THE EFFECT OF SOY FLOUR AS A NATURAL ANTIOXIDANT ON FLAXSEED IN YEAST BREAD

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ABSTRACT

The effect of soy as a natural antioxidant against flaxseed rancidity in yeast breads was tested. Variables included: control (100% bread flour); yeast bread with 15% flax meal in place of part of the total bread flour; yeast bread with 15% flax meal and 5% soy; and yeast bread with 15% flax and 10% soy. Objective and sensory tests were used to evaluate breads. Peroxide values indicated that the hydroperoxides in breads increased during the first four weeks of the study, and then decreased, as would be expected as breads are exposed to more elements with time. Moisture content was not significantly different between the breads. Breads containing flax were significantly firmer ($p < 0.02$) in texture. Breads containing flax were also significantly lower in volume ($p < 0.005$) and significantly darker in crumb color ($p < 0.01$). The level of 10% soy contributed to a significantly darker crust color ($p < 0.04$). Quantitative descriptive analysis (QDA) found the level of 10% soy also contributed to an increased stale taste and aftertaste, firmer texture, coarser crumb, and drier loaf ($p < 0.05$). Musty aroma was not significantly different among breads and all breads containing flax had an increased grainy taste ($p < 0.0001$). Soy was found to have no significant antioxidant effect on the prevention of flaxseed rancidity in yeast breads.

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Chapter 1: Introduction

The incidence of chronic diseases such as cancer, heart disease, and diabetes mellitus has steadily increased over the past few years causing the American public to gain interest in disease prevention, mainly through diet and exercise. Functional foods have come into the spotlight for this reason. Consumption patterns of Americans change as new health information about certain foods becomes available. Functional foods have a positive effect on health when consumed on a regular basis as part of a varied diet (1).

Soybeans have been widely recognized for their health benefits for some time, while other functional foods such as flaxseed have only more recently come under investigation for their potential health benefits. Phytochemicals present in flaxseed and soybeans were once considered non-nutritive substances but are now recognized for their positive implications on health. The creation of other food products that contain the phytochemicals present in functional foods has also begun in the recent past, adding to the health foods already available.

Consumption of functional foods, and other health foods, has been on the rise in recent years as consumers' focus has become more health oriented. "Other health foods" includes organic food products that have also become more prevalent in mainstream grocery stores, as well as foods containing labeling that indicates they are all natural, such as "no preservatives added". Consumers have become more concerned with natural foods, and foods that contain only natural ingredients. Most consumers also desire natural antioxidants more than their chemical counterparts due to the rising concern that diseases such as cancer may be caused by unnatural ingredients (2).

The number of studies conducted to better understand the role of functional foods and how they work has also increased in the past few years, leading to increased media and consumer interest. Studies have been conducted to determine the actual components of functional foods that are responsible for health benefits, the exact mechanisms of action causing functional foods to work, the relationships among functional foods, and the effects of processing on functional food components that may alter their functionality. In the past few years, research has been more directed towards ways for the public to prevent disease. This research includes a very broad range of not just diet and exercise, but also food safety awareness, such as prevention of food borne illnesses. Functional foods provide an area that presents potential for research on how and why these foods offer such excellent health benefits, as well as how to get the public to consume more of these beneficial foods.

Chapter 2: Review of Literature

2.1 Functional Foods

Functional foods have had a large impact on the role of diet and health. Food components that were once thought to be non-nutritive have since been found to be key in disease prevention and overall maintenance of good health. In the United States and in many other countries there are many definitions for functional foods. However, there is an agreement that the term indicates foods that have a positive effect on health beyond normal nutrition when consumed.

Many descriptions of functional food have been created by various organizations. The American Dietetic Association describes functional foods as foods that “have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis, at effective levels” (1). A functional food is typically a food product that health claims may be made about to increase consumers’ interest and purchases. In the United States a health claim is a claim on a food label that shows a relationship between a “nutrient” in the food and a disease or health-related condition (3). The “nutrient” may be naturally found in a food, added to a food product from another food, added to a food product from somewhere other than the food supply, or added to a food product through the creation of a totally new food. Food processing technologies, traditional breeding, or genetic engineering may be used for these purposes (4). However, some of these methods may reduce the functionality of the “nutrient” and this should be taken into consideration.

2.2 Flax and Soy as Functional Foods

Phytochemicals, nonnutritive substances that help to maintain good health, are considered functional in foods (5). Phytochemicals are found naturally in many plant foods, including flaxseed and soybeans. The phytochemicals in flax are primarily lignans, and the phytochemicals in soy are primarily isoflavones, although other phytochemicals such as saponins and phytates are also found in both. The phytochemicals found in flax and soy have been shown to have beneficial effects on the prevention of cancer, osteoporosis, high cholesterol, and other health problems.

Consumption of flax and soy has been on the rise in recent years since a trend towards healthier living has begun. Many people alter diet and exercise in an attempt to be healthier and this is why flax and soy are becoming increasingly popular. Flax and soy may be eaten alone, or they may also be incorporated into other food products. The functional components of flax and soy, phytochemicals, have also been isolated to add to other food products. Baked goods have been supplemented with flax and soy as well as nutritional drinks, nutritional bars, meat substitutes, and other foods. Incorporation of flax and soy into food products may offer increased health benefits but there are also potential problems that may arise. The effect of processing on flax and soy may deplete some of their functional components, making them less effective or not effective at all. The recipes that flax and soy are incorporated into may need to be altered to accommodate the functional foods. Flax and soy contain no gluten so when incorporated into a baked good such as bread additional gluten or yeast may be needed to allow the bread to rise. Flaxseed also contains high amounts of polyunsaturated fatty acids, which could cause a product, especially baked goods, to go rancid more quickly and precautions must be made to prevent this.

2.3 Flaxseed

2.3a What is Flaxseed?

Flaxseed, also referred to as linseed, is an oilseed that is flat, oval, pointed at one end, reddish-brown in color, smooth, shiny, and slightly larger than a sesame seed (6). Flaxseed has been consumed for over 5,000 years as a food product and for medicinal properties. Flaxseed can currently be found in most natural or health food stores, but products such as muffins and breads containing flaxseed can be found in almost all mainstream grocery stores. Flaxseed has come into the spotlight recently for consideration as a functional food since it is high in protein, vitamins, minerals, soluble fiber, the omega-3 fatty acid alpha-linolenic acid, and phytochemicals such as lignans.

2.3b Flaxseed Usage in Foods

Flaxseed can be incorporated into food products in a variety of ways. The seed may be used whole, milled into flax flour, meal, or powder, or the oil may be extracted from the seed. Flax may be integrated into all baked goods like breads, muffins, and cookies, or it has been used in nutritional bars, meat analogues, processed meats, nutritional drinks, and cereals. Flaxseed bread is currently the most available commercial flax-based product (6). Whole flaxseed gives a nutty flavor and crunchiness to foods and is often used in soups, salads, or as a garnish (7). Flaxseed oil is high in unsaturated fatty acids and will quickly roast and darken when exposed to high temperatures making it unacceptable as a cooking oil. However, solin oil, which is obtained from flaxseed oil, contains less than five percent linolenic acid compared to the greater than fifty percent usually found in flaxseed oil (6). Solin oil is lighter in color, and is acceptable to heat for food preparation.

2.3c Stability of Fatty Acids Found in Flaxseed

Lipid oxidation leads to a loss of nutritional quality, sensory appeal, functionality, and shelf life stability in food products. Loss of sensory appeal is due to the development of off-colors, flavors, and aromas in food products caused by oxidation. Unsaturated fatty acids are less stable than their saturated counterparts since they contain more highly reactive carbon double bonds that are most likely to be oxidized. When oxygen is introduced to a fresh oil it reacts with the unsaturated bonds producing free radicals and hydroperoxides. High temperatures often used for processing of food products accelerate this degradation, and light and moisture also play a role in accelerating oxidation. These primary oxidation products can decompose into secondary oxidation products that are more volatile, such as ketones, aldehydes, and alcohols. These secondary oxidation products are more often associated with the off-colors, flavors, and aromas characteristic of rancid oils and food products (8).

Flaxseed oil is naturally high in unsaturated fatty acids. The fatty acid breakdown of 100 grams of flaxseed is only 9% saturated, 18% monounsaturated, and over 73% polyunsaturated. Sixteen percent (16%) of the polyunsaturated make-up is accounted for by the omega-6 acid, linoleic acid, and 57% of the polyunsaturated make-up is from the omega-3 acid, linolenic acid (6). The highly unsaturated make-up of flaxseed oil makes it an unstable oil, very likely to become rancid with the application of catalysts. The two double bonds present in linoleic acid and the three double bonds present in linolenic acid are highly susceptible to oxygen entering and oxidation occurring. Use of flaxseeds and their oil in food products may create problems resulting from this instability, and the acceptability of flaxseed incorporation into many food products may be questioned. Despite the instability of the unsaturated fatty acids found in flaxseed oil, many benefits are associated with alpha-linolenic acid, and flaxseed in general, and its use continues to increase.

Antioxidants are used to extend the shelf life of foods by preventing the formation of hydroperoxides and other free radicals that lead to the oxidative degradation of food. Antioxidants are especially useful in higher fat foods that go rancid more quickly and then develop off-colors, flavors, and aromas making them less appealing. Rancidity decreases the nutritive quality of a food. Antioxidants may be natural such as vitamin E and ascorbic acid. These are effective, but considered to have short-term effects compared to chemical antioxidants. Chemical antioxidants, which include BHA or BHT, are very effective at preventing rancidity, but may be toxic at high levels. For this and other safety reasons, consumers tend to desire more natural antioxidants in their food products (2).

Since flaxseed is high in alpha-linolenic acid and its stability during high temperatures involved with processing has been questioned, studies were conducted to determine its baking stability. One study heated whole and milled flaxseed to 100°C or 350°C for 60 minutes and then evaluated peroxide values and fatty acid composition, especially alpha-linolenic acid content, to determine oxidative stability (9). No significant changes in either test were reported for both forms of flaxseed after heating, and gas chromatography also found no significant changes, although ground flaxseed was found to be more susceptible to autoxidation.

A second study incorporated flaxseed into muffins as 28.5% of the total recipe and compared their oxidative stability to control muffins with no flaxseed (10). After baking for two hours at 178°C there were no changes in the alpha-linolenic acid, although oxygen consumption was greater for the flaxseed muffins. In a clinical trial flaxseed was again incorporated into muffins to be fed to subjects (11). Alpha-linolenic acid again remained stable during the baking process, and thiobarbituric acid levels indicated no significant oxidative rancidity of the flaxseed muffins.

2.3d Health Benefits of Flaxseed

Flaxseed has recently been shown to have many positive effects on health. The high amounts of protein, soluble fiber, and alpha-linolenic acid, along with other phytochemicals found in flaxseed, offer many health benefits. The protein and soluble fiber known as flaxseed mucilage have been shown to have cholesterolemic, hypolipidemic, and atherogenic effects, as well as to have positive effects on blood glucose metabolism. Other phytochemicals present in flaxseed such as phytates and phytic acid have been shown to have cholesterolemic effects, aid in glucose metabolism, prevent cancer, and act as antioxidants. An important phytochemical present in flaxseed is alpha-linolenic acid, which alone has been shown to reduce abnormal heart rhythms and prevent blood clots (7). In one study subjects who had previously survived a heart attack followed a Mediterranean diet for eight weeks that is high in fruits and vegetables, and therefore high in alpha-linolenic acid. Intake of saturated fat, linoleic acid, and cholesterol decreased while intake of oleic and alpha-linolenic acid increased. Subjects consuming increased alpha-linolenic acid had decreased incidences of recurring heart attacks or any other cardiac problems, and most importantly had decreased mortality (12).

