acid is also important in the diet of adults to support normal brain function and to help to improve learning ability. Maintaining sufficient amounts of omega-3 fatty acids is likely to favor improved cognitive, learning and memory functions (B, 2009).

Effects on cancer. There are many reports on the favorable effects of omega-3 fatty acids on development of certain cancers (Seti, Leikin-Frenkel & Werner, 2009; Sun et al., 2011). It is clear that some effects of PUFAs make them an alternative in prevention and treatment of some cancers (Jiang et al., 1998). Polyunsaturated fatty acids have been reported to:

- Change cell membrane phospholipids,
- Reform cellular functions which may diminish tumor motile/invasive potential,
- Inhibit tumor cells
- Help change the reactivity of tumor cells to chemotherapeutic agents and radiation,
- Play an important role in protecting normal tissue from radiation

Many researchers have reported that omega-3 fatty acids play a significant role in prevention of colon (Valenzuela et al., 2010), breast (Seti, Leikin-Frenkel, & Werner, 2009), renal (Sun et al., 2011), prostate, pancreatic cell and liver cancers (Tayyebi-Khosroshahi et al., 2010).

Omega-3 fatty acids and human immune and inflammatory responses. Berquin et al. (2007) summarized the effects of omega-3 PUFA on human immune and inflammatory responses. Different physiological functions are affected by several eicosanoids, which act as chemical messengers in the immune system (Iwami, Nonomura, Shirasugi, & Niimi, 2011). Leucotrienes, prostaglandins, thromboxanes are derived from long chain PUFAs and play significant roles in regulating inflammation, cytokine discharge, immune reactions, vascular reactivity and allergic development (Uauy & Valenzuela, 2000). In biological membranes the balance between arachidonic acid (AA) an omega-6 fatty acid and EPA, an omega-3 fatty acid can be managed by dietary supplementation.

Omega-3 fatty acids play an important role in the production of powerful, hormone-like substances called prostaglandins. Prostaglandins help regulate blood pressure, blood clotting, nerve transmission, inflammatory and allergic responses, the functions of the kidneys and gastrointestinal tract, and the production of other hormones (Wall, Fitzgerald & Stanton, 2010).

Recommended intake of DHA and EPA. Most consumers pay little attention to the recommended daily intake of essential nutrients like omega-3 fatty acids. Guidelines for daily intake of omega-3 fatty acids vary by gender and the agency making the recommendations (Kroes et al., 2003). Health and Welfare Canada advise a daily level of omega-3 fatty acids from 1.0-1.8 g /day. The distinction between the individual omega-3 PUFAs was not recognized (Roman, Schreher, MacKenzie, & Nathanielsz, 2006). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommened a combined daily usage of DHA and EPA at 0.22 g/day. The British Nutrition Foundation (BNF) advised a combined daily intake of DHA and EPA at 1.1 g/day for adult females and 1.4 g/day for adult males. For infants, the United States Institute of Medicine (IOM) published a recommended daily intake of omega-3 fatty acids, including DHA, of 0.5 g/day. Consumption of EPA and DHA up to 3g/day is safe as declared by The United States Food and Drug Administration (USFDA).

Deterioration in Quality of Omega-3 Fatty Acid During Storage

Oxidative deterioration of omega-3 fatty acids. Unsaturated, especially polyunsaturated oils, can oxidize and form unpleasant tasting by-products. Lipid oxidation leads to decomposition of fatty acids in a three-step process that result in the formation of aromatic ketones and aldehydes. The steps are as follows:

(1) **Initiation.** A hydrogen is abstracted from methylene carbon located between the double bonds in the 1,4 pentadiene complex in the polyunsaturated fatty acid chain, leaving an alkyl radical(L·) on the methylene carbon (Figure 1). The radical can delocalize along the chain resulting in a conversion of the 1,4 pentadiene to conjugated double bonds along the chain. The formation of radicals during lipid oxidation in the initiation step occurs at a higher rate for polyunsaturated than monounsaturated fatty acids because of the higher number of double bonds in the chain. The carbon-hydrogen bond is weaker on the methylene carbon compared to adjacent carbons making the hydrogen easier to abstract (Damodaran, Parkin, & Fennema, 2007).

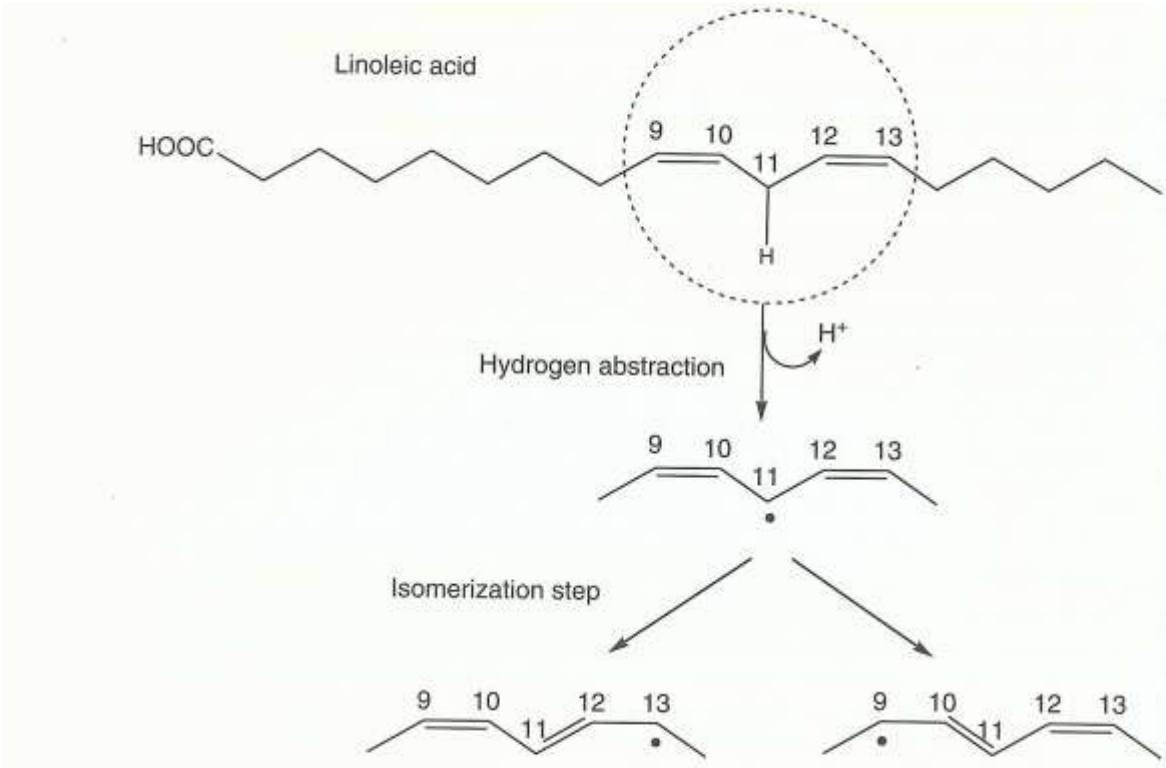

Figure 1. Lipid oxidation for linoleic acid in the initiation step (from Damodaran, Parkin, & Fennema, 2007).

