

The Effect of Antioxidants on Flaxseed Stability in Yeast Bread

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ABSTRACT

The antioxidants BHA, BHT, and ascorbic acid were added to flaxmeal breads to prevent rancidity. Six types of yeast leavened breads were evaluated: control (100% bread flour), flaxmeal (15%) bread, and flaxmeal (15%) bread that contained 0.01% respectively of BHA, BHT, BHA and BHT, and ascorbic acid. Vital wheat gluten was added in all the flaxmeal breads. Chemical, objective and sensory tests were used to evaluate the breads. The crumb texture of all the experimental breads was significantly softer ($p \leq 0.05$) than the control breads, but the control breads were significantly moister ($p \leq 0.05$) than the flaxmeal breads that contained BHA and BHT, separately. No significant differences ($p > 0.05$) were found in loaf volume of the control bread and the experimental breads. The crumb color of the experimental breads was significantly darker ($p < 0.0001$) due to the incorporation of flaxmeal. The acid value of the flaxmeal breads was significantly higher ($p \leq 0.05$) than the control breads. No significant differences ($p > 0.05$) were found in peroxide values between the control breads and experimental breads after eight weeks. The QDA sensory tests showed that breads containing BHA or in combination with BHT were moister, chewier and had the least noticeable stale taste when compared to the control breads. Ascorbic acid was not as effective as BHA or a combination of BHA and BHT in preventing lipid oxidation, but produced the softest bread. This study showed that flaxmeal breads made with BHA and BHT provided the best protection against lipid oxidation and produced a moist and chewy bread.

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Table of Contents

Abstract	ii
Acknowledgement	iii
Table of Contents	iv
List of Tables	vii
List of Figures	ix
List of Equations	x
Chapter 1: Introduction	1
Chapter 2: Review of Literature	2
2.1 Functional Foods	2
2.1.1 Emergence of Functional Foods	2
2.1.2 Flaxseed as a Functional Food	3
2.2 Flaxseed: History and Overview	4
2.3 Varieties of Flaxseed	4
2.4 Nutrient Composition	4
2.5 Flaxseed and Health	8
2.5.1 Flaxseed and Cardiovascular Disease	8
2.5.2 Flaxseed and Cancer	9
2.6 Uses of Flaxseed	9
2.6.1 Processing of Flaxseed	11
2.6.2 Use of Flaxseed in Food Products	11
2.6.3 Food Products That Use Flaxseed	13
2.7 Stability of Flaxseed in Foods	14
2.7.1 Fatty Acid Profile	14
2.7.2 Effects of Lipid Content and Stability	14
2.8 Bread	16
2.8.1 Bread Ingredients	16
2.8.2 Sponge Dough Method	17
2.8.3 Bread Staling	17

2.9 Enzymes: Lipoxygenase and Lipase.....	17
2.9.1 Lipoxygenase	18
2.9.2 Lipase	19
2.10 Antioxidants.....	20
2.10.1 Synthetic antioxidants: BHA and BHT.....	20
2.10.2 Natural Antioxidants	22
2.10.3 Uses of Antioxidants in Foods.....	24
2.11 Measuring Antioxidant Activity.....	25
Chapter 3: Justification and Purpose of the Study	27
Chapter 4: Materials and Methods	29
4.1 Experimental Design	29
4.2 Sponge Dough Method	29
4.3 Lipid Extraction and Gas Chromatography	31
4.4 Chemical Tests.....	32
4.4.1 Acid Value	32
4.4.2 Peroxide Value	33
4.5 Physical Tests	33
4.5.1 Moisture	33
4.5.2 Texture	34
4.5.3 Color	34
4.5.4 Volume	34
4.6 Sensory Evaluation	36
4.7 Statistical Analysis	36
Chapter 5: Results and Discussion	38
5.1 Texture.....	38
5.2 Moisture	40
5.3 Volume	41
5.4 Color	44
5.5 Acid Value	46
5.6 Peroxide Value	50
5.7 Sensory Evaluation.....	58

5.8 Nutritional Analysis.....	62
Chapter 6: Summary, Conclusions, and Recommendations for Future Research	68
6.1 Summary and Conclusions	68
6.2 Recommendations for Future Research.....	72
References	74
Appendix A: Adapted Flax Meal Bread Recipe	81
Appendix B: Consent/Permission Form	82
Appendix C: Scorecard (example)	84
Appendix D: Acronyms, Abbreviations and Conversions	85
Vita	86

List of Tables

Table 1: Flax Varieties	5
Table 2: Color Values	35
Table 3a: The texture mean (n=30) and standard deviation (texture mean) of all bread samples during a 5-week period.....	39
Table 3b: The comparison of texture mean between the control samples and experimental samples during a 5-week period	39
Table 4a: The mean percent moisture (n=48) and standard deviation (moisture mean) of all bread samples during an 8-week period.....	42
Table 4b: The comparison of moisture between control samples and experimental samples during an 8-week period.....	42
Table 5a: The mean percent volume (n=48) and standard deviation (mean volume) of all bread samples during an 8-week period.....	43
Table 5b: The comparison of volume between the control samples and experimental samples during an 8-week period.....	43
Table 6a: The mean crumb (Hunter L-value) color (n=48) and standard deviation (mean crumb color) of all bread samples during an 8-week period.....	47
Table 6b: The comparison of volume between the control samples and experimental samples during an 8-week period.....	47
Table 7a: The mean crumb (Hunter b-value) color (n=48) and standard deviation (mean crumb color) of all bread samples during an 8-week period.....	48
Table 7 b: The comparison of mean crumb color (Hunter-b value) between the control samples experimental samples during an 8-week period.....	48
Table 8a: The mean acid value (n=24) and standard deviation (mean acid value) of all bread samples during a 1-week period.....	49
Table 8b: The comparison of mean acid value between the control samples and experimental samples during a 1-week period	49
Table 9: Lipid Analysis of Flaxseed and Oxidation rates of free fatty acids present.....	51
Table 10a: Peroxide value (n=48) of all bread samples during each week for 8-weeks.....	57

Table 10b: The mean peroxide value (n=48) and standard deviation (mean peroxide value) of all bread samples during an 8-week period.....	57
Table 10c: The comparison of mean peroxide value between the control samples and experimental samples during an 8-week period.....	57
Table 11a: The mean QDA scores for bread characteristics between the experimental breads and control breads (regular yeast and flax) during a 4-week period.....	60
Table 11b: The comparison of mean QDA scores for bread characteristics between the experimental breads and control breads (regular yeast and flax) during a 4 week period.....	60

List of Figures

Figure 1: Structure of α -linolenic acid and linoleic acid.....	7
Figure 2: The formation of enterodiol (ED) and enterolactone (EL) from SDG.....	10
Figure 3: The processing of flaxseed.....	12
Figure 4: Structures of BHA and BHT.....	21
Figure 5: The structure of L-ascorbic acid (Vitamin C).....	23
Figure 6: Flowchart: Sequence of steps followed during the research study.....	30
Figure 7: The change in Hunter L-values of all bread types over an 8-week period.....	45
Figure 8: The change in Hunter b-values of all bread types over an 8-week period.....	45
Figure 9: Free radical autoxidation.....	53
Figure 10a: The development of mean peroxide values in all bread samples over an 8 week period.....	55
Figure 10b: The peroxide values in each bread sample over an 8 week period.....	56
Figure 11a: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 1.....	63
Figure 11b: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 2.....	63
Figure 11c: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 3.....	64
Figure 11d: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 4.....	64
Figure 11e: A Spider Plot showing a summary of the mean sensory (intensity) QDA score of various bread attributes for each bread type over a 4 week period.....	65
Figure 12: Nutrition Label for Flaxmeal Bread without antioxidants.....	67
Figure 13: Nutrition Label for Regular Yeast Bread without antioxidants.....	67

List of Equations

Equation 1: Totox Value.....	26
Equation 2: Acid Value.....	32
Equation 3: Peroxide Value	33
Equation 4: Moisture	34

Chapter 1: Introduction

The importance of nutrition and exercise to maintain and/or improve the quality of life has been increasing over the years due to the high prevalence of chronic diseases, such as cardiovascular disease and cancer. The use of certain food items to improve overall health has been an important part of research in this area. Over the years, investigators have found that various foods can be used to improve overall health. The research focus in nutrition science has shifted from dealing with nutrient deficiencies and nutritional adequacy to the identification of biologically active components in foods that have potential health benefits, i.e. improving overall health (22). The result of these findings was the development of the concept of functional foods. Functional foods are “foods that contain specific minerals, vitamins, fatty acids, dietary fiber, and include foods that have biologically active substances, such as phytochemicals or other antioxidants (22).”

Functional foods have been researched since the 1980s. However, their use has increased recently as the importance of improving one’s health has increased. Some examples of functional foods are low-fat milk, cereal with added folic acid, oatmeal/bran/whole oat products, fruits and vegetables. These functional foods have been shown to reduce the risks of osteoporosis, neural tube defect, reduced cholesterol and cancer risks, respectively. One functional food that is currently being researched for its health benefits is flaxseed. Flaxseed contains a high amount of dietary fiber, omega-3 oils (specifically, alpha-linolenic acid), and anticarcinogenic lignans. Thus, flaxseed has been shown in previous research to reduce the risks of cancer and cardiovascular diseases (43).

Chapter 2: Literature Review

2.1 Functional Foods

The term “functional foods” is a concept that was developed “in Japan in the early 1980s when, faced with escalating health care costs, the Ministry of Health and Welfare initiated a regulatory system to approve certain foods with documented health benefits in hopes of improving the health of the nation’s aging population (37). In 1991, these foods, in turn, were recognized as “foods for specified health use or FOSHU for short (68). A universally accepted definition for the term “functional foods” does not exist. As a result, several authors use different definitions for the term functional food. For example, the Food and Nutrition Board of the Institute of Medicine in the United States defined a functional food as “any food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains (34).” It also has been (34) noted that functional foods may claim specific health benefits because they are considered part of the diet. The definition used by the Food and Nutrition Board of the Institute of Medicine is very similar to the definition by the American Dietetic Association (1) presented in their position statement, except the ADA included three main points in their position paper (56):

- “Functional foods may be whole, fortified, enriched, or enhanced foods,
- To have beneficial effect on health, a functional food would have to be consumed as part of varied diet on a regular basis, at effective levels,
- It’s likely that all foods are functional at some physical level.”

2.1.1 Emergence of functional foods

The demand for health and longevity, quick solutions, natural products and preventive measures (84) has caused the emergence of functional foods over the past several years. The development of functional foods is the result of several factors,

- “increasing health costs (84), self-efficacy and autonomy in health care, an awareness and desire to enhance personal health (2),
- advancing scientific evidence that diet can alter disease prevalence and progression (2) and consumer health awareness and choice (84).”

Over the past several years, the concept of functional foods has developed in the areas of food science and nutrition. Schneeman noted that there may be a connection between food and disease, such that, the lack of four different vitamins caused pellagra, beri-beri, and rickets (75). This early research helped promote more

investigations in finding or developing foods that have functional roles in disease prevention. As a result of previous research the concept of functional foods was developed. In turn, this helped in the development of enriched and fortified foods, such as enriched flour to help prevent pellagra and foods enriched with folic acid to help prevent neural tube defects (75). However, functional foods should not be used to cure disease or illness, but rather as part of the treatment because disease or illness is the result of the dietary pattern, lifestyle and heredity of a person (75). Thus, many foods can be considered a functional food, such as, oat bran, vegetables and fruits. These foods are considered functional foods because they have been shown to reduce the risk for coronary heart disease and cancer (2).

2.1.2 Flaxseed: A Functional Food

A functional food that has received much attention over the past several years is flaxseed. Flaxseed has been shown to have beneficial effects in the prevention and/or treatment of cancer and cardiovascular disease (43). In early research, flaxseed flour was used in bread (17), banana nut muffin and oatmeal cookies (4). In more recent investigations, ground flaxseed was incorporated into pasta (spaghetti and macaroni) and the effects of processing and cooking on the lipid content and stability of α -linolenic acid of ground flaxseed were studied (52). Current research on functional foods has identified flaxseed as a potential functional food because of its high sources of phytochemicals. Flaxseed contains a high concentration of α -linolenic acid and lignans (82) and is an essential source of high-quality protein and soluble fiber and has potential as a source of phenolic compounds (59). It also has been suggested (82) that these substances in flaxseed have anticancer effects. Thus, flaxseed may be used in preventing cancer development. The development and use of functional foods, specifically flaxseed, may increase due to potential health benefits from these foods.

As mentioned earlier, flaxseed is rich in alpha-linolenic acid (ALA, an omega-3-fatty acid) and has a high lignan content. Omega-3-fatty acids are considered essential fatty acids because of their roles in lowering cholesterol and reducing the risk of certain types of cancer and coronary heart disease. Flaxseed is also the richest source of lignans because it contains high levels of the lignan precursor, secoisolariciresinol diglucoside (SDG) which has been shown to control lupus nephritis in humans (63). However, other components of flaxseed have been shown to have health benefits. For example, flaxseed oil by itself was shown to reduce blood cholesterol levels while atherosclerosis was decreased and platelet adhesiveness was reduced when flaxseed was combined with vitamin E (59). As a result of these health claims, the development of functional foods, specifically, the use of flaxseed in products will increase in the future.

2.2 Flaxseed: History and Overview

Flax is an ancient food that has been used in Europe and Asia since 5000-8000 B.C. to make linen cloth and as early as 650 BC Hippocrates cited flaxseed for its medicinal purposes (45). Flax (*Linum usitatissimum*) is a herbaceous blue flowering plant that is grown in Canada primarily for its oil-rich seeds (24). The seeds of flax are smooth, tiny and a light to reddish brown color. Flax is cultivated for two main purposes: fiber production and for the seed. Linen is made from the fiber portion of the flax stem. Flaxseed, on the other hand is used in two different ways. First, flaxseed can be processed to extract the oil, which then can be used in salad oils, paints, stains and linoleum. Second, flaxseed can be added to cereals, pasta, yogurt, and salads and can be incorporated in baked goods, such as breads and muffins (25). Flaxseed provides a nutty flavor, crunchy texture, and a reddish-brown hue when incorporated into baked products. In addition to its baking properties, flaxseed is also known for its health benefits. Flaxseed also provides many nutrients, including protein, essential fatty acids, vitamins, minerals and dietary fiber. Furthermore, flaxseed is a rich source of lignans. Lignans are a type of phytoestrogen that may protect against certain types of cancer by interfering with estrogen metabolism (23).

2.3 Varieties of Flaxseed

Seed flax and fiber flax are the two types of flax that are commonly grown in the United States, Canada, Europe and Asia. Most seed flax is grown in the upper Midwest of the United States and the Prairie Provinces of Canada while fiber flax is grown in Europe and Asia. Each type of flax has different uses. For example, seed flax is used to produce linseed oil and linseed meal. Linseed meal is used as livestock feed while seed flax stems are used to make fine paper. In contrast, fiber flax is used to make fine linen cloth. Another difference between seed flax and fiber flax is that seed flax varieties are short, multiple branched and selected for high speed production while fiber flax varieties are very tall with few branches that are used in low seed production (13). Flax can be divided into flax varieties and solin varieties and each variety contains different kinds of flax. All flax varieties registered in Canada are brown-seeded. The solin varieties are yellow-seeded and have less than 5% linolenic acid content (53). Flax is divided into categories related to their maturing stages (Table 1).

2.4 Nutrient Composition

The major nutrients in flaxseed are essential fatty acids, lignans, dietary fiber, protein, minerals and vitamins. These nutrients have been either linked to possible health benefits or help maintain the body's normal physiological functions.

Table 1: Flax Varieties ⁽⁵³⁾

Flax Varieties	Examples
Medium <i>early</i> maturing varieties	Linott
Medium maturing varieties	AC Emerson, Andro, Cathay, CDC Normandy, Neche, Norlin, NorMan, Omega, Pembina, Somme, Vimy
Medium <i>late</i> maturing varieties	AC Linora, Flanders, Linola 959
Late maturing varieties	AC McDuff, Linola 947, McGregor

Flaxseed contains high amounts of linoleic acid (LA) and alpha linolenic acid (ALA) (Figure 1). Linoleic acid and alpha linolenic acid are examples of polyunsaturated fatty acids. Polyunsaturated fatty acids have more than one double bond in their carbon chain and are divided into two categories; omega-6-fatty acids (e.g. linoleic acid) and omega-3-fatty acids (e.g. alpha linolenic acid). The differences between these fatty acids are due to the position of the first double bond on the carbon chain counting from the methyl end (Omega 6 and Omega 3) (Figure 1), the number of double bonds, and their function. Also, polyunsaturated fatty acids are considered essential fatty acids because these fatty acids are acquired from the diet. Thus, linoleic acid and alpha linolenic acid are important for normal growth, skin and hair growth, cholesterol metabolism and reproductive performance.

Lignans are also present in high concentrations in flaxseed. Lignans are examples of phytoestrogens. Phytoestrogens are compounds that are found in plants that have been shown to have estrogenic effects. As a result, lignans can help prevent hormone-sensitive cancers by disrupting estrogen metabolism in animals and humans. The main plant lignan in flaxseed is secoisolariciresinol diglycoside (SDG) which is the precursor of enterdiol, a mammalian lignan (82). SDG is converted to enterdiol by the bacterial flora in the colon. The possible anticancer effects of flaxseed may be related to the antioxidant activity of SDG. Therefore, flaxseed may be used to prevent cancer.

The last major nutrients found in flaxseed are dietary fiber, proteins, minerals and vitamins. Dietary fiber consists of the plant cell wall polysaccharides and lignin. These components are indigestible in the human body due to the lack of necessary enzymes. There are two types of dietary fiber; water soluble fiber (e.g. mucilage gums) and water insoluble fiber (e.g. lignin and cellulose). Each type of dietary fiber plays a different role in overall nutrition. Water soluble fiber helps lower blood cholesterol levels and maintains blood glucose levels. A research team (21) showed that insoluble fiber helped improve lactation and prevented constipation by increasing fecal bulk and reducing bowel transit time. Flaxseed contains approximately 28 g total dietary fiber per 100g on a dry weight basis (83). As a result, flaxseed is a good source of dietary fiber and may have a role in the prevention of cardiovascular disease.

Flaxseed contains approximately 20 percent protein on a dry weight basis (87) and has a similar amino acid profile to that of soybean. Flaxseed contains many essential amino acids, such as valine, leucine, isoleucine, phenylalanine, tryptophan, lysine, arginine, histidine, methionine and cysteine (89). Arginine, glutamine and histidine are “known to have strong effects on the immune functions of the body” while cysteine and methionine can help reduce certain forms of colon cancer by enhancing the body’s antioxidant

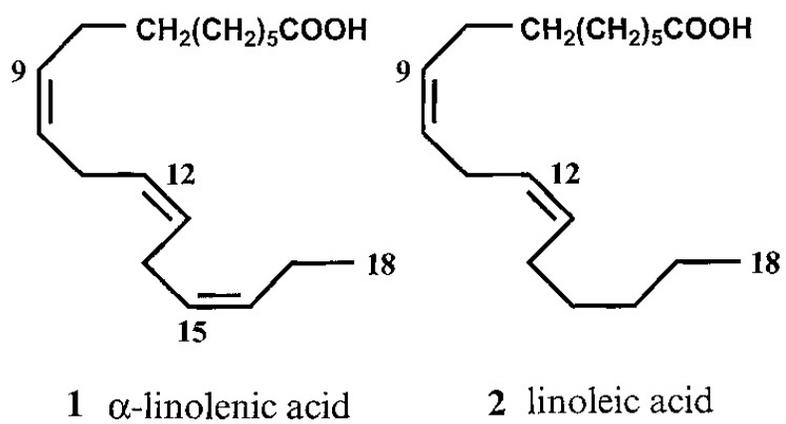


Figure 1: Structure of α-linolenic acid and linoleic acid ⁽⁸⁹⁾

levels (59).

Flaxseed is also a good source of minerals and vitamins; especially potassium, magnesium, phosphorus, iron, copper and zinc. Minerals and vitamins are necessary in maintaining the body's normal physiological functions, such as regulating enzyme activity, fluid balance and growth and may act as an antioxidant. In conclusion, flaxseed contains several nutrients that are very important in maintaining normal body functions and may provide health benefits, such as; reducing the risks of cardiovascular disease, cancer and diabetes.