Of all of the phytochemicals present in flaxseed, lignans currently have the most media and consumer interest. Flaxseed is the most abundant source of lignans, ranging from 75 to over 800 times more lignan production than other foods as measured in flaxseed flour and meal (13). The mammalian lignans found are enterodiol and enterolactone, which are structurally similar to estrogen and exhibit estrogenic and antiestrogenic activities, as well as antitumor, antioxidant, and antimitotic activities. Studies have shown that lignans are present in urine in a dose-dependent response (14), and also present in plasma after increased consumption regardless of whether the flaxseed was ingested raw or processed (15). Eighteen normally cycling women were fed flaxseed powder for three menstrual cycles and increased urinary lignan and phytoestrogen excretion was seen, as well as an increase in the luteal phase (LP) length of the menstrual cycle (16). The authors of this study felt that the increased LP of the menstrual cycle could be related to a decrease in hormonally dependent cancers. Other studies have been conducted to determine the effect of flaxseed on hormonal cancers. In one study, rats fed 5% flaxseed flour had significant reductions in tumor size in mammary cells, on average by 66.7%, although the results were not consistent (17). In another study rats fed a diet supplemented with 5% flaxseed flour had the greatest reduction in nuclear aberrations and cell proliferation in mammary cells, as compared to a control basal diet or supplementation with 10% flaxseed flour, or 5% and 10% flaxseed meal (18). Results of these studies show that mammary cancer may be reduced with the use of flaxseed, but more studies need to be done and results need to be more conclusive. Mice fed a basal diet supplemented with 2.5%, 5%, or 10% flaxseed saw decreased numbers, volume, and cross-sectional area of lung tumor growth, but only the 5% and 10% flaxseed supplemented diets had significant decreases (19). These findings indicate that flaxseed supplementation may be useful in lowering the risks of other cancers, not just hormonally dependent cancers. Flaxseed has also been shown to lower blood lipid levels, including total cholesterol, LDL cholesterol, apolipoprotein B, and apolipoprotein A-I in human subjects after daily consumption of muffins made with 50 grams of defatted flaxseed for three weeks (20). In another study involving human subjects significant decreases in total cholesterol, apolipoprotein B, apolipoprotein A-I, and non-HDL cholesterol were seen after daily consumption of 40 grams of flaxseed for three months (21). Decreases in LDL cholesterol and triglycerides were also seen, but were not significant. This study also looked at bone metabolism and found no significant effects after flaxseed supplementation.

2.4 Soybeans

2.4a General Facts About Soy

Soybeans have been consumed for thousands of years, and are important in many different cultures. Soybeans are considered functional foods since they contain high amounts of protein, complex carbohydrates, soluble fiber, micronutrients, and phytochemicals. Soybeans are also the richest source of the phytochemicals called isoflavones (22). Availability of soybeans is high in the United States since the U.S. produces almost half of the world's soybeans and they are the second most important cash crop (23). However, consumption of soybeans is not as high among the U.S. population. Consumption of soy products such as soymilk and meat alternatives does appear to be on the rise as Americans become more health conscious since soy contains many beneficial nutrients and has been shown to lower cholesterol, prevent cancer and osteoporosis, and may even have a positive effect on menopause and diabetes.

2.4b Soy Usage in Foods

Soybeans may be used to create a variety of products. The bean may be used whole, milled into soybean flour, meal, or powder, ground into a paste, or the oil may be extracted from the bean. Soy powder is used in many nutritional beverages and soybean oil makes up a large portion of typical vegetable oils. Traditional soybean dishes can be made including miso, tempeh, and natto that all involve the use of fermented soybeans. Soy can be used to create soy sauce, soymilk, and tofu. Tofu is used as a meat substitute in many recipes and to create lower fat meat analogues and processed meats. Soy is also used to create many baked goods like breads, muffins, and cookies, or it has been used in cereals and nutritional bars. Soy isolate, flakes, film, and concentrate can also be made from soy primarily for use in studies (24).

Processing of soy products affects the availability of certain components that are thought to provide health benefits such as isoflavones. Soybeans were inoculated and allowed to ferment to create tempeh in one study, and fermentation resulted in reductions of genistein and daidzen in their glycoside forms (25). Further processing to create a tomato-based soup with the soy products resulted in more reductions of daidzen and genistein. However, urinary excretion of isoflavones was increased indicating that the form of isoflavones remaining in the products was more available to the body.

Processing of soy to create different products must be closely monitored to prevent the loss of beneficial components. Certain procedures such as washing soy flour with an alcohol and water mixture to remove taste and color may also remove all phytochemicals present (26).

2.4c Antioxidant Effects of Soy

Soy has natural antioxidant activity due to many of its components including phenolic compounds and isoflavones (8). In one study three concentrations (0.1 uM, 1 uM, and 10 uM) of each of the isoflavones genistein, daidzen, equol, and O-desmethylanglolensin were added to blood samples to test for antioxidant activity on blood lipids (27). All isoflavones inhibited lipid oxidation, and the minimum concentration was 1 um for genistein and daidzen and 0.1 um for equol and O-desmethylanglolensin. Equol and O-desmethylanglolensin were the most effective antioxidants. Fermented soy products have been shown to increase the occurrence of isoflavones and to also increase the isoflavones' antioxidant activity, perhaps due to enzymes present during this process (24). Certain soy components are known for their estrogenic and antiestrogenic activity in the possible prevention of menopause and cancers, but some soy components also exhibit antioxidant activity that may be beneficial in preventing disease.

Just as soy exhibits antioxidant activity in the body, antioxidant activity of soy may also be exhibited in food products. Food products that are high in fat are especially prone to oxidation, which can be expedited by oxygen, heat, light, moisture, and metals, but slowed by antioxidants. One study looked at the effect of soy protein isolate antioxidant on ground beef (28). The beef was ground with metal plates and iron was added which promotes oxidation, and tests were later performed to determine rates of oxidation. Ground beef with 900 ppm of the antioxidant was more effective at preventing oxidation than 300 ppm or than the control ground beef, showing that in certain amounts soy can be an effective antioxidant.

2.4d Health Benefits of Soy

Soybeans have many different components that may provide health benefits to consumers, including isoflavones, saponins, and phytic acid. Isoflavones, however, have received the most attention in studies. The primary isoflavones found are genistein and daidzein. Once digested, intestinal bacteria further breaks down daidzen into equol, dihydrodaidzen, and O-desmethylanglolensin. Genistein is metabolized into dihydrogenistein and 6'-hydroxy-O-desmethylanglolensin. All humans, however, do not produce equol, although a maximal clinical effect is noticed in humans who are "equol-producers" (29). Equol-producers have been classified as people who have plasma concentrations greater than 83 nmol/L or excrete more than 1,000 nmol/L in their urine. Only 30 to 50 percent of the population excrete equol in their urine after daily soy consumption. It has been suggested that equol production may determine the effectiveness of soy in disease prevention, although other factors determine soy's effectiveness as well.

All isoflavones are known to exhibit estrogenic and antiestrogenic activity. Phytoestrogens weakly bind to estrogen receptors in people with normal estrogen levels, which is beneficial in preventing hormone-dependent cancers. However, in low estrogen environments such as postmenopause these weak estrogens may actually stimulate cancer growth (30). With normal estrogen levels, isoflavones bind to estrogen receptors in the nucleus of cells to create certain biological effects that have proved useful in preventing menopause symptoms and hormone-dependent cancers such as breast and prostate cancers.

Many studies have looked at the effect of soy on different cancers, whether hormone-dependent or not. A review of 26 animal studies was conducted to determine the effect of soy or soy isoflavones on various cancers, and 17 of the 26 studies showed a preventative action of soy on both hormone-dependent and non-hormone-dependent cancers (31). The results of this review indicate that soy has an overall protective effect against most cancers, but this cannot be stated without doubts due to inconsistent data. One study looked at the effect of just genistein on breast cancer risk, and found that it decreased the frequency and number of tumors in young rats (32). Genistein is not only a major isoflavone found in soy, but it is also a trypsin inhibitor, which is thought to also play a role in cancer prevention. Soybean also activates other active protease inhibitors, such as the Bowman-Birk inhibitor that have also been shown to prevent cancers (33).

Soy has also proved useful in preventing some of the side effects of menopause. One study determined the effect of soy on hot flashes associated with menopause. Forty postmenopausal women took 60 grams of isolated soy protein daily for twelve weeks, while 39 women received a placebo. Women consuming soy protein isolate daily noticed a significant reduction of 30 to 50 percent in the number of hot flashes, which was not noticed in women consuming the placebo (34). The risk of osteoporosis is increased after menopause, and soy has been shown to alleviate symptoms of osteoporosis as well.

Soy alleviates osteoporosis in the same way that it alleviates menopause by binding to estrogen receptors. A review of the effects of soy isoflavones on bone tissue found that many animal studies have been conducted and have provided considerable evidence that soy improves bone mass and bone density (35). Human studies have not been conducted as frequently as animal studies, but in one study 40 grams of soy protein isolate enriched with isoflavones was given daily for 24 weeks to 24 perimenopausal women (36). Lumbar spine bone mineral density and bone mineral content did not decline in subjects, and with regression analysis a significant positive effect on bone loss was noted showing that soy protein plus isoflavones helps slow bone loss. Other factors also appear to affect bone loss besides diet, including initial bone mass, body mass, and bone turnover that must be taken into account. Dose and duration of intake of soy appear to be some of the main factors affecting outcomes in most clinical trials (37).

Although many of soy's benefits are still being investigated, soy has become well known for its cholesterol-lowering ability, although the exact mechanism for this change is still uncertain. The Food and Drug Administration recently approved a heart health claim for food labels on soy products if they contain at least 25 grams of soy protein per serving (26). In a meta-analysis of 38 human clinical trials, consumption of soy was associated with a decrease in serum total cholesterol, LDL cholesterol, and triglycerides (38). There was also a nonsignificant increase in HDL cholesterol. Another review of human clinical trials with soy found the same results on total and LDL cholesterol and triglycerides, but found no effect on HDL cholesterol (39).

There are many proposed mechanisms for the cholesterol-lowering effect of soy including increased bile acid excretion, increased hepatic metabolism of cholesterol, altered hormone concentrations, and various components of soy such as amino acids, peptides, fiber, saponins, phytic acid, trypsin inhibitors, and isoflavones (40). Many of these various soy components have been isolated to determine if they alone exhibit effects. It has been proposed in some studies that the isoflavones found predominantly in soy are responsible for cholesterol reduction, along with other health benefits (41). One study also considered the effect of soy fiber on health by examining soy flour, soy protein isolate plus soy fiber, soy protein isolate plus cellulose, and a placebo (42). Total cholesterol was lowest in both soy protein isolate groups; LDL cholesterol was lowest in the soy protein plus soy fiber group; apolipoprotein B was lowest in the soy protein plus cellulose group. These results do not associate soy fiber as the main reason for cholesterol reduction. Another study considered the effect of soy stanols on health by providing subjects with lemonade or egg whites that contained 625 mg of stanols (43). Stanols reduced cholesterol absorption when given in both forms. When given in the lemonade, stanols reduced total and LDL cholesterol showing that stanols do have an effect on cholesterol reduction but that may not be the only component responsible. For total health benefits of soy, the exact component from soy or mechanism responsible is difficult to pinpoint and more studies need to be conducted.