(2) **Propagation**. Oxygen is involved in the propagation step of lipid oxidation (Figure 2). The alkyl radical formed on the fatty acid in first step can react with triplet oxygen (atmospheric oxygen) in this step to form a peroxyl radical (LOO˙). The peroxyl radical can abstract a hydrogen from another fatty acid to form a fatty acid hydroperoxide (LOOH) plus a second alkyl radical on the second fatty acid (Damodaran et al, 2007)

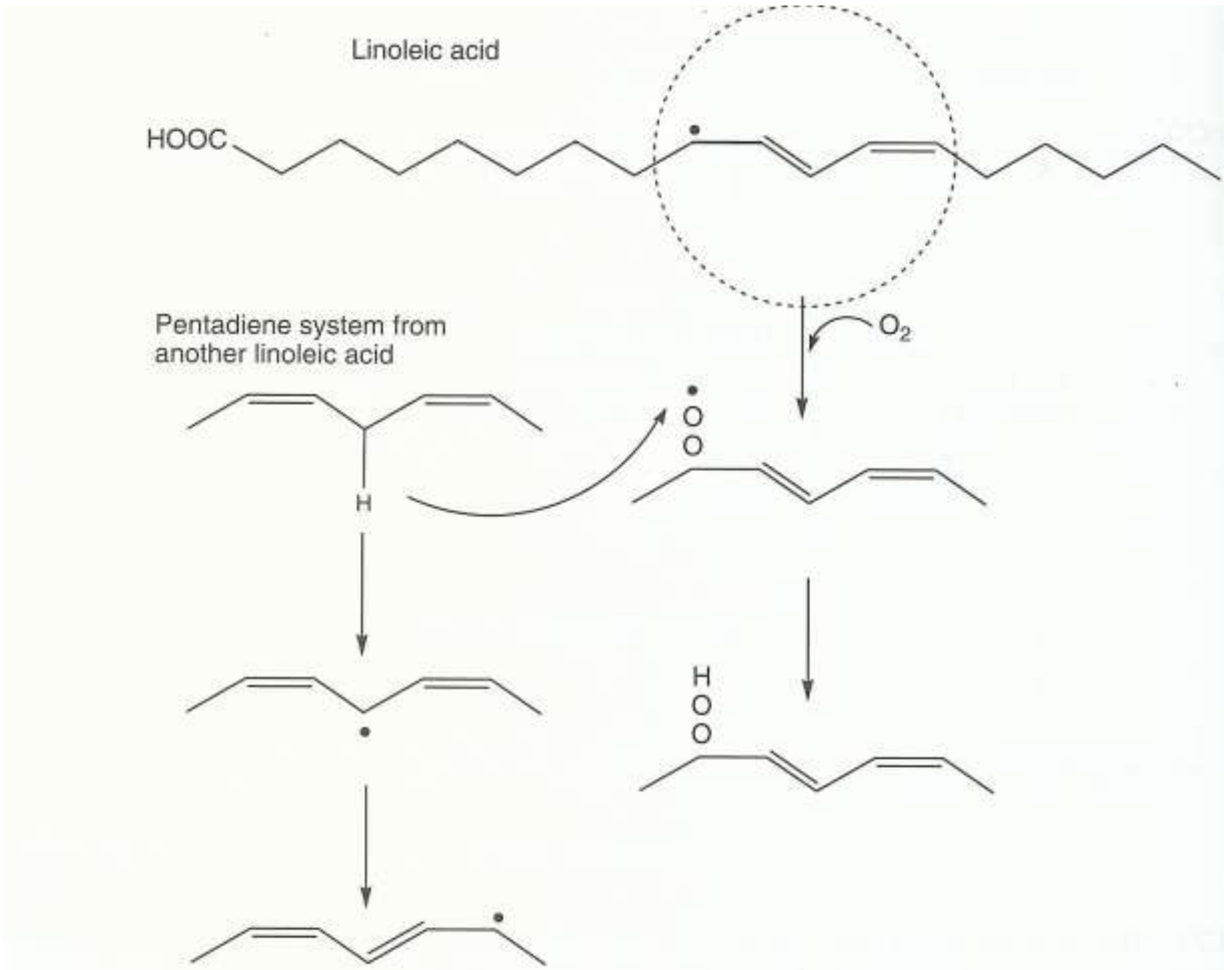

*Figure 2.*_Lipid oxidation for linoleic acid in the propagation step (after Damodaran, Parkin, & Fennema, 2007).

(3) **Termination.** This step can terminate lipid oxidation when radicals on two separate unsaturated fatty acids react to form nonradical, polymerized species. The more unsaturated the oil, the faster it will oxidize because there are more "methylene hydrogens available for abstraction from the fatty acid" (Damodaran et al, 2007).

An alternate series of reactions leading off flavovrs in the oil can also occur prior to termination if the conjugated hydroperoxyl undergoes a β-scission reaction on either side of the hydroperoxyl. This reaction leads to the formation of a variety volatile ketones and aldehydes responsible for the familiar off-flavors and odors associated with oxidized lipids.

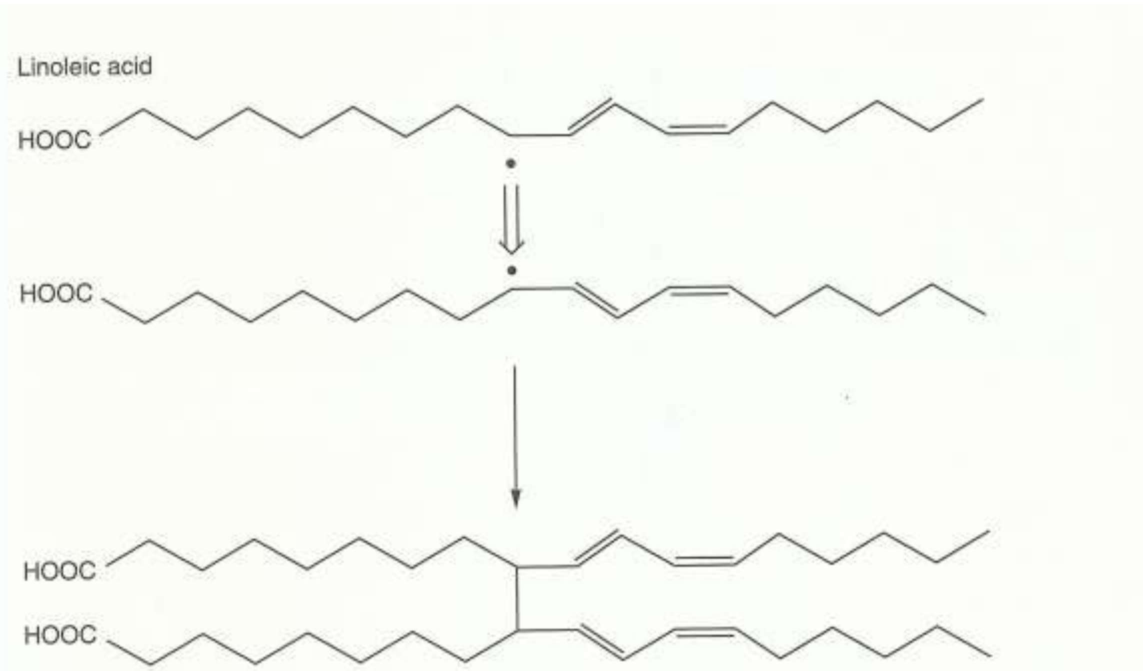

Figure 3. An example of lipid oxidation in the termination step under conditions of low oxygen concentrations (Damodaran, Parkin, & Fennema, 2007).