2.5 Flaxseed and Health

Cancer and cardiovascular disease are the two leading causes of death in the United States. According to the American Heart Association, cardiovascular diseases were responsible for 927,448 deaths in 2002 in the United States (8) while the American Cancer Society predicted that cancer will claim about 570,280 Americans (i.e. 1 of every 4 deaths) in the 2005 (5). Cancer and cardiovascular diseases have been linked to poor diet and nutrition. For example, of the 570,280 deaths, about 190,094 deaths will be related to nutrition, physical inactivity, obesity, and other lifestyle factors, which all can be prevented (5). Proper nutrition has been shown to help reduce the risks of cancer and cardiovascular disease. In previous research, it has been shown that diets in high fruits and vegetables, legumes, whole grains and fish may have protective roles against different cancers and cardiovascular disease (43). As mentioned before, flaxseed has been shown to reduce the risk of cardiovascular disease and cancer.

2.5.1 Flaxseed and Cardiovascular Disease

Cardiovascular disease, in particular coronary heart disease is caused by the atherosclerosis, which is the narrowing of the arteries due to fatty build-up of plaque (8). There are two types of lipoproteins, low density lipoprotein (LDL) and high density lipoprotein (HDL). LDL is known as the bad cholesterol because when combined with other substances it can form plaques that can clog the arteries. HDL is known as the good cholesterol because at high levels, it may have a protective role against heart attacks because HDL may carry cholesterol away from the arteries and back to the liver and helps remove excess cholesterol from plaque in arteries (8).

Flaxseed contains alpha-linolenic acid (ALA) and lignans which have been shown to help reduce the risks of cardiovascular disease and cancer. In one study, 10 young, healthy men and women consumed flaxseed muffins that provided 50g flaxseed/d for four weeks. The results from this study showed that plasma total cholesterol was reduced by 6%; LDL cholesterol was reduced by 9%, and plasma HDL and triglycerides did not change (21). ALA helps reduce the risk of cardiovascular diseases in two ways: by modifying

membrane phospholipids and interfering with eicosanoid production. A diet that contains flaxseed has been shown to help increase the ALA content of blood phospholipids, triglycerides and/or cholesterol esters. This increase in the omega-3-fatty acid content of membrane phospholipids will, in turn, help increase membrane fluidity and alter membrane function. As a result of these changes, the risk for cardiovascular diseases is reduced. ALA also interferes with the reaction that produces arachidonic acid, which is the precursor of eicosanoids. Eicosanoids help promote platelet aggregation and vasoconstriction. Thus, by reducing the production of eicosanoids may help decrease the risks for cardiovascular disease.

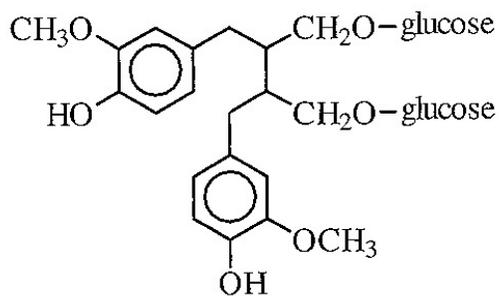
2.5.2 Flaxseed and Cancer

Cancer is a term used to describe a group of disease characterized by uncontrolled growth and spread of abnormal cells (5). Cancer is caused by external factors (i.e. tobacco, chemicals, and radiation) and internal factors (inherited mutations, hormones, and immune conditions). According to the Cancer Facts & Figures 2005, the American Cancer Society predicts that 570,280 Americans will die of cancer this year and of those about a third of those deaths will be related to nutrition, physical inactivity, obesity and other lifestyle factors that can be prevented (5). Flaxseed is a very good source of the lignan, secoisolariciresinol diglucoside (SDG). The intestinal microflora breaks down SDG into enterolactone and enterodiols (Figure 2). Lignans are a type of phytoestrogen, which is an estrogenic compound, found in plants that are structurally similar to endogenous estrogens (79). Thus, lignans may protect against hormone-sensitive cancers by interfering with sex hormone metabolism. In a recent study (19), mice were injected with human breast cancer cells. Mice were fed a basal diet for 8 weeks. At the end of 8 weeks, the mice were separated into two groups: Control (basal diet) and 10% flaxseed (FS) diet. The main results from this study (19) were the FS diet reduced the tumor growth rate and reduced metastasis by 45%. While it has been shown that lignans inhibited the growth of human mammary tumor cells, reduced mammary tumor initiation and inhibited estrogen synthetase activity (79) current research is not conclusive.

2.6 Uses of Flaxseed

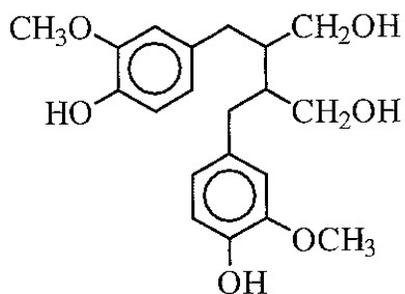
In addition to its possible health benefits, flaxseed is also considered a low carbohydrate food because of its limited amount of digestible carbohydrates. In recent years, diets such as, the Atkins diet and South Beach diet encourage weight loss by restricting or limiting carbohydrate intake, respectively. Flaxseed has a low glycemic index (GI) which indicates how fast blood glucose is raised when compared to a standard food. Thus, foods with a low GI take longer to digest and to be absorbed by the body. This causes the slow increase of blood glucose and insulin levels. As mentioned earlier, flaxseed is also a good source of dietary fiber, low in saturated fat and high in omega-3 fats.

Secoisolariciresinol Diglycoside (SDG)

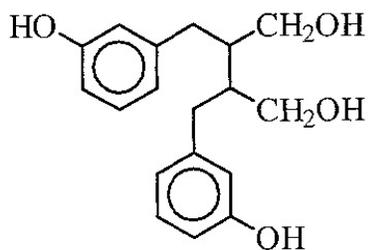


Hydrolysis

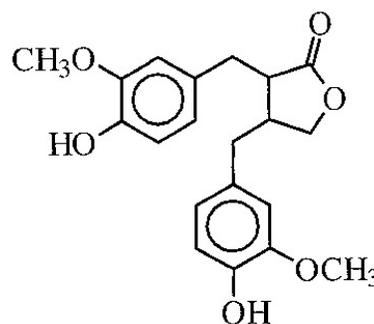
Secoisolariciresinol (SECO)



Enterodiol (ED)

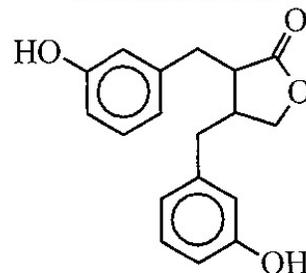


Matairesinol (MAT)



Dehydroxylation
Demethylation

Enterolactone (EL)



Oxidation

Figure 2: The formation of enterodiol (ED) and enterolactone (EL) from SDG ⁽⁶⁶⁾

Flaxseed (whole or ground) may be added to breads, cereals, breakfast drinks, soups, fiber bars, muffins, cookies and cakes. Flaxseeds provide a nutty flavor, a yellow (solin variety) or reddish-brown (flax variety) hue when incorporated into food products.

2.6.1 Processing of Flaxseed

Flaxseed is processed before it is used and/or incorporated into other products. A summary of processing steps that flaxseed can undergo can be seen Figure 3. The processing that flaxseed undergoes depends on how the flaxseed will be used. For example, if the whole flaxseed will be used in a breakfast cereal, the flaxseed is cleaned than then sent to the cereal manufacturer for blending into the cereal formulations (42). However, if the flaxseed is used in baked goods, the flaxseed undergoes further processing steps. After the seed is cleaned, the flaxseed is either cracked by a finely corrugated set of steel rollers or slightly crushed by a set of smooth rolls (42). Further processing is not required if the processed seed is incorporated directly in baked breads. However, there is a 12% inclusion limit. In addition, more processing steps are required if the flaxseed will be used in a designer food or health-food markets, such as “toasting or packaging in a tin-foil/oxygen-barrier bag where the oxygen has been flushed by carbon dioxide or nitrogen before the bag is evacuated (42).” Either carbon dioxide or nitrogen is used because flaxseed contains a high level of alpha-linolenic acid which contributes to rancidity due to oxidation.

Milled flaxseed meal is another by-product of flax and is the result of the production of crude vegetable oil from oilseeds by pressure or solvent extraction (42). The milled flaxseed can be used as is, or it can undergo further processing, such as cold-pressing and defatting using hexane or carbon dioxide extraction. Cold-pressing is a process used to remove most of the oil, so that the resultant flax meal has a residual oil content of about 12 percent. Previous research has shown that milled flaxseed is very stable under normal storage conditions and during prolonged baking (38). As a result, the use of milled flaxseed (i.e. meal or oilseed residue) is preferred over flaxseed oil in baked products because milled flaxseed has a minimal loss of alpha linolenic acid during baking and long-term storage is possible because there is little to no oxidation (42).

2.6.2 Use of flaxseed in food products

As mentioned before, flaxseed has been shown to decrease the risks of cancer and cardiovascular diseases. By incorporating flaxseed or one of its derivatives (i.e. milled flaxseed) into food products, such as baked goods, consumption of flaxseed may increase, which in turn will help decrease the risk of cancer and cardiovascular diseases.

When flaxseed is used as a baking ingredient, it provides a reddish-brown hue, nutty flavor and a crunchy texture in bakery products. Whole flaxseeds and milled flaxseed can be used in bread products; for

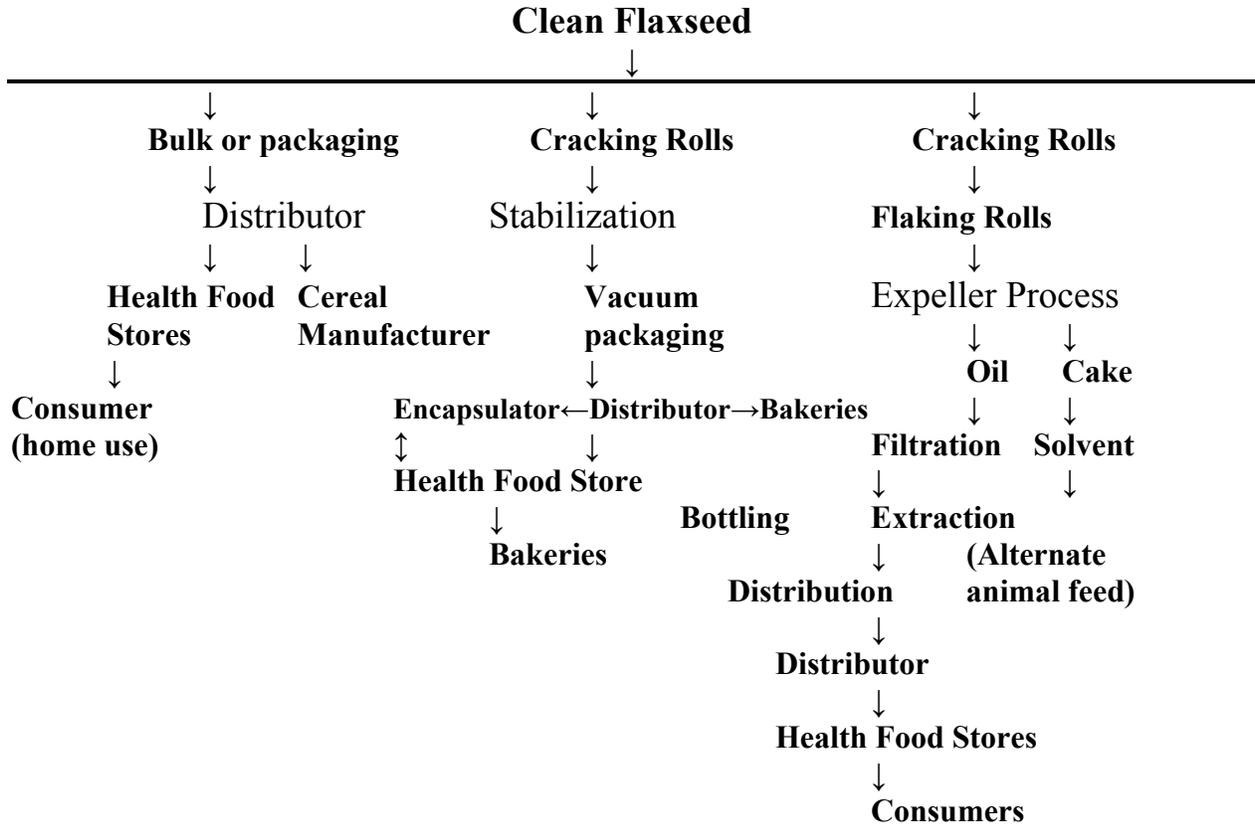


Figure 3: The processing of flaxseed⁽⁴²⁾

example, whole flaxseeds can be used to coat bagels. When milled flaxseed meal is used in bread products, some adjustments with the other ingredients are necessary to ensure a bread product with a satisfactory taste. For example, 25% more yeast is usually needed so that the proof time, texture and consistency of the bread is similar to a regular yeast bread (45). Also, since milled flaxseed meal has more fiber, more water is needed. However, a benefit of using milled flaxseed meal is that due to its high fat content, it acts as a fat replacer (45).

2.6.3 Food products that use flaxseed

Flaxseed is a versatile product that has several functions: as an ingredient in foods for human consumption; as an ingredient for animal and poultry feed; flaxseed oil, specifically linseed oil is used as a diluent in paints and coatings and flax fiber is used for paper products. As a food ingredient, flaxseed can be used in baked products and can be incorporated in ready-to-eat cereals, breakfast drinks, salad toppings, biscuits, meat extenders, crackers, soups, bagels and pastas (13).

In a one study (4) flaxseed was incorporated in a banana-nut muffin recipe and in an oatmeal cookie recipe. The purpose of this study “was to describe the palatability characteristics and overall acceptability of banana nut muffins and oatmeal cookies prepared with ground flaxseed (4).” The treatments that were used were flour, 30% or 33% ground flaxseed and 50% ground flaxseed (as a replacement for flour). The ingredients for each treatment were kept relatively similar by adjusting the amount of flaxseed used. Thus, 30% of ground flaxseed was used in the muffins while 33% of ground flaxseed was used in the cookies.

After using each treatment to make the banana nut muffins and oatmeal cookies, the foods were rated using the 9 point hedonic scale by ninety untrained, randomly selected college student panelists. The main results from this study were that none of the treatments were sweet enough, the control banana nut muffin and oatmeal cookies were paler when compared to the treatment groups and the muffins that “were rated with nearest to the optimal flavor” contained 50% ground flaxseed (4). The treatments that used 30% ground flaxseed and 50% ground flaxseed in the banana nut muffins were rated more acceptable than the control muffin that contained all-purpose flour. Similarly, for the oatmeal cookies, the treatment groups (33% and 50% ground flaxseed) were rated near optimum in tenderness and flavor when compared with the control oatmeal cookies. These findings suggest that the treatment groups are comparable to the control group in relation to sensory attributes which is important because of the beneficial components (i.e. ALA and fiber) that flaxseed imparts when used in baked products.

2.7 Stability of Flaxseed in Foods

Linoleic and linolenic acids are considered polyunsaturated fatty acids because each contain multiple double bonds, 2 and 3, respectively. The chemical profile (structure and number of double bonds) of a fatty acid is related to its reactivity. It is known that the more polyunsaturated a fatty acid (i.e. more double bonds); it is highly reactive due to its increased susceptibility to lipid oxidation.

2.7.1 Fatty acid profile

The fatty acid content of flaxseed is important regarding functionality in foods and health benefits in a food product. Flaxseed is composed of about 41% fat on a dry-weight basis and is low in saturated fat (9% of total fatty acids), moderate in monounsaturated fat (18%) and rich in polyunsaturated fat (73%) (83). The two major polyunsaturated fatty acids in flaxseed are alpha-linolenic acid (ALA) and linoleic acid. The main differences between these fatty acids are that alpha-linolenic acid makes up about 57% of the total fatty acids in flaxseed and is an example of an omega-3 fatty acid while linoleic acid makes up about 16% of the total fatty acids and is an example of an omega-6 fatty acid (83).

2.7.2 Effects of Lipid Content and Stability

Since flaxseed contains a high amount of alpha-linolenic acid, which is highly unsaturated fatty acid with three double bonds, flaxseed is susceptible to lipid oxidation by autoxidation and/or lipoxygenase (52). Autoxidation is a process that results in the formation of hydroperoxides (LOOH) from the interaction between oxygen and lipid radicals which are generated by heat and light. Next, the “oxidized alpha-linolenic acid can undergo a hemolytic cleavage of the LOOH and single bonds adjacent to the LOOH to form carbonyl compounds, alcohols and aldehydes such as hexanal which is a decomposition product of linoleic acid (52).” In one study (50), it was reported that hexanal contributed to an off-flavor in the breads when flaxseed was used.

Several studies have been conducted to determine the oxidative and storage stability of flaxseed in food products. One study (20) measured the changes in alpha-linolenic acid under various conditions to help determine the stability of whole and ground flaxseed when used alone or when incorporated in a muffin mix. The treatments used to determine the stability of flaxseed were heating the flaxseed samples at either 122°C (at two minute intervals for twelve minutes) or 178°C (at 30 minute intervals for 1.5 hours.) or storing the flaxseed samples at room temperature for 280 days with 12-hour light/dark cycles. These treatments helped determine flaxseed’s stability by measuring the percent oxygen consumption. The results of this research were:

- Whole flaxseed was resistant to autoxidation and ground flaxseed was more susceptible to autoxidation than isolated flaxseed lipids;

- There was not a significant decrease in alpha-linolenic acid when flaxseed flour was used in a muffin mix (20);
- A possible relationship was found between the particle size of ground flaxseed and oxidative stability. It was shown that large-sized ground flaxseed had faster oxygen consumption rate while small-sized ground flaxseed had slower oxygen consumption rate. This may be due to the space between the flaxseed particles; the more space, the faster the oxygen traveled which resulted in increased oxygen consumption. Conversely, the smaller the particle size, less space which results in decreased oxygen consumption (20) ;
- Lastly, all three samples, whole flaxseed, ground flaxseed and lipid extracts were all stable at room temperature for 280 days with 12-hour light/dark cycles.

The authors suggested that the other ingredients may play a role in the oxidation of alpha-linolenic acid. Thus, future research is needed to identify a relationship between baking ingredients (such as wheat flour, oils and baking powder) and flaxseed oxidation.

Researchers (50) measured the storage stability of two samples of milled flaxseed: Linott and a mixture of varieties. In this study, a trained sensory panel was used to evaluate the milled flaxseed and bread. The storage stability of the flaxseed samples were evaluated by packing one kilogram of milled flaxseed “into a triple layer medium-weight paper bag with a 1.5-ply plastic liner similar to what is used commercially. These bags were then stored at $23 \pm 2^\circ\text{C}$ and evaluated at 0, 33, 66, 96, and 128 days for chemical, sensory and volatile indicators of quality (50).”

The main results of this study were as follows:

- Over the 128 day storage period, there were “no significant changes ($p \leq 0.05$) in peroxides or conjugated double bonds found for either the mixed variety or Linott samples nor were there differences found between the mixed variety or Linott sample (50);”
- The amount of free fatty acids increased in the Linott samples, but remained unchanged in the mixed variety sample as storage time increased;
- However, there was an increase in the total amount of volatile compounds in the mixed variety while there was no increase in the Linott samples;
- The trained “sensory panelists were not able to detect a difference in odor characteristics among the fresh or stored flaxseed samples for either the mixed variety or Linott variety” which is consistent with the results mentioned earlier;

- Also, the panelists were unable to detect a flavor difference between bread made with 0-day stored milled flaxseed and 128-day stored milled flaxseed (50).”

The authors of this study suggested that the stability of both flaxseed samples in the storage conditions used in this lab may be due to “endogenous antioxidants in the milled flaxseed that prevented oxidation of the unsaturated fatty acids and the corresponding off-flavors (50).” Thus, future research to identify these endogenous antioxidants would be helpful.

2.8 Bread

There are two types of breads; quick breads and yeast breads. The main differences between these types of breads are the kinds of breads products made, the method used (i.e. muffin method and sponge dough method), and the ingredients used. Biscuits, cakes and muffins are some common examples of quick breads while French bread, whole wheat bread and Danish pastries are considered yeast breads. The methods and ingredients used to create a bread product are related to the outcome of the product. Flaxmeal is considered a heavy ingredient and does not contain gluten. As a result, the sponge dough method is used to help incorporate the flaxmeal into the bread dough and vital wheat gluten is added to help with the texture and volume of the flaxmeal bread.