2.5 Breadmaking Basics

Breadmaking has been performed for centuries, dating back almost 12,000 years ago. The Egyptians, Greeks, and Romans have all been noted to have prepared bread as a part of their daily lives. What began as a crude method of food preparation has now turned into an art and a science. Today there are different methods of breadmaking that create hundreds of different types of bread. The common ingredients found in bread are flour, yeast, water, salt, and possibly a few other ingredients such as eggs or milk. In the past few years consumers' interest in variety breads has been on the rise. Variety breads are those created with ingredients other than traditional bread ingredients, such as flax meal or soy flour. Variety breads offer flavor and texture changes to the consumer, as well as increased nutritional benefits (44).

Flour contains proteins, which create the framework for bread. When water is added to flour to create a sponge and dough, gluten is created. Gluten helps create the bread structure due to its major constituents, glutenin and gliadin. Glutenin is responsible for the cohesiveness and mixing tolerance of the dough, while gliadin allows for the expansion, elasticity, and rising of the dough. Some flours, such as bread flour, are made from hard wheat that has increased gluten to yield a higher loaf. Flour strength, which determines the quality of gluten, has the greatest impact on baking quality (45). The amount of gluten developed obviously depends on the type of flour used, and also on gluten development. Kneading the dough aids gluten development by stretching out the gluten, while certain bread ingredients may interfere with gluten development. Fat coats the proteins to interfere with gluten development, which creates a tender product. Sugar also interferes with gluten development, but too much fat or sugar will retard the development. Not only does gluten create the bread framework but it also has a gas retaining ability to aid in the fermentation process.

Yeast is used to begin the fermentation process. While wild yeast was once used, commercial yeast has been available for the past century to accelerate the process. *Saccharomyces cerevisiae* is the type of yeast most often used to create bakers yeast that is used in breadmaking. Strains of *Saccharomyces cerevisiae* are carefully selected, grown, and occasionally crossbred to ensure bread products will have only the most desirable characteristics (46). Compressed or cake yeast and active dry yeast are two different types of bakers yeast that are created differently to withstand different storage conditions and temperature ranges. Active dry yeast is created from a strain that is known for its ability to retain activity when dried, giving it more storage stability. Active dry yeast has been considered better for use with the sponge dough method (47). Variations of active dry yeast have been developed which are very fast acting. Fast acting yeasts come from a specially selected strain, and from recent advances in genetics and drying technology (46). Fast acting yeasts help bread to rise faster, but are considered by some to give poor flavor and keeping quality to the final product (48). To begin fermentation, the yeast consumes sugars and begins to release alcohol and carbon dioxide. While sugar acts as food for the yeast, salt helps to control yeast activity. As fermentation continues a sour smell results from the gases, along with the creation of tiny air pockets, bubbles, that cause the bread to rise. Too much yeast or an incorrect water temperature used to activate the yeast will result in a poor final product.

2.5a The Sponge Dough Method

There are many methods of breadmaking, while the two most common are the straight dough method and the sponge dough method. The sponge dough method of breadmaking allows the fermentation process to begin sooner. All of the yeast and some sugar for food are combined with a portion of the flour and water to create the sponge and to begin fermentation. The sponge may have a long fermentation process dependent on yeast level, amount and type of flour used, and bread quality expectations (49). The sponge is typically considered ready when it “drops,” when the gases begin to leak out. The remainder of the ingredients is then added to the sponge and dough is created. This “headstart” to the fermentation process allows bonds to begin to develop between the glutenin and gliadin, which creates a framework for the bread. The sponge dough method does not require much kneading, if any at all, since the headstart to the fermentation process allowed strong bonds to already form.

In comparison to the straight dough method, the sponge dough method takes more time since it allows the fermentation process to begin early, it creates a more flavorful dough with better keeping quality, and allows the dough to ripen more, weakening the flour and creating a softer product (50). Most importantly the sponge dough method can be used to incorporate weaker flours into a bread. The sponge dough method has been found to give the best results with strong flours that can be broken down and softened during the long fermentation step (51). Weak flours have low-gluten which does not provide stability and allow the bread to fully rise. However, the sponge dough method can be started using a more hardy flour, and once fermentation has begun and the amount of yeast has multiplied, a weaker flour may be added to the dough, creating an improved final product (50). The sponge dough method is more tolerant of variations in a bread recipe than other breadmaking methods (52).

2.5b Bread Staling

Many characteristics besides the final appearance such as flavor, color, gloss, texture, volume, moistness, and crumb stability are considered when determining loaf quality (53). After baking the loaf begins to stale and many unwanted characteristics start to develop in bread. When bread stales it often becomes firm and loses crumb stability, as well as flavor and mouthfeel, which makes it undesirable to consumers (54). There are two different stalings that occur in a bread loaf: crust and crumb staling (53).

Crust staling mainly occurs due to inappropriate storage while crumb staling occurs due to starch. During baking, the amylose fragment of starch gelatinizes completely, but the amylopectin fragment does not and it is this branched fragment that retrogrades after baking (55). However, bread that is stale can be reheated and will soften again making it appear fresher (54). This process shows that the firmness and dryness of a loaf do not represent the actual moisture content of the loaf (53). When reheated water again enters the amylopectin fragments causing them to swell and the loaf to regain tenderness. This process only lasts for a short time before bread retrogrades again. Moisture movement may then be a more accurate term for why staleness occurs. Although many ways to prevent staling from occuring have been tested, no method has been found to be truly successful. Freezing bread when it is still fresh will prevent it from staling until it is removed from low temperatures and brought back to room temperature where staling will resume (54).

Ingredients other than starch, such as gluten, are considered to play a role in staling as well. A low-protein loaf stales faster than a high-protein loaf because the high gluten content of the high-protein loaf helps to conserve moisture. Increased moisture in a loaf will prevent staling from occuring for a short time, as moisture will take longer to move. Loaf volume has been shown to affect staling. Decreased staling occurs in loaves with increased volume, and this may be due in part to gluten content. Quantity and quality of flour affects staling due to its role in the creation of gluten (55). Loaves made with strong flours keep better than those made with weak flours due to the increased gluten. Tender loaves keep longer than tough loaves for the same reason. Other factors may affect staling, such as baking temperature and time, and absorption during baking

(54). A high baking temperature for a long period of time, and high absorption during baking all lead to increased staling. For these reasons, the amount of staling that will occur later can be somewhat more controlled during the baking process. Storage conditions, and especially storage temperature, can also greatly affect staling of bread.

Chapter 3: Justification

Flax and soy are both functional foods that have become increasingly popular among consumers due to their health benefits. Both are considered functional due to their content of phytochemicals. Soybeans contain not only isoflavones, but they also contain small amounts of lignans, the phytochemical found predominantly in flax. Flax and soy both have beneficial effects that may also prevent diseases, including cancer and cardiovascular disease (56). Flax and soy have many similarities, such as both have unique characteristics that aid in their functionality. Flaxseed is very high in alpha-linolenic acid and alpha-linoleic acid. These polyunsaturated fatty acids have been shown to have a positive effect on health related issues, such as heart health. Soy, however, has been shown to possess natural antioxidant activity that may combat oxidative degradation that could lead to disease inside the body, and could also benefit the shelf life of products that it is incorporated in.

Based on these facts it seems appropriate that flax and soy, for additional benefits, should be combined in certain food products such as baked goods. Besides increased health benefits, it appears that these two functional foods would work synergistically together as the antioxidant activity naturally found in soy would inhibit rancidity of the polyunsaturated fatty acids in flaxseed. The polyunsaturated fatty acids prevalent in flaxseed offer many health benefits, but also have the potential problem of being unstable due to the presence of double bonds. This instability contributes to the problem of rancidity, which causes off-flavors, colors, and aromas in a food product. Consumers not only have become more health-conscious in recent years, but they also desire a food product that provides sensory appeal with an increased shelf life.

There have been very few studies conducted to date that examine the effects of processing on functional foods, and few studies have been conducted thus far to determine if the combination of flax and soy in a food product do increase health benefits and work synergistically together to create a better quality product with increased shelf stability. The National Cancer Institute launched a five-year program in 1992 to investigate how to incorporate cancer-preventing phytochemicals into processed foods, while paying close attention to natural synergistic relationships between phytochemicals and the effects of processing on phytochemicals (57). Soybeans were utilized in the studies but unfortunately, flaxseed was not one of the six chosen foods to be evaluated. Therefore, the relationship between flax and soy will not be determined by the NCI study. The effects of both flax and soy on disease states, including obesity and diabetes (58) have been investigated to some extent, but with separate studies being analyzed. One review investigated the effects of both lignans and isoflavones on various disease states, but soybeans were used as the only source (59). Literature indicates very little research that explores the effects of both flaxseed and soybeans together. Breads and muffins, and possibly other baked goods, appear to be useful vehicles for flax and soy experimentation. However, due to the low-gluten content of flax meal and soy flour their incorporation into baked goods may contribute to poor quality product bread. Methods must be explored that allow flax meal and soy flour to be incorporated into baked goods while maintaining high quality characteristics.

The purpose of this study was to utilize both flax meal and soy flour in a yeast bread to achieve optimum functionality of the two ingredients. Therefore, the objectives of this study were:

(A) To incorporate both flax meal and soy flour into a bread as partial replacements for flour.

(B) To determine the effectiveness of soy as a natural antioxidant in maintaining the keeping quality in the flax meal / soy flour bread.

(C) To evaluate the physical and sensory quality of the of the flax meal / soy flour bread.

Chapter 4: Materials and Method

4.1 Sponge Dough Method

The AACC Method 10-11, Baking Quality of Bread Flour - Sponge-Dough, Pound-Loaf Method, was adapted (60). To create the sponge, ten grams of Fleischmann's® yeast (Burns Philp Food, Inc; Fenton, MO) was activated for five minutes with ten grams of Domino® sugar (Domino Foods, Inc; Yonkers, NY) and 140 mL of tap water. Sixty-four (64) grams of King Arthur Unbleached Special Bread Flour (King Arthur Flour Co; Norwich, VT) was added to the yeast mixture to create the sponge. After fermentation, the balance of the bread flour, Bob's Red Mill® flaxseed meal (Bob's Red Mill Natural Foods; Milwaukie, OR), Bob's Red Mill® low-fat soy flour, Bob's Red Mill® vital wheat gluten, tap water, Domino® sugar, Morton® salt (Rohm and Haas Co; Chicago, IL), and Crisco® vegetable oil (The J.M. Smucker Co; Orrville, OH) were incorporated to create a dough. The sponge dough method has been found to be most appropriate when "heavy" ingredients that prevent gluten development, such as the flax meal and soy flour in this recipe, are used. This is also the reason why vital wheat gluten was added to increase the bread volume, and counteract the "heavy" ingredients. Calcium proprionate (Niacet Corp; Niagara Falls, NY) was also added to prevent mold. Calcium proprionate has GRAS status as a chemical preservative in accordance with good manufacturing practices listed under Title 21 (Food and Drugs) of the Code of Federal Regulations last revised April 1, 2004.

