

**The Effect of Xanthan Gum and Guar Gum on Enhancing the
Quality and Preventing Lipid Rancidity in Yeast Bread
Supplemented with Flaxseed**

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ABSTRACT

This study examined the effects of guar gum and xanthan gum on flax supplemented breads through objective and sensory testing. Breads containing flaxseed and gums were found to have a significantly ($p < 0.05$) higher water activity than the control bread. Control bread was also found to have a higher ($p < 0.05$) volume while flax breads containing guar gum had a significant ($p < 0.05$) decrease in volume. Control bread and bread containing guar gum were significantly ($p < 0.05$) harder in crumb texture. Breads with flax and xanthan gum displayed a significant ($p < 0.05$) amount of springiness. While there was no significant ($p > 0.05$) difference in peroxide values among bread samples, control bread had a lower ($p < 0.05$) anisidine value indicating a decrease in hydroperoxide breakdown. While not significant ($p > 0.05$), bread containing xanthan gum had a lower anisidine value than the other treatments. Sensory analysis found bread with both gums to be moister ($p < 0.05$) and have a strong ($p < 0.05$) yeasty aroma and fresher flavor. Control bread was found to have the least ($p < 0.05$) yeasty aroma and taste significantly ($p < 0.05$) less bitter but more stale.

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Chapter 1: Introduction

Recent trends in health have led consumers to demand more from their food than just sustenance and nutritional value. Consumers are looking for added health benefits from the foods they eat and are changing their diets to include foods that do. These foods are known as functional foods, and many foods are being studied to classify them as such.

The aim of a functional food or functional ingredient is to promote health and well-being. Functional foods and products are defined as having disease preventing and/or health promoting benefits in addition to nutritive value. Increasingly, consumers are becoming more aware of the fact that their health and quality of life can be greatly influenced by habits such as diet and exercise (Southon, 2000). The estimated potential market for functional foods is 60 billion pounds annually for Britain. The definition and role is not perfectly clear though, leading to multiple variations and names such as nutraceutical, vitafood, pharmafood, designer food, etc. There are four main recognizable ways to obtain a functional food: 1) eliminate a component that has a negative physiological effect, 2) increase the concentration of a component that may have a beneficial effect, 3) add a novel ingredient seen as advantageous such as a vitamin, and 4) replacing fully or partly a negative component with a neutral or positive one that does not adversely affect the nutritive value. Bacteria and microorganisms can fall into the category of functional foods, as can ingredients that have always been present in foods and foods common to the diet, like milk. Plants are considered functional foods, providing sterols and dietary fiber; vitamins and minerals can be considered functional, such as folic acid. Components such as good fatty acids like polyunsaturates and omegas

are considered to make a food functional. The positive effects of any and all of these components lead to and justify health claims, which promote their consumption (Gibson and Fuller, 1998).

Flaxseed is one food that is considered functional. Flaxseed is an oilseed that has been in the human diet for hundreds of years. Flaxseeds can be eaten whole, ground, and milled into flour. This flexibility allows for incorporation into many foods and areas in the diet. It's health benefits are numerous and widespread, from lowering cholesterol and heart disease, to preventing and treating numerous cancers, maintaining diabetic glucose levels and responses, and even reducing symptoms of menopause (Morris, 2001).

Chapter 2: Review of Literature

1. Functional Foods

The position of the American Dietetic Association (ADA) (Anonymous, 2004) is that functional foods (including whole foods and fortified, enriched or enhanced foods) have a potentially beneficial effect on health when consumed as part of a varied diet on a regular basis and at effective levels. Although functional foods remain undefined under current US food regulations, they are usually understood to be any potentially healthful food or food ingredient that may provide a health benefit beyond the traditional nutrients it contains. The term “functional” implies that the food has some value leading to health benefits, including reducing risks for diseases. A random telephone survey of US consumers conducted by the ADA supported the belief that a significant percentage of consumers are interested in their diet and potential health improvements it may contain. About 50% of those surveyed identified functional foods such as soy and berries as having “health related effects”. Another survey by the International Food Information Council reported that 94% of consumers agree that certain foods have health benefits beyond basic nutrition and 85% expressed interest in learning more about these foods (Anonymous, 2004).

The American Council on Science and Health recently reviewed the scientific evidence for the benefits of functional foods and established a Likert-type scale for classification ranging from very strong evidence to weak evidence. The scientific evidence for functional foods and their physiologically active components can be categorized into four areas: 1-clinical trials, 2- animal studies, 3-experimental in-vitro laboratory studies, and 4-epidemiological studies (Anonymous, 2004).

A majority of the current evidence for functional foods lacks well-designed clinical trials; however the evidence from the other types of studies is substantial. For foods with health claims, there is scientific agreement that a diet-disease relationship exists. Basic examples of functional foods that fall into this category are foods rich in soluble fiber which have been associated with a reduced risk of coronary heart disease, fruits and vegetables that reduce cancer risks and coronary heart disease, and soy protein that lowers cholesterol and cardiovascular disease (Anonymous, 2004).

2. Flaxseed

Flaxseed, also known as linseed (*Linum usitatissimum* L.), is an edible oilseed/grain that was a major dietary staple before the Industrial Revolution. However, due to its limited shelf life, it is not commonly part of the modern day diet (Demark-Wahnefried et al., 2001). Canada is the world's largest producer of flaxseed, growing about 38% of the world's total production (Hosseinian et al., 2004). Flaxseed contains 41% fat, 28% dietary fiber, and 21% protein. Flaxseed is low in saturated fat and high in polyunsaturated fat. The amino acid profile of flaxseed resembles that of soybeans. Its roots can be traced back to 3,000 BC, when it was cultivated by the Babylonians and is currently grown all over the world. The ancient Greeks and Romans used flaxseed for its laxative effects and ability to relieve gastric distress (Morris, 2001).

There are two main types of flaxseed, one is grown for its seed use and is considered an oilseed variety while the other is grown specifically for fiber production used in the textile industry (Sylvester, 2001). Flaxseed has recently gained attention as a “functional food” because of its unique nutrient profile and potential effects on

risk/treatment of cardiovascular disease and some cancers. Flaxseed is the richest source of plant based omega-3 fatty acids (Demark-Wahnefried et al., 2001). It is also the richest source of the mammalian lignan precursor secoisolariciresinol diglycoside (Tou and Thompson, 1999). Today, it is being researched for health benefits such as blood cholesterol lowering, protection against heart disease and stroke, prevention of cancers, mediation of the immune system, blood glucose control in diabetics, and hormonal profiles of menopausal women (Morris, 2001)

Oomah, et al. (1997) found that lipoxygenase enzyme in flaxseed varied from 1.6 g/kg to 6.0 g/kg according to type. Lipoxygenase enzyme is widely distributed in plants. It catalyzes the hydroperoxidation of polyunsaturated fatty acids and esters. Because many products of these reactions are aromatic, the presence of lipoxygenase activity in many foods can affect their properties, such as color, texture, functionality, and nutritive value-particularly during storage periods. These effects can be desirable as well as undesirable.

3. Flaxseed and Health

3a. Flaxseed and Cancer

Flaxseed has been shown to have positive effects on preventing cancers and reducing the growth of cancerous tumors in the breast, prostate, colon and skin. Prostate carcinoma is the leading cancer among American men. Breast cancer is the most prevalent malignancy and the second leading cause of death from cancer in American women. Flaxseed is the richest source of the plant lignan secoisolariciresinoldiglycoside

(SDG), a phytoestrogen, and also α -linolenic acid, which is an n-3 fatty acid. SDG has been shown to inhibit the initiation, promotion, and progression of breast cancer in rats. Products of α -linolenic acid metabolism have also been shown to inhibit breast cancer growth (Chen et al., 2002).

Epidemiological data suggest that diets high in phytoestrogens are responsible for the low incidence of breast cancer in Asian women, but the mechanisms have not been explored. Angiogenesis, the generation of new capillaries from preexisting vessels, is one of the most important events in tumor growth, progression and metastatic dissemination. Vascular endothelial growth factor (VEGF) appears to be a key factor in promoting tumor angiogenesis, and has been found in more breast cancer tissue than normal breast tissue. Also, high VEGF levels have been found to correlate with poor prognosis and decreased overall survival (Dabrosin et al., 2002).

Breast Cancer

Rao et. al. (2000) fed TG.NK transgenic mice (which express the c-neu breast cancer oncogene and are useful in evaluating the delay/prevention of breast cancer) a diet of 0.05-0.2 ml of flaxseed oil, 50-200 mg/kg melatonin, or a combination of 0.1 ml flaxseed oil and 50 mg/kg of melatonin for 30 weeks. The diet containing 0.2 ml flaxseed produced some delay in the growth of mammary tumors. The combination diet was found to significantly decrease both the number of tumors and the tumor weight compared to flaxseed alone. Results concluded that flaxseed oil might delay the growth of mammary tumors if the omega-6:3 PUFA ratio of fat consumed is close to 1 as in the 0.2 ml diet.

Chen et al. (2002) studied the effect of flaxseed on the growth and metastasis of breast cancer cells in the nude mice model. Twenty-four nude mice were given access to a high-fat basal diet (BD) alone or the same diet supplemented with full-fat flaxseed (FS). Supplementation incorporated 10% fresh ground flaxseed into the corn oil base diet. Food intakes were recorded and body weight was monitored weekly. After seven days of acclimatization on the BD diet, mammary fat pads of the mice were inoculated with 1×10^6 breast cancer cells. Mice continued on the BD diet for 7 weeks when they were randomized into two groups of similar weight and tumor size. One group stayed on BD; the second was changed to FS. At week 15 all mice were examined for body weight, primary tumor weight and volume, and weights of major organs. No significant differences in food intake or body weight were found. Palpable primary tumor growth rate was significantly reduced ($P < 0.05$) in the FS group after 1 week of diet separation, and this reduction continued until week 14. The final tumor volume and weight at week 15 were lowered by 27% and 15%, respectively in the FS group, however this finding was not significant. There was reduction in total metastasis incidence in the FS group, but was only significant in lymph node metastasis. A significant 25% lower rate of cell proliferation was detected in the FS group compared with the BD group. This study provides experimental evidence of an inhibitory effect of flaxseed on the growth and metastasis of breast cancer in nude mice.

Another similar study (Dabrosin et al., 2002) on nude mice determined whether a FS supplemented diet affected tumor growth, metastasis and extracellular vascular endothelial growth factor (VEGF). Mice were inoculated and randomized as in the previous study. Mice in the 10% FS diet were fed for only 6 weeks before examination.

Body weight, tumor weight, macroscopic metastasis and VEGF were assessed. No significant differences in body or organ weights were found. Tumor growth rate was significantly lower ($P<0.05$) in tumors exposed to the FS diet, however none of them went into complete remission. The incidence of macroscopic distant metastasis was significantly decreased ($P<0.05$) in mice fed the FS diet. Levels of VEGF in extracellular fluid were decreased in FS diet tumors compared with the BD tumors, however the difference was only significant in the large tumors weighing more than 2.5 grams. Results concluded that a dietary supplementation with 10% flaxseed decreased the tumor growth rate and metastasis, as well as significantly lowering levels of VEGF in large tumors. The results of lowering VEGF were the first of their kind at time of publication.

An earlier study (Tou and Thompson, 1999) was performed to determine the effect on mammary glands (cancer risk) of exposing virgin rats to diets containing either 10% flaxseed (10F), 5% flaxseed (5F), flaxseed oil (FO) or pure SDG at the level found in 5% flaxseed. These diets were given to rats during gestation and lactation, after weaning, or continuous from gestation. Twenty-eight pregnant rats were randomized into control, 5F, 10F, FO, or SDG groups. At weaning, female offspring were grouped to continue the diet of their mother. Mammary gland structures and onset of puberty were examined in relation to the diets. Gestation and lactation as well as lifetime exposure to 10F and SDG significantly reduced ($P<0.05$) the terminal end bud (highly proliferative structures) density and enhanced alveolar bud (less proliferative) density compared with the control group. Gestation and lactation and lifetime exposure to 5F significantly reduced ($P<0.05$) terminal bud density but had no significant effect on alveolar bud density. Lifetime and gestation and lactation exposure to 10F resulted in an earlier age of

puberty onset, but no effect on estrous cycles. Lifetime and gestation and lactation exposure to 5F and SDG delayed age of puberty onset and reduced the number of estrous cycles. The progressive differentiation of terminal end buds into alveolar buds is accentuated by each estrous cycle, therefore reducing cancer risk. These results suggest that gestation and lactation is a critical period for inducing structural changes in the mammary gland, and flaxseed was found to reduce the risk of cancer when consumed during this period.