The amount of peroxides or the amount of ketones and aldehydes present in the oil represents the degree to which the oil has been oxidized. Ketones and aldehydes resulting from β-scission occur after peroxide formation and are the volatile chemicals responsible for the unpleasant flavors. Peroxides formed early in the oxidation reaction are transient and do not present off-flavor. Recognized methods for determining oxidative stability of oils are the Schaal Oven test or the Active Oxygen Method (AOM) (Tan, Man, Selamat, &Yusoff, 2002).

Health Hazard of Oxidized Lipids

Lipid oxidation not only results in off-flavor or rancid odors, but it also reduces the nutritional quality of food products. Many ketones, aldehydes and other secondary reaction products of lipid oxidation are considered a health hazards because they are associated with heart disease, cancer, membrane damage, and aging conditions (Alamed, McClements, & Decker, 2006). Lipid oxidation can produce compounds such as acrolein, 4-hydroxyl-2 nonenal, and malonaldehyde. These compounds are connected to diseases related to aging, cataracts, and atherosclerosis (Nwanguma et al.,1999).

A striking example of the health hazards associated with oxidized lipids occurred in Japan, when oxidized fat and oil caused food poisoning in people who ate instant noodles (Gotoh et al., 2005). While there were no reported deaths from these incidents, many people suffered nausea, abdominal pain, fatigue, diarrhea, and headache. The level of lipid oxidation in degraded noodles that produced food poisoning was approximately 101.5 meq/kg peroxide (PV) (Gotoh et al., 2005).

Level of oxidation in lipids can be determined by measuring induction periods, amount of peroxide formed (peroxide value), or the amount of ketones formed (thiobarbeturic acid reactive substances –Tbars). Analytical methods can detect oxidation prior to the start of off-flavor and they can evaluate the oxidative stability of oil. The initial phase of lipid oxidation is called the induction period. Oxidation proceeds slowly and at a uniform rate during the induction period. After the induction period, oxidation of lipids rapidly leads to a deterioration of oils and formation of polymers and volatile compounds (Moller & Loft, 2010).

Utilization of Antioxidants

Mechanism of antioxidant action. An antioxidant may be defined as a substance which inhibits the rate of the reaction oxidation (Min, 1998). Antioxidants act by scavenging free radicals in "resonance stabilized" aromatic compounds like butylated hydroxy toluene (BHT) or butylated hydroxy anisole(BHA). They are particlulary effective in the initiation and early propogation phase of lipid oxidation. Antioxidant reacts with free radicals to slow the process by donating a proton and then distributing the resulting free radical within the aromatic ring structure. The resultant "antioxidant radical" is much less reactive and retards subsequent oxidative reactions (Stansby, 1967; Cadenas & Packer, 1996).

Utilization of natural antioxidants. Usage of synthetic antioxidants has been limited due to their suitability in oils and fats and by restrictions in food applications. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and teriary-butyl hydroquinone (TBHQ) have been effectively used as synthetic antioxidants in many oxygen sensitive food products (Suja et al., 2004). However, these compounds may be linked to health issues and for this reason Japan, Europe, and Canada have not allowed their use for food applications (Iqbal & Bhanger , 2005). Considering safe alternatives, there is a trend among food scientists to use natural antioxidants rather than synthetic counterparts as safer antioxidants (Iqbal & Bhanger 2005).

Major Natural Antioxidants

The most widely used natural antioxidants are tocopherols, citric acid, and ascorbic acid. Spice extracts and anthocyanin rich berries (cherry, blackberry, strawberry, raspberry, crowberry, blueberry, bilberry/wild blueberry, black currant) have been evaluated as antioxidants in food systems (Sowndhararajan, Rajendrakumaran, & Manian, 2010). Anthocyanins and other phenolic compounds found in these plants are potentially powerful antioxidants, but they have not been fully examined in whole food systems.

As antioxidants, polyphenolic compounds protect cells from free radicals. These polyphenol compounds, including anthocyanins, produce the rich blue color to blueberries. The amount of polyphenoles in blueberries is higher than that of other fruits. Blueberries protect cells from damage or degenerative diseases, which are caused by oxygen free radicals during respiration. Low-bush and wild blueberries have the ability to diminish free radical production in red blood cells, which circulate throughout the body, and high-bush or cultivated blueberries inhibit oxidation (Larson, 1997).

Effectiveness of food or biological antioxidants is measured by the Oxygen Radical Absorbance Capacity (ORAC Test), the ability of a biological substance to inhibit the oxidative reduction of a fluorescent molecule usually fluorescine dye. Blueberries typically rank very high in the ORAC test meaning they are capable of inhibiting free radical oxidation of sensitive molecules (Giacalone et al., 2011). Like all phenolic antioxidants, aromatic compounds in blueberries compete with other molecules for peroxyl radicals and retard oxidative degradation (Vanzani, Rossetto, De Marco, Rigo, & Scarpa, 2011). They inhibit oxidation by stopping initiation and propagation by reacting with alkoxyl radicals preventing the breakdown of hydroperoxides (Frankel, 1996).

Citric acid is usually added to vegetable oil after deodorization to diminish or lower the rancidity of lipids in foods because it chelates transition metals such as iron and copper in the carboxyl and hydroxyl groups of the citric acid molecule. Herbs and spices also act as antioxidants in processed food (Tsimidou et al.,1995).

Extensive research has been conducted on catechins in tea as antioxidants. Green tea leaves contain up to 36% of polyphenols on a dry weight basis. Extracts of green tea have proven to be powerful natural antioxidants. Tea catechins help to diminish the oxidation of marine oil. Tea catechins are active scavengers of free radicals and also act as metal chelators (Wanasundara & Shahidi, 1998; Zandi & Gordon, 1999; Krings et al., 2000).