Sponge:
Bread Flour...............64 g
Water (variable)........140 mL
Yeast, compressed....10 g

Sugar.........................10g

The sponge was mixed with a paddle attachment for approximately five minutes, or until the mixture was smooth. The sponge was then allowed to ferment for approximately one hour, or until the sponge dropped.

Dough:
Bread Flour.....................216 g (w/ 5% soy flour) or 198.5 g (w/ 10% soy flour)
Flax Meal........................52.5 g (15%)
Soy Flour........................17.5 g (5%) or 35 g (10%)
Water (variable).............70 mL (w/ 5% soy flour) or 80 mL (w/ 10% soy flour)
Wheat Gluten...................9 g
Sugar...............................7.5 g
Salt..................................7 g
Vegetable Oil..................10.1 g
Calcium Proprionate…...0.20 g

Dough ingredients were added to the sponge while mixing with a hook attachment for approximately 8 minutes. The dough was allowed to rise in a lightly greased bowl for one hour, or until a finger imprint remained. The dough was then removed and divided into three equal portions, about 200 g each. Loaves were formed and placed into greased pans. Bread was then allowed to proof in a Servolift Eastern proofing oven (Servolift Eastern Corp; Boston, MA) for 30 to 45 minutes at approximately 32°C, or until a finger imprint remained. Bread was baked in a calibrated oven at 220°C for 20 minutes.
The breads prepared were (a) regular yeast bread with 100% bread flour, (b) yeast bread plus 15% flax meal in place of regular bread flour, (c) yeast bread plus 15% flax meal and 5% soy flour, and (d) yeast bread with 15% flax meal plus 10% soy flour. Eighty-four (84) total loaves were prepared. Breads were cooled for a minimum of three hours before being placed in commercial polyethylene bread bags purchased from a local bakery (Our Daily Bread; Blacksburg, VA) and stored in plastic storage containers to keep out light for the duration of the study. Humidity and room temperature status were

monitored by the use of a Dickson temperature and humidity data logger (Model TR320, Dickson, Inc; Chicago, IL) kept inside a plastic storage container with the breads. Physical tests were conducted weekly, and that is the only time breads were removed from the storage containers. Nutritionist Pro™ software (version 2.2.16, First DataBank, Inc.; San Bruno, CA) was also used to determine the nutritional analysis of each type of bread.

4.2 Lipid Extraction and Gas Chromatography

All chemicals were purchased from Fisher Scientific (Fisher Scientific International; Pittsburgh, PA). Internal standards were purchased from Sigma (Sigma-Aldrich, Inc; St. Louis, MO).

Total lipids and triglycerides were extracted from the flaxseed using an adapted Folch method (61) to determine the actual amount. For total lipid extraction, one gram of flax meal was homogenized with 20 mL of chloroform:methanol (2:1) in a 50 mL teflon tube, and then centrifuged for four minutes at 2000 rpm. The upper layer was removed and the bottom layer was transferred to a new teflon tube and washed with four mL of 0.9% NaCl solution, vortexed for one minute, and then centrifuged for four minutes at 2000 rpm. Layers were again separated by adding chloroform: methanol: distilled water (3:48:47), and this process was repeated until the upper layer, including the "fluff", was totally separated from the bottom layer. The bottom layer was dried under nitrogen and then 10 mg of lipid extract was transferred to a glass test tube. One mL of boron triflouride in methanol and three mg of C17 standard were added to the ten mg of extract. The extract was then heated to 100°C for 45 minutes and cooled for five minutes. To this, two mL of pentane and one mL of distilled water was added, and then vortexed for one minute and centrifuged for four minutes at 2000 rpm. Layers were again separated and the top layer was dried under nitrogen and dissolved in 500 uL hexane. A gas chromatography vial was filled with the solution and nitrogen was blown over the top before sealing. The vial was injected into a gas chromatography autoinjector and run with internal standards to determine the total lipids. Shimadzu Model GC14A (Shimadzu Corp.; Columbia, MD) was the gas chromatography autoinjector used in this study. A SP2330 capillary column that was 30 meters long X 0.32 ID, and injector B were used. The column reached a temperature of 150-205°C at 5°C per minute. Injector temperature was 220°C and detector temperature was 230°C. Run time was set for 30 minutes. Flow rate for helium was one ml/minute, for make-up gas (helium) was 50 ml/minute, for air was 300 ml/minute, and for hydrogen was 30 ml/minute with a split ratio of 1:8. The sensitivity was 10^{-1} and the attention was six. The standard used was Supelco RM-1 and the internal standards used were five mg of C17 (heptadecanoic acid) and rapeseed oil. Internal standards were diluted by placing 125 mg of standard in 25 ml of chloroform, and five mg of this solution was then placed in a one ml aliquot for autoinjection.

4.3 Peroxide Value Rancidity Test

The rancidity test performed was adapted from AOCS Method Cd 8-53, Peroxide Value Acetic Acid-Chloroform Method (62): method for the measurement of hydroperoxides formed in initial stages of oxidation. This test evaluates the degree of lipid breakdown, and also evaluates soy’s effectiveness as an antioxidant. Bread samples were tested weekly to assess rancidity.

Portions of each bread type were broken up and homogenized with chloroform: methanol (2:1). Ten grams of the extracted material was combined with 50 mL of an acetic acid:chloroform solution (3:2) in a 500 mL Erlenmeyer flask. The mixture was vortexed and one mL of saturated potassium iodide solution was added with a pipette. The solution stood for two minutes in the dark and 100 mL of distilled water was immediately added to end the reaction. The solution was titrated with 0.1 N sodium thiosulfate until the yellow color of iodine disappeared. Starch indicator solution was added when needed and titrations continued until all blue color disappeared. A blank was used as a control. The formula $PV = \frac{(S - B) \times N \times 1000}{W}$, was used to calculate the PVO value where S = mL of sodium thiosulfate to titrate fat sample, B = mL of sodium thiosulfate to titrate blank, or 0, N = normality of sodium thiosulfate, or 0.1, and W = weight of sample, or ten grams.

4.4 Moisture

Moisture was measured with a Brabender Moisture Tester (C.W. Brabender® Instruments, Inc; South Hackensack, NJ). Ten grams of sample from the middle of each pup loaf was placed on a pan and put into the moisture tester. Samples were heated until moisture equilibrium was reached, about one and a half hours, and then reweighed for moisture loss. The formula below was used:

$$\frac{\text{Wt. Before Heating} - \text{Wt. After Heating}}{\text{10 grams}} \times 100 = \text{Percent Moisture}$$

4.5 Crust and Crumb Color

Color of both the crust and crumb was measured with a Minolta CR-300 Colorimeter (Minolta Corp; Ramsey, NJ). After the colorimeter was zeroed, the pup loaf was placed under the colorimeter and tested for crust brightness: L (0=black; 100=white), redness: a (+a = red; -a = green), and yellowness: b (+b = yellow; -b = blue). The loaf was then cut into slices with ½ an inch thickness and tested for crumb color.

4.6 Texture

Texture was measured with a Stevens LFRA Texture Analyzer (Texture Technologies Corp; Scarsdale, NY). Slices of bread measuring ½ an inch in thickness were placed onto the texture analyzer platform and plunger #5 was used to test for firmness at a speed of two mm / sec traveling a distance of ten mm.

4.7 Volume

Volume was measured using a loaf volumeter (63). A pup loaf of each bread type was covered in plastic wrap and placed inside the volumeter. Volume was then determined by using displacement with rapeseeds.

4.8 Sensory Analysis

Quantitative Descriptive Analysis (QDA) was used to evaluate the quality characteristics of the different bread types (64). QDA test results can monitor changes in the sensory characteristics of a product. Panelists were eight volunteer graduate students from the Human Nutrition, Foods, and Exercise Department. Two half-hour training periods were conducted to specially train the panelists to recognize stale bread and rancid flax, and also to develop a scale for recording intensities and word anchors for each scale category. Panelists determined descriptive terms of each scale category to help in quantification of the flax meal and soy flour bread product. At these sessions panelists were given informed consent forms and told the details of the study (Appendix A). The descriptors derived by the panelists were: musty aroma, aftertaste, stale taste, moisture content, grainy taste, crumb texture, and softness. A scorecard was created with a 9-point category scale as the quantification tool and the descriptors to be rated on line scales with extremes at each end (e.g. absence of characteristic = 1 and strong presence of characteristic = 9) (Appendix B). Since a 9-point scale was used the results were quantitative and generated data that could be analyzed and used to monitor changes in sensory characteristics of the breads over time. The sensory panel completed QDA tests once weekly for four weeks on each bread sample. The panelists were seated in a sensory booth in Wallace Hall, room 335. The bread was sliced at one-inch thickness, plated on matching white paper plates, and covered with clear plastic wrap prior to the panelists' arrival. A new random three-digit number was given to each bread sample each week to prevent bias. Panelists were given bread samples one at a time with a cup of water to rinse between samples.

4.9 Statistical Analysis

A consulting team appointed by the consulting department within the Statistics Department of Virginia Tech helped to determine the experimental design of this study. Statistical Analysis System (SAS®) (SAS Institute, Inc; Cary, NC) was used for the statistical analysis. A mean approach was used for volume, crust color, and crumb color based on a smaller number of valid observations, while a block approach was used for texture, moisture, and peroxide value since more observations were able to be used. The two “blocks” were one eight weeks of bread testing and then a second eight weeks of bread testing where nothing was altered but new loaves were prepared. Contact with the consulting team was continued throughout the study to determine statistical results for reporting.

One-way analysis of variance (ANOVA) was used to compare the mean values recorded weekly for each of the bread characteristics. A significance level of $P \leq 0.05$ was used. Tukey-Kramer test was used to compare least significant differences for characteristics among weeks and among bread types. The GLM procedure and Tukey-Kramer test was used to compare the values from sensory panelists.

Chapter 5: Results and Discussion

5.1 Volume

Physical tests were conducted on the breads weekly for eight weeks to assess changes in quality. These tests were for volume, texture, moisture, crust color, crumb color, and peroxide value. Breads prepared contained either 100% bread flour, or 15% flax meal or 15% flax meal and various percentages (5% and 10%) of soy flour in place of part of the regular bread flour. Volume was determined by displacement with rapeseeds. Results of this study found that the flax and / or soy breads were significantly lower ($p \leq 0.005$) in volume than the regular yeast bread (Table 1). Breads containing flax and/or soy would be expected to be lower in volume than the regular yeast bread since flax meal and soy flour are both heavier ingredients and both contain little to no gluten. Gluten provides a framework for bread by providing strength and cohesiveness (45), and both gluten quantity and quality affect loaf volume.

Vital wheat gluten was added to the flax and/or soy breads in this study to assist with gluten content in the breads containing flax and / or soy. Vital wheat gluten is wheat protein separated from wheat starch usually with a combination of centrifuge, washings, and screenings. In washing, dough is made and manipulated under a running stream of water. This washes out the starch and other soluble parts of the dough, and leaves behind the mass of gluten. The gluten is then dried and processed into a fine powder (65). Powdered vital wheat gluten rapidly absorbs water making it very elastic and similar to regular gluten. It helps to create an increase in yield, dough strength, shelf life, and dough cohesiveness.