Prostate Cancer

It has been suggested that potential cancer preventing effects of flaxseed may be enhanced with a fat-restricted diet. In a pilot study (Demark-Wahnefried et al., 2001) this was explored for prostate cancer. Twenty-five men with prostate cancer who were awaiting prostatectomy were put on a low fat (20% kilocalories or less), flaxseed enhanced (30 g/day) diet. This diet ranged from 21 to 77 days, with an average of 34 days because patients only stayed in the study until their scheduled surgery. Baseline and follow up (1-3 days before surgery) levels of prostate-specific antigen, testosterone, free androgen index, and total serum cholesterol were determined. After surgery, tumors were biopsied for proliferation rates and apoptotic index. Total serum cholesterol, total testosterone, and free androgen index all significantly decreased in patients. Both the proliferation rate and apoptosis were significantly associated with the number of days on the diet. Data from this pilot study suggest that the flaxseed/fat-restricted diet appeared to influence several biomarkers associated with prostate cancer.

Demark-Wahnefried et al. (2004) also looked at the potential effects of flaxseed consumption on benign prostate tumors and circulating levels of prostate-specific antigen (PSA), total testosterone and cholesterol in 15 men. These subjects were waiting to undergo repeat prostate biopsy and were assigned to follow a low-fat (less than 20% of calories), flaxseed supplemented (30g/day) diet for 6 months. Serum samples were taken and analyzed at baseline and after the 6 months. Reports from the original and repeat biopsies were compared and proliferation rates quantified. PSA and cholesterol levels were significantly ($P=0.0002$ and 0.012 , respectively) decreased. No statistically significant change in testosterone was found. The proliferation rates in the tumors decreased significantly ($P=0.0168$). These results concluded that a flaxseed supplemented, low fat diet may affect the biology of the prostate and associated biomarkers.

Flaxseed supplementation was also explored for effects on the prostate cancer mouse model (Lin et al. 2002). A total of 135 male mice were randomized into two groups, control and 5% flaxseed by weight supplementation. Half of the mice in each group were treated for 20 weeks and the other half 30 weeks. Upon completion of treatment, prostatic lobes, seminal vesicles, emptied bladder, lungs, lymph nodes, and grossly abnormal tissues were collected for histological evaluation. All of the control mice developed prostate cancer, while only 97% of the flaxseed mice did. There were no significant differences in tumors found in the 20-week treatment mice. At 30 weeks the flaxseed treated mice had significantly less ($P=0.01$) aggressive tumors. The prevalence of lung and lymph node tumors differed, but not significantly. After 20 weeks there was a significant difference ($P<0.0001$) in cellular proliferation between the control and

experimental groups. Similar results continued in the 30-week treatment groups. This study concluded that a diet supplemented with 5% flaxseed inhibited the growth and development of prostate cancer in the mouse model.

3b. Cholesterol

Diets that include flaxseed are being studied for their ability to lower cholesterol and plasma lipids. High plasma enterolactone (a metabolic product of flaxseed) has been shown to be associated with low risk of acute coronary events in men (Tarpila et al., 2002). Hypercholesterolemia is a risk factor for the development of heart attack and stroke. Hypercholesterolemia increases the cholesterol concentration of platelets, PMNLs, and endothelial cells; all of which lead to atherosclerosis. Oxygen free radicals (OFRs) have been implicated in the development of hypercholesterolemic atherosclerosis. These OFRs are produced in part by polymorphonuclear leukocytes (PMNLs) and monocytes. Flaxseed has been shown to suppress this production (Prasad 1997). In both men and women, coronary heart disease is the major cause of morbidity and mortality. The risk for coronary heart disease has been strongly correlated with elevated blood cholesterol levels. The risk drastically increases for women at the onset of menopause. Recent findings suggest that lipoprotein is a powerful predictor of heart attack and has been shown to modulate the risk of coronary heart disease in patients with high cholesterol (Arjmandi et al., 1998).

Gambus et al. (2001) fed rats bread in which the wheat flour was replaced with either 10 percent or 13 percent milled flax seeds. Bread diets were consumed for 19 days, and then rats were examined for average cholesterol concentration, low-density

lipoprotein (LDL) fraction, and hypoglycemic effects. All breads, regardless of percentage, containing flax seeds reduced total cholesterol and LDL cholesterol by 47.0 and 48.5 percent respectively. No significant hypoglycemic effect was found.

Effects of high alpha-linolenic acid flaxseed were studied (Cunnane et al., 1993) in women. Subjects consumed 50 g of ground, raw flaxseed a day for 4 weeks. Flaxseed was found to raise alpha-linolenic acid and other long chain n-3 fatty acids in both plasma and erythrocyte lipids. It also lowered serum total cholesterol by 9 percent and LDL cholesterol by 18 percent. These effects were also observed with 12 g flaxseed provided in 50 g of raw flour, which suggests that alpha-linolenic acid is highly bioavailable from ground flaxseed. Significant decreases (27%) in postprandial blood glucose responses were also found.

In a study of young healthy adults (Cunnane et al., 1995), 50 g of flaxseed was consumed daily for 4 weeks. Alpha-linolenate increased significantly in adipose tissue, and plasma LDL cholesterol was reduced by 8 percent. Bowel movements per week increased by 30 percent as well.

Tarpila et al. (2002) studied the effects of flaxseed supplementation as part of a daily diet on serum lipids, fatty acids, and plasma enterolactone. This study was carried out in a controlled, double blind, cross over design by 80 volunteers. Subjects were on both diets (control diet A and flaxseed diet B) for 4 weeks each, separated by a 4-week washout period. In addition, 18 volunteers remained in an open part of the study for 4 more months. Dietary intakes, basic blood values, serum lipids, fatty acids and enterolactone were measured at baseline and after each diet. During the open study, measures were made after 2 and 4 months. The percentage of flaxseed supplemented test

food accounted for 20% of energy intake. There was a significant increase in serum α -linolenic acid, eicosapentaenoic acid and docosahexaenoic acid during the flaxseed diet. Serum enterolactone concentration also doubled. The effects on enterolactone continued to be seen during the open study period. However, there were no significant changes in serum lipid values during either diet. One explanation given for this effect is that flaxseed oil has not been shown to have any hypolipidemic effect; only ground flaxseed can lower both total and LDL cholesterol and triglycerides. In this study, a slight but insignificant reduction in total and LDL cholesterol and triglycerides was noticed, particularly during the open phase. Also in the open phase, a slight but non-significant increase in high density lipoprotein (HDL) cholesterol was shown.

Prasad (1997) investigated the effects of dietary flaxseed on high cholesterol diet-induced atherosclerosis, lipid profiles, and OFR-producing activity of PMNLs in rabbits. A total of 30 New Zealand white rabbits were assigned to 4 groups; control (8), 7.5g/kg body weight flaxseed (8), 1% cholesterol (6), and cholesterol + flaxseed (8). After 8 weeks on the diets, aortas were removed for assessment of atherosclerotic plaques. Blood samples were also taken for measuring triglycerides, total cholesterol, and PMNLs at baseline, and weeks 4 and 8. Basal serum triglyceride levels were similar in all groups. Serum cholesterol levels increased in the cholesterol and cholesterol/flaxseed groups. Atherosclerotic plaques were not observed in the control and flaxseed groups. The aortic surface of the cholesterol group was almost completely covered with plaques. The plaques from the cholesterol/flaxseed group were significantly smaller than the cholesterol group. Flaxseed reduced the development of high cholesterol diet-induced atherosclerosis by 46%. The severity of the plaques was associated with

hypercholesterolemia. PMNL activity increased in the cholesterol group, decreased in the flaxseed group, and remained unchanged in the other two. These results suggest that hypercholesterolemia increases OFR producing activity of PMNLs and that dietary flaxseed decreases the OFR production both in control and hypercholesterolemic rabbits. However, this study did not conclude that flaxseed was effective in lowering serum cholesterol, hypothesized to correlate with high amounts of α -linolenic acid, lignans or both.

Another investigation was conducted to assess the effects of dietary Type II flaxseed on high cholesterol diet-induced atherosclerosis and serum lipid values in rabbits (Prasad et al., 1998). Type II flaxseed has similar oil content as regular flaxseed but has only 2-3% of α -linolenic acid content, which tested the previous hypothesis. Rabbits were grouped as in the previous study, supplementing Type II flaxseed for ordinary flaxseed. Samples of blood and aortas were taken as before, with the addition of measuring LDL cholesterol, HDL cholesterol, and very low-density lipoprotein (VLDL) cholesterol. Triglycerides decreased in the control group and increased in the cholesterol/flaxseed group. Serum total cholesterol and LDL cholesterol levels increased in both cholesterol and cholesterol/flaxseed groups, remaining unchanged in the other two. VLDL cholesterol levels increased significantly in the flaxseed/cholesterol group, while HDL levels decreased significantly. HDL increased in the flaxseed group. Similar aortic plaque results were found as in the previous study, with Type II flaxseed reducing the development of atherosclerosis by 69%. Results suggest that a reduction in hypercholesterolemic atherosclerosis by Type II flaxseed could be due to decreases in serum levels of total cholesterol and LDL cholesterol, but could also be due to other

factors, rejecting the previous hypothesis that α -linolenic acid prevents cholesterol-lowering activity.

Arjmandi et al. (1998) performed a double blind crossover study to compare the effects of whole flaxseed and sunflower seed on the lipid profile of 38 postmenopausal women. Women were provided with 38 g of either treatment in the form of breads and muffins. Each treatment period lasted 6 weeks with a 2-week washout period in between. Blood samples were collected at baseline, and weeks 6, 8, and 14. Significant ($P < 0.001$) reductions in total cholesterol were observed for both treatments. However, only flaxseed was able to significantly lower LDL cholesterol levels. Serum HDL cholesterol and triglyceride levels were unaffected by either treatment. The LDL:HDL ratio decreased at borderline significance ($P < 0.053$) with flaxseed. Lipoprotein concentrations, a strong predictor of cardiovascular disease, were significantly ($P < 0.05$) lowered by flaxseed.

3c. Kidney Disease

Whole flaxseed has been found to be beneficial in the treatment of renal disease. Male rats were fed synthetic diets containing either flaxseed oil or corn oil for 8 weeks. Renal inflammation, fibrosis, proliferation, cystic change, and oxidized-low density lipoprotein were assessed. Flaxseed diets increased positive lipid levels in the blood and kidneys, lowered cystic change by 29.7 percent, fibrosis by 21.7 percent, proliferation by 18.7 percent and oxidized LDL by 31.4 percent. Serum creatinine was significantly lower in the flaxseed diet as well (Ogborn et al., 2002).

3d. Menopause

Lignans and isoflavones, both found in flaxseed, are phytoestrogens that have been shown to exert hormonal effects. Estradiol is the biologically active estrogen that is most often associated with breast cancer and maintenance of the skeleton. Serum estradiol is converted into 2-hydroxyestrone and 16 α -hydroxyestrone, of which 16 α -hydroxyestrone has been shown to increase proliferation of breast cancer cells. It is also associated with increased bone mineral density in postmenopausal women.