2.8.1 Bread Ingredients

The main ingredients of yeast bread are flour, liquid, salt, sugar and yeast. Yeast breads are considered “the staff of life” because of its basic ingredients (55). Other ingredients that may be used are sugar, gluten, fat and antioxidants. Each ingredient plays a role in the making of bread, such as: bread flour (sets structure), yeast (leavening agent), salt (for flavor and controlling the rate of yeast growth), water (moisture), sugar (for flavor, food for yeast and browning), gluten (provides elastic and extensible properties), fat (tenderness) and antioxidants (e.g. BHA/BHT and citric acid, used to extend the shelf-life). These ingredients also play a role in the staling of bread. One author (61) referred to staling as “the gradually decreasing consumer acceptance of bread due to all the chemical and physical changes that occur in the crust and crumb during storage.” An example would be the formation of off-flavors and aromas due the oxidation of fat in bread. However, antioxidants such as, BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), ascorbic acid and citric acid have been used in various foods to decrease or prevent lipid oxidation.

2.8.2 *Sponge Dough Method*

Flaxmeal can be incorporated into several food systems, such as cookies and muffins. Another food system that flaxseed may be used in is yeast breads. One author (18) noted that bread products have played a significant role “in contributing nutrition to the diet, by providing protein, starch, fiber, vitamins and minerals.” Thus, incorporating flaxmeal into a bread system may help promote additional health benefits.

There are two main methods to making yeast breads; straight-dough method and sponge dough method. However, the sponge dough method is used when incorporating heavier ingredients, such as, flaxmeal into the bread dough. By using this method, it will help the texture of the heavy dough. (44). The sponge dough method is a two-step process that includes:

- Sponge formation: During this part, the yeast is activated and fermentation occurs with the addition of bread flour. Fermentation occurs as the yeast ferments the sugars to form carbon dioxide and alcohol;
- Dough formation: The dough is kneaded to help form an elastic substance throughout the dough, which will help entrap carbon dioxide and in turn help with gluten development.

2.8.3 *Bread Staling*

Bread is characterized as a solid foam that is unstable and elastic. Since bread is an unstable food product it has a short shelf life. As a result, bread stales faster and is susceptible to mold growth and development of undesirable aromas, flavors and texture. Thus, additives (i.e. antioxidants) are used to extend the shelf life of bread products.

After bread is removed from the oven, the staling process begins. A research team (11) defined staling as “decreasing consumer acceptance of bakery products caused by the changes in crumb other than those due to spoilage organisms.” The staling of bread may be caused by starch retrogradation and the interaction between the bread ingredients, which may include flour, sugar, shortening, milk, salt, yeast, water, a mold inhibitor (calcium propionate) and an antioxidant. The staling process of the bread system at the molecular level is not completely understood due to the interactions between the bread ingredients.

2.9 Enzymes: Lipoxygenase and Lipase

In addition to staling, bread is also susceptible to (oxidative) rancidity due to the deterioration of fat by lipid oxidation. Lipid oxidation can also be caused by enzymes (i.e. lipoxygenase). Since flaxmeal has a high amount of unsaturated fatty acids, it contains double bonds which are vulnerable to oxygen attack. The rate of lipid oxidation is related to the degree of unsaturation, such that, a higher degree of unsaturation increases the rate of lipid oxidation.

Lipid oxidation consists of three phases: initiation, propagation and termination. Lipid oxidation is initiated by a fatty acid, such as linoleic acid. During initiation, free radicals are formed which lead to the formation of hydroperoxides, which is unstable. Hydroperoxides decompose to form compounds, such as hexanal, pentanal and malonaldehyde which are responsible for off-flavors and aromas.

To help extend the shelf of breads, additives and preservatives, such as antioxidants (i.e. BHA, BHT and ascorbic acid) are added to the bread doughs prior to baking. Antioxidants help extend the shelf life of bread by interfering with the initiation stage, so that secondary oxidation products are not formed (64).

2.9.1 Lipoxygenase

Lipid oxidation in oilseeds can be caused by several factors, such as, light, air, high temperature, enzymes, microorganisms, trace metals and the presence of free fatty acids (80). One enzyme that causes lipid oxidation is lipoxygenase. Lipoxygenase is an enzyme present in plant (30) and animal tissues, such as fish and shellfish. Lipoxygenase also “catalyzes the oxidation of linoleic and linolenic acids, esters and triglycerides leading to the formation of aldehydes, ketones and alcohols which are responsible for beany flavor in products like soy milk (85).” Since flaxseed contains a high amount of alpha-linolenic acid, flaxseed is susceptible to lipid oxidation by lipoxygenase. Thus, foods that contain lipoxygenase may undergo oxidative rancidity. One author (28) noted that lipoxygenase is concentrated in the germ of cereal grains and reacts quickly when water is mixed with the cereal grain. This is due to the rapid uptake of oxygen due to lipoxygenase-catalyzed oxidation of the linolenic and linoleic acids. The results of lipid oxidation are fatty acid hydroperoxides which are converted to hydroxyl fatty acids (73), ketones and aldehydes (80) and other aromatic compounds. These degradative compounds, in turn, may affect color, texture, functionality and the nutritive value of foods (60).

Extensive research concerning lipoxygenase in soybeans has been conducted, whereas, research on lipoxygenase content in flaxseed has been limited. However, a recent study by Oomah (60) tested the effects of environmental conditions, specifically, cultivar, location and year interaction on the lipoxygenase content of the flaxseed samples. The results of this research were:

- There was a significant difference in lipoxygenase content of defatted flaxseed meal between cultivars. For example, the mean lipoxygenase content ranged from 1.6g/kg for the cultivar NorLin to 6g/kg for the cultivar Linola 947 (the solin-type flax). Thus, “lipoxygenase content in flaxseed was cultivar specific;

- The effects of the environment (year and location) on cultivars were studied. The researchers concluded that there was a significant relationship ($p < 0.0001$) between environmental effects (year and location) and cultivar with respect to lipoxygenase content.

Oomah (60) concluded that a “large variability exists in the lipoxygenase content of flaxseed cultivars.” As a result, the lipoxygenase enzyme can be altered using breeding programs. The reduction of lipoxygenase levels in flaxseed may help improve the nutritive value, storage stability and utilization of flaxseed. Therefore, the positive effects of the omega-3-fatty acids (e.g. protective functions related to coronary heart disease) may be enhanced with decreased levels of lipoxygenase.

2.9.2 Lipase

Another enzyme that can cause rancidity in foods is lipase. Lipase is an enzyme responsible for catalyzing the hydrolysis of fat to produce free fatty acids and glycerol (55). These free fatty acids may affect the aroma and flavor in the foods. The series of reactions that result in the formation of free fatty acids and glycerol is known as hydrolytic rancidity. Hydrolytic rancidity in cereal (grains) is important for two reasons:

- The polyunsaturated free fatty acids formed during hydrolytic rancidity “are the precursors of both volatile and non-volatile off-flavored (28).
- Free fatty acids have negative effects on the functional properties of many cereal based products (28).

Wheat (bran) lipase is concentrated in the bran and bran contains a higher amount of free fatty acids than wheat germ. Since wheat germ contains a significant amount of oil, it is unstable and accumulates free fatty acid. Thus, a mixture of germ and bran is more unstable than bran alone (28).

Bran lipase is active at moisture levels below 5% while most enzymes in normal milling products are active between (10-15%). Research (28) showed that bran lipase has a maximal activity when the water activity (A_w) = 0.85 (or 17% moisture). As a result, free fatty acids accumulate in unstabilized milling products. At normal moisture levels (10-12%) bran lipase is heat stable. It was shown that wheat bran can be held at 80°C for several days without affecting lipase activity (28). However, lipase activity was inactivated within 10 minutes at 100 °C in a 50/50 w/w bran-water mixture and when autoclaved at 15 psi for 10 minutes (28).

Oats, like flaxseed have a high amount of unsaturated fatty acids (49) and have high lipase content. When oats were cooked at 90-100 °C for a few minutes at a moisture level greater than 12%, lipase activity is inactivated. However, if oats are not heat treated, the amount of free fatty acids formed reach an unacceptable level within 2-3 days.

Researchers (49) conducted a study to evaluate heat treatment on lipid stability in processed oats. The authors found “that the lower the residual lipase activity in whole kernels or kernel fractions, the higher was the oxidation of lipids and evolution of volatile oxidation products during prolonged storage of the dry fractions (49).” When the lipase activity was totally eliminated through heat treatment, “the amount of headspace hexanal detected after 12-months of storage was 5 to 7 times larger than in the non-heat treated bran (49).” The authors noted that the oxidation of polar lipids was related to the hexanal formation. However, if heat treatment was not used, “the oxidation of unsaturated fatty acids in polar lipids did not occur even during prolonged storage (49).”

2.10 Antioxidants

The use of antioxidants may help decrease lipoxygenase levels in oilseeds. Food antioxidants are a group of chemicals that are used to help extend the shelf-life of various food products. Natural antioxidants are present in foods, but upon processing or storage, these antioxidants are inactivated. As a result, synthetic antioxidants have been added to foods. Before antioxidants (natural or synthetic) can be incorporated into foods, several requirements must be fulfilled. These requirements are as follows (64):

- “The antioxidant should be soluble in fats;
- it should not impart foreign color, odor, or flavor to the fat even on long storage;
- it should be effective for at least 1 year at a temperature of 25-30°C;
- it should be stable to heat processing and protect the finished product (carry-through effect);
- it should be easy to incorporate; and
- it should be effective at low concentrations.”

2.10.1 Synthetic antioxidants: BHA and BHT

The shelf stability in foods can be extended with the use of antioxidants. The most common antioxidants that are used in food products are BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene). BHA and BHT are examples of synthetic antioxidants and are also considered primary antioxidants (Figure 4). The functions of primary antioxidants are:

- donating a hydrogen or electrons to free radicals to terminate the free-radical chain reaction and form more stable products (3),
- reacting with lipid radicals to form a lipid-antioxidant complexes,
- reacting with a lipid-free radical to delay or inhibit the initiation step, and

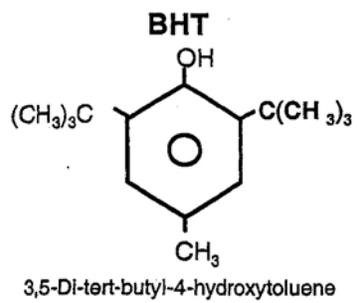
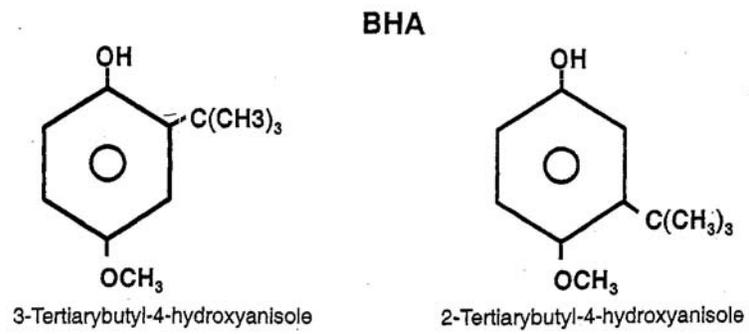


Figure 4: Structures of BHA and BHT ⁽¹⁶⁾

- reacting with the peroxy or alkoxy radicals to help delay the propagation step

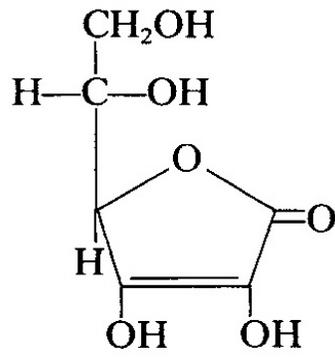
BHA (tertiary-butyl-4-hydroxyanisole) is a white waxy solid that is a mixture of two isomers; 2-tertiary-butyl-4-hydroxyanisole (2-BHA) and 3-tertiary-butyl-4-hydroxyanisole (3-BHA) while BHT is a white crystalline solid that is also known as 3, 5-Di-tertiary-butyl-4-hydroxytoluene. BHT and BHA have similar characteristics, such as, good carry-through properties (i.e. the ability of an antioxidant to survive a processing step to impart stability to the finished product) and can be used in packaging materials (3; 16). BHA enhances the stability of baked goods due to its stability at pH values above 7.0 and has been shown to work synergistically with BHT, propyl gallate and ascorbic acid. When BHA was used with BHT and propyl gallate, it was shown to increase the stabilities of dry breakfast cereals, wheat germ meal and rice bran. A combination of BHA and ascorbic acid helped retard “lipid and pigment oxidation in raw ground beef for up to 8 days of refrigerated storage in oxygen-permeable film (32).” BHT can be used in dry breakfast cereals, chewing gum base, sausage products and snack foods. It is known that when BHA and BHT are combined, it provided better protection against oxidation than when either antioxidant was used alone (16). For example, researchers (81) showed that a combination of BHA and BHT helped stabilize walnuts (shelled), peanuts and peas.

2.10.2 Natural antioxidants

Natural antioxidants can also be used to increase the shelf life of foods. The shelf stability in foods can be extended with the use of antioxidants. Some natural antioxidants include ascorbic acid (Vitamin C), citric acid, spices and herbs (e.g. rosemary and sage). Ascorbic acid (Figure 5) can function as an antioxidant, a metal chelator, or as an oxygen scavenger (27). As an oxygen scavenger, ascorbic acid removes oxygen from food and results in the formation of dehydroascorbic acid from the oxidation of ascorbic acid. Rosemary extract has been shown to have natural antioxidant properties by inhibiting oxidation in salad dressings, poultry products, and processed food. Other sources of natural antioxidants are sage, yam flour and extracts from amla, drumstick leaves and raisins.

The use of natural antioxidants in foods has been on the rise due to increased health concerns of consumers and increased popularity of natural food products. The other benefits of incorporating natural antioxidants are the use of a “natural food” label and the amount used in foods is not limited, unlike synthetic antioxidants.

As with BHA and BHT, ascorbic acid is more effective when used in combination with another antioxidant. When BHA and ascorbic acid were used together, it helped retard “lipid and pigment oxidation in raw ground beef for up to 8 days of refrigerated storage in oxygen-permeable film (32).”



L-Ascorbic acid
(Vitamin C)

Figure 5: Structure of L-Ascorbic acid (Vitamin C) ⁽⁷⁶⁾

2.10.3 Use of Antioxidants in Foods

Researchers (64) have noted several antioxidants that are used in foods, such as ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), citric acid and propyl gallates. Each of these antioxidants is an example of different kinds of antioxidants, which are grouped together based on their function.

The two main groups of antioxidants are primary antioxidants and secondary/synergistic antioxidants. Primary antioxidants include phenols (e.g. gallates) and hindered phenols (e.g. BHA and BHT) while secondary/synergistic antioxidants include oxygen scavengers (e.g. ascorbic acid) and chelating agents (e.g. citric acid).

Several studies have been conducted to determine the effectiveness of various antioxidants on lipoxygenase activity in soy products. Researchers (85) studied the effects of various levels of antioxidants (i.e. propyl gallate, BHA and BHT) with acidic compounds, such as, ascorbic acid, EDTA, citric acid and ascorbic acid) on the lipoxygenase levels in soy milk. Two levels of pH (pH=6.8 and pH-9.0) were used during the study because soybeans contain three different isozymes (i.e. L-1, L-2 and L-3) that are active at pH 9.0 and pH 6.8 (L-2 and L-3) respectively. The researchers (85) concluded that:

- “propyl gallate was the only antioxidant to become increasingly effective as the concentration was raised from 320 to 640mg/liter and it showed the best inhibition of the antioxidants used in the study;”
- At pH 6.8, the activity of L-1 was inhibited when ascorbic acid and citric acid were combined with propyl gallate (i.e. when ascorbic acid and propyl gallate were used together and when citric acid and propyl gallate were used together).”

The authors noted that ascorbic acid and citric acid are known to have synergistic effects with phenolic antioxidants and are common ingredients that can be added to soy milk. Also, when propyl gallate is combined with either ascorbic acid or citric acid lipoxygenase activity in soy milk may be controlled.

Lee and Lillard (48) evaluated the effects of esculetin on lipoxygenase activity in soybean extracts. Esculetin is found in the barks of *Aesculus hippocastanum* and *Fraxinus japonica Blume* and was found to inhibit lipoxygenase activity in rat platelets (77). The purpose of this study was “to determine the concentration of esculetin required to inhibit lipoxygenase activity in soybeans and to explain the mechanism of its ability to inhibit lipid oxidation (48).” Three concentrations of esculetin were combined with soybean extracts to determine the relationship between the concentration of esculetin and its effect on lipoxygenase activity. These concentrations were 1.8×10^{-4} M, 3.3×10^{-4} M and 5.7×10^{-4} M. Also, the inhibition effects of esculetin were

compared with two antioxidants, BHA and α -tocopherol. BHA or butylated hydroxyanisol is an example of a phenolic compound and is commonly used as a synthetic antioxidant because of its ability to donate a hydrogen and form a stabilized radical to inhibit lipid oxidation (48). Due to BHA's good carry-through properties, it has been used for the stabilization of fats in baked and fried products (92). Alpha tocopherol is also a phenolic compound, but it is an example of a natural antioxidant that has been shown to delay the decomposition of hydroperoxides. Its role as an antioxidant is "by donating the hydrogen of the hydroxyl group to the lipid peroxy radical (92)."

The main results of this study were:

- Increased concentrations of esculetin caused a decrease in lipoxygenase activity. Specifically, there was a significant difference ($p < 0.05$) between the 1.8×10^{-4} M esculetin solution with the other two esculetin solutions and this solution had the least effect on lipoxygenase activity;
- The comparison between esculetin, BHA and α -tocopherol on their effects on lipoxygenase inhibition showed that esculetin had a significant effect ($p < 0.05$) on lipoxygenase activity.

The authors noted that esculetin's possible role as a reducing agent may be the reason for its ability as an effective inhibitor of lipoxygenase activity. Thus, as a reducing agent, it "reduces the catalytic active ferric lipoxygenase to the inactive ferrous form (48)." The authors concluded that esculetin has a radical scavenging property that inhibits lipoxygenase activity, but further research is needed to determine esculetin's inhibition mechanism.

2.11 Measuring Antioxidant Activity

As mentioned earlier, antioxidants have been used in various food systems as protective agents against rancidity. The factors that can affect the activity of an antioxidant are temperature, food composition, food structure, processing, storage and the availability of free oxygen (30). Thus, the effectiveness of antioxidants can be measured using several tests, such as peroxide value, p-anisidine value, and TBA (thiobarbituric acid value).

The peroxide value test is one of the most common chemical methods used to measure the oxidative deterioration of oils, but it should be used in conjunction with another test, such as para-anisidine to get the full picture of the oxidation process. This test usually involves, the titration of an oil sample containing potassium iodide in a chloroform or isooctane/acetic acid mixture that results in the hydroperoxides. These hydroperoxides, in turn, oxidizes iodide to iodine, which is determined by how much sodium thiosulfate is used during the titration (30).

The para-anisidine (*p*-anisidine) value test is usually used in conjunction with the peroxide value test. Para-anisidine is a useful reagent because it will react with aldehydes and yield oxidation products with an absorbance at 350nm. In this test, one gram of a sample of fat is mixed in a isooctane solution (100mL) with *p*-anisidine (0.25% in glacial acetic acid) and the absorbance of this solution is measured. The oxidation products that result from this test are unsaturated aldehydes (2-alkenals), which absorb strongly at an absorbance of 350m. Thus, this test is used because of its sensitivity to these oxidation products. However, this test is not able to differentiate between volatile and non-volatile products. As a result, this test is usually used along with the peroxide value to help describe the total extent of oxidation by the Totox value. The non-volatile carbonyls present in processed oils, as well as, any other oxidation compounds developed during storage are measured using the Totox value which is determined using the following equation (72):

Equation1:

$$\text{Totox value} = \text{Anisidine Value} + 2\text{PV (Peroxide value)}$$

Another test that is useful in measuring antioxidant activity is the thiobarbituric acid (TBA) value test. In this test, thiobarbituric acid is used to react with malonaldehyde, which in turn “form red condensation products that absorb between 532-535nm (30).” The problem with this test is that the reaction is not specific and other factors may influence the absorbance. For example, 2, 4- alkadienals have a strong absorption at 532nm and food components, such as proteins, Maillard browning products and sugar degradation products. As a result, the values of this test are known as TBA reactive substances or TBARS.