Sensory Evaluation

Sensory evaluation is defined as the study of judging and ranking the quality of a product by the use of human senses such as smell, sight, touch, taste and hearing (Kiokias, Varzakas, & Oreopoulou, 2008). Sensory analysis is often conducted in conjunction with objective instrumental analyses in an attempt to correlate subjective responses of human subjects with a measurable chemical of physical property of food. Both testing methods are very important in characterization of food products (Stone, 2004). Application of the hedonic scale method is one type of subjective testing. The aim of this method is to use a hedonic scale to characterize and quantify how human subjects respond to certain sensory aspects of food. In hedonic testing, samples are given to the subject to analyse how much he likes or dislikes the product or specific product attributes (Stone, 2004). Experienced as well as unexperienced or untrained members are involved in hedonic testing (Lawless, 2010). The information given to the subjects is limited, and no attempt is made at direct response. A separate scale is used for each sample in a test. The hedonic scale presents nine different intensity levels ranging from like extremely to dislike extremely. Because many different types of the scale can be employed, variations in the scale type can lead to changes in the distribution of responses and statistical rigor used to analyze the results (Lawless, 2010). The ratings acquired on a hedonic scale may be affected by the conditions of the test being held, subject characteristics, and the opinions or assumptions of the panellist. This could have a profound effect on the researcher and the results (Piggott, 1988).

This study explores the effects of DHA on shelf life and sensory properties of peanut based toddler foods. It also examines the role of natural, food antioxidants like blueberries on the rate of lipid oxidation in DHA fortified toddler supplements.

Chapter III: Methodology

Materials

Peanut butter. Crunchy peanut butter, produced from Valencia peanuts was purchased from the Menomonie Food Co-operative Market, Menomonie, Wisconsin.

Omega-3 fatty acid. Docosahexaenoic acid produced by Martek/DSM (Parsippany, NJ) was the omegy-3 fatty acid used in this experiment. It is a rosemary-free algal oil containing a minimum 35% DHA. The oil was supplied by the Food and Nutrition Department at the University of Wisconsin-Stout. The oil was stored frozen under a N_2 atmosphere until the experiments were conducted. It was melted in warm water prior to use. In addition to docosahexaenoic acid, Martek DHA- HM contains algal oil, high oleic sunflower oil, sunflower lecithin, tocopherols and ascorbyl palminate (as antioxidants).

Dried wild blueberries. Dried, organic wild blueberries were purchased from Trader Joe's (an organic food store) located in Woodbury, Minnesota. It contained wild blueberries, sugar, and sunflower oil.

Corn syrup solids (CSS). Medium course 36 DE corn syrup solids (Cargil, Inc. Wayzata, Minnesota) were used as a partial replacement for sucrose to lower the sweetness in the Plumpy Nut-like product. The corn syrup solids were low in sweetness and had medium coarse particle size, low hygroscopicity, and moderate reducing sugar potential.

NFDM (non-fat dried milk powder). Low heat, non-fat dried milk powder was purchased from Mainstreet Ingredients (La Crosse, Wisconsin).

Canola oil and sucrose. Canola oil and sucrose were typical commercial grade purchased from the local food store in Menomonie, Wisconsin.

Formulations used to prepare the three peanut butter supplements are listed in Table 1. All of the ingredients were weighed using an analytical balance, placed in a standard Kitchen-Aide blender, and blended until the mixture achieved a smooth, paste like texture. The blended products were placed into 60 ml, plastic containers, sealed and stored in dry, ambient conditions while waiting for sensory analysis.

Table 1

Formulas for Peanut Butter Based Toddler Food Fortified with DHA Omega-3 Fatty Acid With and Without Dried Blueberries

Ingredient	Control (%)	Control + DHA (%)	Control + DHA + Blueberries (%)
Peanut Butter	41.00	40.60	36.33
Corn Syrup Solids	15.00	15.00	13.29
Non-fat Dry Milk	14.00	14.00	12.40
Canola Oil	10.00	10.00	8.86
Sucrose	20.00	20.00	17.72
DHA	0.00	0.40	0.40
Dried Blueberries	0.00	0.00	11.00
Total	100.00	100.00	100.00

Products were evaluated by sensory panels immediately after mixing (Day 0) and after one day (Day 1), three days (Day 3) and eight days (Day 8) at ambient temperature. Panels consisted of 25, 32, 38, 46 people on Day 0, Day 1, Day 3, and Day 8 respectively. Panelists were recruited from the students in the Food Science and Nutrition Department at the University of Wisconsin-Stout. For each shelf life time, the three samples of Plumpy Nut (control, control with omega-3 fatty acids and control with omega-3 fatty acids plus dried blueberry) were rated for development of off-flavor, changes in texture, intensity of peanut flavor, and preferential ranking.

Sensory analysis tests were completed after receiving approval from Institutional Review Board at the University. Subjects were asked to taste and evaluate each of the three peanut butter supplement formulas based on off-flavor, texture, peanut flavor and overall liking. Specifically subjects randomly presented one of the peanut butter formulas and were to rate the level of off-flavor from none to very high, the texture from dry and grainy to smooth and creamy, and the peanut flavor from low to strong. This was repeated for each of the formulas. After trying all three samples, a final question asked the panelists to order the three different formulas from one (like most) to three (like least). A sample sensory ballot is shown in Figure 4.

Subjects were screened for those who liked peanut butter, and precautionary methods were taken to verify that the participants were above 18 years of age, not allergic to peanut or dairy products, and not pregnant,. This was done using a recruiting questionnaire. The sensory test done was an effective test in which subjects ranked the most acceptable product according to attribute and their overall preference. The products were stored and evaluated according to the schedule shown in Table 2.

Table 2

Shelf Life Holds and Taste Panel Evaluation Schedule for the Control and Treated Peanut Butter Supplements

Sample	Code	Day 0	Day 1	Day 3	Day 8
Peanut Butter Supplement (control)	512	Taste panel	Taste panel	Taste panel	Taste panel
Peanut Butter Supplement with Omega-3 Fatty Acids	408	Taste panel	Taste panel	Taste panel	Taste panel
Peanut Butter Supplement with Omega-3 Fatty Acids and Dried Blueberries	168	Taste panel	Taste panel	Taste panel	Taste panel

Statistical Analysis

Data collected from the taste panels were analyzed for statistical significance using analysis of variance (ANOVA) on XLSTAT Statistical Software. Tukey's comparison test was used to identify differences between the samples between samples at P< 0.05.

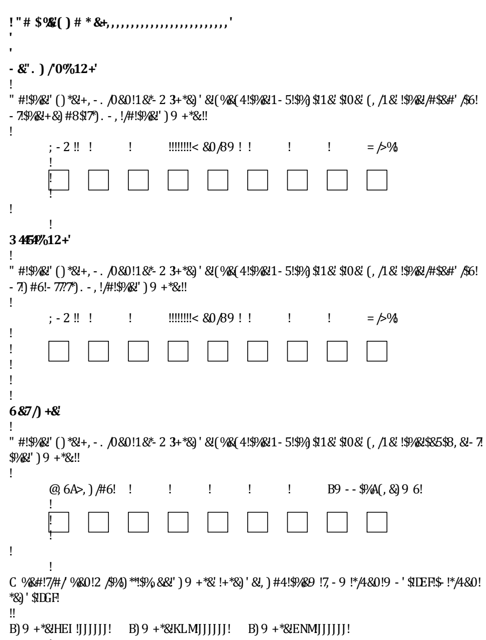

Figure 4. A sample of the questionnaire presented to panelists evaluating the peanut butter supplements.