Brooks et al. (2004) compared the effects of consuming equal amounts of flaxseed or soy on estrogen metabolism and biochemical markers of bone metabolism in postmenopausal women. The diets of 46 women were supplemented with placebo, 25 g soy flour, or 25 g ground flaxseed in muffins for 16 weeks. Blood and 24-hour urine samples were taken at baseline and at the end. Samples were analyzed for phytoestrogens, estrogen metabolites 2-hydroxyestrone and 16 α -hydroxyestrone, serum hormone estradiol, estrone, and estrone sulfate, and biochemical markers of bone metabolism. Concentrations of 2-hydroxyestrone increased significantly in the flaxseed group. Also in the flaxseed group, the ratio of 2-hydroxyestrone to 16 α -hydroxyestrone was positively correlated with urinary lignan excretion. No significant change in serum hormones or biochemical markers of bone metabolism was found.

4. Lipid Oxidation

Lipid oxidation occurs when electrons are removed from an atom or group of atoms. At the same time, there is a corresponding reduction reaction that involves the addition of electrons to a different atom or group of atoms. Oxidation reactions may or

may not involve the addition of oxygen atoms or the removal of hydrogen atoms from the substance being oxidized. Lipid oxidation is one of the major causes of food spoilage. Autoxidation, a reaction with molecular oxygen via a self-catalytic mechanism, is the main reaction involved with oxidative degradation of lipids. The reaction proceeds by free radical mechanisms usually formed by exposure to light and/or air. This first step is referred to as Initiation, which is followed by Propagation where hydrogen atoms are abstracted from positions α to fatty acid double bonds on the lipid structure. This step produces a free radical, onto which oxygen is then added forming peroxy radicals, which can then be reduced to hydroperoxides. Propagation can occur over and over again until substrates are used up, resulting in the last step, Termination (Nawar, 1996).

Flaxseed is high in linolenic acid, an 18 carbon and 3 double bond fatty acid that is very susceptible to oxidation, leading to flavor reversion (rapid oxidation, offensive odors, and rancidity). These flavor reversion problems make flaxseed nearly impossible to store, making it undesirable for use in food products (Hosseinian et al., 2004). In addition, oxidative reactions can decrease the nutritional value of food, and certain oxidation products are potentially toxic. Rancidity can be controlled with the use of antioxidants. Antioxidants are substances that can delay onset, or slow the rate of oxidation. They do this by inhibiting the free radical formation in the initiation step, or interrupting propagation of the free radical chain.

5. Flaxseed Stability

In a study (Malcolmson et al., 2000) of stability and quality of milled flaxseed two samples of flax (Linott and a flax mixture) were stored at $23\pm 2^{\circ}\text{C}$ for 128 days in paper bags with plastic liners. Samples were evaluated at 0, 33, 66, 96, and 128 days for chemical, sensory, and volatile components. No samples showed a significant increase in peroxide values or conjugated double bonds. Total volatiles increased in the mixed sample, but not in the Linott sample. A trained sensory panel could not detect any differences in odor among fresh or stored samples. No flavor differences were found in breads made with 0 and 128 day samples. These results suggest that flaxseed can be stored for up to 4 months without detriment to quality.

Stability of whole and ground flaxseed either alone or as an ingredient in muffin mix was studied by Chen et al. (1994). Oxygen consumption and changes in alpha linolenic acid were measured under different conditions such as heating at different temperatures and long-term storage (280 days with 12 hour light/dark cycles). Ground flaxseed absorbed the most oxygen. Muffin mix containing 28.5 percent flaxseed was found to be stable at room temperature along with both forms of flaxseed.

Research (Lee et al., 2003) was conducted to determine the effects of boiling, boiling then refrigerating, and boiling, refrigerating, then microwaving on the cooked quality and stability of lipids in macaroni made with 15% ground flaxseed. Boiling increased brightness but decreased redness and yellowness of the macaroni. After boiling, cooking loss was lower and cooked firmness was greater for macaroni dried at ultra high temperature (90°C) than at low temperature (40°C). Flaxseed macaroni had lower cooking loss than did regular macaroni. Firmness was greatest with boiled

macaroni, and least firm with boiled-refrigerated-microwaved macaroni. Boiling reduced extractable lipid content of the macaroni. Free fatty acid content was greatest with uncooked macaroni and least with boiled refrigerated and microwaved. Boiling, refrigerating, and microwaving did not affect conjugated diene content in lipid extracted from the flaxseed macaroni, regardless of drying temperature.

White et al. (1999) examined the quality changes in yellow-seeded low linolenic acid solin, brown-seeded standard and brown-seeded high linolenic acid flaxseed during 6 months of storage at different seed temperatures and moisture contents. Storage temperatures were 35, 30, 25, 20, 15, and 10°C with moisture contents of 8.0, 9.5, 11.0, and 12.5% (wet mass basis). Three jars of each cultivar at each moisture level were subjected to each of the chosen temperature regimes for the duration of the experiment. Three other jars (replicates) at each moisture level of each cultivar; together with one jar containing air dried seed were kept at -15°C as controls. Jars were sampled at the beginning of the experiment and every four weeks for 20 weeks, for a total of six times. Free fatty acid levels, fatty acid composition, moisture content, and off odors were determined. The oil composition of both cultivars changed only slightly during storage at the highest moisture content of 12.5%. Palmitic acid was the only fatty acid that changed significantly ($P < 0.05$), increasing slightly while the other fatty acid levels did not change. The standard and high linolenic acid cultivars with lower stearic acid content also had higher oleic acid contents. The reverse was true for the solin cultivars, where higher stearic acid content correlated with higher oleic acid content. Free fatty acid levels increased with increasing moisture, higher temperatures, and time. The dry grade for flax

is 10.0%, but some deterioration occurred at 9.5% at 30 and 35°C. All cultivars stored well at 8% moisture for 6 months.

6. Natural Antioxidants

Gums have been studied for their antioxidant effects, although they are usually used for texture enhancing. Their antioxidant activity may be due to metal chelation and oxygen consumption, and their viscosity increasing effects. Xanthan gum, pectin, guar gum, and tragacanth gum are recognized as antioxidants (Reische et al., 1998).

Guar gum is the ground endosperm of seeds from the Guar plant *Cyamopsis tetragonolobus*. A galactomannan is the main component of the endosperm. The polysaccharide of guar gum is guaran. Commercial guar gum usually contains 80-85% guaran. Guar gum hydrates and becomes viscous very quickly when dispersed in water and heated. Guar gum is a neutral molecule and is therefore compatible with most other food substances. Guar gum does exhibit interactions with starches and other gums such as xanthan. The interaction results in a synergistic increase in viscosity. The prime function of guar gum in foods is to bind water; it also improves mouthfeel, reduces chewiness, improves mixing tolerances, and improves shelf life through moisture retention.

Xanthan is produced as a defense mechanism of the bacteria *Xanthomonas campestris*. It is widely used as a food gum, with changes in the strain of bacteria used and the conditions grown under determining the properties. Solutions of xanthan have high viscosities and are highly pseudoplastic. It is excellent for generating and stabilizing

emulsions and suspensions. These characteristics are maintained over a wide range of pH values and temperature ranges (Whistler and BeMiller, 1997).

Shimada et al. (1992, 1994) performed studies on the antioxidant activity of xanthan gum in soybean oil. The first study was performed in cyclodextrin (CD) emulsion (1.5 %) with soybean oil containing tocopherols and not. Tocopherols were determined by high performance liquid chromatography (HPLC). Pyruvate group content of xanthan was determined and removed by heating with oxalic acid and sodium chloride. Emulsions were autoxidized at 37°C for 50 days. Incubation of the soybean oil without tocopherols was carried out at 37°C for 47 hours. Peroxide value (POV) was determined for oils at intervals. Thiobarbituric acid (TBA) value was measured, hydrogen-donating activity estimated, and Fe²⁺ activity was measured. Lipid peroxidation is accelerated at acidic pH by metal ions such as Fe²⁺. When xanthan (0.5 %) was added to the water phase in the CD emulsion, oil autoxidation was strongly suppressed. The viscosity of the water phase in emulsion was found to be independent of oxidation rate. The antioxidative activity of xanthan was not high in the absence of tocopherols, indicating that there may be a possibly synergistic action between the two. Xanthan showed hardly any hydrogen-donating activity. Fe²⁺ binding ability was observed for xanthan and increased with the concentration. This high activity may be due to its unique structure as well as its negative charge. An increase in the binding activity corresponded to that of pyruvate content, depyruvated xanthan did not inhibit autoxidation effectively. Results concluded that xanthan used as an emulsion stabilizer inhibited strongly the autoxidation of soybean oil in the CD emulsion system and that xanthan suppresses oil peroxidation synergistically in the presence of tocopherols.

The second study investigated the Fe²⁺ and binding site on xanthan in more detail, again in CD emulsion. Two Fe²⁺ systems were prepared, one with sodium ascorbate and one with fructose. These additions were made to maintain iron in the Fe²⁺ state. At intervals, POV was determined and TBA value measured. Iron analyses were done with or without ascorbate, depending on whether data for total Fe or only Fe²⁺ were desired. Concentrations of Fe²⁺ added to xanthan solutions (0.05% w/v) differed from .25 to 5.00 mM. The Fe²⁺ induced oxidation of soybean oil proceeded rapidly without xanthan during storage but was slowed by the addition. These results indicate that xanthan contributes to the inactivation of Fe²⁺ ions by chelation and inhibits the induced oxidation of soybean oil in the emulsion, however other antioxidative mechanisms of xanthan may exist.

In a study (Nag, 2000) on natural antioxidants, capsicum powder was found to be effective. Oil was extracted from dried flaxseed and sampled during air oxidation for peroxide value and TBA reactive substance values. Control oil showed a sharp increase in peroxide value, indicating oxidation. Oil with added capsicum showed a constant peroxide value. These effects were the same with increasing temperature. Results were similar for TBA values. As a note, product flavor was found to be acceptable when used as a salad oil.

Another natural antioxidant was developed for flaxseed in the Western part of the world, particularly India and Asia (Bera et al., 2004). This antioxidant is made from the extract of *Carum copticum*, commonly referred to as ajowan. With the incorporation of the soluble extract in the form of powder into oils, oxidation was prevented. After storage for a year with the antioxidant, the odor, taste, and chemical properties of the oil

were the same. This antioxidant is rather inexpensive, readily available, and consumed in large quantities already.

Other interesting natural antioxidants have been found in sesame seeds (*Sesamum indicum* L.). Two of these include sesamol and more recently sesamolinol. Toshihiko et al. (1985) reported the lignan-type antioxidant sesamolinol to be more active than vitamin E. This study was performed to report the structural identification after isolation from the sesame seed.

7. Gums and Texture

Many additives are used in the baking industry to improve dough-handling properties, increase quality of fresh bread, and extend shelf life of stored bread. Gums, however, are not as commonly used in the baking industry as they are in the food industry. They are able to control the rheology and texture of aqueous systems throughout the stabilization of emulsions, suspensions and foams. They are also able to modify starch gelatinization and to extend the overall quality of the product during time (Rosell et al., 2001).

Rosell et al., (2001) analyzed the behavior of doughs containing different gums in mixing, fermentation, and baking; as well as the influence they had on the bread properties. Gums used included sodium alginate, κ -carageenan, xanthan, and hydroxypropylmethylcellulose (HPMC). Farinograph characterized the water absorption, dough development time, stability, mixing tolerance index, and elasticity. Extensograph results characterized the resistance to constant deformation after 50 mm of stretching, extensibility, the ratio of the two, and work input. Alveograph parameters recorded

included tenacity, dough extensibility, curve configuration ratio, and the deformation energy. Rheological characteristics measured were maximum dough height, the time at which the dough reached maximum dough height, loss in dough height at the end of the test, the time of maximum gas formation, the time at which gas starts to escape from the dough, and the gas retention. An oven rise recorder was used to determine the baking quality. Bread quality analysis included: weight, volume, specific volume, crumb firmness, crumb water activity, and moisture. Water absorption was increased by the gum addition, amount depending upon specific gum. Xanthan and alginate considerably increased dough consistency and produced the strongest dough. Elasticity of the dough was decreased with the addition of all the gums. The gums increased the dough extensibility and decreased the ratio of deformation to extensibility. Xanthan gum was the only addition that did not reduce the work input necessary for the deformation. Xanthan increased the tenacity of the dough the most, increasing its ability to retain gas. Xanthan had the most impact on alveograph properties of the dough, especially in dough resistance and extensibility. Xanthan caused a pronounced drop in dough height during fermentation. Time of maximum dough development was increased with all the gums and loss of volume as a percentage was greatly reduced indicating improvement in dough stability. Time of maximum gas formation was decreased by xanthan and alginate. The gums decreased final rise of the bread during baking. Xanthan gum was found to increase the crumb firmness of the fresh bread. Results concluded that xanthan gum and alginate produce the best effects at dough level, but other gums produced better effects on bread quality.