Table 1. Mean (n=9) Volume for each Bread Treatment over an 8-Week Period

Treatments	Mean Volume (cm^3)	Standard Deviation
Control Bread*	1434 a	$\pm$0.59
Flax Bread*	1329 b	$\pm$0.59
5% Soy Bread*	1347 b	$\pm$0.59
10% Soy Bread*	1295 b	$\pm$0.59

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter are not significantly different at $p \geq 0.05$

The AACC Method 10-11 (60), sponge dough method was also used in this study to account for the heavier ingredients with lower gluten levels. The sponge dough method begins fermenting sooner and allows more time for the bread's framework to develop, and therefore, flours with less gluten can still be used and a satisfactory loaf may still be achieved (50). However, the results (Table 1) suggest that the addition of vital wheat gluten and the use of the sponge dough method did not alleviate the problem of bread loaves with decreased volume since regular yeast bread was significantly higher in volume than breads containing flax and/or soy. Also, no significant differences ($p > 0.05$) were noted between the volumes of breads over the period of eight weeks, as the loaves did not shrink considerably.

A study by Dhingra and Jood (66) blended soy flour (either full-fat or defatted) and barley flour with wheat flour to study the effect on volume and other characteristics of bread. The researcher's findings agreed with the current data that the addition of heavier ingredients with lower gluten levels, such as barley and soy flours, play a role in decreasing loaf volume.

5.2 Moisture and Texture

During baking, gelatinization causes starch granules to swell and lose their crystalline structure. As soon as baking is complete staling begins as starch molecules begin to recrystallize, or retrograde. The amylose fraction of starch rapidly undergoes retrogradation and will have completed the process by the time the bread is cooled to room temperature (67). Amylopectin is involved in the staling and firming that occurs during storage.

Moisture is not actually lost from the bread as it stales, but moisture migrates to other areas of the bread. First moisture migrates from the crumb of the bread to the crust, which makes a once crisp crust become soft. Moisture may also migrate among starch components in the bread, from gluten to starch and vice versa (68). Often analytical moisture tests reveal that the moisture content is the same over time since a loss of moisture did not occur, but a migration of moisture occurred (69). Bread has an initial crumb moisture content that is between 35% and 45% (68). Results (Table 2) indicated values slightly below this range. In addition, there were no significant differences ($p > 0.05$) among the bread varieties, nor were there any significant ($p > 0.05$) changes that occurred in moisture content over the eight week period (Table 2).

As amlyopectin recrystallizes, the branched molecules join together forming a more rigid structure and creating a firmness in the crumb of the bread (68). As staling continues over time, the firmness in bread texture also increases. Moisture migration from the crumb to the crust will also create a firmer crumb. Texture was measured as the resistance to penetration of the bread. The texture of the regular yeast bread was significantly softer ($p < 0.02$) than the breads containing flax and/or soy (Table 3). Staling and moisture migration appeared to occur more readily in breads containing flax meal and soy flour that resulted in a firmer texture. Since the volume of breads containing flax and/or soy was also significantly lower, a more compressed crumb may also have contributed to a firmer texture. Additionally, there were also significant differences ($p < 0.003$) in the texture of the breads over the eight week period (Fig. 1). Bread from the first week was significantly softer in texture than bread from all remaining

Table 2. Mean (n=16) Percent Moisture for each Bread Treatment over an 8-Week Period

Treatments	Mean Percent Moisture (%)	Standard Deviation
Control Bread*	33.60 a	±0.05
Flax Bread*	33.40 a	±0.05
5% Soy Bread*	32.90 a	±0.05
10% Soy Bread*	32.50 a	±0.05

*Control Bread: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

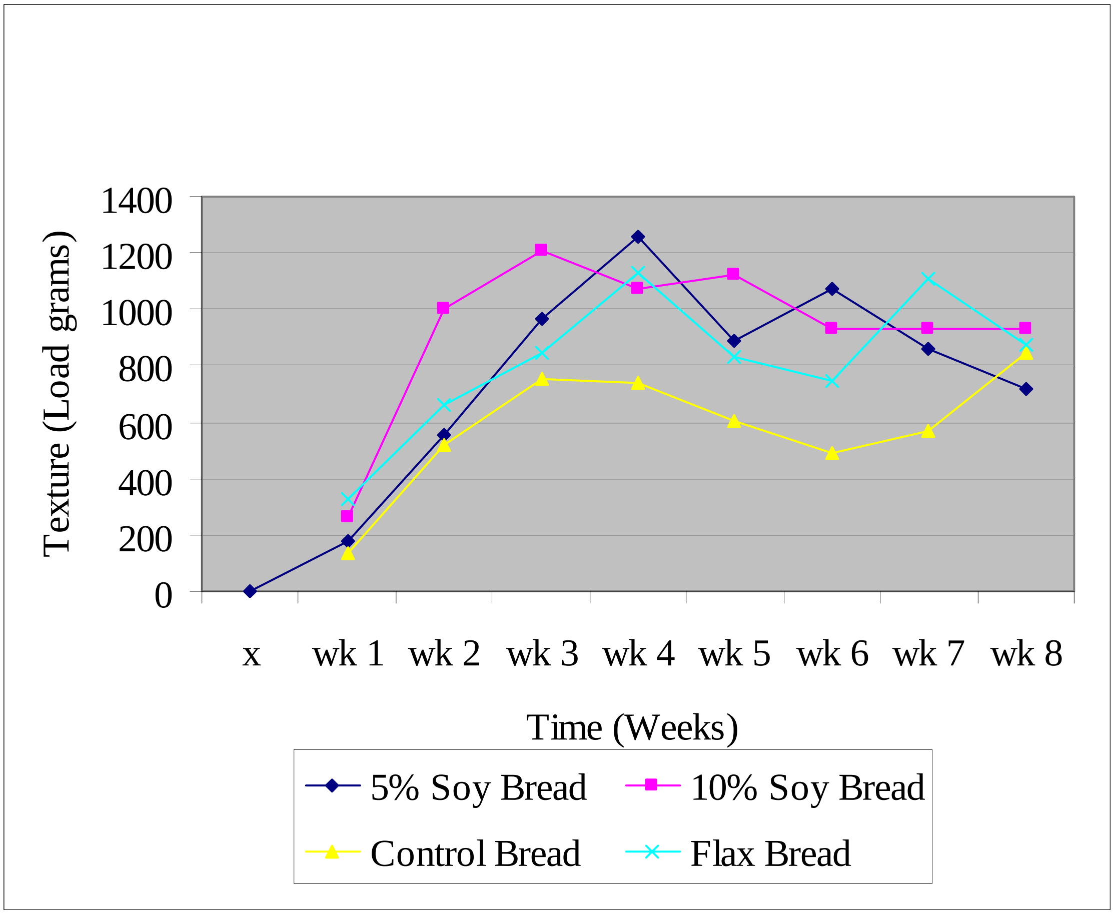
Values with the same letter are not significantly different at $p \geq 0.05$

Table 3. Mean (n=16) Texture for each Bread Treatment over an 8-Week Period

Treatments	Mean Texture (Load grams)	Standard Deviation
Control Bread*	582.56 a	$\pm$147.11
Flax Bread*	816.25 b	$\pm$147.11
5% Soy Bread*	812.25 b	$\pm$147.11
10% Soy Bread*	934.10 b	$\pm$147.11

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter are not significantly different at $p \geq 0.05$



*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Figure 1. Effect of Eight Weeks of Storage on Texture of Breads

weeks. This is due to the staling of breads that occurred with time, as the moisture migrated and retrogradation occurred giving the crumb a firmer texture. There were no significant differences in the texture of breads during any of the remaining weeks ($p > 0.05$).

5.3 Crust Color and Crumb Color

Crust color is affected by Maillard browning. The Maillard reaction is a nonenzymatic browning reaction that involves the amino group of a protein or amino acid and the carbonyl group of a simple sugar. Therefore, the amount of starch and protein in a bread formula will affect the rate of darkening. Of the amino acids, lysine participates strongly in the Maillard reaction producing a dark brown color (70). Therefore, lysine-rich products, such as soy flour, which has 44% lysine-rich protein, will darken more than others when baked (69). The flax bread was significantly lighter in color than the other breads, while the 10% soy bread was significantly darker than the others ($p < 0.04$) (Table 4). These results agree with the principle that lysine-rich products have increased browning. However, bread with the addition of 5% soy was only significantly different from flax bread ($p < 0.02$), but not the control bread. Flax bread had the lightest crust color because flax meal does not participate in the Maillard reaction as much as bread flour and soy flour do. Therefore, flax bread lacked the simple sugars necessary to react with the amino groups and produce a darkening in color. There were no significant differences ($p > 0.05$) between the crust colors of the breads over the eight week period. After the initial browning caused by a Maillard reaction during baking, the crust color did not appear to darken or lessen with time.

Table 4. Mean (n=9) Crust Color for each Bread Treatment over an 8-Week Period

Treatments	Mean Crust Color (L value)	Standard Deviation
Control Bread*	45.76 b	$\pm$3.72
Flax Bread*	49.66 a	$\pm$3.72
5% Soy Bread*	42.87 b	$\pm$3.72
10% Soy Bread*	41.16 c	$\pm$3.72

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter are not significantly different at $p \geq 0.05$

L = 0 = black, 100 = white

Crumb color does not undergo Maillard browning, as noted with the crust. Instead, crumb color would be more affected by the ingredients in the formula. The regular yeast bread was significantly lighter ($p < 0.0001$) in crumb color than the breads with the addition of 15% flax meal or 15% flax meal and 5% or 10% soy flour (Table 5). Since both flax meal and soy flour are darker than regular bread flour it would be expected that breads containing one or both of those ingredients would differ in color. The crumb color of the 5% soy bread was also significantly darker ($p < 0.01$) than the flax meal bread.

5.4 Peroxide Value

Besides being low in carbohydrates and high in protein, flaxseed contains high amounts of heart-healthy omega-3 and omega-6 fatty acids. The flax meal used for bread production in this study contained 2.9 mg of stearic acid (C18), 22.4 mg of oleic acid (C18:1), 14.7 mg of linoleic acid (C18:2), and 0.3 mg of linolenic acid (C18:3) (Table 6) based on the lipid extraction and gas chromatography analysis methods (61). Although high levels of unsaturated fatty acids such as oleic, linoleic, and linolenic acids are desirable for health benefits, the double bonds present in unsaturated fatty acids are less stable and therefore more easily oxidized. Linoleic acid is considered twenty times more susceptible to oxidation than oleic acid due to the presence of a 1,4-pentadiene structure (71). The two double bonds present in linoleic acid are also doubly activated. Linolenic acid, which contains two 1,4-pentadiene structures and three double bonds, is even more susceptible than linoleic acid. Exposure to catalysts such as high temperatures and light will speed up the breakdown process.

Table 5. Mean (n=9) Crumb Color for each Bread Treatment over an 8-Week Period

Treatments	Mean Crumb Color (L value)	Standard Deviation
Control Bread*	75.51 a	±6.43
Flax Bread*	63.65 b	±6.43
5% Soy Bread*	61.69 c	±6.43
10% Soy Bread*	62.94 bc	±6.43

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter are not significantly different at $p \geq 0.05$

L = 0 = black, 100 = white

Table 6: Flax Meal Lipid Analysis Results

Fatty Acid	Amount Present in Flax meal Used (mg/g)
Stearic Acid: C18	2.9
Oleic Acid: C18:1	22.4
Linoleic Acid: C18:2	14.7
Linolenic Acid: C18:3	0.3

Soybeans are naturally high in fat like flaxseed but when ground into soy flour the beans are most often dehulled and defatted to lower the fat content. The soy flour used in this study was low-fat, although a non-fat version is available (defatted). The soy flour was extruded in order to remove fat. Although no chemicals or solvents were used, this process still may have decreased the antioxidant potential of the soy flour.