Chapter 3: Justification and Purpose of the Study

The current recommendation by the American Dietetic Association (6) is to consume 6-13 ounce equivalents per day (depending on your caloric needs) from the grains group with at least 3 ounce equivalents from whole grain products and the other 3 ounce equivalents from other types of grains. Overall, these foods provide complex carbohydrates, vitamins, minerals and fiber. Thus, foods from the grains group are an important part of a balanced diet. However, popular low carbohydrate diets, such as the Atkins diet, suggest consuming fewer refined carbohydrates and limiting the consumption of sugary foods, breads, pasta, and starchy vegetables in one's diet. As a result, people who are on the Atkins diet or similar diets tend to consume less foods with carbohydrates. Thus, these people will consume less vitamins, minerals and fiber. However, the role of vitamins, minerals and fiber are important in maintaining overall health. By increasing the awareness of the role that carbohydrates play in foods and health, the use of different grains in foods, such as flaxseed to increase fiber, may promote carbohydrate consumption.

Flax (*Linum usitatissimum*) is an annual, herbaceous blue flowering plant that is primarily grown in Canada, but is also grown in Argentina, China, India, Poland, Romania, Russia, Uruguay and USA (North Dakota and Minnesota). Flaxseed can be identified by their variety (Table 1) and their seed color. The seed color is determined by the amount of pigment in the outer seed coat-the more pigment, the darker the seed. The two colors of flaxseed are brown and yellow. Brown-colored flaxseed is the most common flaxseed grown in Canada and is high in alpha-linolenic acid (ALA) while there are two types of yellow-colored flaxseed: omega and solin. Omega has a high level of ALA like brown-colored flaxseed while solin has a low level of ALA. In addition to ALA, flaxseed also contains linoleic acid (an omega-6-fatty acid), lignans, dietary fiber, proteins, minerals and vitamins. Alpha-linolenic acid (ALA) is a polyunsaturated fatty acid (omega-3 fatty acid) while lignans are types of phytoestrogens; both have been shown to share protective roles against cancer and cardiovascular diseases. Another health benefit of flaxseed is that it is a good source of fiber which in turn helps maintain blood glucose and lowering of blood cholesterol. Flaxseed is also a very good source of proteins, vitamins, and minerals which help maintain normal body functions.

Flaxseed has several functions: as a food ingredient, an ingredient in animal and poultry feeds, a diluent (linseed oil) in paints and coatings, and in paper products (flax fiber). As a food ingredient, flaxseed can be added to or incorporated in several products, such as, ready-to-eat-cereals, breakfast drinks, salad toppings, biscuits, muffins, meat extenders, crackers, soups, bagels and pastas. Flaxseed can also be used in baked goods, such as bread, because of its minimal loss of alpha-linolenic acid during baking. However, due to its

high levels of ALA, flaxseed is susceptible to lipid oxidation by autoxidation and/or via lipoxygenase activity. Both processes result in the formation of carbonyl compounds, alcohols, aldehydes and ketones, which result in off-flavors and aromas. However, antioxidants have been used to help slow down these detrimental effects in foods. Antioxidants are a group of chemicals that are used to help extend the shelf-life of various products. Some common antioxidants that are utilized in food products to halt rancidity are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and ascorbic acid.

In this study, ascorbic acid, BHA, BHT, and a combination of BHA and BHT were incorporated into the flax meal breads to evaluate their effectiveness in preventing oxidative rancidity. Each antioxidant is known to have good-carry through properties in baked goods and a synergistic relationship exists between BHA and BHT (i.e. their combination in retarding lipid oxidation is more effective together than when each antioxidant is used separately). Thus, the utilizing antioxidants in foods will help increase the shelf life of a product. Therefore, the objectives of the study were:

- to examine the effect of flaxseed on the baked quality of the yeast bread;
- to examine the effect of various antioxidants, BHA, BHT and ascorbic acid on preventing rancidity in the flax yeast bread;
- to determine the acceptability of the flax yeast bread through sensory analysis.

Chapter 4: Materials and Methods

4.1 Experimental Design

Bread samples were made in one of the food labs in the HNFE Department at Virginia Tech. The antioxidants that were used were ascorbic acid (DNP, China), 3-butylated hydroxyanisole (BHA) (Eastman Chemical Company; Kingsport, TN), and butylated hydroxytoluene (BHT) (Eastman Chemical Company; Kingsport, TN). The effectiveness of these antioxidants in maintaining the shelf life of the flax meal bread was determined. A combination of BHA and BHT was used to evaluate their synergistic effect. For each antioxidant, approximately 0.01% (of the total weight of the bread) was used in the bread samples. In the bread samples with both BHA and BHT, 0.005% of each antioxidant was used in those bread samples. Six types of breads were used: control (100% bread flour), yeast bread with 15% flaxmeal and yeast breads with 15% flaxmeal that contained individually: BHA, BHT, BHA and BHT, and ascorbic acid. The study was designed as a randomized block design. A total 144 bread samples (48 * 3 subsamples) were made within a one week period. Then, the bread samples were stored in plastic containers for 8 weeks at room temperature and were removed each week to determine quality aspects using objective and sensory analyses. Calcium propionate (Niacet Corporation, Niagara Falls, NY) was used in the bread samples to avoid development of mold over the testing period. Figure 6 is a flow chart to illustrate the steps followed during this study.

4.2 Sponge-Dough Method

A modified version of the sponge-dough method was used to make yeast breads that contained flax meal. The formula used to make the flax meal bread was adapted from a procedure from the American Association of Cereal Chemists (7) (Appendix A). The sponge-dough method involves a two-step process that includes the formation of a sponge and then the formation of the dough. In the first part, a sponge is formed by combining part of the granulated sugar (Domino Foods, Inc; Yonkers, NY) and water with active dry yeast (Fleischmann's®, Burns Philip Food, Inc; Fenton, MO) in a mixing bowl, and then the mixture is activated for five minutes. Next, a portion of the bread flour (King Arthur Flour Co; Norwich, VT) is combined with this mixture and then allowed to ferment for 1.5 hours. In the second part, a dough is formed by combining the salt (Morton® ionized salt; Rohm and Haas Co; Chicago, IL), vital wheat gluten (Hodgson Mill, Inc; Effingham, IL), flax meal (15% of the total amount of bread flour) (Bob's Red Mill Natural Foods, Inc; Milwaukie, OR), the balance of the sugar, bread flour, water, and an antioxidant or combination of antioxidants (BHA, BHT, and ascorbic acid) and the sponge from the first part. These ingredients are mixed with a dough hook for

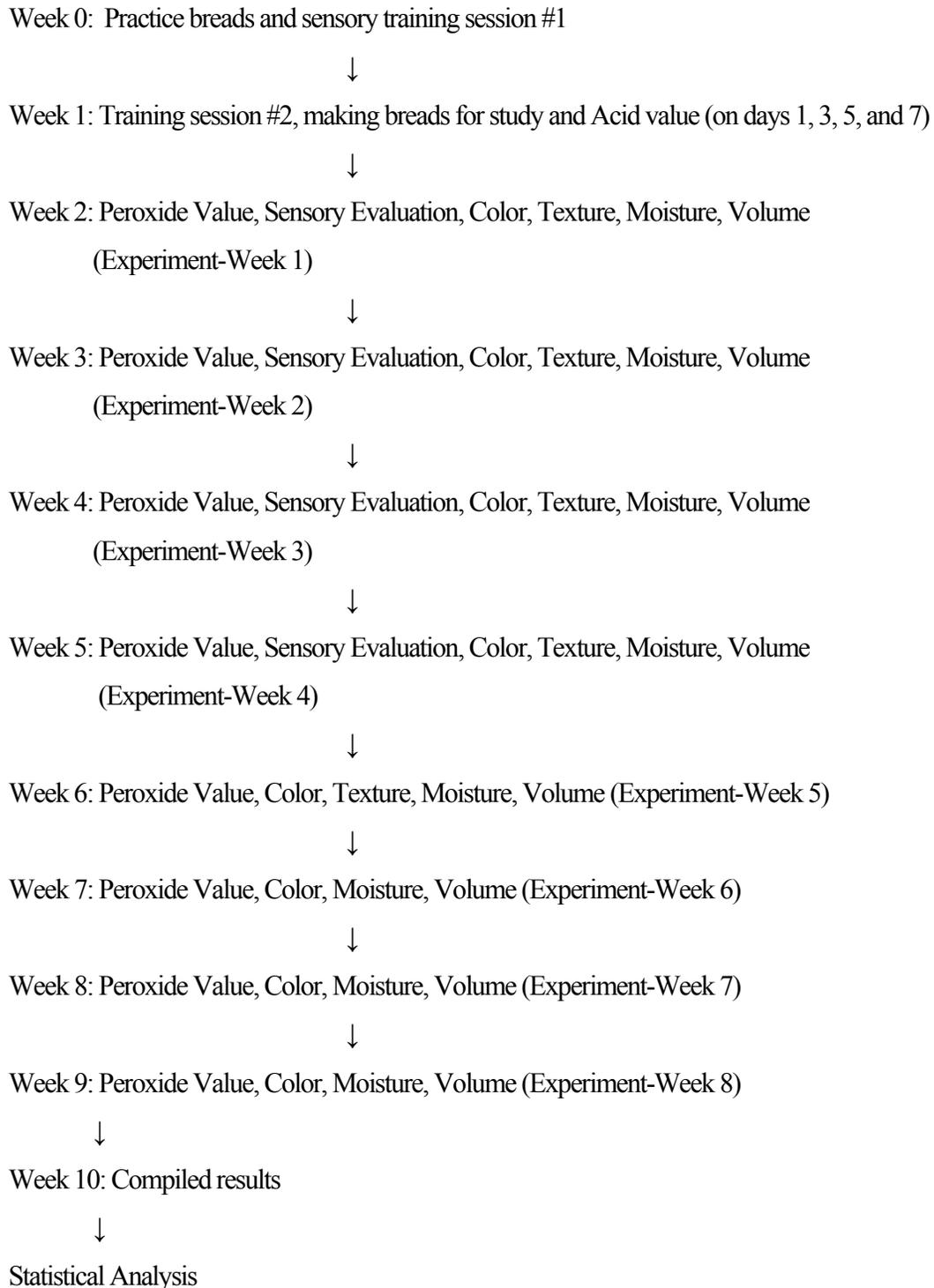


Figure 6: Flowchart: Sequence of steps followed during this research study

thirteen minutes and then placed in a greased bowl. The bowl is covered and the mixture is allowed to ferment for an hour or until a fingerprint remains in the dough. After the dough has fermented for an hour, the dough is divided into three equal parts (about 185g for each loaf). Each part was rolled and shaped into loaves and placed in a pup pan, then allowed to rise for an hour in a Servolift Eastern proofing oven (Boston, MA) prior to baking in a preheated (425°F) oven. After the oven is preheated, all the loaves were baked for twenty minutes. Loaves were cooled thoroughly for 3 hours and placed in polyethylene bags and stored at room temperature for sampling.

4.3 Lipid Extraction and Gas Chromatography

The chemicals that were used in this procedure were purchased from Fisher Scientific (Fisher Scientific International; Pittsburgh, PA) while the internal standards used for the gas chromatography were purchased from Sigma (Sigma-Aldrich, Inc; St. Louis, MO). An adapted version of the Folch Method (26) was used to extract the total lipids and triglycerides were extracted from the flaxseed. During lipid extraction, one gram of flax meal was placed in a 50mL teflon tube and homogenized with 20 mL of chloroform:methanol (2:1). This mixture was then centrifuged for four minutes at 2000 rpm. After centrifugation, two layers were formed. The upper layer was discarded while the bottom layer was transferred to a new teflon tube. Next, the solution was washed with four mL of 0.9% NaCl solution, vortexed for one minute, and then centrifuged for four minutes at 2000 rpm. A mixture of chloroform: methanol: distilled water (3:48:47) was used to separate the layers. This step was repeated until the upper layer was separated from the bottom layer. Next, nitrogen was used to dry the bottom layer. When this was completed, 10 mg of lipid extract was transferred into a glass test tube. Then, one mL of boron trifluoride in methanol and three mg of C17 fatty acid standard were added to the glass tube. The extract was heated for 45 minutes at 100°C and then cooled for five minutes. Next, two mL of pentane and one mL of distilled water were added to the glass tube, vortexed for one minute and then centrifuged for four minutes at 2000 rpm. Layers were again separated. This time the top layer was dried under nitrogen and then dissolved in 500 uL hexane.

A Shimadzu Model GC14A (Shimadzu Corp; Columbia, MD) gas chromatograph was used in this study. A gas chromatography vial was filled with the solution and before it was sealed nitrogen was blown over the top of the seal. Next, the vial was injected into a gas chromatography auto-injector and run with internal standards to determine the total lipids. A SP2330 capillary column that was used was 30 meters long * 0.32 ID. The column reached a

temperature of 150-205°C at 5°C per minute while the injector temperature was 220°C and detector temperature was 230°C. The run time was set for 30 minutes. Flow rates for helium, make up gas, air and hydrogen were 1ml/min, 50ml/min, 300ml/min and 30ml/min, respectively, with a split ratio of 1:8. The sensitivity was 10^{-1} and the attenuation was six. The internal standards used were five mg of C17 (heptadecanoic acid) while rapeseed oil was the standard used for identifying the fatty acid profile and elution times. A mixture containing a solution of 125 mg of standard and 25 ml of chloroform was used to dilute the internal standards. Five mg of this solution was then placed in a one ml aliquot for auto-injection.

4.4 Chemicals Tests

Acid value and peroxide value were two chemical tests used in this study. Acid value was used to detect the presence of free fatty acids while peroxide value was used to detect the presence of hydroperoxides in the breads.

4.4.1 Acid Value

A piece of flax meal bread (without the crust, about 5g) was broken down into pieces and placed in a plastic tube with 20mL of chloroform and 10mL of methanol and homogenized using Kinematica -Polytron homogenizer (Switzerland). A blank was conducted simultaneously. Next, this mixture was filtered through a Whatman No. 4 filter paper into a beaker. Then, 5 g of the filtered flaxmeal solution was measured into a 250mL Erlenmeyer flask. Fifty milliliters of an ethanol-petroleum ether solution (1:1 v/v) and 3 drops of phenolphthalein solution were added to the filtered solution. This mixture was titrated with 0.1N alcoholic (ethanol) potassium hydroxide solution until a permanent faint pink color appeared. The following is the equation that was used to calculate acid value (Equation 1) where mL of alcoholic KOH used in the titration, normality = 0.1 and weight of oil sample = 5.00 ± 0.05 g.

Equation 2:

$$\text{Acid Value} = \frac{\text{mL alc. KOH solution used in the titration} * \text{normality of alc. KOH solution} * 56.1}{\text{Weight of oil sample in grams}}$$

4.4.2. Peroxide Value

The rancidity test that was used was the peroxide value (acetic acid-chloroform method) and the procedures that will be followed are adapted from the AOCS Official Method Cd 8-53 (9) and the Folch method (26).

A piece of flax meal bread (without the crust, approximately 10g) was broken down into pieces and placed in a plastic tube with 20mL of chloroform and 10mL of methanol and homogenized using Kinematica -Polytron homogenizer (Switzerland). A blank was conducted simultaneously. Next, this mixture is filtered through a Whatman No. 4 filter paper into a beaker. Then, 10g of the filtered solution was measured into a 500mL Erlenmeyer flask and combined with 30mL of acetic acid and 20mL chloroform. Next, a potassium iodide solution was made by mixing 10g of potassium iodide in 6.0mL of water and placed in a brown glass bottle and then placed in the dark for two minutes. After that, 1mL of the potassium iodide solution was added to the filtered solution in the Erlenmeyer flask and mixed rapidly for 15-20 seconds; the mixture was allowed to stand in the dark for two minutes. When two minutes were complete, 100mL of distilled water was added to the Erlenmeyer flask, mixed, and then titrated with a 0.1N sodium thiosulfate solution until the solution became a pale yellow. Once the solution turns a pale yellow color, several drops of a starch solution were added and titrated until the blue color disappeared. The following equation was used to calculate the peroxide value (Equation 3) where S = mL of sodium thiosulfate used to titrate fat sample, B = mL of sodium thiosulfate to titrate blank, N = normality of sodium thiosulfate, or 0.1, and W = weight of sample, or ten grams.

Equation 3:

$$\text{Peroxide Value (PV)} = \frac{(S - B) * N * 1000}{W}$$

4.5 Physical Tests

Moisture, color and volume were objective tests used to measure the effects of flaxmeal and antioxidants on the quality characteristics of bread during storage (over an eight week period). A Stevens LFRA Texture Analyzer (Scarsdale, NY) was used to measure the texture of the bread samples were during a five week period.

4.5.1 Moisture

The moisture levels of the bread samples were measured using a Brabender Moisture Tester Analyzer SASS 692 (C.W. Brabender Instruments, Inc., South Hackensack, New Jersey). The crumb of the bread

samples were cut into small pieces approximately ½” by ½”, then placed in a Teflon-lined metal pan with a fork and weighed to 10 grams with a top loading balance (Fisher Scientific XL-500 Top Load Balance #13028824, Denver Instrument Company, Arvada, CO). Also, the Brabender moisture tester analyzer was heated for one hour and set to 130°C prior to analysis. Then, the sample pans were placed into the Brabender and the door latched. The bread samples were dried for an hour. After the samples were dried, each bread sample was weighed again. The difference between the pre-drying weight post-drying weight was divided by the original weight (before drying) and multiplied by 100 to determine the percentage of moisture in the bread samples, such that:

Equation 4:

$$\text{Percent (\%) Moisture} = \frac{\text{Weight of sample before heating} - \text{Weight of sample after heating}}{\text{Weight of sample before heating}} \times 100$$

4.5.2 Texture

The texture of the bread samples were measured by the Stevens LFRA Texture Analyzer (Scarsdale, NY). The probe, TA-9, was programmed to travel a distance of 5mm with a speed of 2mm/sec with a normal cycle. The readings were made by cutting the bread samples, so that each sample is ½ inch thick. Ten samples from each type of bread for each week (for five weeks) were used during the study.

4.5.3 Color

The color of the flax meal bread samples was measured using the Minolta Colorimeter CR300 Series (Mahwah, NJ). Hunter L, a, and b values were measured. The L value differentiates the varying levels of black (L=0) and white (L=100) while the “a” and “b” values is determined by the value. For example, when the “a” value is positive (+), then the color is red while a negative (-) a value suggests that the color is green. Also, when the “b” value is positive (+), then the color is yellow while a negative (-) b value suggests that the color is blue (Table 2). Ten slices (½ inch slices) from the inner portion of the bread were used for the color samples. Then, the ten measurements were averaged.

4.5.4 Volume

A volumeter was used to measure the volume of the bread samples. A plastic wrap was used to cover the bread before it was placed in the volumeter. Volume was measured by using displacement with rapeseeds.

Table 2: Color Values

Color	Minolta Colorimeter CR-300 series (L, a, and b values)
Red	is a positive (+) a
Green	is a negative (-) a
Yellow	is a positive (+)b
Blue	is a negative (-)b
Black	L=0
White	L=100

4.6 Sensory Evaluation

The sensory qualities and of the bread products were determined using a Quantitative Descriptive Analysis (QDA). Eight panelists were selected to evaluate the bread samples. There were two-1 hour sessions to train the panelists on selecting the attributes that were used to evaluate the bread samples. During these sessions, the panelists were informed about the study and their responsibilities as panelists.

The first session consisted of instructing the panelists about sensory evaluation procedures, the significance of sensory analysis to the study, and then the panelists tasted the flax bread samples to derive the descriptors that were to be evaluated. The panelists also signed consent forms (Appendix B). At the second session the panelists became familiar with the derived scorecard (Appendix C) using the descriptors from the first session.

The panelists evaluated the bread samples once a week for four weeks. The panelists were seated in a sensory booth in Wallace 335. Red clearphane film (Highlander; Highland, IL) was placed over the sensory lights, so that the panelists could not discriminate between bread samples due to color. The samples were prepared prior to the panelists' arrival to prevent drying of the bread samples. The samples were prepared in a separate room, so that the panelists were unaware of sample order. A random three-digit number was assigned to each group of bread samples every week. The panelists were given one slice of bread from each group, with a total of six slices per panelist. The panelists were instructed not to converse during the sensory test to help prevent bias. The panelists evaluated each bread sample using the chosen descriptors and then the data was quantified for each descriptor. Data was quantified by measuring from left to right on the line where the panelist marked when making his/her decision.

