

EFFECT OF FLAXSEED FIBER ON BREAD QUALITY
OBTAINED FROM FROZEN DOUGH

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MASTER OF SCIENCE

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ABSTRACT

Fiber in frozen bread dough is thought to reduce the loss of yeast vitality and improve nutrition and quality of bread. The objective of this study was to determine the applicability of flaxseed fiber in frozen bread dough. Fiber was extracted from two sources of brown flaxseed and added to bread formulas at 0%, 1% and 3%. Dough was blast chilled and kept frozen until thawed at scheduled intervals. Thawed dough was proofed, baked into bread. Bread was tested analytically and by a sensory panel. Loss of overall quality was observed overtime. Breads containing flaxseed fiber had significantly larger loaf volumes (cc) and reduced firmness (g). A trained sensory panel detected significantly lower crumb firmness and stale flavors in breads containing flaxseed fiber. These results suggest flaxseed fiber has the potential to improve the perceived quality of frozen bread dough.

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TABLE OF CONTENTS

ABSTRACT	iii
ACKNOWLEDGEMENTS	iv
LIST OF TABLES.....	x
LIST OF FIGURES	xi
1.0. INTRODUCTION.....	1
2.0. LITERATURE REVIEW.....	3
2.1. Overview of flaxseed.....	3
2.2. Composition of flaxseed.....	3
2.2.1. Lipid.....	4
2.2.2. Protein.....	5
2.2.3. Carbohydrate.....	5
2.2.4. Minor Components.....	6
2.3. Flaxseed Fiber	6
2.3.1. Extraction	6
2.3.2. Composition.....	7
2.3.2.1. Protein.....	7
2.3.2.2. Carbohydrate.....	8
2.3.3. Functional Properties.....	8
2.3.4. Applications.....	10
2.4. Frozen bread dough	11

2.4.1. Applications.....	11
2.4.2. Processing.....	11
2.4.2.1. Mixing	12
2.4.2.2. Fermentation.....	12
2.4.2.3. Freezing	13
2.4.3. Ingredients	14
2.4.3.1. Flour.....	14
2.4.3.2. Yeast.....	17
2.4.3.3. Dough additives	18
2.4.3.3.1. Oxidants.....	18
2.4.3.3.2. Non-fat dry milk (NFDM).....	18
2.4.3.3.3. Vital wheat gluten (VWG).....	19
2.4.3.4. Hydrocolloids	19
2.4.3.5. Shortening/lipids.....	20
2.4.3.6. Sugar.....	20
2.4.3.7. Sodium chloride (salt).....	20
2.5 Sensory analysis	21
3.0. HYPOTHESIS.....	23
4.0. OBJECTIVE	24
5.0. MATERIALS AND METHODS.....	25
5.1. Materials	25

5.1.1. Flaxseed.....	25
5.1.2 Reagents	25
5.2. Methods.....	26
5.2.1. Flaxseed mucilage extraction	26
5.2.2. Dough formation.....	27
5.2.3. Proofing and bread baking.....	29
5.2.4. Bread quality	30
5.2.4.1. Loaf volume	30
5.2.4.2. Texture analysis	30
5.2.4.3. C-Cell imaging	31
5.2.4.4. Water activity	31
5.2.4.5. Sensory analysis	31
5.2.5. Crude flaxseed fiber analysis.....	34
5.2.6. Statistical analysis.....	34
6.0. RESULTS AND DISCUSSION.....	35
6.1. Crude Flaxseed Fiber Analysis.....	35
6.1.1. Extraction yield.....	35
6.1.2. Proximate analysis	36
6.1.2.1. Protein.....	36
6.1.2.2. Dietary fiber	36
6.2. Objective Flaxseed Fiber Bread Quality.....	37

6.2.1. Loaf Volume	37
6.2.1.1. Treatment effect	37
6.2.1.2. Time effect	40
6.2.2. Firmness.....	42
6.2.2.1. Treatment effect	42
6.2.2.2. Time effect	44
6.2.3. Water activity.....	45
6.2.3.1. Treatment effect	45
6.2.3.2. Time effect	46
6.3. Subjective Flaxseed Fiber Bread Quality.....	47
6.3.1. Crumb Firmness	47
6.3.1.1. Treatment effect	47
6.3.1.2. Time effect	47
6.3.2. Stale Flavor	48
6.3.2.1. Treatment effect	48
6.3.2.2. Time effect	49
6.3.3. Overall acceptability	49
7.0. CONCLUSION	50
8.0 FUTURE WORK.....	51
9.0. LITERATURE CITED.....	52