The effects of sodium alginate, κ -carageenan, HPMC, and xanthan gum on fresh bread quality and potential in retarding the staling process during storage were investigated by Guarda et al. (2004). Moisture, ash, and proteins were determined. Farinograph characteristics determined were water absorption, dough development time, and stability. Viscoelastic properties of deformation energy, tenacity, and curve configuration ratio were determined using the alveograph. Physiochemical characteristics measured included weight, volume, specific volume index, width/height ratio, moisture content and crumb texture. Sensory analysis was performed by a panel of trained judges using semi-structured scales scoring 1 (lowest) to 5 (highest). The attributes evaluated were visual appearance, aroma, flavor, and crunchiness. Breads were considered acceptable if their mean value for overall acceptability were equal or above 3. Xanthan gum exhibited the highest increase in curve configuration ratio and deformation energy in dough. Specific volume of baked bread significantly improved, with xanthan being the second best improver after HPMC. Gum addition also increased the moisture content significantly ($p < 0.05$). Xanthan and κ -carageenan both produced an increase in the width/height ratio. The addition of xanthan was also found to increase the hardness of the bread crumb. All sensory attributes received scores higher than 3 in all the gum containing breads, however breads with xanthan received the smallest score. Breads with the addition of gums showed a lower loss of moisture content, therefore higher water retention compared to the control. Xanthan bread showed the highest hardness increase after 24 hours of storage. These results conclude that no general effect can be attributed to gums, since each effect is related to a certain gum. HPMC was found to be the best bread improver, with regard to its effect on fresh bread quality and anti-staling properties.

8. Flaxseed Acceptability

Alpers and Sawyer-Morse (1996) performed a study aiming to describe the palatability characteristics (color, tenderness, and flavor) and overall acceptability of banana nut muffins and oatmeal cookies prepared with ground flaxseed. Three treatments were tested, 30% regular flour, 33% flaxseed, and 50% flaxseed. Three replications were completed for each treatment. Ninety untrained, randomly selected college students rated muffins and cookies using 9-point hedonic scales. Limited significant differences were found in palatability characteristics of the banana nut muffins. Panelists thought that the addition of flaxseed, which provided a brown hue, gave the muffins a “whole grain” look. Both the 50% and 33% flaxseed muffins were rated as more acceptable than the control muffin. However, all muffins were found to be not sweet enough. Similarly, few significant differences were found in palatability characteristics of the oatmeal cookies. Panelists did rate the 33% flaxseed cookies as slightly more acceptable than the control cookies. These results suggest that using 30%-50% ground flaxseed will produce acceptable oatmeal cookies and banana nut muffins that differ little in sensory attributes from those made with all-purpose flour.

Gambus et al. (2003) investigated the quality of cookies and muffins containing brown and yellow flaxseeds. Experiments were conducted using Canadian hermit cookie and bran flax muffin recipes, however instead of adding 8% flax to cookies and 11% flax to muffins as called for, 11 and 9% were added respectively. Sensory properties of the cookies and muffins were not affected; in fact average scores were better than good. The increased flax improved nutritional values for both baked goods by increasing protein and

dietary fiber amounts. Levels of α -linolenic acid, usually low in the diet, were significantly increased as a proportion of total fatty acids. No significant differences were found between the nutritional values of the brown and yellow flaxseeds. The shelf life of the muffins was only 7 days, but the cookies' shelf life was approximately 2 months.

9. Bread Baking

Breadmaking is a centuries old traditional craft practiced in any country capable of growing or importing wheat. There are a number of central themes that all bread products have in common; the mixing of wheat flour, water, yeast and other functional ingredients (also known as improvers) and the expansion of the dough mass through the generation of carbon dioxide gas. The relationship between mixing and dough development is still not fully understood. There are a few basic steps that define the breadmaking process, they are: the mixing of wheat flour and water, together with yeast and salt, and other specified ingredients in appropriate ratios, the development of gluten structure in the dough and incorporation of air bubbles during mixing, continued gluten development and creation/modification of flavors during fermentation, dividing and shaping dough into desired shape, proofing of dough pieces, and final expansion and fixation of structure during baking (Cauvain, 2003).

Wheat is the second largest crop in the United States, however from the nutritional standpoint it outranks all other cereals in importance. Wheat has excellent storage stability and yields excellent flour when milled. The unique properties of wheat proteins, which upon wetting and mixing yield a continuous, extensible gluten matrix,

permit the production of yeast-leavened light bread that has served mankind as a principal food source for centuries. The formation of gluten is an essential component of the breadmaking process. *Triticum aestivum*, also known as common wheat, comprises about 92% of total wheat production. When milled, wheat is blended for desired protein contents and optimum baking performance. The blend is cleaned and tempered, then undergoes a series of grinding operations to produce flour. These operations separate and remove the bran covering and the germ from the endosperm and obtain maximum extraction of the endosperm without excessive damage to the starch granules. Glutenin, which is closely associated with wheat quality for bread making due to its contribution of elasticity to doughs, makes up about 35 to 45% of the flour protein. Glutenin molecules of high molecular weight result in long mixing times and high dough stability and are generally present in greater amounts in good quality wheats. Gliadin proteins are responsible for strengthening dough systems, and are associated with glutenin proteins. Glutenin and gliadin together make up the wheat gluten. The principal carbohydrates of wheat flour are starch, dextrans, pentosans, and sugar. Damage to starch granules has a negative effect on gas production during fermentation, formation of dextrans during baking, and the level of baking absorption. The sugar found in flour is used by yeast to convert into glucose and fructose, of which glucose is directly fermentable. Water-soluble pentosans form an irreversible gel that adds rigidity to the dough and maintains cell structure of bread by preventing the coalescence of gas bubbles during fermentation and baking. Wheat flour lipids constitute about 1.5% by weight of flour and exert an important influence on dough properties, baking behavior, and bread staling (Pylar, 1988b).

Water plays an essential role in baking by providing, either by itself or with other substances, the necessary medium for the physical, chemical, biochemical, and biological reactions that underlie transformation of raw materials into finished baked goods. It also influences the over-all quality and palatability of the goods. Although it appears to be a very simple substance, it is actually a highly complex one with many unique properties that determine its functionality (Pylar, 1988c). The properties of the dough will vary according to the level of added water. Too little and the dough will be firm and difficult to form, producing breads that have small volume and poor appearance. Too much and the dough will be soft and also difficult to form; it will flow during proofing and give poor quality bread. The ‘optimum’ level of water is really the maximum quantity that will go into the dough and still be moldable and give acceptable quality. This amount depends on the properties of flour used.

Baker’s yeast (*Saccharomyces cerevisiae*) comes in a number of different forms. The yeast produces carbon dioxide gas to expand the dough at its various processing stages, particularly during proofing and the early stages of baking (Cauvain, 2003). Gas is produced during fermentation, the enzymatic conversion of carbohydrates into ethanol and carbon dioxide under anaerobic conditions within yeast cells. However, under aerobic conditions very little ethanol is produced (Pylar, 1988a). Build up of carbon dioxide in the dough causes it to rise and provides many air bubbles with which bread structure forms.

A basic function of salt in bread dough is to contribute flavor but it also has an inhibiting effect on the formation of gluten during mixing (Cauvain, 2003). Bread without salt has an insipid and flat taste and is usually undesirable to consumers. Salt has

also been shown to have such further flavor enhancing effects as enhancing fullness in mouthfeel, increasing sweetness perception, masking possible off flavors, and most importantly, improving the flavor balance. Salt also inhibits yeast activity, as well as activity of potential spoilage organisms. Lastly, salt functions to strengthen and tighten the gluten of the dough partly by inhibiting proteolytic enzymes (Pylar, 1988c).

The sponge and dough method is commonly used to make bread in the United States. Only the sponge part of the ingredients is fermented. This method consists of a two step process in which part of the total quantity of flour, water, and other ingredients such as yeast and flavorings from the recipe are mixed to form a homogenous soft dough referred to as the sponge. The sponge then rests for a prescribed amount of time, depending on flavor requirements. The sponge is then mixed with the remainder of the ingredients to form homogenous dough. The final dough is then immediately processed. In many cases the addition of the sponge changes the rheological character of the final dough sufficiently to not require any additional resting time. Flours used in typical sponge-dough production are strong, with protein contents not less than 12% (Cauvain, 2003).

Chapter 3: Justification

Baked goods are eaten widely around the world, with bread being one of the most commonly consumed. The many health benefits of flaxseed make it a very desirable food to incorporate into the daily diet of consumers. In order to maximize acceptance, the best mode of incorporation would be in a baked good using the milled version. The whole and ground seeds have a nutty flavor that not all consumers may like, but in a baked good, such as bread, the flax flavor is reduced from being milled and mixed with the other ingredients.

An issue facing a baked good product made with flaxseed is texture. Milled flaxseeds are not as soft as flour and have different mixing and baking properties than flour. The addition of a texturizing agent to this baked good may be necessary. Gums, such as xanthan gum and guar gum, have been found to improve texture, moisture, and volume of bread. Also, these gums have been shown to have antioxidant properties. Their addition to the formula would serve a dual purpose.

Another problem with the use of flaxseed is its tendency towards oxidative rancidity. Products such as baked goods are not shelf-stable for very long. They would require additional storage conditions such as refrigeration or need to be eaten quickly. Another solution would be the addition of an antioxidant to the product. Both consumers and the Food and Drug Administration (FDA) favor natural antioxidants, with their amounts not being as closely monitored as chemical antioxidants. Xanthan and guar gums are possible natural antioxidants that require further investigation.

There has been little research regarding the use of flaxseed in food products. The health contributions of flax have been investigated, but the incorporation of it into food is still premature. Additionally, the stability of flax due to the presence of polyunsaturated fatty acids is still a challenge to research, especially when extending shelf life of the product. The incorporation of natural gums into the flax bread offers a product with improved texture and shelf-life stability.

Therefore, it seems justifiable to produce a baked good, such as bread, from milled flaxseeds including the adjustments necessary to make it more acceptable to consumers. This bread would qualify as a functional food, imparting all the health benefits of flaxseed in a daily diet and still possess quality attributes.