Chapter IV: Results and Discussion

At the beginning of the storage period, right after production, all samples were perceived to have similar peanut flavor (hedonic values around 6) with no detectable off-flavors as measured by hedonic scores below 2 (Table 3). The sample made with dried blueberries had a noticeable fruit flavor and the blueberry paste resulted in a drier, slightly courser texture. Over the eight-day holding period, the flavor of the control peanut butter supplement did not change with consistent hedonic scores below 2 (Table 3). There was no detectable off-flavor perceived by the panelists at any time during the eight days in storage at 21°C. Product fortified with DHA developed detectable off-flavor within 24 hours, and at day 3 there were significant differences in the level of detectable rancidity between the control and the samples with DHA. By day eight the off-flavor in the DHA-containing sample reached very high levels with average hedonic scores above 7. The peanut butter supplement with DHA and dried blueberries developed detectable off-flavor at a slower rate than samples with DHA and no blueberries. Off-flavor was detectable in DHA/blueberry samples at day 3 and reached significant levels with a hedonic of 4.32 at day 8 (Table 3). Oxidation of DHA in the peanut butter supplement was retarded by the addition of dried blueberries, but not enough to prevent development of off-flavor during the trial period.

Table 3

The Hedonic Scores for Sensory Evaluation for Off-Flavor in Peanut Butter Supplements With and Without DHA

Days in Storage	Control	Control + Omega-3 Fatty Acid	Control + Omega-3 + Dried Blueberries
0	1.13	1.50	1.21
1	1.18	2.68	1.40
3	1.32	4.75	2.40
8	1.59	7.35	4.32

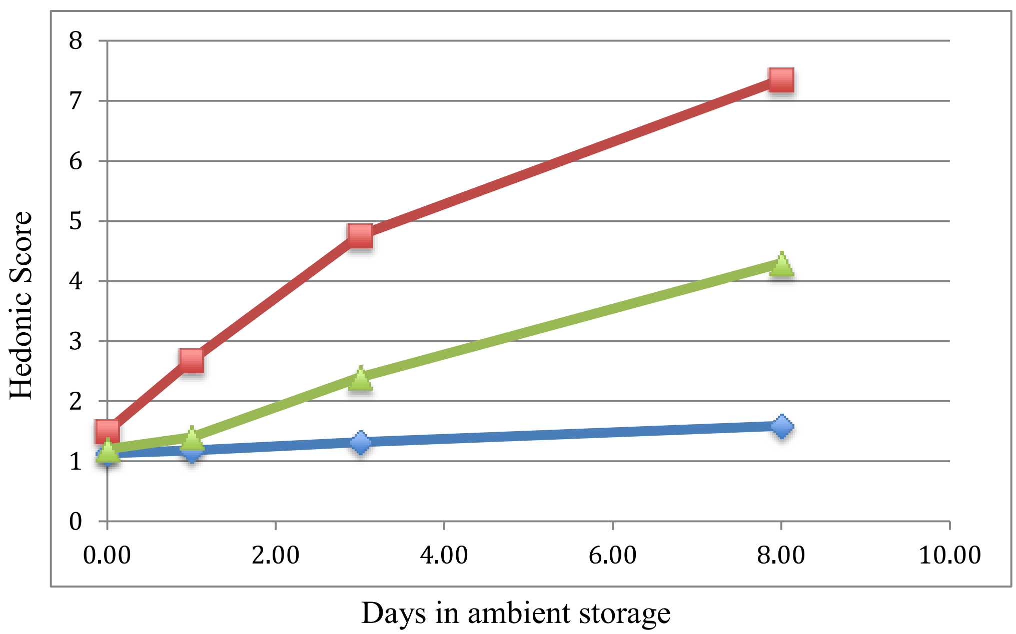

Figure 5. The effects of DHA on off-flavor development in peanut butter supplements.

Intensity of peanut flavor in the control peanut butter supplement did not change during the trial period. Hedonic levels remained slightly above 6 for all control samples tasted over the eight-day period (Table 4). Samples with DHA had similar peanut flavor intensity but the flavor declined slightly with time. Peanut flavor intensity in samples with both DHA and blueberry was less than the control, beginning with hedonic values below 6 and declining to 3.89 at the end of the trial. The overall change in perceived peanut flavor for all three samples is depicted in Figure 5.

Table 4

The Hedonic Scores for Sensory Evaluation for Peanut Flavor Intensity in Peanut Butter Supplements With and Without DHA

Days in Storage	Control	Control + Omega-3 Fatty Acid	Control + Omega-3 + Blueberries
0	6.25	6.55	5.57
1	6.27	5.72	5.02
3	6.46	5.51	4.86
8	6.73	5.32	3.89

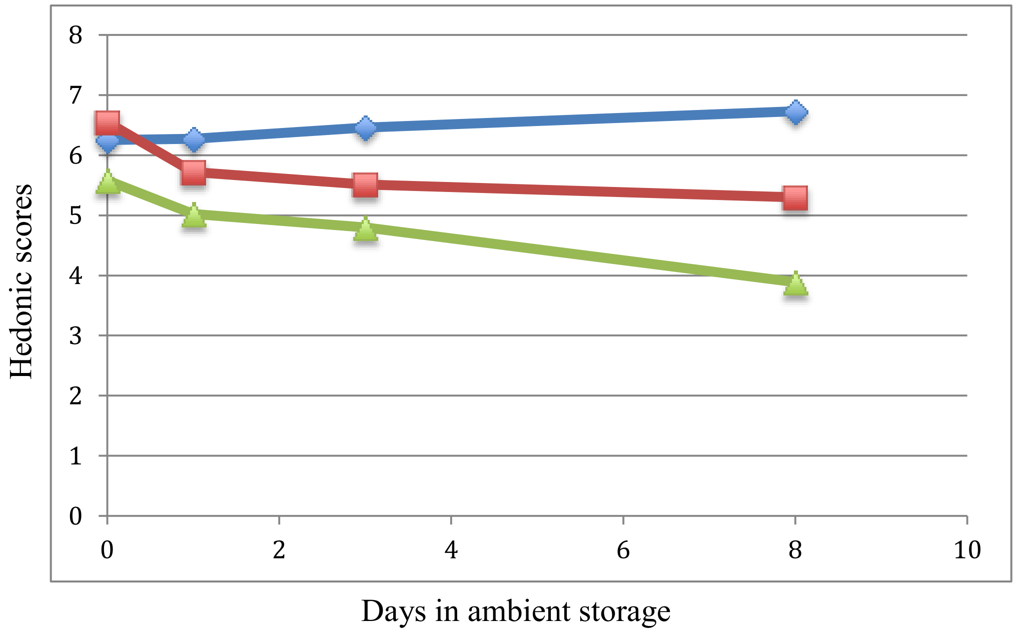

Figure 6. The effects of DHA on peanut flavor intensity in peanut butter supplements.