4.7 Statistical Analysis

The experimental design for this study was determined by a consulting team within the Statistics Department of Virginia Tech. SAS® (Statistical Analysis System) (SAS Institute, Inc.; Cary, NC) was used for the statistical analysis. The consulting team helped analyze the statistical results throughout the study.

The tests for color, volume, peroxide value and acid value, volume and texture were analyzed by a completely randomized design using analysis of variance (ANOVA). For this group of data, a general linear model (GLM) was used and a Bonferroni adjustment was made to the data. The data from the sensory evaluation was analyzed by a randomized complete block design using analysis of variance (ANOVA). The judges and time were the blocks for this design. For this group of data, a mixed procedure was used and a Bonferroni adjustment was made to the data. All data was analyzed by comparing the effects of the antioxidants.

The mean values recorded for each test (i.e. texture, moisture, volume, color, acid value, peroxide value, and sensory evaluation) were compared using a one-way analysis of variance (ANOVA). Significance was determined using a p-value of less than or equal to 0.05 ($p \leq 0.05$). The least significant differences (of each test from each week and from each bread type) were compared using the Tukey-Kramer test while the data from the sensory evaluation was compared using The GLM procedure and Tukey-Kramer test.

Chapter 5: Results and Discussion

5.1 Texture

Staling (11) has been defined as “a term which indicated decreasing consumer acceptance of bakery products caused by a change in crumb other than those resulting from the action of spoilage organisms.” The staling of bread may be caused by starch retrogradation, cross linking between partially solubilized starch and gluten, partial drying and glassy-rubbery transition. Bread undergoes several changes as it stales, such as crumb firming, moisture changes, crust softening and flavor losses and changes.

Starch is composed of amylose (linear molecules) and amylopectin (branched molecules) which are both composed of D-glucose. However, the structural differences between amylose and amylopectin result in different chemical and physical properties which will affect their behavior during baking. As bread bakes, starch granules swell and amylose diffuses out due to the gelatinization of the starch. After the bread is removed from the oven and cooled to room temperature, the bread begins to stale.

Amylose recrystallizes (or retrogrades) rapidly when it cools after gelatinization while amylopectin retrogrades slowly during storage to make the crumb firmer. The firmness in bread texture increases as bread stales over time. In this study, the texture of the bread samples was measured over a five week period. The texture was measured as the amount of force (load grams) that was needed to penetrate the surface of the bread samples. The more force that was required to penetrate the bread samples, the firmer the bread. As bread stales, it undergoes textural changes, such as a softer crust and firmer crumb. The staling of bread may be due to starch retrogradation (10) which involves the interaction between amylose and amylopectin. In general, the control (regular yeast bread) samples were firmer than the experimental bread samples (which includes the flaxmeal control samples). When the control samples were compared to the experimental samples, the experimental samples were significantly ($p \leq 0.05$) softer (Tables 3a & 3b). When the flaxmeal control samples were compared to the experimental samples there were no significant differences in firmness (Tables 3a & 3b).

Protein content of flour has been cited as a factor affecting crumb firmness (33). Flaxseed contains 20 g protein in 100 g of dry flaxseed (24). Researchers (41) suggested that an inverse relationship exists between protein content and staling during storage. It has been suggested (91) that high levels of protein may have an anti-firming effect. Due to the high protein content in flaxseed, the flaxmeal bread samples were softer than the control (regular yeast) bread samples.

Table 3a: The texture mean (n=30) and standard deviation (texture mean) of all bread samples during a 5-week period

Type of bread	Texture mean	Standard deviation (Texture mean)
Regular Yeast (Control 1)	964.3	284.9
Ascorbic acid	643.5*	184.9
BHA	675.8*	207.1
BHT	631.4*	143.8
BHA and BHT	744.8*	234.4
Flax (Control 2)	702.0	159.3

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

Table 3b: The comparison of texture mean between the control samples and experimental samples during a 5-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Significant	Not significant
BHA	Significant	Not significant
BHT	Significant	Not significant
BHA & BHT	Significant	Not significant

In this study, different antioxidants were evaluated to determine their effectiveness in preventing lipid oxidation and thus the onset of rancidity. However, the antioxidants used in this study also had an effect on crumb texture, such that there was a significant effect ($p \leq 0.05$) on the crumb texture between the experimental bread samples and the control (yeast bread) samples (Table 3a & 3b). Specifically, the experimental breads that contained either ascorbic acid or BHT were softer than the other experimental breads except during week 3 when ascorbic acid had a higher texture value. BHT is a synthetic antioxidant that has good carry-through properties in foods and is used in packaging materials. Thus, it is able to endure processing steps, such as frying or baking and imparts stability to the baked product (16). However, there has been controversy over the use of synthetic antioxidants due to its toxicity. In previous research, it was shown that a carcinogenic effect developed in rats that consumed high amounts of BHA and BHT (62). As a result, natural antioxidants, such as ascorbic acid are being used as a preservative in foods.

Bread is characterized as a solid foam that is unstable and elastic. Since bread is an unstable food product, it has a short shelf life. As a result, bread stales faster and is susceptible to mold growth and development of undesirable flavors and texture. Thus, additives such as antioxidants are used to extend the shelf life of bread products. In general, ascorbic acid had a significant effect on texture in breads during storage (Tables 3a & 3b). Early research (40) has shown ascorbic acid as a reducing agent, but in a later study it was shown that the oxidation product of ascorbic acid, dehydro-L-ascorbic acid functioned as an oxidant and flour improver (57). In a study conducted by Zentner (93) it was proposed that the softening effect of ascorbic acid may be due to the change in the water binding properties of gluten. Researchers (39) found that BHA and BHT reduced mixing requirements and ascorbic acid softened bread dough that was continuously mixed. In a study conducted by Gujral (33), ascorbic acid was added at 20ppm. The firmness of the bread samples decreased to 10.94N in fresh bread crumb and 34.44N in 72hr stored bread. Thus, it was shown that ascorbic acid reduced the extensibility and increased elasticity, giving a better shape and fine texture to finished breads (18). Gujral (33) also showed that wet gluten and ascorbic acid lowered the crumb firmness while barley flour increased firmness.

5.2 Moisture

The moisture content in bread changes as bread stales over time. The moisture content was expressed as the percentage of water present in the bread samples. Moisture content in bread is limited to 38 percent (31). It has been shown that the moisture content of bread influences the staling rate (78), such that, bread with

higher moisture content will have a slower staling rate. Thus, an inverse relationship exists between the moisture content and staling rate.

In this study, the moisture content of the bread samples was measured over an eight week period. There were significant differences ($p \leq 0.05$) in moisture between the control (regular yeast) bread and flaxmeal breads that contained BHA and BHT, separately (Tables 4a & 4b). There were no significant differences ($p > 0.05$) in moisture levels in the experiment bread samples when compared to regular flaxmeal bread (Tables 4a & 4b).

One of the most significant changes during baking is the redistribution of the water in the dough. Bread staling is due to the moisture transfer from crumb to crust and due to the starch recrystallization during storage (54). The firming of bread is due to the changes in physical orientation of branched amylopectin molecules of starch within the swollen granule. The amylopectin chains gradually come together and align with one another through intramolecular bonds. This results in an increase in rigidity of the internal structure of swollen starch granules which causes crumb hardening (61).

Based on the mean percent moisture (Tables 4a & 4b), it appears that the control (regular yeast bread) sample had the highest moisture content while the experimental breads that contained BHA and BHT (separately) had the lowest moisture content. Overall, these results would suggest that the regular yeast bread had a slower staling rate than the experimental breads due to its higher moisture content.

As a result, the regular yeast bread samples should be softer (i.e. lower texture values) than the experimental breads. However, the results from this study showed that the regular yeast bread samples were firmer than the experimental breads overall (Tables 3a & 3b). A research team (69) noted that the rate of firming is dependent on the type of bread, such that the firming rate of white bread is faster than variety breads. The researcher's findings agreed with the current data that the firming rate was faster in regular yeast bread than in the flaxmeal breads.

5.3 Volume

There were no significant differences ($p > 0.05$) in volume between both control samples and the experimental breads (Tables 5a & 5b). The sponge dough method was used to prepare the breads in this study. The sponge-dough method involves a two-step process that includes the formation of a sponge and then the formation of the dough. This method is commonly used to help improve the texture of heavy doughs (44).

Table 4a: The mean percent moisture (n=48) and standard deviation (moisture mean) of all bread samples during an 8-week period

Type of bread	Moisture mean	Standard deviation (Moisture mean)
Regular Yeast (Control 1)	33.53	0.0124
Ascorbic acid	32.58	0.0143
BHA	32.34*	0.0084
BHT	32.38*	0.0100
BHA and BHT	33.26	0.0110
Flax (Control 2)	33.38	0.0175

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

Table 4b: The comparison of moisture between control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Not significant	Not significant
BHA	Significant	Not significant
BHT	Significant	Not significant
BHA & BHT	Not significant	Not significant

Table 5a: The mean percent volume (n=48) and standard deviation (mean volume) of all bread samples during an 8-week period

Type of bread	Mean volume	Standard deviation (Mean volume)
Regular Yeast (Control 1)	1200	135.6
Ascorbic acid	1222	158.9
BHA	1209	116.4
BHT	1188	100.0
BHA and BHT	1209	71.88
Flax (Control 2)	1231	141.3

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

Table 5b: The comparison of volume between the control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Not significant	Not significant
BHA	Not significant	Not significant
BHT	Not significant	Not significant
BHA & BHT	Not significant	Not significant

Flour is one of the main ingredients used in making bread and is composed of two proteins, gliadin and glutenin. When flour is moistened and manipulated, gluten is formed. Gluten is responsible for the volume, texture and appearance of baked goods (44). In this study, flaxmeal was used as a partial (15%) replacement for bread flour. Therefore, there is 15% less flour used in the flaxmeal breads, which results in less gluten formation. Flaxmeal is a grain and grains interfere with gluten formation during kneading. Additionally, flaxmeal does not contain any gluten. As a result, vital wheat gluten was added to the flaxmeal breads to help increase gluten content and loaf volume. Due to lower gluten content, flaxmeal breads are expected to be smaller than the regular yeast breads. However, in this study, all the flaxmeal breads except the flaxmeal breads with BHT had higher loaf volumes than the control (regular) yeast bread (Tables 5a & 5b). This indicated that the addition of vital wheat gluten may have helped to increase the volume of the flaxmeal breads.

The main differences in the regular yeast bread and the flaxmeal breads were the ingredients used, such as flaxmeal, antioxidants and gluten. Each of these ingredients has been shown to have some effect when used in baked products. For example, due to its high protein content, flaxmeal may have an anti-firming effect, which results in softer breads. When ascorbic acid was added to a bread product, it was shown to reduce extensibility and increase elasticity, which help give a better shape to finished breads (18).

5.4 Color

Color is defined as *visual sensation* caused by the *perceived* wavelength of light reflected off objects. The brown color produced in baked products, particularly on the crust of breads, muffins and cakes is due to Maillard browning or Maillard reaction. Maillard browning is an example of nonenzymatic browning that involves the combination of an amino group (-NH₂) from a protein and an aldehyde group (-CHO) from a reducing sugar, which then leads to the formation of many complex substances (12).

In this study, the crumb color of the bread samples was measured over an eight week period. Only the Hunter L and Hunter b values were reported since these values are commonly used when evaluating the color of baked goods. Figure 7 shows how the Hunter L-values were higher in the regular yeast breads than all of the flaxmeal breads while the same was seen for Hunter b-values (Figure 8). Specifically, the regular yeast breads were whiter in color (higher L-values) and more yellow in color (higher (+) b-values) than all the flaxmeal breads. In general, the Hunter L-values and b-values decreased steadily in all of the flaxmeal bread samples while the Hunter L-values and b-values varied in the regular yeast bread samples during the eight week period.

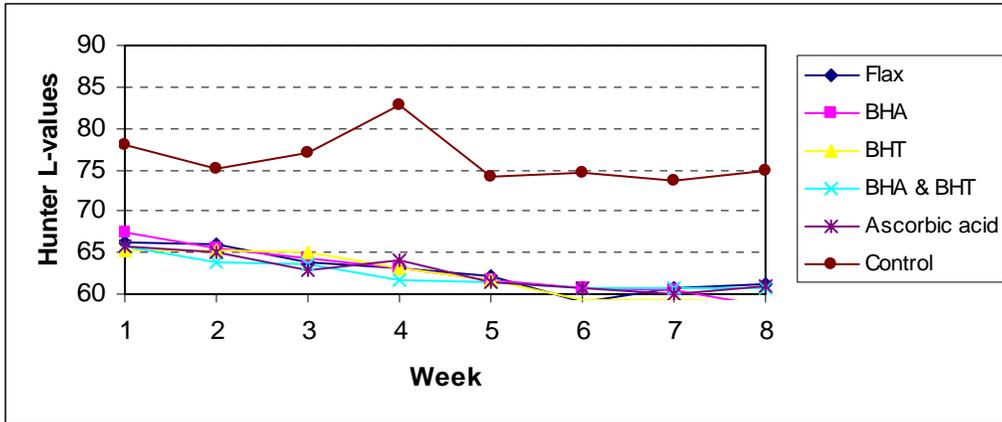


Figure 7: The change in Hunter L-values* of all bread types over an 8-week period
 * L = 0 = black; L = 100 = white

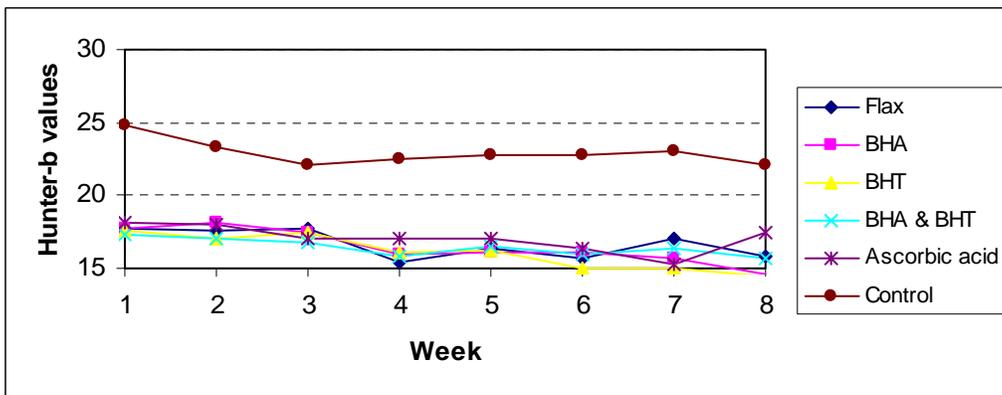


Figure 8: The change in Hunter b-values* of all bread types over an 8-week period
 * +b = yellow; -b = blue

Thus, the Hunter L-values and Hunter b-values were significantly differences ($p < 0.0001$) between the regular yeast bread and all the flaxmeal breads (Tables 6a, 6b, 7a and 7b respectively). However, there were no significant differences ($p > 0.05$) in the Hunter L-values and Hunter b-values when the regular flaxmeal breads (no antioxidants) and experimental flaxmeal breads (with antioxidants) were compared (Tables 6a, 6b, 7a and 7b).

Crumb color is an important physical attribute in baked products. For example, crumb color of red devil's food cake can be affected by altering the pH of the cake batter. Research (47) has shown that when flaxseed powder was used along with Nutrim oat bran as shortening substitutes in cakes that there were "significant effects of the addition of Nutrim oat bran (Nutrim OB) and flaxseed powder on color ($p < 0.01$). Other results from this study showed that the crumb of the control cakes were lighter (higher L-values) in color than the cakes which contained Nutrim OB and flaxseed powder. The researchers' finding agreed with the current data that the crumb color of the regular yeast bread was lighter (i.e. higher L-values) than the flaxmeal breads. However, the current data did not agree with the researchers' finding that increased levels of Nutrim OB and flaxseed powder contributed to a more yellow color (i.e. higher "b" values), since the flaxmeal was held at a constant level in the bread.

5.5 Acid Value

Free fatty acid value (i.e. acid value) is a chemical test used as an indicator of fat hydrolysis, specifically, the presence of free fatty acids. Release of free fatty acids is caused by heat, moisture, metals or enzymes present in the food. This test was conducted only in the first week to determine if free fatty acids were being released and present in the bread. The presence of free fatty acids, especially an unsaturated fatty acid, is an indicator for the onset of rancidity.

Specifically, acid value is "the amount of potassium hydroxide required for neutralization (58)." In general, the higher the acid value the more free fatty acids present which can cause rancidity. In this study, there was a significant difference ($p \leq 0.05$) in acid value when the control (regular yeast) bread sample was compared to the experimental flaxmeal bread with a combination of BHA and BHT (Tables 8a & 8b). However, even though there were no significant differences ($p > 0.05$) in the experimental breads with the flaxseed, acid values were higher than in the regular control bread (Tables 8a & 8b). Thus, these samples would have more free fatty acids which may be vulnerable to lipid oxidation.. In a recent study (65), it was shown that the biscuits that contained one of three plant extracts; amla (*Emblica officianalis*), drumstick leaves (*Moringa oleifera*) and raisins (*Vitis vinifera*) had lower acid values than the biscuits with BHA over a six

Table 6a: The mean crumb (Hunter L-value) color (n=48) and standard deviation (mean crumb color) of all bread samples during an 8-week period

Type of bread	Hunter L-value** (mean)	Standard deviation (Mean Hunter L-value)
Regular Yeast (Control 1)	76.34	2.99
Ascorbic acid	62.63*	2.15
BHA	62.77*	2.92
BHT	62.29*	2.78
BHA and BHT	62.24*	1.89
Flax (Control 2)	62.79	2.55

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

**L = 0 = black; L = 100 = white

Table 6b: The comparison of volume between the control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Significant	Not significant
BHA	Significant	Not significant
BHT	Significant	Not significant
BHA & BHT	Significant	Not significant

Table 7a: The mean crumb (Hunter b-value) color (n=48) and standard deviation (mean crumb color) of all bread samples during an 8-week period

Type of bread	Hunter b-value** (mean)	Standard deviation (Mean Hunter b-value)
Regular Yeast (Control 1)	22.93	2.99
Ascorbic acid	17.04*	2.15
BHA	16.47*	2.92
BHT	16.42*	2.78
BHA and BHT	16.12*	1.89
Flax (Control 2)	16.69	2.55

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

** +b = yellow; -b = blue

Table 7b: The comparison of mean crumb color (Hunter-b value) between the control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Significant	Not significant
BHA	Significant	Not significant
BHT	Significant	Not significant
BHA & BHT	Significant	Not significant

Table 8a: The mean acid value (n=24) and standard deviation (mean acid value) of all bread samples during an 8-week period

Type of bread	Mean acid value (mg KOH/g fat)	Standard deviation (Mean acid value)
Regular Yeast (Control 1)	2.00	2.99
Ascorbic acid	2.50	2.15
BHA	2.25	2.92
BHT	3.25*	2.78
BHA and BHT	2.50	1.89
Flax (Control 2)	2.75	2.55

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

Table 8b: The comparison of mean acid value between the control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Not significant	Not significant
BHA	Not significant	Not significant
BHT	Significant	Not significant
BHA & BHT	Not significant	Not significant

week storage period. This suggested that plant extracts may be more effective as an antioxidant than BHA in controlling lipid oxidation during storage (65).

5.6 Peroxide Value

Flaxseed contains high levels of two polyunsaturated fatty acids, linoleic acid (LA) and alpha linolenic acid (ALA). Both fatty acids are considered essential fatty acids since these fatty acids have to be incorporated in one's diet. The main differences between linoleic acid and linolenic acid are linoleic acid is an omega-6-fatty acid and contains two double bonds while linolenic acid is an omega-3-fatty acid and contains three double bonds. The more double bonds, higher is the reactive rate of these fatty acids. As a result, flaxseed is more susceptible to lipid oxidation by autoxidation. Autoxidation (3) has been defined as "a process in which susceptible lipids are attacked by oxygen leading to complex chemical changes, resulting in the rancidity and generation of off flavors in the food."