The purpose of this study was to examine the effects of xanthan gum and guar gum in flaxseed supplemented breads. The objectives of this study were:

1. to incorporate xanthan gum and guar gum into a bread containing flaxseed to determine their antioxidant activity
2. to determine the effect of xanthan gum and guar gum on texture, moisture, and volume of the flax bread
3. to determine the sensory quality of the breads containing xanthan gum and guar gum

Chapter 4: Materials and Methods

1. Breads

Breads were made using an adaptation of the American Association of Cereal Chemists (AACC) Sponge-Dough Method # 10-11 (AACC, 1983). A summary of the bread formulations can be found in Appendix A. The sponge was formed by mixing 420 ml warm (40.6-46.1°C) water with 30 grams Fleischmann's™ Active Dry Yeast and 30 grams of sugar. This mixture was allowed to rest for 5 minutes to activate the yeast. Then 192 grams of King Arthur® Unbleached Bread Flour (Norwich, VT) was added. This sponge was mixed with the paddle attachment of a Kitchen Aid (St. Joseph, MI) stand mixer for 5 minutes until smooth. The sponge fermented for 90 minutes. After fermentation, the remaining ingredients (control/experimental dependent) were added and mixed for 8 minutes, then the dough was allowed to rise for 60 minutes. For control loaves, the ingredients included: 700.5 grams of bread flour, ~210 ml water, 22.5 grams sugar, 21 grams salt, and 30.3 grams of Crisco® Pure vegetable oil (Orrville, OH). For flaxseed loaves the ingredients were: 566.7 grams bread flour, 133.8 grams Bob's Red Mill® Whole Ground Flaxseed Meal (Milwaukie, OR), ~210 ml water, 27 grams Hodgson Mill® wheat gluten with vitamin C (Effingham, IL), 22.5 grams sugar, 21 grams salt, and 30.3 grams vegetable oil. Experimental loaves also included: 0.6 grams xanthan gum, 0.6 grams guar gum or 0.6 grams xanthan gum and 0.6 grams guar gum. The gums were provided by TIC Gums (Belcamp, MD). Bread dough was divided into eight loaves of approximately 187 grams each, placed in standard pup loaf pans and proofed for another 30 minutes before baking in a 425° Maytag electric oven (Newton, IA) for 15-20 minutes. A total of 16 loaves of each type of bread (regular control, flax

control, guar gum, xanthan gum, guar and xanthan gum) were made, for a total of 80 loaves. All loaves were produced on the same day to maintain continuity. Batches (2) of each type were produced and placed in random order according to randomization sheets provided by the Virginia Tech Statistical Consulting Center (Appendix B). This was done in order to randomize which batches of breads would be tested each week. After production, breads were cooled for 60 minutes and then stored in plastic bags provided by Our Daily Bread Bakery (Blacksburg, VA). Baked bread was stored at ambient room temperature in large plastic containers.

2. Lipid Content

Lipid content of the flaxseed was determined using a modified Folch method (Folch et al. 1957). First, 1 gram of flaxseed was homogenized with 20 ml chloroform:methanol (2:1) for 3 minutes using a Kimatica Polytron homogenizer (Cincinnati, OH). The homogenate was then centrifuged in a Damon/IEC PPR 6000 centrifuge (Needham Heights, MA) set at 5000 RPM, 5°C for 5 minutes. The upper layer was transferred to a new tube and washed with 4 ml of 0.9% NaCl solution. This solution was vortexed on a Fisher Vortex Genie 2 (Bohemia, NY) set on 8 for about 30 seconds then centrifuged again for 5 minutes. This upper layer was siphoned off and discarded. The remaining layer was rinsed with 1 ml chloroform:methanol:water (3:48:47), vortexed, and centrifuged again. This step was repeated up to three times as necessary until the “fluff” was fully removed. The remaining chloroform phase containing lipids was then evaporated under a nitrogen stream.

Oil obtained from the above method was then prepared for gas chromatography. A total of 15 mg was weighed into a 15 ml glass tube with a Teflon™ lined cap. Internal standard (C:17) (Sigma Chemical) was added in the amount of 5 mg along with 1.5 ml chloroform:methanol (1:1) and 0.5 ml 14% boron trifluoride in methanol. The tube was capped after flushing with nitrogen and heated for 45 minutes at 100°C on a Thermolyne heating block (Dubuque, IA). After cooling, 2 ml water and 4 ml pentane were added and this solution was vortexed for 1 minute then centrifuged in a Fisher Centrifric™ centrifuge (Bohemia, NY) set at 3000 RPM for 3 minutes. The upper (pentane) layer was carefully transferred to a clean 5 ml vial and evaporated under nitrogen. Hexane was added in the amount of 100 ul to this residue and then transferred to an autoinjector gas chromatography vial. This vial, and a vial of FAME rapeseed oil standards (Sigma Chemical) were placed into the Shimadzu Model GC14A (Shimadzu Corp., Columbia MD) auto injector gas chromatograph to be measured. This chromatograph contains a SP2330 capillary column measuring 30 meters long by .32 I.D. The column temperature was set to 150-205°C increasing 5°C per minute. The run time was 15 minutes with a flow rate of 1ml/minute helium, 50ml/minute makeup gas, 300ml/minute air, 30 ml/minute hydrogen and a split ratio of 1:8. The sensitivity was 10^{-1} and the attenuation was 6. Lipid content was calculated using a ratio method from the derived printout. Each individual fatty acid area was divided by the total area to obtain the percentage that it was present in the flax sample.

3. Peroxide Value

Peroxides and other products of oxidation were measured using an adaptation of the American Oil Chemists' Society (AOCS) Peroxide Value Acetic Acid-Chloroform Method Cd 8-53 (AOCS, 1998). First, 10 grams of sample were placed into a 250 ml Erlenmeyer flask and 30 ml of acetic acid:chloroform (3:2) solution was added. The flask was swirled to dissolve the sample. This mixture was then homogenized and filtered to remove any solids. Using a pipette, 0.5 ml of saturated KI solution was added then the solution was allowed to stand for 1 minute with occasional shaking. Afterwards, 30 ml of distilled water was added. This solution was titrated with 0.1 N sodium thiosulfate until the yellow iodine color almost disappeared. Droplets totaling 2.0 ml of starch indicator solution were added and the titration was continued until the blue color disappeared. Blank determination of the reagents was also conducted. Peroxide value was calculated using the following equation:

$$\frac{(S - B) * N * 1000}{W}$$

Where: S= ml of sodium thiosulfate to titrate fat sample
B= ml of sodium thiosulfate to titrate blank
N= normality of sodium thiosulfate = 0.1N
W= weight of sample = 10 grams

4. Anisidine Value

Amount of aldehydes present were measured using an adaptation of the AOCS p-Anisidine Value method Cd 18-90 (AOCS, 1998). First, 0.5 grams of sample was weighed into a 25 ml volumetric flask. This was dissolved, homogenized, and diluted to volume with isooctane. This solution was then filtered to remove solids. The absorbance

(abs) of the solution was measured at 350 nm in a cuvette with a Beckman Coulter Du® 530 spectrophotometer (Fullerton, CA), using a reference cuvette filled with solvent as a blank. Exactly 5 ml of the fat solution was placed into a test tube and 5 ml of solvent into a second test tube using a pipette. Exactly 1 ml of the p-anisidine reagent was placed into each tube, which was then shaken. After 10 minutes the absorbance was read of the solvent in the first test tube in a cuvette at 350 nm, using the solution from the second test tube as a blank in the reference cuvette. p-anisidine value was calculated using the following formula:

$$\frac{25 * (1.2\text{abs of fat solution after reaction with reagent} - \text{abs of fat solution})}{\text{mass of the test portion,g}}$$

5. Totox Value

Totox or oxidation value is equivalent to 2 times the peroxide value plus the anisidine value (totox value= 2 x PV + AV). Totox value has been suggested to be an assessment of oxidation in oils. This value was calculated to assess the total lipid oxidation in breads tested (Nawar, 1996).

6. Physical Properties

Volume

Volume of the bread loaves was measured using the rapeseed displacement method. Individual loaves wrapped in plastic wrap were placed in the rapeseed

volumeter, then the seeds were released onto the loaves. Amount of seeds lost from the containment unit are noted, allowing for calculation of volume. Units of volume used are cm^3 (Griswold, 1962).

Water Activity

Water activity of the breads was tested using the Aqualab CX-2 (Pullman, WA). This instrument was calibrated prior to analysis. Samples of bread were placed in small cups and inserted into the instrument. Water activity levels were then reported and recorded when equilibrium is reached inside the instrument. Water activity consists of the amount of free water in a sample. This free water has the capability of migrating in or out of the sample and reacting with other substances (Fennema, 1996).

Crumb Color

Crumb color was measured using the Minolta Colorimeter (Mahwah, NJ). Bread slices of $\frac{1}{2}$ inch thick were placed underneath the hand-held device and scanned. The colorimeter recorded L values: 100 equaling white and 0 equaling black and b values indicating yellow (positive) and green (negative).

Texture

Texture of bread was analyzed using the TA.XT.Plus Texture Analyser (Texture Technologies Corporation, Scarsdale, NY). The machine conducted Texture Profile Analysis (TPA) tests, which 1) compressed the sample at a fixed distance, 2) withdrew to the original sample height as was determined by the trigger force, 3) allowed the sample

to rest/recover for a fixed amount of time, and 4) repeated the compression to the original penetration distance. Based on the sample's performance, hardness (g) and springiness (%) were calculated using the accompanying software. Bread slices of ½ inch thick were placed underneath the probe and then the crumb was compressed. Tests were conducted with a 36mm diameter cylinder probe set to compression distance of 7 mm at a speed of 2 mm/sec. Rest/recover time was fixed at 3 seconds. The trigger force was 1 gram and trigger distance was 1 mm.

7. Sensory Analysis

A trained panel of 8 judges performed sensory analysis. Quantitative Descriptive Analysis method was used to obtain a “map” of the attributes chosen to analyze (Meilgaard et al. 1999). Judges were trained during two separate sessions in which they first sampled breads and discussed attributes they derived. The group then decided on which of those attributes to evaluate, producing the scorecard that utilized a 6-inch line scale (Appendix C). Attributes chosen included texture: moist vs dry; texture/appearance: dense vs airy; aroma: yeasty or sour vs not yeasty or sweet; flavor: bitter vs neutral; and flavor: stale vs fresh. During the second session the judges again sampled breads and performed a practice session with the scorecards. Sensory analysis was performed once a week for four consecutive weeks. During analysis bread samples were given to judges in random order according to randomization assignments made by the Statistical Consulting Center, Virginia Tech. Judges sat in individual testing booths to sample the breads. Bread samples were coded with random numbers at each testing

period to prevent bias from the judges. Water was also provided to cleanse the palette in between sampling

8. Statistical Analysis

The experimental design of the study was determined by a consulting team within the Statistics Department of Virginia Tech. SAS (Statistical Analysis System (SAS Institute Inc., Cary, N.C.)) was used for the statistical analysis. The study uses a randomized complete block design with subsampling that was averaged out in the analysis. The mean values recorded for each test were compared using two-way analysis of variance (ANOVA). Significance was determined using a p value ≤ 0.05 . The least significant differences were compared using the Tukey-Kramer test. All testing, including sensory analysis, was performed on the same day each week to maintain continuity of data.

Chapter 5: Results & Discussion

1. Physical Properties

Crumb Color

Crumb color of the control bread was significantly ($p < 0.05$) whiter than the other bread types (Table 1). This was an obvious difference due to the lack of flaxseed. Seed coat color of flaxseed can range from brown to light yellow (Daun, et al. 2003). Experimental loaves containing the flaxseed had a grainy appearance, with dark brown flecks throughout the crumb and crust. This produced a visible color difference in the bread samples.

Bread containing guar gum was significantly ($p < 0.05$) darker (Table 1). Xanthan gum is white in color, while guar gum possesses a creamy yellow color. Both gums were added directly to the flour and when in contact with water and allowed to gel, xanthan gum has good transparent properties and guar gum has fair transparent properties (Hoefer, 2004). This could have had a color effect on the bread samples. Rao, et. al (1984) found that breads containing gums had a distinct improvement in the crust color from dull brown to golden brown. The fair transparent properties of guar gum decreased the white color of the two breads it was included in by adding a cloudy color element.