Dried blueberries had a significant effect on the textural quality of the peanut supplement. The average texture hedonic for the control sample began at 4.8 and rose to 7.18 after 8 days, suggesting that the product became creamier with time (Table 5). Dried sugars and proteins added to the formula partially hydrated over time leading to the improvement in texture. Sugars can also act as plasticizers that develop a softer mouth feel in the product. A similar change was seen for the samples with DHA. They increased from a hedonic of 4.35 to 5.38 during storage. Dried blueberries, because of their particulate nature and dietary fiber content, competed for water in the formula and had an immediate effect on texture, lowering the hedonics to 2.94 suggesting a drier, grainier product. Over the course of the experiment, hedonic scores for the samples made with dried blueberries dropped to 1.45 indicating that the product became drier over time as the minimal amount of moisture equilibrated throughout the sample. The overall change in texture for all three samples is depicted in Figure 6

Table 5

The Hedonic Scores for Sensory Evaluation for Peanut Flavor Intensity in Peanut Butter Supplements With and Without DHA

Days in Storage	Control	Control + Omega-3 Fatty Acid	Control + Omega-3 + Dried Blueberries
0	4.80	4.35	2.94
1	5.77	4.63	2.09
3	6.62	5.48	1.64
8	7.18	5.58	1.45

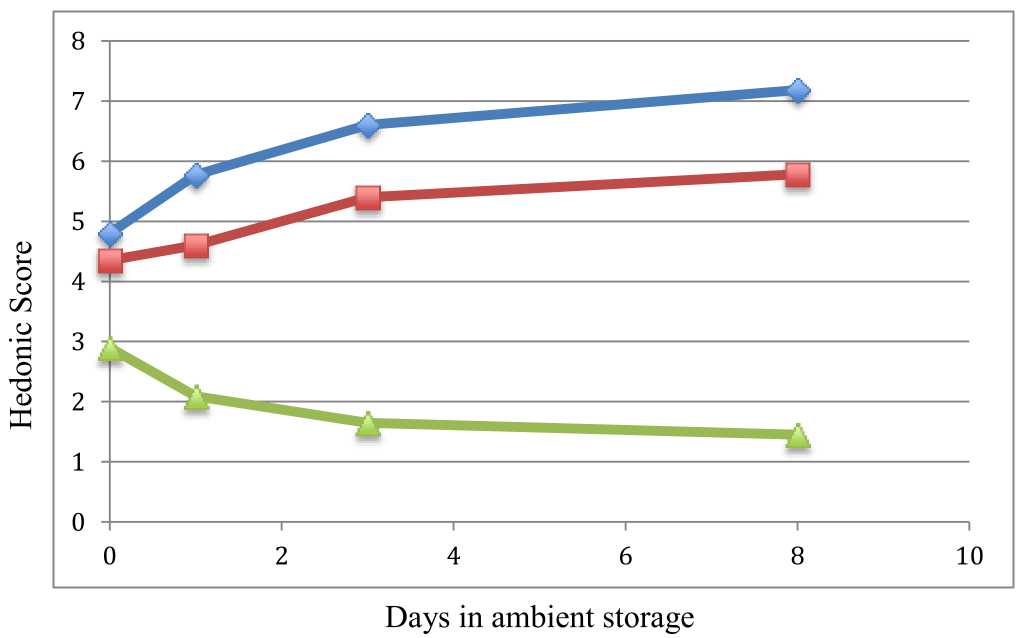

Figure 7. The effects of DHA and dried blueberry on texture in peanut butter supplements.

When asked to rank each sample on a scale of 1-3, panelists consistently chose the control peanut butter supplement. It remained between 1.0 and 1.3 over the course of the experiment (Table 6). One signifies most preferred while three is least preferred. The sample fortified with DHA had a preference similar to the control at the start of the storage period, but became least preferred at the conclusion of the test eight days later. The samples containing both DHA and dried blueberries started least preferred and retained the same level of dislike throughout the trial period. The decrease in preference for the DHA-containing sample reflects the increase in off-flavor presumably a result of lipid oxidation over time in storage. The perceived dislike for the DHA/blueberry sample appears to be a combination of the panelists' objection to dry texture and blueberry flavor compounded by the, albeit lower, development of rancid flavors from oxidized DHA.

Table 6

Preference Rating for the Peanut Butter Supplement Containing DHA and DHA Plus Dried Blueberries

Days in Storage	Control	Control + Omega-3 Fatty Acid	Control + Omega-3 + Dried Blueberries
0	1.0	2.1	2.3
1	1.0	2.1	2.5
3	1.2	2.6	2.6
8	1.3	2.7	2.6

Chapter V: Conclusions

Addition of omega-3 fatty acids like DHA to shelf stable foods like Plumpy Nut nutritional supplements results in rapid oxidation of the unsaturated fatty acids and development of rancid off-flavors. Natural anti-oxidants like anthocyanins in blueberries can retard the rate of oxidation, but when added as part of a dried fruit preparation they are insufficient for stabilization of peanut butter supplements fortified with omega-3 fatty acids over long periods of ambient storage. Additionally, dried blueberries or any other dried fruit can alter the textural qualities of the peanut butter supplement making it potentially less palatable to young children.

Options for fortifying peanut butter-based supplements with omega-3 fatty acids include the use of oil seeds like ground flaxseed in which the fatty acids remain compartmentalized and protected from damaging oxygen species or other free radicals. Similar to peanuts, ground versions of oil seeds would be compatible with the texture and flavor profile of peanut based supplements. A useful extension of this work would be to conduct similar studies using ground flaxseed preparations in place of algal DHA. If successful a search of indigenous oil seeds in the targeted regions for use could lead to likely candidates for fortification. This would meet the an additional objective of sourcing and producing foods including nutritional supplements in the regions where they will be used, thereby increasing food security and supporting local economies.

While Plumpy Nut has had an enormous impact on the survival of toddlers in developing countries, it's most important contribution is that of a model for future nutritional aids. Not all communities like or eat peanut butter. In addition there is a limit to the amount of peanut butter like products a child can consume. It is very dehydrating and can become difficult to swallow. Low water activity and limited molecular mobility is important in shelf stable foods. The inclusion of caloric density creates the additional burden of palatability. With Plumpy Nut as model, other calorically dense food type should be explored, especially the sweet or candy like products indigenous to a particular region. Most of these products are reasonably shelf stable and bring with them the familiar palatability appreciated by local children. These may serve as bases, like sweetened peanut butter, from which to construct stable and nutritious supplements.