The flaxmeal that was used in this study was Bob's Red Mill[®]-Whole Ground Flaxseed Meal (Bob's Red Mill Natural Foods, Inc; Milwaukie, OR). Before the flaxmeal was used in the bread samples, the lipids were extracted and gas chromatography was used to analyze the fatty acid profile of the flaxmeal. An adapted version of the Folch Method was used to extract the flaxmeal lipids (26). Table 9 shows the lipid content of the flaxmeal that was used in this study. The unsaturated fatty acids that were present in the flaxmeal were oleic acid, linoleic acid and linolenic acid. The oxidative stability of a fatty acid decreases as it becomes more unsaturated (58). Thus, the oxidative rate of linolenic acid (C18:3) is double to that of linoleic acid (C18:2) (Table 9). As a result, linolenic acid is more unstable and vulnerable to lipid oxidation. The flaxmeal used in this study was processed March 2004 and bread production began at the end of May 2004. Since initial peroxide values of the flaxmeal were not measured there was no certainty that the flax meal was not undergoing rancidity. However, every effort was made to minimize the amount of rancidity in breads during storage. After the breads were cooled for at least three hours, the breads were placed in commercial polyethylene bread bags and then stored inside plastic storage containers to prevent exposure to moisture and light.

Table 9: Lipid analysis of flaxseed and oxidation rates of free fatty acids in flaxseed

Name of fatty acid	Chemical Formula	Amount Present in Flaxmeal (mg/g)	Oxidative rates
Stearic Acid	C18	2.9	
Oleic Acid	C18:1	22.4	1
Linoleic Acid	C18:2	14.7	12
Linolenic Acid	C18:3	0.3	25

Oxidative rancidity is a chemical change due to lipid oxidation that results in the unpalatable odors and off-flavors in oil (35). Lipid oxidation or autoxidation involves a series of reactions that results in the formation of free radicals and peroxides, which can lead to rancidity. The addition of antioxidants in foods hinders the oxidation process by donating either a hydrogen atom or an electron, which will attract free radicals. Thus, the autoxidation process is halted and the shelf life of foods is extended for longer periods of time. Figure 9 shows how free radicals ($R\cdot$) are formed during autoxidation and are represented by $R\cdot$ (36). During the first stage of lipid oxidation, oxygen attacks the double bond in free fatty acids of an oil sample to form peroxides. Thus, the more unsaturated a fatty acid is (i.e. more double bonds), the more susceptible it is to oxidative rancidity. In addition to oxygen, heat, light and trace metals can affect the rate of oxidation (16) and result in the formation of free fatty acids.

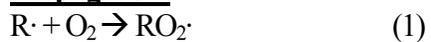
However, the rate of lipid oxidation can be slowed down with the addition of antioxidants in an oil sample. Antioxidants are chemical compounds that protect food from lipid oxidation by scavenging free radicals via donation of an electron or hydrogen atom, or by deactivation of metal ions and singlet oxygen. Thus, antioxidants can be used to preserve food by retarding rancidity or discoloration due to lipid oxidation. There are two groups of antioxidants: primary and secondary. Primary antioxidants or “free radical terminators” interrupt the free-radical chain of oxidative reactions by contributing hydrogen from the phenol hydroxyl groups, themselves forming stable free radicals which do not initiate or propagate further oxidation of lipids (74).” Examples of primary antioxidants are BHA and BHT. Ascorbic acid (i.e. Vitamin C) is an example of an oxygen scavenger (helps transfer hydrogen atoms).

Antioxidants (BHA, BHT, a combination of BHA & BHT and ascorbic acid) were incorporated in the experimental flaxmeal bread samples to evaluate their effect on controlling rancidity. Food antioxidants are a group of chemicals that are used to help extend the shelf-life of various food products. Flaxmeal contains linolenic and linoleic acids which are both polyunsaturated fatty acids. The number of double bonds and its position within the molecules is directly related to the susceptibility of oxidation (15). Lipid oxidation occurs when oxygen attacks the double bonds in fatty acid and results in the formation of hydroperoxides, and the subsequent degradation of the hydroperoxides into secondary products, such as aldehydes and ketones.

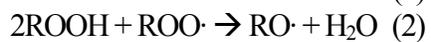
Initiation:



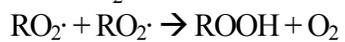
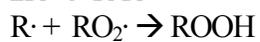
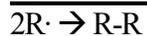
Propagation:



Branching:



Termination:



Where: R = Fatty acid radical

ROOH = Fatty acid hydroperoxide

RO₂· = Peroxy radical

RO· = Alkoxy radical

Figure 9: Free radical autoxidation ⁽³⁶⁾

The effectiveness of four antioxidants (BHA, BHT, BHA/BHT and ascorbic acid) on preventing rancidity in the experimental breads was evaluated by using a peroxide value test over an eight week period. In general, the food has a chance to become rancid when as the peroxide value is increased. Peroxide values peak and decrease over time (Figures 10a and 10b) and that is why peroxide values were monitored over an eight week period. However, the initial peroxide values for the flaxmeal could not be evaluated before the beginning of the study. Therefore, it was hard to determine which stage the peroxide values of the samples were in during the study. There were no significant differences ($p>0.05$) in peroxide values between both control samples and the experimental breads (Tables 10a & 10b).

Based on these results, the experimental breads made with BHA and with BHA & BHT together had the lowest peroxide values. Thus, these experimental breads were less rancid. The experimental breads that used BHT and ascorbic acid separately had higher peroxide values. As a result, breads made with BHT and ascorbic acid had more of a tendency towards rancidity. These results were similar to findings from previous research (29). When a 250ppm mixture of antioxidants (BHT, BHA, propyl gallate and citric acid) was added to pure soybean oil (PSBO) it was more effective in hindering rancidity when compared to a blend of oils (soybean and palm kernel oils) (29). It has been reported that when BHT is used in combination with BHA or propyl gallate, it was highly effective in retarding lipid oxidation (3). Ascorbic acid when used in combination of BHA was reported to be effective in retarding lipid oxidation in raw ground beef for up to 8 days of refrigerator storage in oxygen-permeable film (3). However, ascorbic acid had different antioxidant trends under different oxidation conditions, such that ascorbic acid was more effective than BHA and BHT when soybean oil was oxidized at higher temperatures (27). A research team (51) showed that 200ppm BHA was more effective in preventing oil oxidation and in storage stability than 200 ppm BHT in refined, bleached and deodorized (RBD) palm olein during the deep-fat frying of potato chips. However, two spices were also evaluated. It was shown that 200ppm rosemary and 200ppm sage possessed some antioxidant activity, such that oleoresin rosemary was more effective than BHA while sage was more effective than BHT in retarding oil oxidation. In a more recent study (65), three plants were used as sources of natural antioxidants. The extracts from amla, drumstick leaves and raisins were added to biscuits. The results of this study showed that biscuits treated with amla, drumstick leaves and raisins had lower peroxide and acid values after 6 weeks than the control biscuits and biscuits treated with BHA (65). These studies showed the possibility of using natural antioxidants, such as extracts of rosemary, sage, amla, drumstick leaves, and raisin in inhibiting lipid oxidation in foods rather than using synthetic antioxidants.

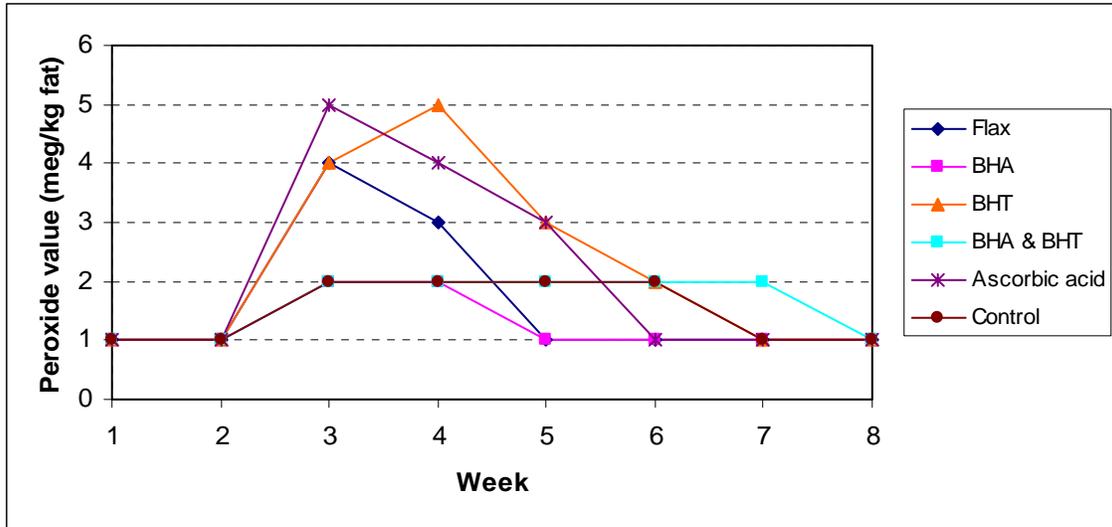


Figure 10a: The development of mean peroxide values in all bread samples over an 8 week period

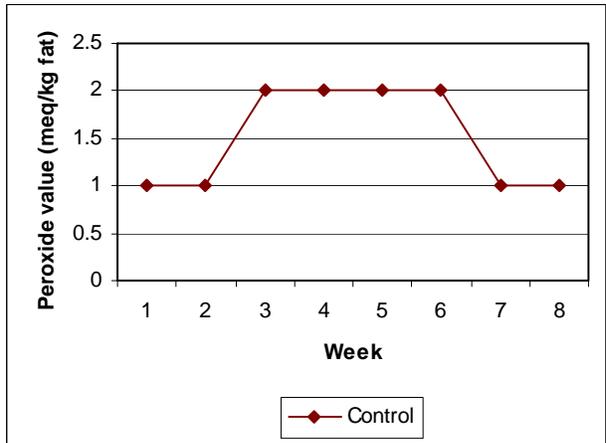
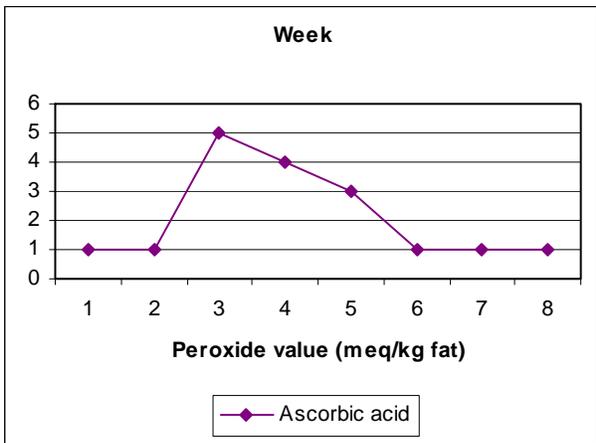
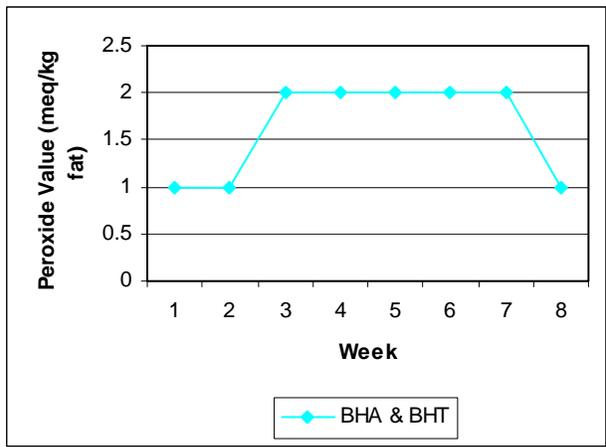
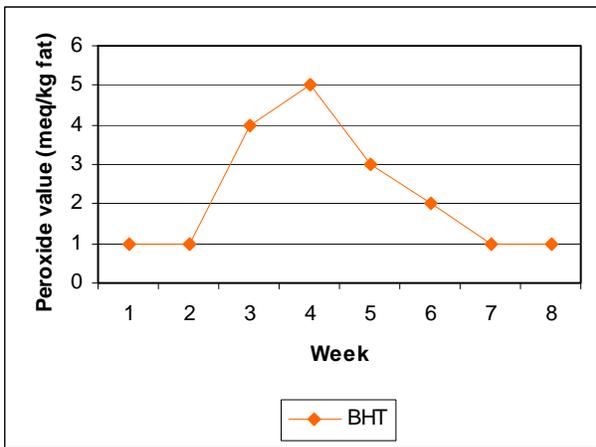
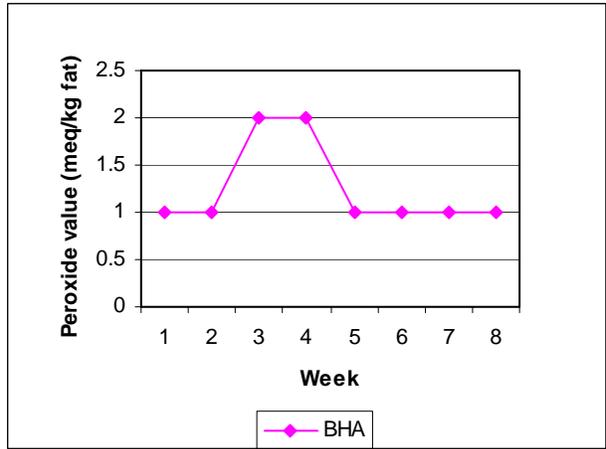
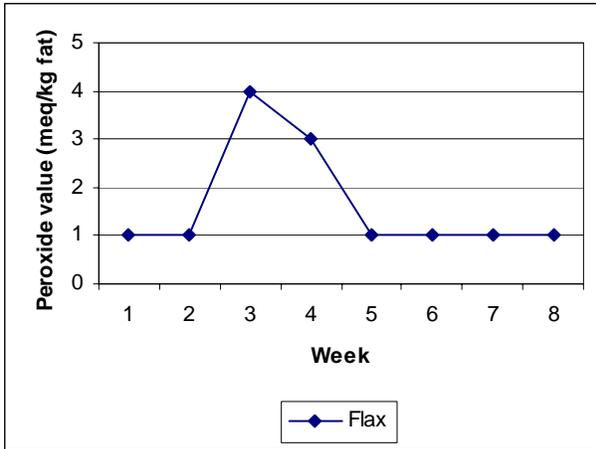


Figure 10b: The peroxide values in each bread sample over an 8 week period

Table 10a: Peroxide value (n=48) of all bread samples during each week for 8-weeks

Time	Peroxide Value meq/kg fat						
	Week	Flax	BHA	BHT	BHA & BHT	Ascorbic acid	Control
1	1	1	1	1	1	1	1
2	1	1	1	1	1	1	1
3	4	2	4	2	5	2	2
4	3	2	5	2	4	2	2
5	1	1	3	2	3	2	2
6	1	1	2	2	1	2	2
7	1	1	1	2	1	1	1
8	1	1	1	1	1	1	1

Table 10b: The mean peroxide value (n=48) and standard deviation (mean peroxide value) of all bread samples during an 8-week period

Type of bread	Mean Peroxide Value (meq/kg)	Standard deviation (Mean Peroxide Value)
Regular Yeast (Control 1)	1.50	0.53
Ascorbic acid	2.13	1.64
BHA	1.25	0.46
BHT	2.25	1.58
BHA and BHT	1.63	0.52
Flax (Control 2)	1.63	1.19

* Marked values are significantly different at $p \leq 0.05$ from the control or flax, respectively

Table 10c: The comparison of mean peroxide value between the control samples and experimental samples during an 8-week period

Comparison with	Control 1 (Yeast)	Control 2 (Flax)
Ascorbic Acid	Not significant	Not significant
BHA	Not significant	Not significant
BHT	Not significant	Not significant
BHA & BHT	Not significant	Not significant

5.7 Sensory Evaluation

The current definition of *sensory evaluation* is “a scientific method used to evoke, measure, analyze, and interpret those responses to products as perceived through the senses of sight, smell, touch, taste, and hearing (46).” In this study, the sensory attributes of regular yeast bread (control), regular flaxmeal bread and flaxmeal breads with antioxidants (i.e. Ascorbic acid, BHA, BHT and BHA/BHT) were evaluated by eight panelists during a 4-week period. The sensory attributes that were evaluated during this study were aroma, grainy taste, aftertaste, stale taste, moisture content, crumb texture and softness. Each sensory attribute was evaluated on a 1 to 9 scale. Then, the means of each attribute for each bread sample were used to form a spider plot which is a graphical representation of a quantitative descriptive analysis or QDA.

Quantitative Descriptive Analysis is a descriptive analysis that uses line scales, replicated experimental designs, consumer-oriented descriptive terminology, and use of analysis of variance (ANOVA) (46). ANOVA is used to indicate differences between samples, panelist agreement and discrimination, and reliability of replicates. A small panel is used and generated the terms that were used throughout the experiment. Panelists worked independently of each other and worked in separate booths to help minimize influences from other panelists. After all the data was collected, it was presented graphically by using a spider-web plot.

The three main senses that were used by the panelists to evaluate the different bread types were aroma, taste and touch. Aroma of a food product is the “fragrance or odor of a product as perceived by the nose from sniffing through the external nares (46).” The panelists used their sense of smell to evaluate the aroma of the different bread types to determine whether or not the breads emitted a musty aroma. Next, the panelists used their sense of taste. Taste was related to in terms of flavor. Flavor is defined as a “blend of taste and smell perceptions noted when food is in the mouth (55).” The sense of taste was used to evaluate the flavor of the different bread types to determine whether or not the bread samples had a grainy taste, an aftertaste or a stale taste. The last sense that the panelists used in this study was their sense of touch. Touch was related in terms of texture. The term *texture* can be related to the appearance of the food and to how it is perceived in the mouth or mouthfeel. In this study, the panelists evaluated the moistness or lack thereof of the breads, as well as, the crumb texture (mouthfeel) and the softness or hardness of the different bread types.

Aroma, flavor and texture of foods are affected as bread stales. As a result of bread staling, a musty aroma, a strong aftertaste and stale taste may become more noticeable upon increased storage time. Moisture is redistributed from the crumb to the crust during bread staling and results in a coarse crumb texture (mouthfeel) and, overall, a dry and hard bread.

In this study, there were significant differences ($p \leq 0.05$) noted in aroma, grainy taste, aftertaste, and moisture content when the regular yeast (control) bread and the experimental breads were compared (Tables 11a & 11b). There was also a significant difference ($p \leq 0.05$) in softness when the flaxmeal bread with ascorbic acid and the regular yeast (control) bread were compared, but there were no significant differences in softness in the other experimental breads (Tables 11a & 11b). Also, there were no significant differences in stale taste and crumb texture when the control and experimental breads were compared. However, there were no significant differences in any of the sensory attributes when the regular flaxmeal breads were compared to the experimental flaxmeal breads (Tables 11a & 11b).

The sensory panelists described the bread aroma as either strong musty or not musty. Overall, as the storage time increased, there was increase in the sensory scores in all bread samples. The regular flaxmeal bread had a stronger musty aroma (which may be related to lipid oxidation) than the regular yeast bread and the experimental breads. The use of antioxidants in the experimental breads had a significant effect ($p < 0.0001$) on the aroma of the breads. Varying levels (70) of different antioxidants, ascorbic acid (500mg/kg), BHA (200mg/kg), BHT (200 mg/kg) and BHA + BHT (100 + 100 mg/kg) were used in soyaspread and evaluated the sensory acceptability (aroma) in the soyaspread at room temperature and refrigerated temperature. They found that ascorbic acid was the best in retarding the development of rancidity in the soyaspread, especially in the refrigerated samples. However, in soyaspread samples that were stored at room temperature, BHA worked better than the other antioxidants by preventing rancid odors for up to 12 days. The main difference between the regular yeast bread and the experimental breads was the use of flaxmeal and/or the use of antioxidants. Flaxmeal contains a high level of polyunsaturated fatty acids which are susceptible to lipid oxidation. As a result, hydroperoxides, aldehydes and ketones are volatile compounds formed and their volatility is dependent upon their structure length. These volatile compounds affect the aroma of the flaxmeal breads during an increased storage time. Thus, aroma was used as an indicator of rancidity.