The crumb of control bread was also significantly more ($p < 0.05$) yellow than the other breads (Table 1). The breads containing guar gum and a combination of both gums were significantly more ($p < 0.05$) yellow than the flax and xanthan breads. The cloudy color of the gelled guar gum plus its yellow color produced an increased yellow color in the bread. These results agree with Mandala (2005), who found that guar gum produced higher 'b' hue values, indicating more yellowness. Mandala (2005) also concluded that

Table 1. Mean Water Activity (a_w) (n=16), Crumb Color (n=32), and Volume (n=16) of Bread Containing Flaxseed and Gums

Type		a_w	Crumb Color		Volume cm ³
			L	b	
Control	Mean	0.914b	76.594c	22.598c	640.938a
	Standard Deviation	±0.013	±0.568	±0.273	±78
Flax	Mean	0.926a	61.918b	16.204a	608.44ab
	Standard Deviation	±0.014	±1.05	±0.291	±78.54
Guar	Mean	0.926a	60.839a	16.549b	548.75c
	Standard Deviation	±0.01	±1.8	±0.217	±65.7
Xanthan	Mean	0.927a	61.892ab	16.058a	576.875bc
	Standard Deviation	±0.013	±0.748	±0.239	±74.786
Both	Mean	0.926a	62.023b	16.459b	586.875b
	Standard Deviation	±0.014	±1.487	±0.423	±85.71

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

L: 100= white; 0= black

b: +b= yellow; -b=green

the addition of gums produced higher intensity values in all samples, especially those containing guar gum.

Volume

Control bread was significantly higher ($p < 0.05$) in volume (Table 1). While differences between control bread and bread with flaxseed were not significant at $p < 0.05$, they did exist at a $p = 0.09$. Cereal grains and gums are considered to be “high fiber” ingredients. The addition of these ingredients to bread can cause a decrease in volume. Grains usually interfere with gluten development and affect the structure and baking quality. Therefore, there is a decrease in bread volume and crumb elasticity. Wheat bran at a substitution level of 20% has been found to produce a decrease in loaf volume of 19% (Salmenkallio-Martilla, et al. 2001). One suggested cause of these negative effects is the dilution of the gluten network, which impairs gas retention. Another possible cause is the disruption that the grains cause in the starch-gluten matrix and restriction on gas cell expansion. Gluten comprises a complex mixture of proteins differing in size and structure. These proteins are classified as gliadins and glutenins and are responsible for the variations in baking quality. Gluten quality and composition determine dough viscoelasticity and performance. Thus, the formation of gluten is key. It requires hydration of the proteins and the application of energy through the process of kneading (Cauvain, 2003).

Flax bread containing guar gum had a significant ($p < 0.05$) decrease in volume (Table 1). From the data, the addition of gums did not improve volume, except xanthan appeared to have a more positive effect than guar. Acs et. al (1996) found that the addition of guar gum and xanthan gum in corn-starch based breads produced a significant

increase in bread volumes with xanthan gum being the best. In this study, while xanthan gum did produce a higher volume than guar gum, the bread containing a combination of both gums produced the highest volume of the three. Perhaps a synergistic union between these two gums was present.

Gums were initially added in breads to improve gas retention and oven spring, but reported effects have been contradictory (Katina, 2003). When gums encounter water, they act like a sponge and rapidly absorb the water surrounding it, swelling to many times its original size. When the gum reaches a certain size, the molecules are completely hydrated and begin to untangle themselves and float off into the solution. At this point, the swollen gum particle becomes smaller from the outside in (Hoefer, 2004). This can enhance the volume of baked good products. Valli et. al. (1997) found that overmixed dough laminated with a hydrocolloid solution consistently produced higher volume pastries. Gums can also be used to replace gluten in gluten-free baked goods (Acs et. al, 1996).

Water Activity

Water activity (a_w) is a measurement of the movement of moisture within or out of a sample. Control bread was found to have a significantly lower ($p < 0.05$) water activity than the other bread types (Table 1). The quality of bread owes much to the special properties of the proteins and starch that are present in the flour and water is key in the transformations that take place in the loaf as it bakes. The organoleptic qualities of all baked goods change during storage and many of the changes result in staling. These changes are most often linked with the movement of water both within and out of

the product matrix. Bread has the highest water levels of virtually all baked products. The average moisture content for bread ranges from 38%-44%. Typical water activity for bread is between 0.90 and 0.97. Within limits, the higher the moisture content, the fresher the bread will be perceived by the consumer. Bread crumb with higher moisture is usually softer and easily compressed, while bread crumb with too much moisture does not spring back after compression (Cauvain and Young, 2003). The change of water activity which takes place in bread crumb is an important issue. Water activity decreases with crumb aging and parallels that of the corresponding water content (Czuchajowska and Pomeranz, 1981). Higher water activities indicate a greater tendency for water to escape and move freely within the crumb. During aging, water is expected to move within the crumb from regions of less-bound state to regions of more-bound state. This implies that a decrease in water activity would occur as bread ages. In mixed bread dough, gluten, soluble proteins, and pentosans are responsible for adsorbing much of the water. When dough is heated during baking, some water is involved in starch gelatinization, but a significant fraction of it remains attached to those other components, which compete for the available moisture (Schiraldi and Fessas, 2001).

Reductions in water activity are obtained by dissolving low molecular weight molecules such as fructose or salt in the water, while gums have a higher molecular weight and are not as efficient at lowering water activity. Gums are best described as “water organizing”: a substance that retains water, binding it while still maintaining a high water activity (Hoefler, 2004). This agrees with results found in bread samples containing gums. Water activity levels were significantly higher ($p < 0.05$) in breads containing xanthan gum, guar gum, and a combination of the two (Table 1). These

results differ from those of Walter and Seeger (1990) who found that the addition of guar gum to Hawaiian ethnic foods did not have any appreciable impact on water activity at 26.7°C. Pettitt (1982) concluded that the ability of xanthan gum to retain moisture and form a complex with starch is of importance and inhibits retrogradation.

Texture

Textural properties are highly dependent upon physical properties, microstructure, and chemical components (Stanley, 1987). Control bread and bread containing guar gum were significantly ($p < 0.05$) harder than breads containing flax, xanthan gum, and both gums (Table 2). Hardness, a texture characteristic, is defined as a product that displays substantial resistance to deformation or breaking (Bourne, 2002). The firmness of fresh fiber enriched bread has been reported to be 41% higher compared with bread without the bran (Laurikainen, et al. 1998). This is contradictory to results found in this study, where control breads were harder than breads with flaxseed. Starch retrogradation and the transfer of moisture from starch to gluten can lead to textural changes in bread, which include an increase in firmness and toughness and a decrease in springiness (Bourne, 2002). However, this agrees with water activity results (Table 1) that showed control bread to have a low water activity, indicating lower moisture content that would equate to bread that is less soft in texture.

Gums provide two qualities to the creation and improvement of texture. These qualities are thickening (make more viscous) and gelation (giving a “cuttable” texture). Guar gum is a thickening agent. Thickening agents provide viscosity in a food system,

Table 2. Mean (n=48) Hardness and Springiness Results for Bread Crumb Containing Flaxseed and Gums

Type		Hardness g	Springiness %
Control	Mean	1724.159b	88.742b
	Standard Deviation	±663.6	±2.82
Flax	Mean	1396.922a	89.939c
	Standard Deviation	±469.872	±1.963
Guar	Mean	1876.707b	87.599a
	Standard Deviation	±661.407	±2.605
Xanthan	Mean	1448.279a	89.542bc
	Standard Deviation	±523.937	±2.09
Both	Mean	1469.042a	86.821a
	Standard Deviation	±546.578	±2.704

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

but are not capable of suspending particulates. They do slow the settling of particulates or the rising of oil droplets in solutions (Hoeﬂer, 2004). These effects contributed to the bread containing guar gum to be harder than the other bread types. However, these results disagree with water activity results. Guar gum was found to have a higher ($p < 0.05$) water activity than control bread, which would indicate higher moisture and increased softness. These results also disagree with those of Mettler and Seibel (1995) who found that bread crumb remained softer with the addition of guar gum due to high water content. Sidhu and Bawa (2001) found that higher levels of xanthan gum incorporated into bread improved quality by softening the crumb. Their results agree with the results in this study where xanthan gum contributed to a significantly softer texture.

Springiness measures the elasticity of a sample, or how much the original structure of the sample was broken down by compression (Smewing, 1999). Breads with flax and xanthan gum displayed a significant ($p < 0.05$) percent of springiness than the other bread types (Table 2). Addition of cereal grains to bread also produces a moister and shorter dough and a crumb that is tense and non-elastic (Katina, 2003). This is contradictory to reported results in which bread containing flax were found to have a significant percentage of springiness. Xanthan gum is a known thickening and gelling agent. Gelling agents form links between their molecules, building a three-dimensional lattice in a food system. The result is that particulates or oil droplets become permanently trapped in the lattice and do not separate out. These combined effects of xanthan gum led to an increase in springiness. When combined with guar gum, the two exhibit a synergistic viscosity increase (Hoeﬂer, 2004). The thickening effect of guar

gum contributed to a firmer bread with reduced springiness. Guar gum has been found (Mettler and Seibel, 1993, 1995) to produce a soft bread that is lacking in sufficient elasticity.

2. Lipid Content of Flaxseed Meal

Results from GC analysis of flaxseed used in this experiment provided the lipid profile. A summary of the profile can be found in Table 3. These results agree with those of flaxseed found from the 2002 flaxseed harvest (Daun et al. 2003), whereas linolenic acid is present in the highest amount with the other fatty acids in corresponding amounts. The level of α -linolenic acid found in flaxseed harvested in 2002 ranged between 52- 63%. Traditional flaxseed has very high levels of α -linolenic acid, usually making up greater than 50% of the total fatty acid profile. Other fatty acids include about 5% of palmitic, 3% stearic, 18% oleic, and about 14% linoleic (Daun et al. 2003).

Flaxseed is characterized by being comprised of about 45% oil and 55% meal on a dry basis. There is controversy in the methodology used to determine the fat content of flax. The physical and chemical structure of flaxseed makes it relatively difficult to extract the oil from the seed. Therefore, the methods most often used in analyzing the fat in a food, including those used for food labeling purposes, give inaccurately low values when applied to flaxseed. There can be discrepancies in values up to 5%. The unsaturation level varies with seed variety and environment. Certain altered species have been bred containing α -linolenic acid with levels lower than 3%, commonly referred to as solin (Daun et al. 2003).

Table 3. Fatty Acid Profile of Flaxseed Used¹

Fatty Acid	Structure	Amount %
Linolenic	18:3	54.8
Oleic	18:1	19.7
Linoleic	18:2	15.1
Stearic	18:0	4.4
Palmitic	16:0	6.0

¹Flaxseed: Bob's Red Mill® Whole Ground Flaxseed Meal (Milwaukie, OR)

The fatty acid composition of fats and oils influence oxidation. Specifically, the more unsaturated a fatty acid is (higher number of double bonds), the more susceptible it is to degradation by oxygen. Linoleic acid and α -linolenic acid have 2 and 3 double bonds, respectively, which can come under direct attack from oxygen (Min, 1998). The number, position, and geometry of double bonds affect the rate of oxidation. Relative rates of oxidation for arachidonic, linolenic, linoleic, and oleic acids are approximately 40:20:10:1, respectively. *Cis* fatty acids oxidize more readily than *trans*, and conjugated double bonds are more reactive than nonconjugated (Nawar, 1996).

3. Rancidity

Rancidity can be caused by changes that occur from a reaction of lipids with atmospheric oxygen, or oxidative rancidity. Another way rancidity can occur is due to hydrolytic reactions that are catalyzed by enzymes, or hydrolytic rancidity. Rancidity occurs when a food containing solid or liquid fat comes in contact with microorganisms, oxygen, temperature, exposure to light and metals, and/or moisture. This contact results in forming free fatty acids, which are responsible for rancidity. Bakery foods with low water activity are susceptible to water-activity related spoilage due to autoxidation which occurs rapidly in products with water activities of less than 0.3 and higher than 0.5. Lipases present in products are most active at higher water activities (Cauvain and Young, 2003).