References

Abeywardena, M. Y., & Patten, G. S. (2011). Role of 3 longchain polyunsaturated fatty acids in reducing cardio-metabolic risk factors. *Endocrine, Metabolic & Immune Disorders - Drug Targets, 11*(3), 232-246.

Alamed, J., McClements, D. J., & Decker, E. A. (2006). Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids. *Food Chemistry, 95*(4), 585-590. doi:10.1016/j.foodchem.2005.01.041

B., A. V. (2009). Docosahexaenoic acid (DHA), an essential fatty acid for the proper functioning of neuronal cells: Their role in mood disorders. *Grasas y Aceites, 60*(2), 203-212.

Berquin, I. M., Min, Y., Wu, R., Wu, J., Perry, D., Cline, J. M., . . . Chen, Y. Q. (2007). Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. *Journal of Clinical Investigation, 117*(7), 1866-1875. doi:10.1172/JCI31494

Briefel, R. R., Reidy, K., Karwe, V., Jankowski, L., & Hendricks, K. (2004). Toddlers' transition to table foods: Impact on nutrient intakes and food patterns. *Journal of the American Dietetic Association, 104*, 38-44. doi:10.1016/j.jada.2003.10.025

Bruegel, M. (2011). From the crisis of food to food in crisis. *Gastronomica, 11*(3), 40-52.

Cadenas, E., & Packer, L. (1996). *Handbook of antioxidants* New York : M. Dekker.

Cole, Greg M.Qiu-Lan MaFrautschy,Sally A. (2010). Dietary fatty acids and the aging brain. *Nutrition Reviews, 68*, S102. doi:10.1111/j.1753-4887.2010.00345.x

DeMar, J.,James C., Ma, K., Bell, J. M., & Rapoport, S. I. (2004). Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of omega-3 polyunsaturated fatty acids. *Journal of Neurochemistry, 91*(5), 1125-1137. doi:10.1111/j.1471-4159.2004.02789.x

Dunstan, J. (2007). Omega-3 fatty acids in pregnancy. *Nutridate, 18*(3), 1.

Dyer, C. (2010). Two US charities challenge company's patent on plumpy'nut. *BMJ: British Medical Journal (Overseas & Retired Doctors Edition), 340*(7756), 1055-1055.

Enserink, M. (2008a). The peanut butter debate. *Science, 322*(5898), 36.

Enserink, M. (2008b). The peanut butter debate. *Science, 322*(5898), 36.

Erickson, M. C. (1998). Lipid oxidation of muscle foods. In C. C. Akoh, & D. B. Min (Eds.), *Food lipids: Chemistry, nutrition, and biotechnology* (pp. 297-332). New York, NY: Marcel Dekker.

Fox, M. K., Pac, S., Devaney, B., & Jankowski, L. (2004). Feeding infants and toddlers study: What foods are infants and toddlers eating? *Journal of the American Dietetic Association, 104*, 22-30. doi:10.1016/j.jada.2003.10.026

Frankel E. N. 1996. Antioxidants in lipid food and their impact on food quality. *Food Chemistry*, 57, 51-55.

Friesen, R. W. I.,Sheila M. (2009). Dietary arachidonic acid to EPA and DHA balance is increased among canadian pregnant women with low fish intake. *Journal of Nutrition, 139*(12), 2344. doi:10.3945/jn.109.112565

Ghosh, D. (2009). Effect of preventive supplementation on young children in niger. *JAMA: Journal of the American Medical Association, 301*(21), 2208.

Giacalone, M., Di Sacco, F., Traupe, I., Topini, R., Forfori, F., & Giunta, F. (2011). Antioxidant and neuroprotective properties of blueberry polyphenols: A critical review. *Nutritional Neuroscience, 14*(3), 119-125. doi:10.1179/1476830511Y.0000000007

Gogus, U., & Smith, C. (2010). omega-3 omega fatty acids: A review of current knowledge. *International Journal of Food Science & Technology, 45*(3), 417-436. doi:10.1111/j.1365-2621.2009.02151.x

Gotoh N., Watanabe H., Osato R., Inagaki K., Iwasawa A., & Wada, S. (2005). Novel approach on the risk assessment of oxidized fats and oils for perspectives of food safety and quality. I. Oxidized fats and oils induces neurotoxicity relating pica behavior and hypoactivity. *Food and Chemical Toxicology*, *article in press*.

Hampshire, K., Casiday, R., Kilpatrick, K., & Panter-Brick, C. (2009). The social context of childcare practices and child malnutrition in niger's recent food crisis. *Disasters, 33*(1), 132-151. doi:10.1111/j.1467-7717.2008.01066.x

Hollis, P. (2010). Regardless of who gets credit, plumpy' nut remains a miracle. *Southeast Farm Press, 37*(23), 4.

Hraš A. R., Hadolin M., Knez Ž., & Bauman, D. (2000). Comparison of antioxidative and synergistic effects of rosemary extract with α -tocopherol, ascorbyl palmitate and citric acid in sunflower oil. *Food Chemistry, 71*, 229-233.

Iqbal S., & Bhanger, M. I. (2005). Stabilization of sunflower oil by garlic extract during accelerated storage. *Food Chemistry*, *article in press*.

Iwami, D., Nonomura, K., Shirasugi, N., & Niimi, M. (2011). Immunomodulatory effects of eicosapentaenoic acid through induction of regulatory T cells. *International Immunopharmacology, 11*(3), 384-389. doi:10.1016/j.intimp.2010.11.035

Kiokias, S., Varzakas, T., & Oreopoulou, V. (2008). In vitro activity of vitamins, flavonoids, and natural phenolic antioxidants against the oxidative deterioration of oil-based systems. *Critical Reviews in Food Science & Nutrition, 48*(1), 78-93. doi:10.1080/10408390601079975

Krebs, N. F. (2007). Food choices to meet nutritional needs of breast-fed infants and toddlers on mixed Diets[1,2]. *Journal of Nutrition, 137*(2), 511S.

Kreiter, T. (2008). AMAZING OMEGA-3s. *Saturday Evening Post, 280*(4), 28.

Kroes R., Schaefer E. J., Squire R. A., & Williams G. M. (2003). A review of the safety of DHA 45-oil. *Food and Chemical Toxicology*. *41*, 1433-1446.

Larson, R. A. (1997). *Naturally occurring antioxidants*. Boca Raton: Lewis Publishers.

Lawless, H. T. (2010). *Sensory evaluation of food principles and practices* New York: Springer.

Leaf, A. (2006). Prevention of sudden cardiac death by omega-3 polyunsaturated fatty acids. *Fundamental & Clinical Pharmacology, 20*(6), 525-538. doi:10.1111/j.1472-8206.2006.00438.x

Linneman, Z., Matilsky, D., Ndekha, M., Manary, M. J., Maleta, K., & Manary, M. J. (2007). A large-scale operational study of home-based therapy with ready-to-use therapeutic food in childhood malnutrition in Malawi. *Maternal & Child Nutrition, 3*(3), 206-215. doi:10.1111/j.1740-8709.2007.00095.x

Min, D. B. (1998). Lipid oxidation of edible oil. In C. C. Akoh, & D. B. Min (Eds.), *Food lipids: Chemistry, nutrition, and biotechnology* (pp. 283-296). New York: Marcel Dekker.