Table 11a: The mean QDA scores for bread characteristics between the experimental breads and control breads (regular yeast and flax) during a 4 week period

Parameter	Control 1 (Yeast)	BHA	BHT	BHA & BHT	Ascorbic acid	Control 2 (Flax)
Aroma	3.98	5.39	5.65	6.00	5.50	6.19
Grainy taste	3.06	5.20	5.29	5.77	5.67	5.45
Aftertaste	3.70	5.07	4.82	5.47	5.44	5.28
Stale taste	5.01	4.40	4.00	4.86	4.26	4.37
Moisture content	6.78	5.10	5.67	5.35	5.42	5.38
Crumb texture	5.43	4.28	5.17	5.39	4.63	4.92
Softness	5.48	4.58	4.78	4.92	4.40	5.06

Aroma: No musty aroma = 1, Strong musty aroma = 9

Grainy taste: No grainy taste = 1, Strong grainy taste = 9

Aftertaste: No aftertaste = 1, Strong aftertaste = 9

Stale taste: No stale taste = 1, Strong stale taste = 9

Moisture content: Moist = 1, Dry = 9

Crumb texture: Smooth & Chewy = 1, Course & Rough = 9

Softness (tactile): Soft & Springy = 1, Hard & Rough = 9

Table 11b: The comparison of mean QDA scores for bread characteristics between the experimental breads and control breads (regular yeast and flax) during a 4 week period

Comparison Parameters			Experimental Treatments			
Parameter	Control 1 (Yeast)*	Control 2 (Flax)	BHA	BHT	BHA & BHT	Ascorbic acid
Aroma	3.98	6.19	5.39*	5.65*	6.00*	5.50*
Grainy taste	3.06	5.45	5.20*	5.29*	5.77*	5.67*
Aftertaste	3.70	5.28	5.07*	4.82*	5.47*	5.44*
Stale taste	5.01	4.37	4.40	4.00	4.86	4.26
Moisture content	6.78	5.38	5.10*	5.67*	5.35*	5.42*
Crumb texture	5.43	4.92	4.28	5.17	5.39	4.63
Softness	5.48	5.06	4.58	4.78	4.92	4.40*

* Marked values are significantly different at $p \leq 0.05$ from the control (regular yeast) samples

Taste was evaluated using the terms grainy taste, aftertaste and stale taste. As with aroma, the sensory scores increased over time in all the bread samples. Overall, the sensory scores for grainy taste and aftertaste increased over increased storage time in all the bread samples (Tables 11a & 11b). There were significant differences in grainy taste and aftertaste due to bread type. Thus, the use of antioxidants may have played a role in the prevention of rancid flavors due to lipid oxidation. However, in a study conducted by Man and Tan (51) there were no significant changes in flavor upon storage (14 weeks) when BHA (200ppm) and BHT (200ppm) were used separately in fried potato chips.

Grainy taste was described as either no grainy taste or a strong grainy taste. The grainy taste of the breads was due to the inclusion of flaxmeal as a partial substitution for flour in the flaxmeal bread. As a result, the regular yeast bread (no flax) had a significantly lower ($p < 0.0001$) sensory score (3.06) for grainy taste when compared to the experimental breads (Tables 11a & 11b).

Aftertaste was described by the sensory panelists as no aftertaste or strong aftertaste. The sensory scores of aftertaste for all bread types were higher as storage time increased. The control (regular yeast) breads had the lowest aftertaste scores when compared to the experimental flaxmeal breads and regular flaxmeal breads (Tables 11a & 11b). Thus, all the flaxmeal breads had a stronger aftertaste than the control (regular yeast) breads. As a result, the use of flaxmeal and antioxidants had a significant effect on aftertaste.

Panelists also evaluated the stale taste of the breads. The sensory scores for stale taste were also higher as storage time increased. The control (regular yeast) bread sample had the highest stale score (5.01) of all the bread samples (Tables 11a & 11b). Since flaxmeal was used, it would be expected that the flaxmeal breads would have a stronger stale taste due to high (polyunsaturated) fat content of flaxmeal. However, the use of antioxidants may have prevented the onset of a rancid odor due to lipid oxidation. As a result, the control bread was perceived to have a stronger stale taste than the regular flaxmeal bread and experimental bread samples.

Bread undergoes several changes as it stales, such as crumb firming, moisture changes and crust softening. Overall, the control (regular yeast) bread was the least moist (6.78), had the coarsest bread texture (5.43) and the hardest texture overall (5.48) when compared to all the flaxmeal breads (Tables 11a & 11b). The moisture content of bread is affected by increased storage time. As bread stales during storage, the moisture in the bread is redistributed from the crumb to the crust which is due to the starch recrystallization during storage (54). Therefore, the crumb texture and overall softness of the breads are affected by the moisture content. The sensory scores for moisture content increased over the four week period in all the bread samples, such that there was a significant difference in moisture due to bread type ($p = 0.0018$) and time ($p < 0.0001$). The crumb texture of all the bread

samples was evaluated in terms of mouthfeel. The control (regular yeast) bread samples had the coarsest texture than all the flaxmeal bread samples. In general, the softness scores of all the bread types were higher due to increased storage. Again, the control (regular yeast) bread samples were the hardest in texture while the flaxmeal breads that contained ascorbic acid were the softest among all the bread types. Hence, there was a significant difference in softness over time ($p < 0.0001$).

The sensory results confirmed the results from the objective tests (i.e. texture analysis) which showed that the regular yeast bread samples were harder in texture (Table 3). Thus, these results may reaffirm the positive use of antioxidants in extending the shelf life (i.e. helping the storage stability) of foods.

Spider plots (Figures 11a-11e) were developed to represent the mean sensory (intensity) scores of different bread attributes. From table 11, the regular flaxmeal bread had the strongest aroma (musty) while the sensory scores for grainy taste and aftertaste were very similar in all the flaxmeal breads while the regular yeast (control) breads did not have a grainy taste nor a strong aftertaste. The regular yeast (control) bread samples had the highest scores in stale taste, moisture content (i.e. driest), crumb texture/mouthfeel (hardest) and overall hardness.

5.8 Nutritional Analysis

Due the prevalence of chronic diseases, such as cardiovascular disease and cancer, the importance of nutrition and exercise to maintain and/or improve the quality of life has increased over the years. As a result, many low-fat, fat-free and sugar-free food products, as well as diet supplements have been developed over the years and it is currently estimated that “more than \$40 billion was spent on weight-control pills, gym memberships, diet plans and related foods (88)”

Two popular diets that promise a significant amount of weight loss are the South Beach Diet and the Atkins diet. Both diets are similar by limitation or total restriction of carbohydrates. The current recommendation by the American Dietetic Association (6) is to consume 6-13 ounce equivalents per day (depending on your caloric needs) from grains group which includes breads, cereal, rice and pasta. At least 3 ounce equivalents (or half of total requirement) should be whole grain products while the other 3 ounce equivalents can be from other types of grains. Overall, these foods provide complex carbohydrates, vitamins, minerals and fiber and are an important part of the American diet.

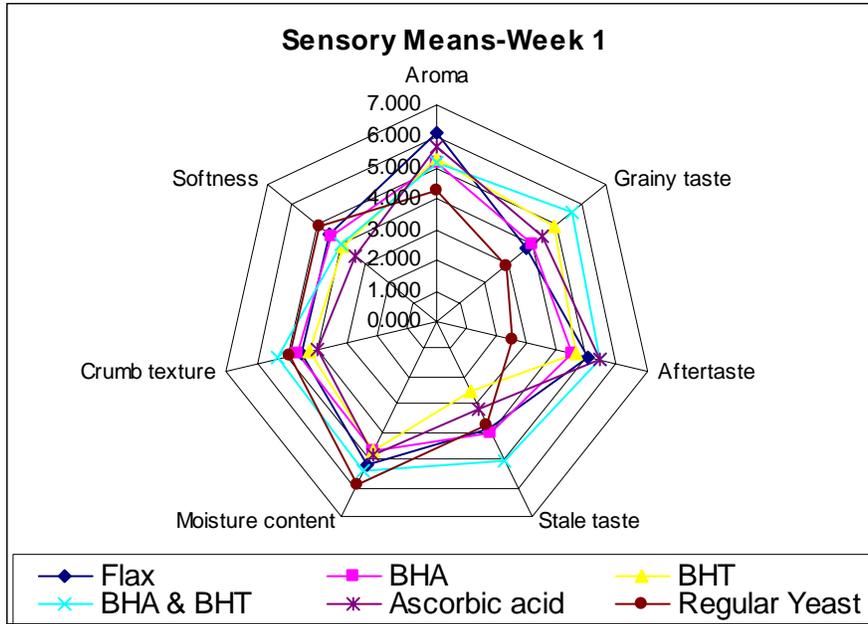


Figure 11a: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 1

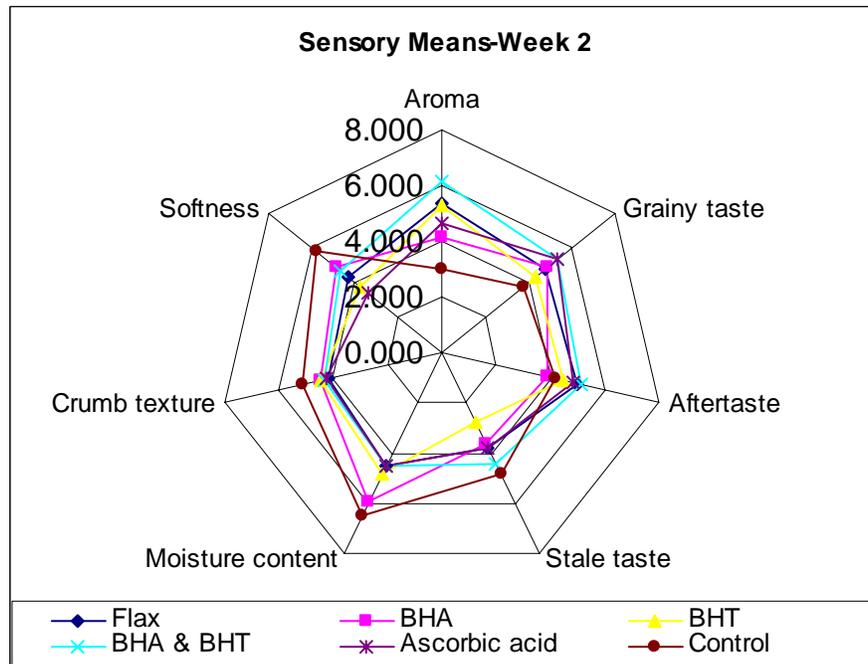


Figure 11b: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 2

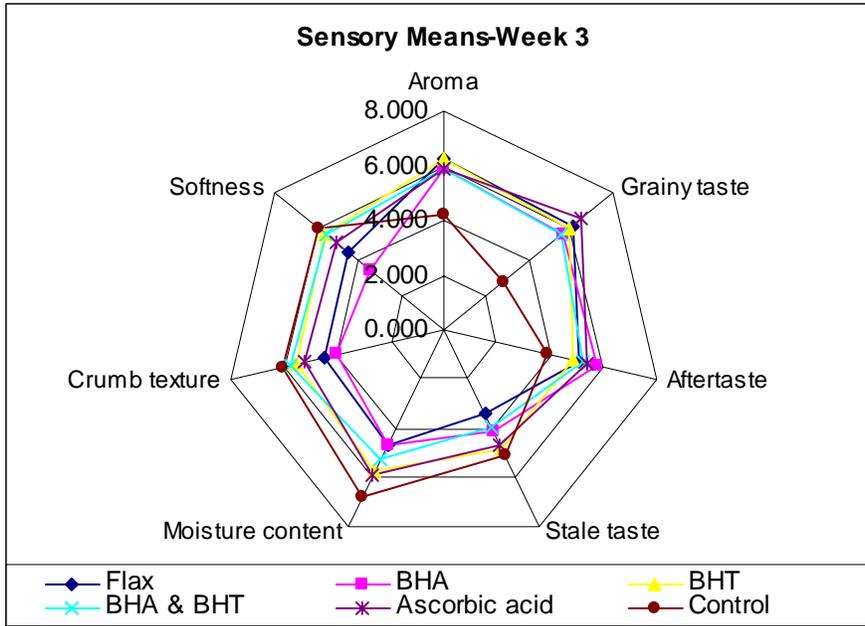


Figure 11c: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 3

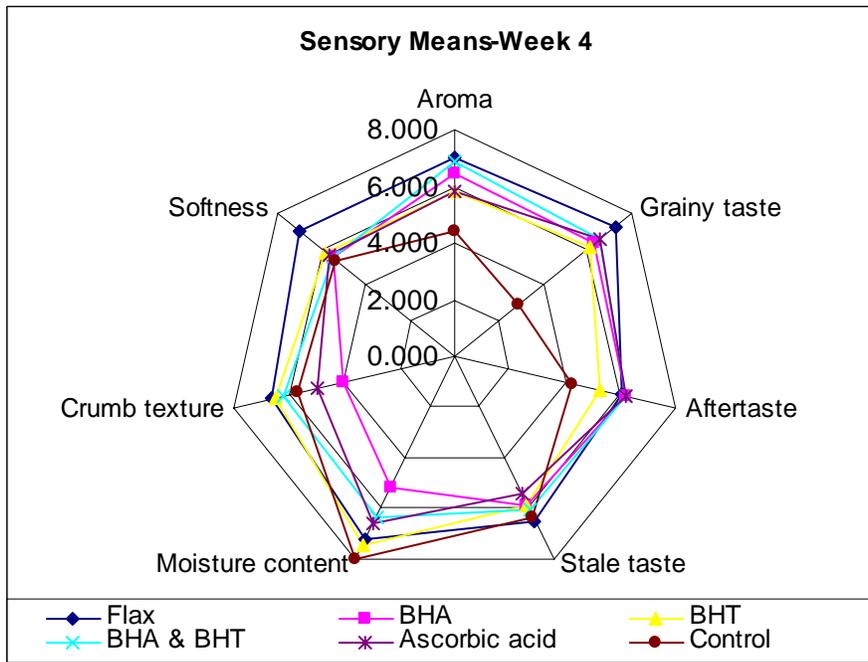


Figure 11d: A Spider Plot showing the mean sensory (intensity) score of various bread attributes for each bread type during Week 4

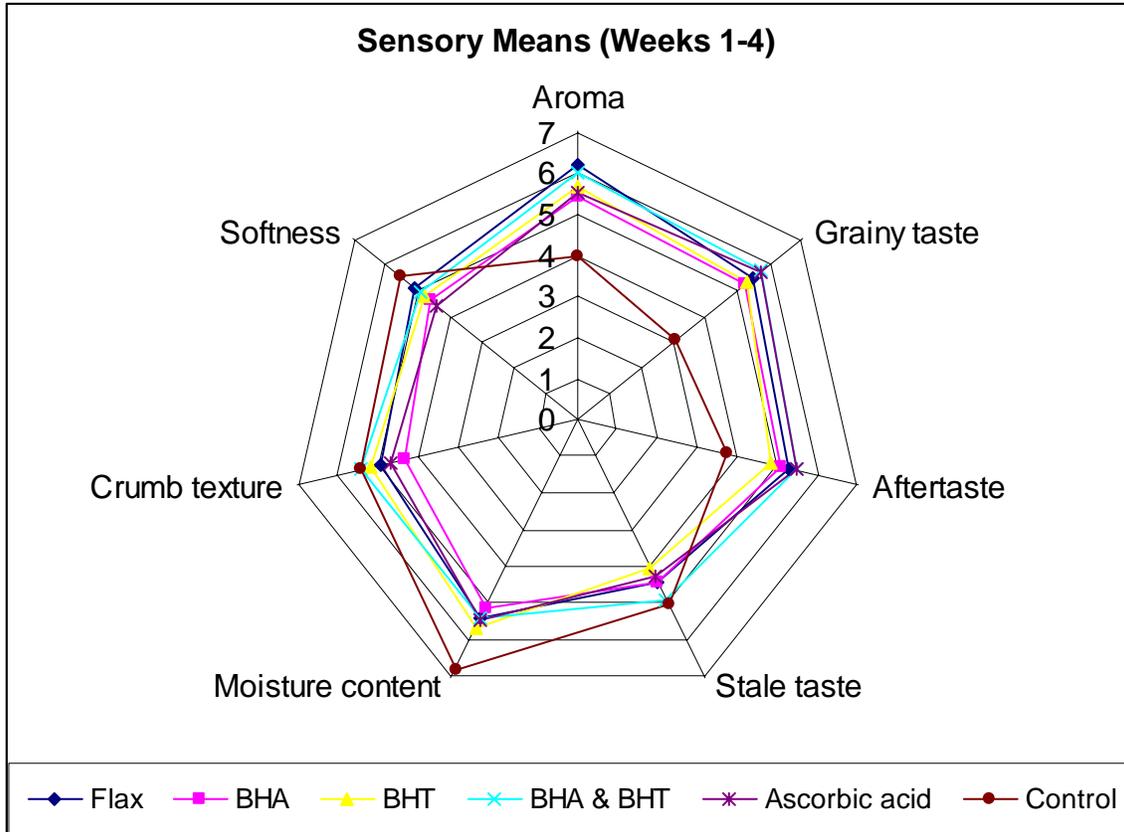


Figure 11e: A Spider Plot showing a summary of the mean sensory (intensity) QDA score of various bread attributes for each bread type over a 4 week period

Aroma: No musty aroma = 1, Strong musty aroma = 9
 Grainy taste: No grainy taste = 1, Strong grainy taste = 9
 Aftertaste: No aftertaste = 1, Strong aftertaste = 9
 Stale taste: No stale taste = 1, Strong stale taste = 9
 Moisture content: Moist = 1, Dry = 9
 Crumb texture: Smooth & Chewy = 1, Course & Rough = 9
 Softness (tactile): Soft & Spring = 1, Hard & Rough = 9

However, the Atkins diet and South Beach diet, suggest restricting carbohydrate intake and consuming fewer refined carbohydrates and limiting the consumption of sugary foods, breads, pasta, and starchy vegetables in one's diet, respectively.

Flaxseed contains high levels of polyunsaturated fatty acids (i.e. linoleic acid and linolenic acid) which are considered essential fatty acids, as well as, a high amount dietary fiber and a high amount of protein. Thus, flaxseed contains several nutrients that are very important in maintaining normal bodily functions and has been shown to provide possible health benefits, such as reducing the risk of cardiovascular disease and cancer. As a result, foods that include flaxseed may be considered high protein foods.

First DataBank Nutritionist Pro™ (San Bruno, CA) software was used to analyze the nutritional content of the breads used in this study and create the nutrition labels seen in Figure 12 and Figure 13. All the flaxmeal breads used in the study followed the same formula. The only difference between the flaxmeal breads was the inclusion of antioxidants. Overall, the inclusion of the antioxidants did not change the total fat, total carbohydrate and protein content of the flaxmeal breads. The flaxmeal breads contained 15% flaxmeal and 85% bread flour while the control (regular yeast) bread just contained 100% bread flour. Figure 12 shows that breads made with flaxmeal had a higher protein content (5.8g/serving) and a higher level of total fat (3.7g/serving) when compared to the control (regular yeast) breads (Figure 13). However, the flaxmeal breads had a lower amount of carbohydrates (25.7g/serving) than the control (regular yeast) breads (27.7g/ serving). The nutritional content of the control bread and flaxmeal bread as reported by First DataBank Nutritionist Pro™ were expected. As mentioned earlier, flaxmeal is high in protein and breads that are made with flaxmeal had a higher amount of protein and lower amount of carbohydrates than breads made from bread flour. Breads made with flaxmeal are also higher in fat which is due to the high amounts of polyunsaturated fats which are considered “good fats” because of their role in reducing the risks of cardiovascular disease. Hence, flaxmeal breads may be consumed by those who are on a low carbohydrate diet, such as, the South Beach diet.

Nutrition Facts	
Serving Size 1.00 slice(s) (61g)	
Amount Per Serving	
Calories	159 Calories from Fat 34
% Daily Value	
Total Fat	3.7g 6%
Saturated Fat 0.5g 2%	
Trans Fat 0.0g 0%	
Cholesterol	0.0mg 0%
Sodium	278.5mg 12%
Total Carbohydrate	25.7g 9%
Dietary Fiber 2.4g 10%	
Sugars 1.9g	
Protein	5.8g
Vitamin A	0% Calcium 2%
Vitamin C	0% Iron 10%

Figure 12: Nutrition label for Flaxmeal Bread without antioxidants
(First DataBank Nutritionist Pro™)

Nutrition Facts	
Serving Size 1.00 slice(s) (61g)	
Amount Per Serving	
Calories	153 Calories from Fat 18
% Daily Value	
Total Fat	2.0g 3%
Saturated Fat 0.3g 1%	
Trans Fat 0.0g 0%	
Cholesterol	0.0mg 0%
Sodium	276.8mg 12%
Total Carbohydrate	27.7g 9%
Dietary Fiber 1.1g 4%	
Sugars 1.9g	
Protein	5.4g
Vitamin A	0% Calcium 1%
Vitamin C	0% Iron 10%

Figure 13: Nutrition label for Regular Yeast Bread
(First DataBank Nutritionist Pro™)

Chapter 6: Summary, Conclusions, and Recommendations for Future Research

6.1 Summary and Conclusions

MarketData Enterprises estimated that “Americans spent more than \$40 billion in 2004 on weight control pills, gym memberships, diet plans and related foods (88).” The interrelationship between food, nutrition and health has led to the development of food products and/or the discovery of potential health benefits of certain foods. It has been reported that “dietary factors are associated with at least 5 of the 10 leading causes of death: coronary heart disease, certain types of cancer, stroke, noninsulin dependent diabetes mellitus and atherosclerosis (14).” Due to increased health concerns, many food products have been developed that contain either little or no fat and/or sugar or have been discovered to have potential health benefits when consumed. Functional foods are foods that provide essential nutrients beyond quantities necessary for normal maintenance, growth and development, and/or provide other biologically active components that impart health benefits or desirable physiological benefits. Flaxseed is an example of a functional food because it contains heart healthy omega-3 and omega-6 fatty acids and phytoestrogens, which have been shown to help reduce the risks of cardiovascular disease and cancer, respectively.