The flax meal used in this study was processed in March 2004 and the soy flour was processed in January 2004 by their respective processor, and bread baking began in May 2004. No initial peroxide values were measured so it cannot be guaranteed that these ingredients had not begun to go rancid before testing began. To limit the amount of rancidity that occurred during storage, breads were placed into commercial polyethylene bread bags after cooling and then stored inside plastic storage containers to be protected from moisture and light. The temperature and humidity inside the plastic storage container were monitored with a Dickson temperature and humidity data logger. DicksonWare™ Software, version 7.8, was used to determine the average ambient temperature recorded was 22.5°C and the average relative humidity was 48.4% throughout the eight weeks.

Peroxides are primary breakdown products that decompose into secondary breakdown products, which cause the off-aromas and flavors most often found in rancid fats (8). Hydroperoxides are formed when a hydrogen atom is removed from a double bond to produce a free radical (71). Oxygen attaches to this free radical and peroxy radicals are produced. A hydrogen atom is taken from another molecule to create a hydroperoxide and new free radicals which will repeat the process of hydroperoxide formation. A peroxide value test was adapted from AOCS Method Cd 8-53, Peroxide

Value Acetic Acid-Chloroform Method (62) to measure and monitor weekly the amount of hydroperoxides formed in the initial stages of oxidation. Peroxide values were evaluated weekly since peroxides are very unstable and begin to decompose as soon as they are formed (71). In the early stages, the rate of formation exceeds the rate of decomposition, but in the latter stages the rate of decomposition will exceed that of formation. When charted, peroxide values will rise until they peak and then begin to drop off until they are no longer detected. If the product is not closely monitored the determination of rancidity onset cannot be established.

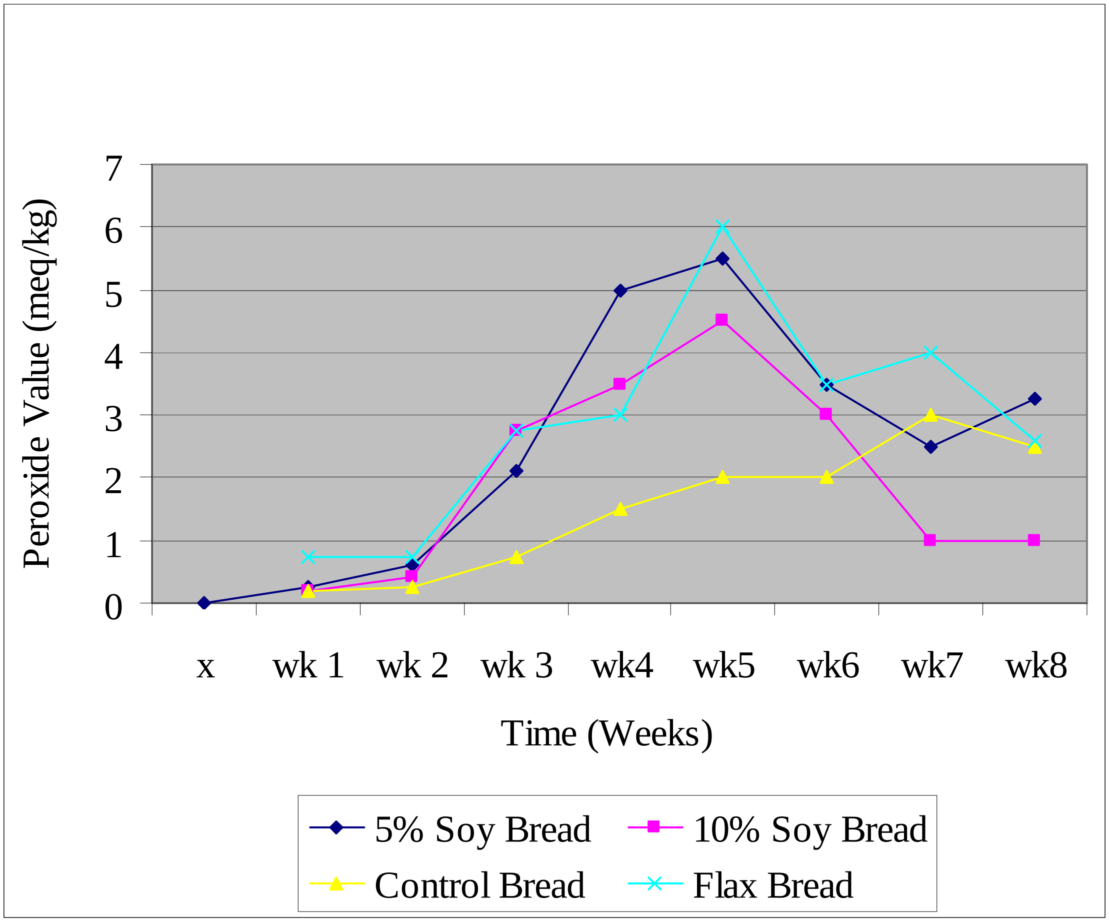
Results from this study indicated that a significant difference in peroxide values only occurred between flax bread and the regular yeast bread ($p = 0.05$) (Table 7). Both breads containing soy were not significantly different ($p > 0.05$) from either the regular yeast bread or the 15% flax meal bread. Based on this result, soy does not appear to prevent rancidity of the polyunsaturated fatty acids found in flax meal. Peroxide values were able to be better controlled when soy flour was added to the bread formula, although the effect was not significant. Analysis of each week shows that bread from week one was significantly different ($p < 0.02$) after weeks four, five, six, seven, and eight. A significant difference was also noted between bread from the second week ($p < 0.02$) and weeks four, five, six, and seven. These results indicate that peroxide values peaked after four weeks when they were first significantly different from baseline, and then began to decline. Bread from week three was only significantly different from week five bread ($p = 0.003$) since peroxide values began to rise in week three but fall after week five (Fig. 2).

Table 7. Mean (n=16) Peroxide Values for each Bread Treatment over an 8-Week Period

Treatments	Mean Peroxide Value (meq/kg)	Standard Deviation
Control Bread*	1.53 a	±0.67
Flax Bread*	2.92 b	±0.67
5% Soy Bread*	2.84 ab	±0.67
10% Soy Bread*	2.04 ab	±0.67

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter are not significantly different at $p \geq 0.05$



*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Figure 2. Effect of Eight Weeks of Storage on Peroxide Values of Breads

Results of this study do not find soy to be an effective natural antioxidant that may be used to extend the shelf life of foods by preventing the formation of hydroperoxides and other free radicals that lead to the oxidative degradation of food. However, a 1994 study by Wu and Brewer (28) did find soy to be a beneficial antioxidant in food products. Soy protein isolate antioxidant (SPIA) was added to ground beef and oxidation was catalyzed by the addition of iron. The addition of 900 ppm of SPIA to beef decreased thiobarbituric acid values, rancid odor, and total volatiles after 16 and 24 hours. A 2004 study by Ulu (73) also supported the antioxidant effects of soy protein isolate in food products. Meatballs were produced with the addition of 0.2% soy protein isolate (SPI), and then cooked and stored. SPI was found to be most effective at preventing lipid oxidation in the cooked meatballs.

Unsaturated fatty acids have multiple double bonds that are less stable than single bonds. A free radical has one or more unpaired electrons and it will take an electron from a vulnerable compound, such as a hydrogen from an unstable double bond, to become more stable itself. Antioxidants either interact with oxidant compounds such as oxygen so that they cannot create damaging free radicals, or they react with free radicals to prevent them from causing further damage (72).

5.5 Sensory Evaluation

The senses of smell, taste, and touch were used by quantitative descriptive analysis (QDA) panelists to evaluate the bread samples. The sense of smell was used by panelists to determine the strength of the musty aroma present, if any, in bread samples. The sense of touch was used to determine the softness or firmness of the breads, and in a slightly different sense of touch, mouthfeel was used to determine if a smooth or coarse crumb texture was present and if the bread was moist or dry. Taste was also used throughout the sensory test to determine if breads exhibited the following characteristics: the strength of the grainy taste, stale taste, and aftertaste of the breads.

During staling a number of changes in sensory characteristics of bread are known to take place. The crumb begins to get firmer and drier, and a deterioration in flavor can be noticed. As flavor deteriorates, some new flavors begin to take place such as sourness and mustiness (68). Panelists were trained to look for sensory changes that indicated a staling of the bread such as increased dryness, firmness, musty aroma, or sour aftertaste, to name a few.

No significant differences ($p > 0.05$) were found in the aromas of all bread types over the four week period (Table 8), although significant differences over time were noted. Bread from week one was found by the panelists to have a significantly stronger musty aroma ($p < 0.04$) than bread from the other three weeks of the sensory test. Over time the aroma of the breads became less inviting to panelists and more of an indicator of staleness and rancidity.

Grainy taste was also described as nutty, wheaty, and floury by panelists in their training session. Control bread had a significantly lower grainy taste ($p < 0.0001$) than the experimental breads (Table 8). The increased grainy taste of the experimental breads is due to the addition of flax meal and soy flour in the bread formulas. The grainy taste of the breads was found to intensify with time as breads from week one and week four were significantly different ($p < 0.02$).

Table 8: Average QDA Scores for Bread Characteristics

	Musty Aroma	Aftertaste	Stale Taste	Moisture Content	Grainy Taste	Crumb Texture	Softness
Control Bread*	3.47 a	3.44 b	4.91 ab	5.59 ab	2.78 a	4.13 a	4.31 a
5% soy Bread*	3.91 a	4.44 b	4.34 a	4.91 a	5.66 b	4.94 ab	4.78 ab
10% soy Bread*	4.09 a	5.56 a	5.97 b	5.94 b	6.09 b	5.44 b	5.56 b
Flax Bread*	3.94 a	4.31 b	5.25 ab	5.75 ab	5.09 b	5.22 b	5.16 ab

*Control: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Values with the same letter within the same column are not significantly different at $p \geq 0.05$

Musty Aroma: 1=none, 9=strong

Aftertaste: 1=none, 9=strong

Stale Taste: 1=none, 9=strong

Moisture Content: 1=moist, 9=dry

Grainy Taste: 1=none, 9=strong

Crumb Texture: 1=smooth, 9=coarse

Softness: 1=soft, 9=firm

An aftertaste was created by an increase in bitter, tangy, and sour notes as the breads staled. The 10% soy bread was found to have a significantly stronger aftertaste ($p < 0.05$) than any of the other breads in this study, although the difference between 10%and 5% soy breads was only slightly significant (Table 8). Aftertaste was found to increase over time as bread from week one had a significantly lower aftertaste ($p < 0.007$) than breads from weeks three and four. The aftertaste of bread from week two was significantly lower ($p < 0.5$) than bread from week four.

The sense of touch was used to determine the softness of the bread samples. The 10% soy bread was significantly firmer than the control bread ($p < 0.01$) (Table 8). Additionally, objective texture results found all the remaining experimental breads to be firmer than the control bread (Table 3). Stale taste of breads was also described during training of QDA panelists as a toughening and firming of the bread. Panelists determined that only 10% soy bread had a significantly stronger stale taste ($p < 0.0008$) than the 5% soy bread over the four week period (Table 8).