The chances of rancidity occurring increases with increasing unsaturation and as the product ages. Rancidity is a three-step process that produces broken down compounds resulting in off flavors and aromas (Figure 1). The first step in the rancidity mechanism

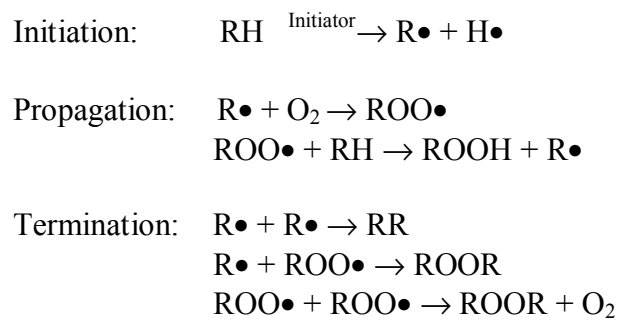


Figure 1. Free Radical Mechanism of Lipid Oxidation¹

¹Shahidi and Wanasundara, 1998

is that free radicals are formed during initiation, then the radicals form peroxyradicals as they combine with oxygen during propagation, lastly, during termination, hydroperoxides are formed which break down into smaller, more volatile substances such as aldehydes, ketones, and hydroxyls.

Adding a low concentration of an antioxidant can inhibit autoxidation. Chain breaking antioxidants can interfere with either chain propagation or initiation, while preventative antioxidants reduce the rate of chain initiation. If the two types work together and cause a better effect than alone, they are synergistic antioxidants (Hamilton, 1994).

3a. Peroxide Value

Peroxides are the initial products formed from and are one indication of rancidity. Peroxide value (PV) monitors the amount of peroxides formed in a food product and is expressed as milliequivalents O₂ per kilogram of fat. When graphed, typical peroxide values form a bell curve indicating increasing peroxides over time reaching a maximum peak then declining. It is important to monitor the product to determine the increase and decline of hydroperoxide formation. This monitoring also indicates the beginning (initiation) and end (termination) of the rancidity process. The highly unsaturated fatty acid profile (89.6%) of the flaxseed used (Table 3) in the breads is very susceptible to rancidity. No significant differences ($p>0.05$) were found among peroxide values for all the breads (Table 4).

Table 4. Mean (n=16) Peroxide, Anisidine, and Totox Values for Bread Containing Flaxseed and Gums

Type		PV meq O ₂ /kg fat	AV	Totox Value*
Control	Mean	6.688a	0.105b	13.48a
	Standard Deviation	±4.423	±0.267	±8.997
Flax	Mean	5.937a	0.379a	12.248a
	Standard Deviation	±3.941	±0.292	±8.094
Guar	Mean	6.625a	0.378a	13.626a
	Standard Deviation	±4.938	±0.377	±10.093
Xanthan	Mean	6.688a	0.293a	13.676a
	Standard Deviation	±5.77	±0.294	±11.666
Both	Mean	5.937a	0.342a	12.217a
	Standard Deviation	±4.041	±0.306	±8.208

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

*Totox value= 2 x Peroxide value + Anisidine value

Malcolmson et al. (2000) found that milled flaxseed showed no significant increase in peroxide values over 128 days of storage at 23°C. However, Chen et al. (1994) found that when flaxseed was heated to 178°C, oxygen consumption increased and α -linolenic acid content decreased. They also found that when incorporated at 28.5% into muffin mix and heated, the mix containing flaxseed consumed oxygen more rapidly than control but the α -linolenic acid content was unchanged. Flaxseed extracts have been reported to have natural antioxidant activity. It has been proposed that the lignans are responsible, but other components such as phenolic acids and tannins may play a role (Hall, 2001).

There are numerous analytical procedures for the measurement of peroxide value. In all cases, the results and accuracy of the test depend on the experimental conditions, as the method is highly empirical. The most common method used, and the one used in this experiment, is based on the iodometric titration that measures the iodine liberated from potassium iodide by the peroxides present in the oil. It has been suggested that the two principal sources of error in these methods are the absorption of iodine from potassium iodide at unsaturated bonds in the fatty acids and the liberation of additional iodine from potassium iodide by oxygen present in the solution (Rossell, 1994). The titration end-point determination of peroxide value has also been shown to fail in measuring low peroxide values and should be replaced with an electrochemical technique in order to measure values ranging from 0.06 to 20 accurately (Rossell, 1994). Peroxide value is a good guide to the quality of fat, and in the view of one author freshly refined fats should have values of less than 1. Fats that have been stored for any period of time after refining may be found to have values of up to 10 before off flavors and aromas are detected (Rossell, 1994).

3b. Anisidine Value

Anisidine value (AV) monitors the amount of aldehydes/alkenals produced in a sample. Aldehydes are the smaller, highly volatile substances formed from the breakdown of peroxides during the termination step of rancidity. The control bread loaves had a significantly lower ($p < 0.05$) anisidine value than the other bread types (Table 4). This could be due to the absence of flaxseed in the bread, which is highly susceptible to rancidity.

Control bread was found to also have significantly lower ($p < 0.05$) water activity level (Table 1), implying that water activity level may have played a role in causing rancidity of bread samples in this study. Breads with a higher water activity were found to have higher anisidine values that resulted from advanced stages of rancidity. Gums were found to have little effect on water activity. Breads containing gums had higher water activities with corresponding higher anisidine values, which could have also increased the chances of rancidity occurring. Under appropriate conditions, as water activity is lowered oxidation can accelerate. The effect of water activity on autoxidation has been explained by proposing that at high levels water interacts with metal catalysts reducing their activity and that water interferes with the decomposition of hydroperoxides; at high water activity hydrogen bonding helps to stabilize the hydroperoxide (Padley, 1994). This was not found to be true in this experiment, where at higher water activities hydroperoxides broke down further as indicated by higher anisidine values.

These results provide an insight into the changes that were occurring on a structural level beyond those of the peroxide testing. While peroxide values were not

significantly different ($p>0.05$), the anisidine values indicate that the peroxides in the flax supplemented breads broke down further than those of the control loaves. While not significant, bread containing xanthan gum had a lower anisidine value than the other experimental loaves.

Anisidine value is an excellent confirmation test to perform after peroxide testing. Graphed anisidine values take on an exponential line, due to the continual cycle of breakdown. Anisidine values correlate with peroxide values by increasing with increasing or decreasing peroxide values that might help indicate which side of the peroxide “bell curve” graph a sample might fall on. Anisidine value testing is particularly useful for oils with low peroxide values and in evaluation of the totox value (Rossell, 1994).

3c. Totox Value

No significant differences were found among totox values for any of the bread types (Table 4). While there were differences in anisidine values for bread samples, the lack of any difference in peroxide values led to the same result in totox values.

Totox value is a combination of the peroxide and anisidine values to achieve an overall assessment of rancidity in a sample. In the totox equation, peroxide value is given in double weighting due to findings of Holm and Ekbom in 1972 that when oil was heated at 200°C under vacuum, 1 peroxide value unit decomposed to give an increase of 2 anisidine value units. This has been rationalized by the fact that peroxides have two oxygens per molecule while aldehydes have only one (Rossell, 1994). The totox value is often considered to have an advantage due to the combination of evidence about the past

history of a food (reflected by the anisidine value) with its present state (evidenced by the peroxide value). Therefore, totox determination has been carried out extensively to estimate oxidative deterioration of food lipids. However, despite its practical advantages, some researchers believe that the totox value does not have any sound scientific basis because it combines variables with different dimensions (Shahidi and Wanasundara, 1998).

4. Sensory Analysis of Bread

Bread containing both gums were found to be significantly moister ($p < 0.05$) by the panelists, while the control bread and guar gum bread were significantly ($p < 0.05$) drier (Table 5). These results for control bread agree with the theory of lower water activity values (Table 1), while for guar gum they contradict it. Control bread had the lowest ($p < 0.05$) water activity (Table 1) than the other breads, indicating lower moisture content. Guar gum had higher water activity levels that would indicate higher moisture content. Cookies made with guar gum were given maximum scores in another study (Kaur et al., 2000) contradictory to sensory results here.

Bread containing flax were found to feel and appear significantly ($p < 0.05$) airy (Table 5). Breads with guar gum and xanthan gum, respectively, were found to be significantly ($p < 0.05$) denser. These results agree with data from physical analyses, whereas, guar gum contributed to a significantly ($p < 0.05$) harder, less springy crumb (Table 2), and produced a significantly low ($p < 0.05$) volume (Table 1). These results indicate a denser bread type. These poor results for bread containing guar gum also agree

Table 5. Mean (n= 32) Sensory Scores for Bread Containing Flaxseed and Gums

Type		Moist vs Dry ¹	Dense vs Airy ²	Yeasty vs Not ³	Bitter vs Neutral ⁴	Stale vs Fresh ⁵
Control	Mean	6.438 b	4.063ab	5.094b	6.219b	4.406a
	Standard Deviation	±2.015	±2.313	±2.006	±1.879	±2.123
Flax	Mean	5.719ab	4.469b	3.875ac	5.188a	5.125ab
	Standard Deviation	±2.174	±2.14	±1.699	±1.712	±2.012
Guar	Mean	6b	3.438a	4bc	4.719a	4.844ab
	Standard Deviation	±2.185	±1.722	±1.901	±2.113	±2.343
Xanthan	Mean	5.25ab	3.313a	3.813ac	5.125a	5.375ab
	Standard Deviation	±1.951	±1.655	±1.595	±1.54	±1.98
Both	Mean	4.844a	3.656ab	3.063a	5.375a	5.656b
	Standard Deviation	±2.142	±2.042	±1.722	±1.862	±1.961

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

¹1=moist 9=dry

²1=dense 9=airy

³1=yeasty 9=not

⁴1=bitter 9=neutral

⁵1=stale 9=fresh

with those of Kaur et al. (2000) who found that panelists awarded lower scores for appearance of bread prepared with guar gum.

Control bread was found to have the least ($p < 0.05$) yeasty aroma to the panelists as compared to the other breads (Table 5). Control bread was also found to taste significantly ($p < 0.05$) less bitter than the other breads (Table 5).

Control bread was found to have a predominant ($p < 0.05$) stale taste over the four weeks, while the bread containing both gums had a fresher ($p < 0.05$) flavor (Table 5). Water activity results (Table 1) concluded that control bread maintained lower levels, an indication of lower moisture or staling. Texture results (Table 2) found the control bread to be significantly ($p < 0.05$) harder, indicating an increase in staling, which agrees with these sensory results.

Figure 2 summarizes results found from sensory testing in a visual radar plot. Pictorially, control bread was found to be the stalest and most neutral tasting, as well as the most yeasty. Bread containing both gums are shown to be moister and not yeasty. Xanthan gum bread is pictured to be the most airy, while flax the most dense. Guar gum bread was found to have a strong bitter taste.

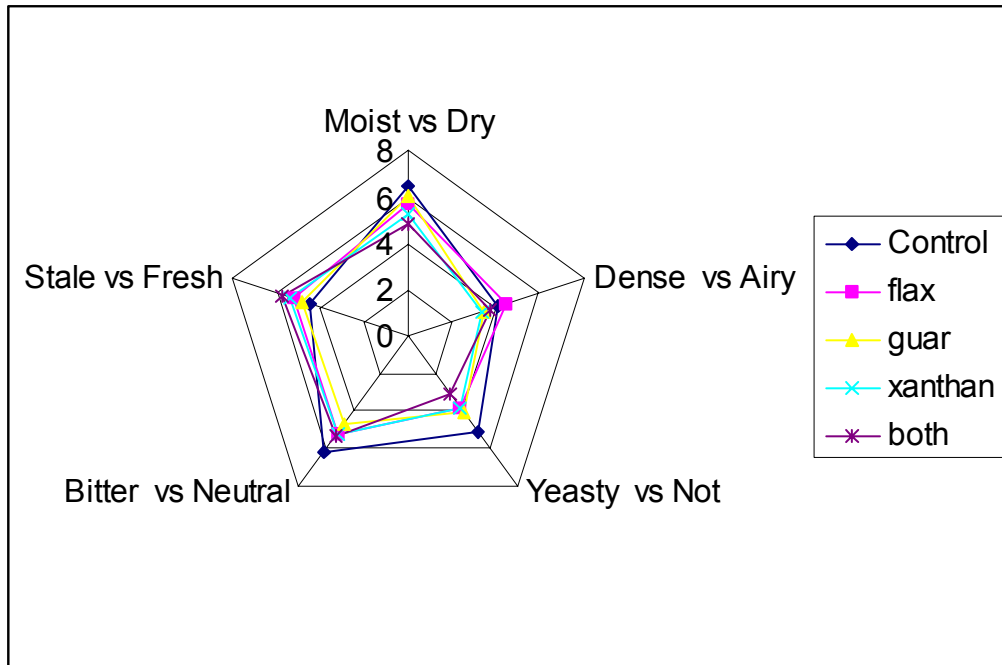


Figure 2. Radar Plot of Mean Sensory Scores for Bread Containing Flaxseed and Gums

Chapter 6: Summary, Conclusions & Recommendations

1. Summary and Conclusions

Flaxseed is gaining popularity in the functional foods spotlight. Reasons for this include its many health benefits and availability. Flaxseed has been found to have positive effects on preventing/treating numerous cancers, cholesterol and heart disease, diabetes, and even symptoms of menopause. Flaxseed can be eaten whole or ground, or incorporated into any number of foods including cereals, drinks, and baked goods.