Flaxseed can be incorporated into bakery products; such as cookies, muffins and breads. However, flaxseed contains unsaturated fatty acids, such as oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3). Linoleic acid and linolenic acid are both polyunsaturated fatty acids. Both acids are more susceptible than oleic acid to lipid oxidation which can cause foods that contain flaxseed to go rancid. Oxidative rancidity is a chemical change due to lipid oxidation that results in the development of unpalatable odors and off-flavors in oil (35). Thus, the more unsaturated a fatty acid is (i.e. more double bonds), the more susceptible it is to oxidative rancidity. The rate of oxidation is affected by oxygen, heat, light, moisture and trace metals. However, the rate of oxidation can be controlled by using food antioxidants.

Antioxidants are chemical compounds that protect food from lipid oxidation by scavenging free radicals via donation of an electron or hydrogen atom, or by deactivation of metal ions and singlet oxygen. Thus, antioxidants can be used to preserve food by retarding

rancidity or discoloration due to lipid oxidation. Antioxidants can be categorized by either their type (i.e. natural or synthetic) or their mode of action (i.e. free radical terminator or an oxygen scavenger). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are examples of synthetic (phenolic) compounds while ascorbic acid (Vitamin C) is considered a natural antioxidant. BHA and BHT are both considered GRAS (generally recognized as safe) substances and are limited to 0.02 percent of the fat or oil content of the food product (15). BHA and BHT are used to delay the effects of oxidation, which include changes in color and the development of odors and off-flavors. Both antioxidants are cost effective and have “good carry-through properties in baked foods (16).” As a result, both these antioxidants can be used in a wide range of food products. It has also been shown that BHA and BHT have a synergistic relationship, such that, when both these antioxidants are used together it helps increase shelf life in the foods.

In recent years there has been interest in the use of natural antioxidants instead of synthetic antioxidants. The use of natural antioxidants in foods is not new. Ascorbic acid and citric acid have been used as a preservative in foods. During recent years, the use of natural food products has become more popular due to increased concerns of using synthetic antioxidants in foods. In current research (65), the extracts of rosemary, sage, amla, drumstick leaves and raisins were used to determine their antioxidants effects in foods.

The purpose of this study was to determine the effectiveness of three antioxidants (i.e. BHA, BHT and ascorbic acid) on flaxseed stability in breads. Flaxmeal was used as a partial replacement for regular bread flour in the breads used in this study. There were 6 different types of breads; regular yeast bread (100% bread flour, control); regular flaxmeal bread (15% flaxmeal, flax control), flaxmeal bread with BHA, flaxmeal bread with BHT, flaxmeal bread with BHA & BHT, and flaxmeal bread with ascorbic acid. Since flaxmeal was used as a partial replacement for regular bread flour, the characteristics of the flaxmeal breads were different compared to the regular yeast breads used in this study. Due to the partial substitution of bread flour with flaxmeal, there was less gluten formation. Gluten is responsible for the volume, texture and appearance of baked goods (44). Thus, these characteristics should be affected in the flaxmeal breads. As a result, vital wheat gluten was added to ameliorate the flaxmeal bread mixture while the sponge dough method was used to help improve the texture of heavy doughs (44).

The texture, moisture, volume, color, acid value, peroxide value and sensory evaluation (QDA) were conducted on the bread samples. Bread is characterized as a solid foam that is unstable and elastic. Since bread

is an unstable food product, it has a short shelf life. As a result, bread stales faster and is susceptible to mold growth and development of undesirable flavors and texture. Calcium propionate was added to the bread mixtures to help prevent mold growth. Rancidity also causes undesirable flavors and odors in foods. In this study, flaxmeal was used and due to its unsaturated fatty acid profile, the flaxmeal breads were also vulnerable to lipid oxidation which results in rancidity. The term staling has been defined as “the decreasing consumer acceptance of bakery products caused by changes in crumb other than those resulting from the action of spoilage organisms (11).” Bread undergoes several changes as it stales. These changes include crumb firming, moisture changes, crust softening and flavor changes. The staling of bread is caused by starch retrogradation or recrystallization of the starch. Starch is composed of amylose and amylopectin. As bread bakes, the starch granules swell and amylose diffuses out. After baking is complete, the bread is taken out of the oven and begins to cool. At this point, the staling process has begun. As the breads begin to cool, the amylose chains recrystallize (i.e. come together) to give bread its initial shape and strength. The amylopectin chains retrograde upon storage to cause the crumb to become firmer.

During the study, all the breads began to stale upon storage. As the breads began to stale, the texture and moisture of the bread samples were affected. In general, the crumb texture of all the experimental breads was significantly softer ($p \leq 0.05$) than the control (regular yeast) breads, except during week 3 when the breads containing BHA and ascorbic acid were firmer than the control bread. Moisture is not lost during staling, but rather is redistributed from crust to crumb. As a result, the crust becomes leathery, but is still soft (55). The control (regular yeast) breads were significantly moister ($p \leq 0.05$) than the flaxmeal breads that contained BHA and BHT, separately.

The volume of the flaxmeal breads, except the flaxmeal bread with BHA, had larger loaf volumes than the control (regular) yeast bread. It was expected that the flaxmeal breads would have a smaller loaf volume since flaxmeal is a grain product that would interfere in gluten development. However, vital wheat gluten was added to make up for the difference. Thus, there were no significant differences ($p > 0.05$) found when the control (regular yeast) bread and the experimental breads were compared.

The crumb color of the experimental breads was significantly darker ($p < 0.0001$) due to the incorporation of flaxmeal in these breads while Maillard browning was responsible for the crust color. Flaxseed contains many essential amino acids, such as valine, leucine, isoleucine, phenylalanine, tryptophan, lysine, histidine, methionine and cysteine (59). The amino group from lysine is known to participate in Maillard browning. Therefore, the crust color of the flaxmeal breads was significantly higher ($p < 0.0001$) than the control (regular yeast) breads.

The acid value in flaxmeal breads was higher than the control (regular yeast) bread. The acid value of all the bread samples was monitored to determine if free fatty acids were present after baking and during the first week of storage. Since flaxmeal has a high fat content, more free fatty acids were released. Thus, the acid value of the flaxmeal breads was significantly higher ($p < 0.05$) than the control (regular yeast) breads. Free fatty acids are involved in rancidity reactions and this was a good indicator to instability in the breads.

The peroxide values of the bread samples began to increase during week two and then decreased during this study. Since peroxide levels generally increase and then decrease over time, the values were monitored over an eight week period. During lipid oxidation, fatty acids degrade and form into hydroperoxides which are unstable and degrade further into aldehydes and ketones. The more hydroperoxides produced, the higher the peroxide value. In this study, the effectiveness of the antioxidants was evaluated by measuring the peroxide values in the bread samples. No significant differences ($p > 0.05$) in peroxide values were found between both control breads and experimental breads. A peroxide value of greater than 2.5 indicates that an oil sample has undergone excessive oxidation (69). However, the mean peroxide values for all bread samples were lower than 2.5. The flaxmeal breads made with BHA and with a combination of BHA and BHT were not as rancid as the other bread samples. Thus, incorporating BHA or a combination of BHA and BHT into baked products may help improve the shelf life of these products.

The sensory characteristics: aroma, taste and touch were evaluated in the bread samples over a four week period. Quantitative descriptive analysis (QDA) was conducted to evaluate the effects of antioxidants on shelf-life stability on the breads. During this study, eight panelists were trained and developed descriptors to describe the bread characteristics. These descriptors were then quantified using a 9-point scale. The results from the QDA showed that when BHA was used alone and in combination with BHT, it provided the best protection against lipid oxidation and the experimental breads were moister, chewier and had the least noticeable stale taste when compared to the control (regular yeast) breads. Ascorbic acid was not as effective as BHA or a combination of BHA and BHT in preventing lipid oxidation. However, breads made with ascorbic acid were the softest. The sensory panelists found that the control (regular yeast) breads did not have a noticeable aroma, grainy taste and aftertaste, but had the strongest stale taste and were drier and firmer when compared to all the flaxmeal breads. There were no significant differences ($p > 0.05$) in the sensory attributes when regular flaxmeal bread and the experimental flaxmeal breads were compared during this study.

The nutritional analysis for both control breads, regular yeast and flaxmeal breads, showed that the flaxmeal breads were higher in fat and protein, but low in carbohydrates (Figures 12 and 13). Thus,

incorporating flaxmeal in breads may appeal to those who follow low-carbohydrate diets, such as the South Beach Diet and Atkins diet.

6.2 Recommendations for Future Research

In our study, flaxmeal (15%) was used as a partial flour replacement in the experimental breads. Flaxmeal contains a high amount of omega-3 and omega-6 unsaturated fatty acids which have been shown to have some health benefits. However, due to the high unsaturated fat content in flaxmeal, products made with flaxmeal have the potential to go rancid. As a result, antioxidants were used to help counteract rancidity by slowing down the rate of oxidation.

The amounts of flaxmeal and antioxidants were predetermined to ensure consumer acceptability and antioxidants were used at specific levels to prevent toxicity. The breads with BHA and a combination of BHA and BHT were the most effective in slowing down the rate of oxidation. However, there were mixed results when the sensory attributes were evaluated. Panelists noted that the breads made with BHA were moister and chewier than the other breads. Also, breads made with a combination of BHA and BHT had a stronger (grainy) taste and aftertaste. Thus, more ways of incorporating flaxmeal and antioxidants into food products, such that the breads are still acceptable, need to be explored further.

Some ideas for further research include making breads with a varying combination of soy flour, flaxmeal, antioxidant(s), and food gums. Soybeans can be used as an antioxidant due to its natural antioxidant activity. Using an equal amount of flaxmeal and soy flour, in addition to antioxidants may produce a bread with acceptable sensory characteristics. The type of flaxmeal can also be changed. There are two varieties of flax: flax and solin. The flax variety is the most common and are brown-seeded while the solin variety are yellow-seeded and contain less than 5% of linolenic acid content. Thus, by using solin in breads may produce breads that have a longer or more stable shelf-life.

Antioxidants are used in foods to help delay the onset of rancidity. BHA, BHT and ascorbic acid are commonly used. BHA and BHT are examples of synthetic antioxidants while ascorbic acid is a natural antioxidant. Synthetic antioxidants tend to function better than natural antioxidants. However, with increased concerns about toxicity and the increased popularity of using all natural ingredients in food products, further research is needed to find additional sources of natural antioxidants to test their effectiveness in delaying lipid oxidation. Current

research (65) has shown rosemary, sage, yam flour and extracts from amla, drumstick leaves and raisins as having a better antioxidant effect than BHA and BHT.

While antioxidants are used to retard lipid oxidation, hydrocolloids can be used to enhance the textural properties of food products. Hydrocolloid (86) has been defined as “a water soluble polysaccharide with high molecular weight that exhibits a variety of functional properties: thickening, gelling, film forming, emulsifying, and adhesion.” Some gums that can enhance textural properties of food products are guar gum, locust bean gum, xanthan gum, alginate, hydroxypropylmethylcellulose (HPMC), and alginate. Guar gum “improves mixing and recipe tolerance in bakery products, as well as, improves shelf life through moisture retention (90),” while locust bean gum has been shown to help “control water absorption and batter and dough rheology in breads (90).” Xanthan and alginate have been shown to help strengthen doughs while breads containing either hydroxypropylmethylcellulose (HPMC) or k-carrageenan had a better specific volume and softer crumb (71). Thus, the use of food gums may help those people who suffer from celiac disease which is a very severe, genetic intolerance to gluten.

Other recommendations for future research include the use of more analyses to determine the effectiveness of antioxidants and gums in breads. In addition to peroxide and acid values, the TBA test, the Kreis test and anisidine value can be used to determine the degree of rancidity. Since moisture is redistributed and not lost in breads during staling, a more effective test to measure moisture would be to measure the water activity to determine the amount of free water in the breads. Thus, this may be useful to show a stronger relationship between the effects of moisture on the onset of rancidity.

Flax is a food product that has many health benefits. Antioxidants are used to enhance the shelf life of food products that contain flax. It has been shown that when BHA and BHT are used together, a synergistic effect is in place. Thus, both these antioxidants should be effective in retarding rancidity. However, since only a limited amount of synthetic antioxidants can be used in foods, natural antioxidants may be a better option to use in foods. However, there is a limited amount of literature on the use of flax, soy, natural antioxidants and gums in breads. More research is needed to determine the effectiveness of using natural antioxidants and gums in breads that contain flax and soy.

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Appendix A: Adapted Flax Meal Bread Recipe

Sponge:

- 10 g active dry yeast (Fleischmann's®, Burns Philip Food, Inc; Fenton, MO)
- 10 g sugar (Domino Foods, Inc; Yonkers, NY)
- 140 mL water
- 64 g unbleached bread flour (King Arthur Flour Co; Norwich, VT)

Directions:

1. Mix the first three ingredients and activate the mixture for 5 minutes.
2. Then, cover and ferment for about an hour and a half (or until sponge drops)

Dough:

- 1 T Crisco vegetable oil (The J.M Smucker Co; Orrville, OH)
- 7.5 g sugar (Domino Foods, Inc; Yonkers, NY)
- 7 g salt (Morton® ionized salt; Rohm and Haas Co; Chicago, IL)
- 9 g vital wheat gluten (Hodgson Mill, Inc; Effingham, IL)
- 233.5 g bread flour (King Arthur Flour Co; Norwich, VT)
- 52.5 g whole ground flaxseed meal (15% flax meal) (Bob's Red Mill Natural Foods, Inc; Milwaukie, OR)
- 60 mL water
- 0.2 g calcium propionate (Niacet Corp; Niagara Falls, NY)
- Other ingredients
 1. .06g BHA (Eastman Chemical Company; Kingsport, TN)
 2. .06g BHT (Eastman Chemical Company; Kingsport, TN)
 3. .03g BHA & .03g BHT
 4. .06g Ascorbic acid (DNP, China)

Directions:

1. Preheat oven to 425°F
2. Mix all the ingredients into a mixing bowl with a dough hook for about 13 minutes.
3. Then, grease bowl, cover and let rise for about an hour (or until fingerprint remains)
4. After an hour, divide the dough into 3 even pieces (usually about 185 g each)
5. Roll and shape each into loaves
6. Next, place the loaves into pup pans and allow to rise for another hour (over an oven as it heats) or until fingerprint remains
7. Bake for 20 minutes

Appendix B: Consent/Permission Form

Virginia Polytechnic Institute and State University Informed Consent for Participation in Sensory Evaluation

Title of Project: The Effect of Antioxidants on Flaxseed Stability in Yeast Bread

Principal Investigator: Katherine Cachaper

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about the effect of antioxidants in yeast breads with flax meal. This study was designed to determine chemical and sensory characteristics between flax meal breads using different antioxidants.

II. PROCEDURES

There will be 4 sessions over a period of 4 weeks lasting about 15-30 minutes per session. You will be presented with 6 bread samples during each session. As a panelist, it is critical to the project that you attend each session. Should you find a sample unpalatable or offensive, you may choose to spit it out and continue to the other samples.

Certain individuals are sensitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you are aware of any food or drug allergies, list them in the following space.

III. BENEFITS/RISKS OF THE PROJECT

Your participation in the project will provide the following information that may be helpful: differences in taste and texture in flax meal breads made with different antioxidants. You may receive the results or summary of the panel when the project is completed. Some risk may be involved if you have an unknown food allergy.

IV. EXTENT OF ANONYMITY AND CONFIDENTIALITY

The results of your performance as a panelist will be kept strictly confidential. Individual panelists will be referred to by a code number for analyses and in any publication of the results.

V. COMPENSATION

You will receive NO compensation for each session completed, or for completion of the entire project.

VI. FREEDOM TO WITHDRAW

It is essential to the sensory evaluation projects that you complete each session in so far as possible. However, there may be conditions preventing your completion of all sessions. If after reading and becoming familiar with the sensory project, you decide not to participate as a panelist, you may withdraw at any time without penalty.

VII. APPROVAL OF RESEARCH

This research project has been approved by the Institutional Review Board for projects involving human subjects at Virginia Polytechnic Institute and State University and by the human subjects' review of the Department of Food Science and Technology.

VIII. SUBJECTS RESPONSIBILITES

I know of no reason I cannot participate in this study which will require: 4 sessions during 4 weeks lasting 15-30 minutes.

Signature/Date

Please provide an address and phone number(s) so the investigator may reach you in case of an emergency or schedule changes.

ADDRESS:

PHONE:

-----**TEAR OFF**-----

IX. SUBJECT'S PERMISSION (for human subject to keep)

I have read the information about the conditions of this sensory evaluation project and give my voluntary consent for participation of this project.

I know of no reason I cannot participate in this study which will require: 4 sessions for 4 weeks lasting 15-30 minutes.

Signature/Date

Should I have any questions about this research or its conduct, I should contact:

Katherine Cachaper / 381-5286
Investigator / Phone

Dr. Frank Conforti / 231-8765
Faculty / Phone

David Moore / 231-4991
Chair, IRB / Phone for Research Division

Appendix C: Scorecard (Example)

Name: _____

Date: _____

Sample Code: _____

Please taste and evaluate all samples. You will have a total of six samples. Take a drink of water after tasting each sample. For each sample, read the definition for each attribute and then rate the sample (according to the degree which the attribute is present) and ***check the appropriate space on the scale***.

Attribute 1: *Aroma* (sweet smell, earthy, fresh, and musty)

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
No musty aroma Strong musty aroma

Attribute 2: *Grainy taste* (wheaty, nutty, and floury)

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
No grainy taste Strong grainy taste

Attribute 3: *Aftertaste* (acid, bitter, cardboard)

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
No aftertaste Strong aftertaste

Attribute 4: *Stale Taste*

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
No stale taste Strong stale taste

Attribute 5: *Moisture content*

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
Moist Dry

Attribute 6: *Crumb texture* (mouthfeel)

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
Smooth, chewy Coarse, rough

Attribute 7: *Softness* (tactile)

1____ 2____ 3____ 4____ 5____ 6____ 7____ 8____ 9____
Soft, springy Hard, rough

Appendix D: Acronyms, Abbreviations and Conversions

Acronym	Definition
ADA	American Dietetic Association
ALA	Alpha-linolenic acid
ANOVA	Analysis of variance
AOCS Official Method	American Oil Chemists' Society
AW	Water Activity
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
ED	Enterodiol
EL	Enterolactone
EDTA	Ethylenediaminetetraacetic acid
FOSHU	Foods for specified health use
GI	Glycemic Index
GLM	General linear model
GRAS	Generally recognized as safe
HDL	High density lipoprotein
LA	Linoleic acid
LDL	Low density lipoprotein
p-anisidine	Para-anisidine
PSBO	Pure soybean oil
QDA	Quantitative Descriptive Analysis
RBD	Refined, bleached, deodorized
SDG	Secoisolariciresinol diglycoside
TBA	Thiobarbituric acid
TBARS	TBA reactive substances

Vita

Katherine F. Cachaper was born on February 14, 1976 in Cleveland, Ohio. She attended Tallwood High School in Virginia Beach, Virginia and graduated in May 1994. She then attended Virginia Polytechnic Institute and State University in Blacksburg, Virginia where she received a Bachelor of Science in Science of Food, Nutrition and Exercise in May 1999 from the Department of Human Nutrition, Foods and Exercise. Upon completion of her undergraduate degree, Katherine worked as administrative assistant at Sentara Bayside and Sentara Leigh Hospitals. She then returned to Virginia Polytechnic Institute and State University to pursue a Master of Science in Foods from the Department of Human Nutrition, Foods and Exercise which she completed in May 2005. Upon graduation, Katherine plans to pursue a career in the food industry.