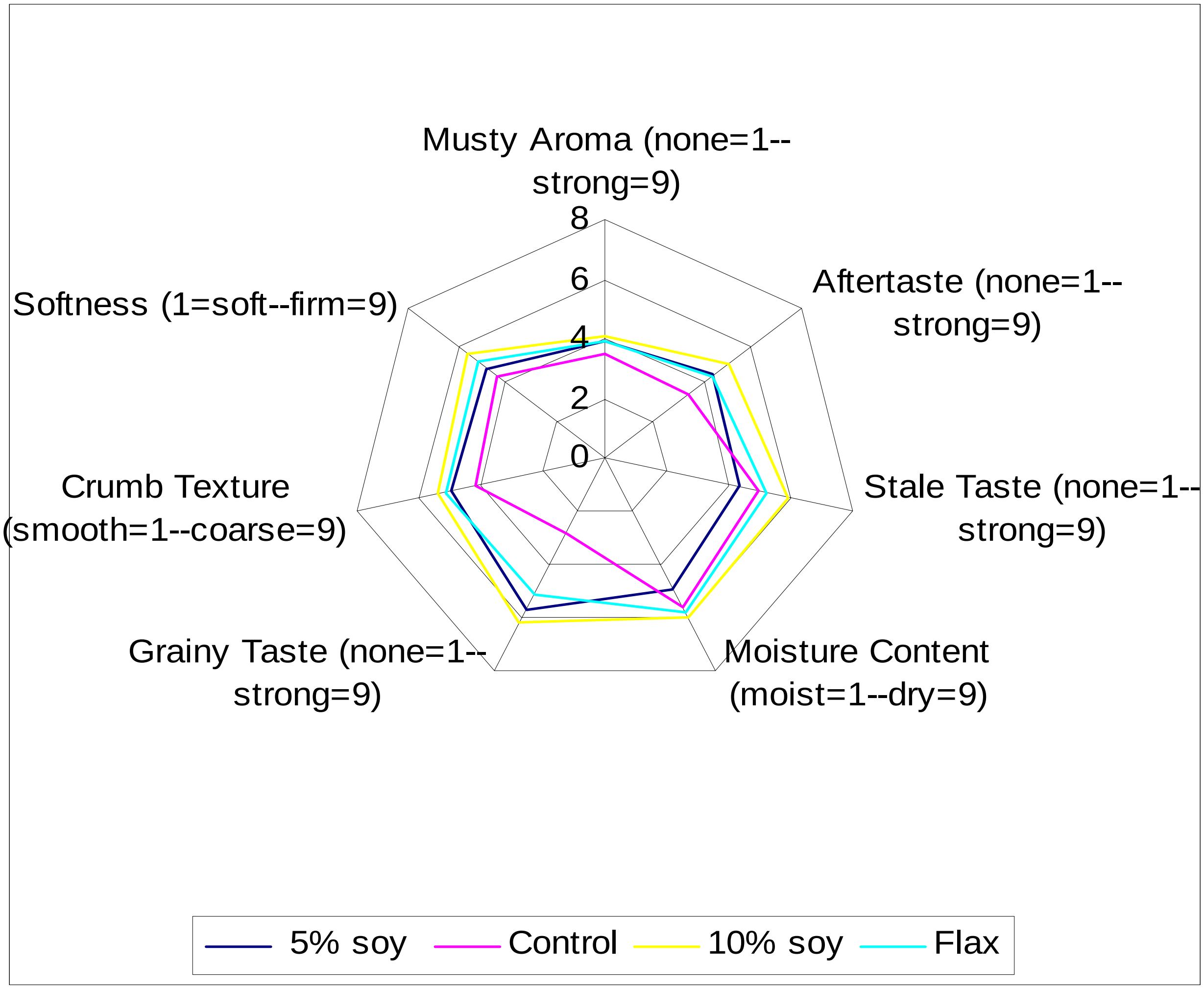
Objective moisture tests do not typically measure changes in moisture content since moisture migrates and is not lost (69). Sensory moisture tests may be more in depth in monitoring the loss of moisture from the crumb. The 10% soy bread was found to be significant drier ($p < 0.05$) than the 5% soy bread (Table 8), but no significant differences between the moisture contents of all the other breads were noted.

Based on mouthfeel, the crumb texture of the control bread was found to be significantly smoother ($p < 0.03$) than that of the 10% soy bread or flax bread (Table 8). Although staling creates a coarser crumb, the addition of flax meal and soy flour into the bread formulas would also contribute to a coarser crumb.

Figure 3 summarizes the results of the QDA tests. The seven different points creating the plot represent the seven bread characteristics that were tested by QDA panelists. The four colored rings in the center of the plot represent the various bread types that were tested, and give the plot the overall appearance of a spider web. From the lines representing the various bread types, we can find the 10% soy bread line farthest from the center and the control bread line closest to the center meaning that 10% soy bread had the highest sensory ratings on the 9-point scales used, while the control bread had the lowest ratings. The lower ratings indicate the least taste and texture changes and consumer dislike, while the highest ratings indicate the most taste and texture changes and consumer dislike. The lines for flax and 5% soy breads alternate. This analysis indicates that flax bread had a higher rating for a certain bread characteristic while 5% soy bread had a higher rating for other characteristics. In summary, however, the addition of 10% soy to bread altered the taste and textures more than was desired. The addition of 5% soy to bread does not appear to alter taste or texture more than was desired since it still appealed to QDA panelists.

5.6 Nutritional Analysis

A recent trend of low carbohydrate diets such as the Atkins diet has captured the attention of many Americans. The Atkins Diet was developed by Dr. Roger C. Atkins upon the scientific principle that if the body does not have any more carbohydrate stores to be used as energy it will use fat stores (74). His diet advises very low carbohydrate intake and high fat and protein intake in order to convince the body to begin using fat stores for energy. The food industry has begun developing many new foods and many



*Control Bread: 100% bread flour
Flax bread: 85% bread flour and 15% flax meal
5% soy bread: 80% bread flour, 15% flax meal, and 5% soy flour
10% soy bread: 75% bread flour, 15% flax meal, and 10% soy flour

Figure 3. Radar plot summarizing the sensory score results of each bread type for various bread characteristics

restaurants are adding low carbohydrate menus to their regular menu in an attempt to appeal to consumers following low-carbohydrate diets. Although low-carbohydrate diets are not condoned by this study, the breads that were created in this study fall under the category of low carbohydrate, high protein foods that would appeal to consumers following these diets.

For this reason Nutritionist Pro software was used to determine the nutritional analysis of each type of bread. Both flax meal and soy flour are naturally high in protein and contain fewer carbohydrates than flour made from wheat, so a loaf made with one or both or these ingredients may appeal to consumers on a low carbohydrate diet. The addition of both flax meal and soy flour to bread also increases the fat content, although it is polyunsaturated fats which are considered heart healthy that cause the increase. Increased fat is also acceptable for a low-carbohydrate, high-protein diet such as the Atkins Diet. Since flax meal and soy flour were used in place of regular bread flour, both flax loaves alone (Fig. 7) and flax loaves containing 5% and 10% soy (Figs. 5 and 6, respectively) were higher in protein and lower in carbohydrates than the control bread (Fig. 4), which contained only regular bread flour. As would be expected, the control bread had the lowest protein content and highest carbohydrate level (per gram). Flax meal contributed a slight increase in protein content, but with the 10% addition of soy flour there were higher protein and lower carbohydrate levels. Breads containing flax and soy are already appealing to consumers due to their abundance of health benefits, but recently flax and soy breads would have high market value for their nutritional content, since they are low in carbohydrates and high in protein.

Nutrition Facts

Serving Size 1 slice (40 g)
Servings Per Container 10

Amount per Serving

Calories 145

	% Daily Value*
Total Fat 2g	2%
Saturated Fat 0.2g	1%
Trans Fat 0 g	
Cholesterol 0mg	0%
Sodium 273mg	11%
Total Carbohydrate 28g	9%
Dietary Fiber 1g	4%
Sugars 2g	
Protein 5g	

Figure 4. Label for Control Bread

Nutrition Facts

Serving Size 1 slice (40 g)
Servings Per Container 10

Amount per Serving

Calories 157

	% Daily Value*
Total Fat 3g	5%
Saturated Fat 0.4g	2%
Trans Fat 0 g	
Cholesterol 0mg	0%
Sodium 276mg	12%
Total Carbohydrate 25g	8%
Dietary Fiber 3g	10%
Sugars 2g	
Protein 6g	13%

Figure 5. Label for 5% Soy Bread

Nutrition Facts

Serving Size 1 slice (40 g)
Servings Per Container 10

Amount per Serving

Calories 158

	% Daily Value*
Total Fat 4g	5%
Saturated Fat 0.4g	2%
Trans Fat 0 g	
Cholesterol 0mg	0%
Sodium 276mg	12%
Total Carbohydrate 25g	8%
Dietary Fiber 3g	11%
Sugars 3g	
Protein 7g	

Nutrition Facts

Serving Size 1 slice (40 g)
Servings Per Container 10

Amount per Serving

Calories 156

	% Daily Value*
Total Fat 3g	5%
Saturated Fat 0.4g	2%
Trans Fat 0 g	
Cholesterol 0mg	0%
Sodium 276mg	11%
Total Carbohydrate 26g	9%
Dietary Fiber 2g	10%
Sugars 2g	
Protein 6g	

Figure 6. Label for 10% Soy Bread Figure 7. Label for Flax Meal Bread

Chapter 6: Summary, Conclusions, and Recommendations

6.1 Summary and Conclusions

As the incidence of chronic diseases in America has become more prevalent, Americans are attempting to become healthier through food and exercise. The consumption of functional foods has been on the rise as a result of this increased health awareness. Flaxseed and soybeans both are considered functional foods because they have many positive effects on health due to substances such as phytochemicals. They have been shown to have positive effects on diseases, such as, heart disease and certain cancers. Research is ongoing to determine their exact mechanisms of action and the other unknown aspects of these functional foods.

However, the incorporation of flaxseed into food products presents the potential problem of rancidity occuring since flaxseed contains many unstable, unsaturated fatty acids. A gas chromatography lipid analysis (Table 6) of the flaxseed used in this study determined that it contained high amounts of oleic acid (C18:1) and linoleic acid (C18:2). Although only a small amount of linolenic acid (C18:3) was present, the three double bonds present in that fatty acid are more easily disrupted by oxidative agents such as oxygen than the two double bonds of linoleic acid or the one double bond of oleic acid. There are many catalysts that speed up the oxidation process that are easily encountered during baking, such as high heat temperatures, moisture, and light. In order to use flaxseed in baked goods and other food products, antioxidants need to be considered.

Antioxidants bind with oxygen and other oxidative agents themselves to prevent the damaging oxidation reaction from taking place. Antioxidants may be natural, such as ascorbic acid and vitamin E, or chemical, such as BHT or BHA. Although chemical antioxidants are typically found to work better than natural antioxidants, many consumers are wary of food ingredients that are not natural. Soy has been found to be a successful natural antioxidant, although more studies have been conducted on the antioxidant activity of soy in the body rather than in food products.