Møller, P., & Loft, S. (2010). Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. *Environmental Health Perspectives, 118*(8), 1126-1136. doi:10.1289/ehp.0901725

Nwanguma, B. C., Achebe A. C., Ezeanyika L. U. S., & Eze, L. C. (1999). Toxicity of oxidized fats II: Tissue levels of lipid peroxides in rats fed a thermally oxidized corn oil diet. *Food and Chemistry Toxicology*, *37*, 413-416.

Onyango, A. W., Receveur, O., & Esrey, S. A. (2002). The contribution of breast milk to toddler diets in western Kenya. *Bulletin of the World Health Organization, 80*(4), 292.

Peanut paste treats malnutrition. (2008). *Current Science, 93*(16), 12.

Piggott, J. R. (John Raymond). (1988). *Sensory analysis of foods* (2nd ed. ed.) London; New York: Elsevier Applied Science.

Polgreen, L. (2008). Niger: Aid group appeals ban. *New York Times,* 8.

Prakash, A., Rigelhof, F., & Miller, F (n.d.). *Antioxidant activity.* Retrieved from www.medalianlab.com

Roman, A., Schreher, S., MacKenzie, J., Nathanielsz, A. P., & Peter, W. (2006). Omega-3 fatty acids and decidual cell prostaglandin production in response to the inflammatory cytokine IL-1β. *American Journal of Obstetrics & Gynecology, 195*(6), 1693. doi:10.1016/j.ajog.2006.04.009

Seti, H., Leikin-Frenkel, A., & Werner, H. (2009). Effects of omega-3 and omega-6 fatty acids on IGF-I receptor signaling in colorectal cancer cells. *Archives of Physiology & Biochemistry, 115*(3), 127-136. doi:10.1080/13813450902905899

Simms, J. T. (2010). Ingenuity, peanut butter, and a little green leaf. *World Policy Journal, 27*(3), 75-77.

Sowndhararajan, K., Joseph, J. M., Rajendrakumaran, D., & Manian, S. (2010). In vitro antioxidant characteristics of different parts of melothria maderaspatana (L.) cogn. *International Journal of Pharmacy & Pharmaceutical Sciences, 2*, 117-123.

Stansby, M. E. (1967). *Fish oils: Their chemistry, technology, stability, nutritional properties and uses.* Westport: The Avi Publishing Company, Inc.

Stark, A. H., Crawford, M. A., & Reifen, R. (2008). Update on alpha-linolenic acid. *Nutrition Reviews, 66*(6), 326. doi:10.1111/j.1753-4887.2008.00040.x

Stone, H. (2004). *Sensory evaluation practices.* Amsterdam; Boston: Elsevier Academic Press.

Suja K. P., Abraham J. T., Thamizh S. N., Jayalekshmy A., & Arumughan C. (2004). Antioxidant efficacy of sesame cake extract in vegetable oil protection. *Food Chemistry*, *84*, 393-400.

Sun, H., Hu, Y., Gu, Z., Owens, R. T., Chen, Y. Q., & Edwards, I. J. (2011). Omega-3 fatty acids induce apoptosis in human breast cancer cells and mouse mammary tissue through syndecan-1 inhibition of the MEK-erk pathway. *Carcinogenesis, 32*(10), 1518-1524.

Tayyebi-Khosroshahi, H., Houshyar, J., Tabrizi, A., Vatankhah, A., Zonouz, N. R., & Dehghan-Hesari, R. (2010). Effect of omega-3 fatty acid on oxidative stress in patients on hemodialysis. *Iranian Journal of Kidney Diseases, 4*(4), 322-326.

Thurow, R. (2005). In battling hunger, A new advance: Peanut-butter paste. (cover story). *Wall Street Journal - Eastern Edition, 245*(71), A1.

Tolasa, Sebnem, Lee, C. M., & Calki, S. (2011). Lipid oxidation and omega-3 fatty acid retention in salmon and mackerel nuggets during frozen storage. *Journal of Aquatic Food Product Technology, 20*(2), 172. doi:10.1080/10498850.2011.556316

Tsimidou, M., Papavergou, E., & Boskou, D. (1995). Evaluation of oregano antioxidant activity in mackerel oil. *Food Research International*, *28,* 431-433.

Uauy, R., & Valenzuela, A. (2000). Marine oils. The health benefits of omega-3 fatty acids. *Nutrition*, *16*, 680-684.

Valentine, R. C. (2010). In CRC P., Valentine D. L. (Eds.), *Omega-3 fatty acids and the DHA principle*. Boca Raton, FL: CRC Press.

Valenzuela, A., Nieto, S., Sanhueza, J., Morgado, N., Rojas, I., & Zañartu, P. (2010). Supplementing female rats with DHA-lysophosphatidylcholine increases docosahexaenoic acid and acetylcholine contents in the brain and improves the memory and learning capabilities of the pups. *Grasas y Aceites, 61*(1), 16-23.

Vanzani, P., Rossetto, M., De Marco, V., Rigo, A., & Scarpa, M. (2011). Efficiency and capacity of antioxidant rich foods in trapping peroxyl radicals: A full evaluation of radical scavenging activity. *Food Research International, 44*(1), 269-275. doi:10.1016/j.foodres.2010.10.022

Varela, S. L., Muniz, F. J. S., & Cuest, C. (1995). Decreased food efficiency ratio, growth retardation and changes in liver fatty acid composition in rats consuming thermally oxidized and polymerized sunflower oil used for frying. *Food and Chemistry Toxicology*, 33, 181-189.

Wall, R., Ross, R. P., Fitzgerald, G. F., & Stanton, C. (2010). Fatty acids from fish: The anti-inflammatory potential of long-chain omega-3 fatty acids. *Nutrition Reviews, 68*(5), 280-289. doi:10.1111/j.1753-4887.2010.00287.x

Wanasundara, U. N., & Shahidi, F. (1998). Antioxidant and pro-oxidant activity of green tea extracts in marine oils: *Food Chemistry, 63*, 335-342.

Weaver, L. T., More, J. A., & Harris, G. (2008). What foods for toddlers? *Nutrition Bulletin, 33*(1), 40-46. doi:10.1111/j.1467-3010.2007.00667.x

Weers, P. M. M., & Gulati, R. D. (1997). Effect of the addition of polyunsaturated fatty acids to the diet on the growth and fecundity of daphnia galeata. *Freshwater Biology, 38*(3), 721-729.

Zivkovic, A. M., Telis, N., German, J. B., & Hammock, B. D. (2011). Dietary omega-3 fatty acids aid in the modulation of inflammation and metabolic health. *California Agriculture, 65*(3), 106-111.