However, the favorable fatty acid profile thought to be partially responsible for its health effects has a detrimental effect on the stability and shelf life of flaxseed. This study found that flaxseed meal contained 54.8% linolenic acid and 15.1% linoleic acid. The high amounts of linoleic and linolenic acids present are known to be more susceptible to autoxidation than saturated fatty acids. This susceptibility can be controlled through the use of antioxidants. Antioxidants can be synthetic, such as BHT and TBHQ, or natural. Natural antioxidants are often preferred due to the harmful limitations many synthetics have. Natural antioxidants are numerous, and research continues to find more in fruits, seeds, herbs, spices, and other plant sources. Gums have also been studied as natural antioxidants. Xanthan gum, pectin, guar gum, and tragacanth gum are thought to have antioxidant activity.

Gums are primarily used as food additives to control rheology and texture by stabilization of emulsions, suspensions, and foams. The use of gums in products such as baked goods is not common and the subject of recent research. This study looked at both the antioxidant activity and rheological effects xanthan gum, guar gum, and a combination of both gums had on yeast bread containing 15% flaxseed.

Bread was chosen as the subject of this research due to its flexibility and acceptability. Bread is a common staple of diets worldwide. Flaxseed was easily incorporated into bread because bread is adaptable to many variations without changing its identity. The sponge dough method for making bread was used in order to achieve an optimum product with heavier ingredients such as flax. Heavy ingredients can weigh bread dough down and alter volume by interfering with the gluten network. The sponge dough method has a longer proving time, which stabilizes the dough and enables sufficient hydration of the gluten molecules, thereby increasing volume.

Crumb color of control bread was significantly ($p < 0.05$) whiter than the experimental breads. This was due to the substitution of flaxseed for 15% of the flour in the experimental breads. Flaxseed exists in a variety of colors, ranging from light yellow to brown. Milled flaxseed used in this study was brown and darkened the color of breads it produced. Bread containing guar gum was significantly ($p < 0.05$) darker than other breads, and more yellow ($p < 0.05$) than breads containing flax alone and xanthan gum. Guar gum has fair transparent properties when hydrated, producing a cloudy color that darkened the bread. Prior to hydration, guar gum was added to flour in powdered form that possessed a creamy yellow color. This enhanced the yellow color of the bread containing guar gum.

Control breads were significantly ($p < 0.05$) higher in volume, however, breads containing flaxseed alone had a very similar volume. This difference is due to the interference grains and gums have on the gluten network responsible for the volume of breads. Use of the sponge dough method was employed to counteract this interference, but did not work completely. Breads containing guar gum had a significant ($p < 0.05$)

decrease in volume when compared to the other bread types. Guar gum is a thickening agent that swells rapidly and absorbs water. This reaction could have been responsible for interfering with the development of gluten by binding available water needed by gluten proteins and thereby causing a decrease in volume

Control bread was found to have significantly lower ($p < 0.05$) water activity than the experimental breads, indicating a decreased movement of water within and out of the bread crumb. Water activities generally correspond with moisture levels, indicating that the control bread sample had decreased moisture content and an increased staling rate than the other breads. Gums were not found to have an impact on the water activity of bread. Gums bind water, while still allowing it to move freely, thus increasing water activity. However, the effect of gums on moisture content was not evaluated and it is not known whether breads containing gums have corresponding water activities and moisture levels. This water binding effect probably altered moisture of the breads.

Control bread and bread containing guar gum were significantly ($p < 0.05$) harder than the other breads. These results for control bread agree with water activity results, which indicate lower moisture availability causing a decrease in softness. However, results for bread containing guar gum contradicted the theory of water activity, in which results showing higher levels would indicate a moist and soft bread. Volume results agree with bread containing guar gum which had a significantly low volume, indicating a more compressed, dense crumb. Guar gum is a thickening agent, which contributed in producing a harder bread. Breads containing flax and xanthan gum had significantly ($p < 0.05$) higher percentage of springiness than the other breads. These results for flaxseed bread contradict the proposed shortening and stiffening effects that grains have

on bread. Xanthan gum is a known thickening and gelling agent, but also has been found by other researchers (Sidhu and Bawa, 2001) to contribute to an increase in springiness.

The fatty acid profile of flaxseed used in this study contained a high percentage of α -linolenic acid (54.8%). Linoleic acid was also found to be present at 15.1%. These results agreed with expected lipid content of flaxseed as well as results from other analyses (Daun et al. 2003). These fatty acids were of primary interest in this study due to their susceptibility to autoxidation. Due to their degree of unsaturation, linolenic acid and linoleic acid oxidize at a rate 20 and 10 times faster, respectively, than that of saturated fatty acids.

Rancidity was examined by peroxide value, anisidine value, and totox value. There were no significant ($p>0.05$) differences found among the breads for peroxide values. However, peroxide testing is not the most accurate test, and that is why other tests were used to evaluate the breads. Anisidine values were used to enhance rancidity testing.

Control bread had significantly ($p<0.05$) lower anisidine values than the other breads, which indicated a decreased breakdown of peroxides that could be attributed to the lack of flaxseed in the bread. These results also contradict the theory of water activity in which higher water activity values are thought to prevent peroxide breakdown. Control bread was found to have the lowest ($p<0.05$) water activity. While not significant, bread containing xanthan gum had the lowest anisidine value of the experimental loaves, indicating it may have some effect on slowing the breakdown of hydroperoxides. There were also no significant ($p>0.05$) differences found in totox value results, which agree with peroxide values. This could be due to the doubled peroxide

value in the tottox equation. Flaxseed has been shown to remain stable for up to four months (Malcomson et. al, 2000), while this study lasted only two. Natural antioxidant sources have also been fractioned from flaxseed (Hall, 2001), which could be responsible for this increased stability and the lack of significant results from rancidity testing found in this study.

Bread containing both gums was rated significantly ($p < 0.05$) moister by sensory panelists, while control bread and bread containing guar gum were rated the driest ($p < 0.05$). These results contradicted those of water activity, where breads containing both gums and guar gum had higher ($p < 0.05$) water activities than control bread, implying higher moisture content as well. Bread containing flaxseed was found to appear and feel significantly ($p < 0.05$) airy, while breads with guar gum and xanthan gum were found to be denser ($p < 0.05$). These sensory results for bread containing guar gum agree with its low volume results and significantly ($p < 0.05$) hard crumb, which would lead to a denser bread. Control bread was found to have the least ($p < 0.05$) yeasty aroma and least ($p < 0.05$) bitter flavor. Panelists rated bread containing guar gum to have a strong ($p < 0.05$) yeasty aroma. Control bread also developed a predominant ($p < 0.05$) stale taste over the four weeks of sensory testing, which agrees with water activity results and texture results. Control bread had the lowest ($p < 0.05$) water activity, implying lower moisture levels and staling. Control bread was found to be significantly ($p < 0.05$) harder, which also indicated staling.

2. Recommendations for Future Research

In this study, xanthan gum and guar gum were examined for their antioxidant activity and effects on quality attributes in breads containing flaxseed. Flaxseed has a highly unsaturated fatty acid profile, which makes it susceptible to rancidity. However, due to its many health benefits, it is desirable to find ways to incorporate it into the diet. While the results from this study were inconclusive, the area of incorporating gums into baked goods is promising and warrants further investigation.

The amount of gums added to bread dough was estimated according to recommendations of TIC and review of similar studies. There is no set appropriate amount, as gums are not a common additive to bread or other baked goods. Research should be conducted to determine what levels of gums would be the most effective at enhancing attributes and providing any antioxidant effect they might possess.

In this study, water activity levels were evaluated. While gums were found to have no effect on lowering water activity, their effect on moisture levels in the breads is unknown. The theory of water activity suggests that moisture content generally corresponds to water activity. However, it is possible that while gums did not alter the water activity they might have altered the moisture content by binding water in the bread. This change could play a role in the rancidity process. In future studies, it would be beneficial to evaluate both water activity and moisture levels to identify the effects gums have on each.

Results from chemical testing were not significant in providing evidence of the autoxidation of flaxseed in the bread. This could have been caused by several factors including the testing method used, the presence of natural antioxidant compounds in the

flaxseed, and/or the possibility that the flaxseed used was rancid before being incorporated into the bread. In order to achieve the most accurate results when performing peroxide value testing it is recommended to use an electrochemical technique that can measure low values. Fresh flaxseed should also be used and should be evaluated for antioxidant activity. This flaxseed should also be monitored by rancidity testing along with bread samples to determine if any difference exists due to the presence of a food system. Since flaxseed has been shown to be stable for long storage, future studies should be conducted for longer than eight weeks, which was the duration of this study. However, the use of bread might limit the length of time available due to its short shelf life.

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Appendix A: Summary of Bread Formulations

Ingredient	Control	Flax	Xanthan	Guar	Both Gums
Sponge:					
Flour	192 g	192 g	192 g	192 g	192 g
Yeast	30 g	30 g	30 g	30 g	30 g
Sugar	30 g	30 g	30 g	30 g	30 g
Water	420 ml	420 ml	420 ml	420 ml	420 ml
Remaining:					
Flour	700.5 g	566.7 g	566.7 g	566.7 g	566.7 g
Water	~210 ml	~210 ml	~210 ml	~210 ml	~210 ml
Sugar	22.5g	22.5g	22.5g	22.5g	22.5g
Salt	21 g	21 g	21 g	21 g	21 g
Oil	30.3 g	30.3 g	30.3 g	30.3 g	30.3 g
Flaxseed	-	133.8 g	133.8 g	133.8 g	133.8 g
Wheat gluten	-	27 g	27 g	27 g	27 g
Xanthan gum	-	-	0.6 g	-	0.6 g
Guar gum	-	-	-	0.6 g	0.6 g

Appendix B: Randomization Sheet

Round	Bread Type	Mixing Order
1	Regular	1
1	Both gums	2
1	Flax	3
1	Guar gum	4
1	Xanthan gum	5
2	Flax	6
2	Xanthan gum	7
2	Both gums	8
2	Regular	9
2	Guar gum	10

Appendix C: Sensory Scorecard

Name _____ Date _____

Sample Code _____

Taste sample(s) provided. Rate according to the attributes listed with the scale given. Water is provided to rinse with.

Thanks ☺

Texture: Moist vs Dry

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

Texture/Appearance: Dense (small air cells) vs Airy/Fluffy (large air cells)

Dense _____ Airy
1 2 3 4 5 6 7 8 9

Aroma: Yeasty (sour) vs Not Yeasty (sweet)

Yeasty _____ Not
1 2 3 4 5 6 7 8 9

Flavor: Bitter vs Neutral

Bitter _____ Neutral
1 2 3 4 5 6 7 8 9

Flavor: Stale vs Fresh

Stale _____ Fresh
1 2 3 4 5 6 7 8 9