The basis behind this study was to determine if soy could act as a natural antioxidant to prevent flax from going rancid if both were incorporated into a food product. Bread was chosen as the food product and the objectives of this study were to incorporate both flax meal and soy flour into a bread as partial replacements for flour, to determine the effectiveness of soy as a natural antioxidant in maintaining the keeping quality in the flax meal / soy flour bread, and to evaluate the physical and sensory quality of the of the flax meal / soy flour bread. Yeast breads were prepared containing 100% bread flour, bread flour with 15% flax meal, or bread flour with 15% flax meal and either 5% or 10% soy flour in place of a portion of the regular bread flour in bread formulas for this study.

Bread baking is considered an art by many, and is a complex process when done from scratch. The decision to use bread as the vehicle for flax and soy substitution came with many problems of its own other than the possible rancidity. Firstly, using flax meal and soy flour in place of part of the regular bread flour altered the outcome of the bread loaf. When compared to regular bread flour, both flax and soy are heavier ingredients and contain significantly less gluten, which creates a framework for the bread. Vital wheat gluten was added to make up for this deficiency, and the sponge dough method was also used in an attempt to strengthen the dough's framework and allow the volume of the experimental breads to match that of the control bread.

Calcium proprionate is a mold inhibitor that was added to the bread formulas, but molds still continued to form after time on some breads and could not be used in this study. Staling also occurs with time, and begins as soon as baking is complete. Starch molecules that have gelatinized and lost their crystalline structure during baking begin to recrystallize, or retrograde. The amylose fraction of starch has retrograded completely once the bread has cooled, but the amylopectin fraction of starch begins to retrograde after storage and time. This brings on a migration of moisture inside the bread which leads to a firming of the crumb. Flavor compounds also change with time creating additional musty and sour flavors which lead to different aromas. During this study, objective and sensory tests were conducted for eight weeks to determine the effect of the addition of flax and soy on bread characteristics.

The volume of the breads containing flax and/or soy was found to be significantly lower ($p < 0.005$) than the volume of the control bread. Both flax meal and soy flour are heavy ingredients that have less gluten than regular bread flour causing a decrease in volume.

Staling typically produces a drier crumb, even though there were no significant moisture changes between the breads ($p > 0.05$). However, analytical tests usually do not measure a change in moisture content since a loss in moisture has not occurred, but a migration of moisture has occurred.

The texture of the breads containing flax and/or soy was found to be significantly firmer ($p < 0.02$) than the texture of the control bread. The addition of coarser ingredients such as flax and soy leads to a firmer crumb, as well as, the role that staling plays in the bread. Moisture migration from the crumb to the crust occurs during staling causing a less crisp crust and a firmer crumb.

The addition of flax and soy also led to a significantly darker ($p < 0.01$) crumb color than that of the control bread. The darkening of the crust however, is affected more by the Maillard browning reaction between protein and starch. Soy flour is very high in the amino acid lysine, which participates effectively in the Maillard browning reaction. This may have attributed to the fact that 10% soy bread was found to have a significantly darker crust color ($p < 0.04$) than the other breads.

Peroxide values of all breads began increasing around week four of the study and then decreased. Hydroperoxides begin decomposing as soon as they are created and secondary oxidation products, such as aldehydes, ketones, or alcohols, begin to be formed. It is due to this factor that a decrease in hydroperoxides, and therefore peroxide value, over time is considered normal. All breads exhibited oxidation activity, while the flax bread exhibited the highest rate of oxidation activity. Flax bread was significantly different in peroxide value ($p = 0.05$) from the regular yeast bread, but the breads containing soy flour were not significantly different from the flax bread or the regular yeast bread ($p > 0.05$). Soy was not found to suppress the oxidation of the unsaturated fatty acids found in flax since the peroxide values of the flax and soy breads were not significantly different from each other, or from the regular yeast bread.

Sensory analysis was also conducted for four weeks to monitor changes in the breads either due to the addition of flax and soy or due to time. Eight panelists were trained during two half hour sessions and they created descriptors for bread characteristics that were used on 9-point scales to monitor the breads. Although 10% soy bread was successful at preventing oxidation, QDA panelists appeared to favor this bread the least. Panelists found the 10% soy bread to have a greater stale taste and to be firmer and drier than the 5% soy bread. The 10% soy bread was found to have a significantly greater aftertaste than all of the other breads. The crumb texture of both 10% soy bread and flax bread had a significantly coarser crumb than the other two breads. Musty aroma was found to be non-significant, and all experimental breads had a significant grainier taste than the control bread.

A nutritional analysis of the breads revealed that the addition of flax and soy creates a high protein, low carbohydrate bread that may also appeal to consumers following low carbohydrate diets, such as the Atkins Diet. The 5% and 10% soy breads both contained the lowest amount of carbohydrates per gram and the two highest amounts of protein per gram (Figures 5 and 6).

Soy was not found to be an effective antioxidant against the rancidity of flax in yeast breads. The addition of 10% soy flour to breads was also found to be unappealing to consumers. Although the addition of more soy flour may work as an effective antioxidant, the sensory attributes of the bread would not be appealing to consumers, based on the QDA results of this test. More effective solutions need to be found to incorporate flax into food products while preventing the rancidity of its many unsaturated fatty acids.

6.2 Recommendations for Future Research

Both flax meal (15%) and soy flour (5% and 10%) were added to bread formulas to replace part of the amount of bread flour to create a nutritious bread. However, flax meal has many unsaturated fatty acids that contribute health benefits, but also have the potential to go rancid. Soy was not found to be an effective antioxidant in suppressing the rancidity of flax. This problem still needs to be investigated, and other effective solutions to the problem of flaxseed rancidity in foods must be found.

The concentrations of flax meal and soy flour used in this study were determined beforehand to maintain the consumer appeal of the bread, while, at the same time, being effective levels to provide their health benefits and antioxidant effects. QDA analysis appeared to find that the addition of 10% soy was not as acceptable to consumers as the addition of 5% soy. A soy flour concentration higher than 10% may be more effective in preventing rancidity, but would not appeal to consumers based on QDA results from this study. Other combinations of concentrations of flax meal or soy flour may be used, such as less flax. Also, other forms of flax and soy may be used. Flax meal and soy flour were used since they are most similar to the bread flour that was substituted.

Certain varieties of flaxseed may contain more or less of the unsaturated fatty acids that lead to rancidity, and would, therefore, affect the outcome of a study such as this. Most flax varieties are brown-seeded, but the yellow-seeded solin varieties have less than 5% of linolenic acid content (7).

The type of soy flour may also be altered. Low fat soy flour was used in an effort to have the most unsaturated fatty acids in the bread formula come from flaxseed. However, the process of defatting the soybeans may actually decrease the antioxidant potential of the soy. A study could be conducted between full-fat soy flour, low-fat soy flour, and non-fat soy flour to determine the effects the defatting process has on antioxidant activity.

Soy flour was tested as an antioxidant in this study in an effort to keep the majority of the ingredients as natural food components. Other natural antioxidants such as vitamin E and ascorbic acid or chemical antioxidants such as BHA or BHT may also be tested in the prevention of rancidity. Other natural antioxidants or chemical antioxidants may work better than soy at preventing the rancidity of flax meal in breads. Natural and chemical antioxidants could also be combined to increase their effectiveness against oxidation of lipids.

In future studies, oxidation of flax meal could be initiated through exposure to a number of different catalysts such as light, oxygen, or iron to better test the effectiveness of soy or other antioxidants. An initial measurement of rancidity must be taken to better determine the effectiveness of antioxidants. Different rancidity tests could also be used to better measure the effectiveness of antioxidants. Tests could be conducted to measure secondary oxidation products such as aldehydes, alcohols, and ketones, and therefore have a better idea of the overall rancidity of a food product.

Since moisture content of foods such as breads does not typically change, but migrates over time, a different moisture test could also be conducted such as water activity. While moisture content measures the total amount of water in a food (bound and free), water activity measures the amount of free water only in a food. This test may be more useful in determining spoilage and rancidity of the breads.

Flax and soy were also used in this study because a possible synergistic relationship is thought to exist between the two ingredients. Flax and soy protect against many of the same diseases, and their health benefits may be increased when they are used together in food products. However, there is very little literature about the use of flax and soy as food ingredients and this, as well as, the relationship between flax and soy, should be explored further.

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Appendix A

Informed Consent Form

Informed Consent for Participation in Sensory Evaluation

Title of Project: The Effect of Soy Flour as a Natural Antioxidant on Flaxseed in Yeast Bread

Principal Investigator: Sarah F. Davis, RD

I. THE PURPOSE OF THIS PROJECT

You are invited to participate on a sensory evaluation panel about bread with flax meal and soy flour. This study was designed to determine differences in the quality of various bread types that occur over time.

II. PROCEDURES

There will be 5 sessions over a period of 5 weeks involving about 30 minutes at each session. You will be presented with approximately 4 samples at 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 other samples.

Certain individuals are sentitive to some foods such as milk, eggs, wheat gluten, strawberries, chocolate, artificial sweeteners, etc. If you 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 helpful: differences in quality of various types of bread over time. 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 code 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 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 Human Nutrition Foods and Exercise.

VIII. SUBJECTS RESPONSIBILITIES

I know of no reason I cannot participate in this study which will require: 5 sessions during 5 weeks lasting 30 minutes.

____

Signature / Date

Please provide address and phone number so investigator may reach you in case of 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 in this project.

I know of no reason I cannot participate in this study which will require: 5 sessions for 5 weeks lasting 30 minutes.

____

Signature / Date

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

Sarah F. Davis / 558-2112
Investigator / Phone

Frank Conforti / 231-8765
Faculty / Phone

Appendix B

QDA Test Scorecard

Name____________________ Date____________________

Sample Code____________________

Taste and evaluate the sample. Read the definition for each characteristic and indicate the degree to which it is present by **checking the appropriate space on the scale**. Take a drink of water after rating the sample.

Attribute 1: <u>Musty Aroma</u> (musty, old, stale aroma)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
No Musty Aroma Strong Musty Aroma

Attribute 2: <u>Aftertaste</u> (bitter, sour, tangy, etc. aftertaste)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
No Aftertaste Strong Aftertaste

Attribute 3: <u>Stale Taste</u> (tough, firm)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
Not Stale at All Very
Stale

Attribute 4: <u>Moisture Content</u>

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

Attribute 5: <u>Grainy Taste</u> (grainy, wheaty, nutty, floury, etc. taste)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
No Grainy Taste Strong Grainy Taste

Attribute 6: <u>Crumb Texture</u> (mouthfeel)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
Smooth Crumb Coarse Crumb

Attribute 7: <u>Softness</u> (tactile)

1______ 2______ 3______ 4______ 5______ 6______ 7______ 8______ 9______
Soft, Springy Hard, Firm

Vita

Sarah F. Davis was born on March 3, 1980 in Harrisonburg, Virginia. She attended Spotswood High School in Penn Laird, Virginia and earned her degree there in May 1998. She then attended Virginia Polytechnic Institute and State University in Blacksburg, Virginia where she received a Bachelor of Science degree in Dietetics and Consumer Foods in December 2001 from the Department of Human Nutrition, Foods and Exercise. Upon completion of her undergraduate degree, Sarah completed a year-long dietetic internship with Virginia Commonwealth University Health System in Richmond, Virginia. She then returned to Virginia Polytechnic Institute and State University to pursue a Master of Science degree in Foods from the Department of Human Nutrition, Foods and Exercise which she completed in December 2004. Sarah passed the registration exam to become a registered dietitian in June of 2003 and plans to combine her nutrition and foods knowledge with a career in the food industry.