LIST OF TABLES

<u>Table</u>	<u>Page</u>
1. Mixolab Chopin + protocol for determining water absorption.	28
2. Formulas for 0%, 1% and 3% flaxseed fiber bread dough based on baker's percent.	30
3. CT3 texture analyzer procedure to determine peak firmness.	31
4. Serving order of samples 1-6 for nine panelists.	34
5. Water amount added to of bread dough formulas made with and without flaxseed fiber.....	46
6. Changes in firmness responses (cm) in baked bread from frozen dough with and without flaxseed fiber, as indicated by sensory panelists.	47
7. Changes in firmness responses (cm) over time for bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.....	48
8. Changes in stale flavor responses (cm) in bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.....	48
9. Changes in stale flavor responses (cm) over time for bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.....	49

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
1. General proximate composition of flaxseed (lipid- 40%, carbohydrate-30%, protein-20%, ash-4%, and moisture-6%) as reported by Daun et al., 2003.	4
2. Affect of freezing rate on the amount of time foods spend in the critical zone during ice crystal formation (adopted from Fellows, 2000).	17
3. Mixolab profile of the control flour (C1= Gluten development; C2= gluten weakening due to over mixing and temperature begins to rise; C3= starch gelatinizes/ peak viscosity; C4= amylose activity/break down; C5= starch retrogrades/final viscosity).....	28
4. Sensory scale used by trained sensory panelist to determine the characteristics of flaxseed fiber bread over 20 weeks.	33
5. Percent loaf volume reduction of breads made from frozen dough with and without flaxseed fiber stored frozen over 20 weeks.....	38
6. Number of holes (px) in breads made from frozen dough with and without flaxseed stored frozen over 20 weeks.Values indicate number of holes after 20 weeks of frozen storage, values with different letters indicate significant differences at ($p < 0.05$).	39
7. Loaf volumes of bread made with and without flaxseed fiber from frozen dough stored over 20 weeks.....	40
8. C-Cell images of breads with or without flaxseed fiber made from frozen dough stored over 20 weeks.....	41
9. Percent increase in firmness (g) of breads made from frozen dough with and without flaxseed fiber stored frozen for various periods of time over 20 weeks.	43
10. Mixolab torque (Nm) at points C1, C2, C3, C4 and C5 for flour with and without flaxseed fiber. See Figure 3, page 28 for description of data collection.	44
11. Water activity (A_w) of bread made from frozen dough with and without flaxseed fiber stored frozen over 20 weeks.	46

1.0. INTRODUCTION

Flaxseed, also known as linseed (*Linum usitatissimum*), is an ancient crop first grown in Turkey over 9,000 years ago (Cullis, 2007). It was used as a source of fiber for linen, food and medicine before being taken to North America during colonization (Vaisey-Genser and Morris, 2003). Today, Canada is the largest producer (Li et al., 2008), with production ranging between two to three million metric tons in the last decade (Wu et al., 2010). In food applications flaxseed is sought by consumers for its dietary (soluble and insoluble) fiber, α -linolenic acid and lignans (Zhang et al., 2009). These nutrients provide numerous benefits including: reducing the risk of stroke, coronary heart disease, lowering blood cholesterol, and aiding in laxation (Vaisey-Genser and Morris, 2003). The antioxidant activity providing protection for cardiovascular disease and cancer also has been reported (Jenkins et al., 1999).

Since its domestication, the functionality of flaxseed has varied greatly. Non-food applications of flaxseed have included: textiles, lamp oil, paint and varnishes, printing ink and plastics (Cullis, 2007). While flaxseed has numerous non-food applications, it is also commercially processed for food applications including: whole flaxseed, milled flaxseed (which can be placed in baked goods) and premade/ready to eat products (Duan, 2003). While flaxseeds are utilizable in food and non-food applications, flaxseed as a food ingredient is not as profitable as flaxseed utilized by the paint industry and other non-food industries (Chen et al., 2006). Therefore, through fractionation, scientists have made flaxseed more utilizable and economically applicable for food use (Oomah, 2003).

Most recently, the functionality of flaxseed fractions in food applications has been investigated. Of particular interest, the soluble fiber fraction of flaxseed has the potential to be a co-product of the flaxseed oil industry suitable for food applications (Oomah, 2003). Pre-treating the

whole flaxseed prior to oil processing can provide a fiber (also known as mucilage) product that can be used in food applications. The fiber fraction can be removed with little loss of oil (Zhang et al., 2009). Alternatively, flaxseed fiber can be removed from the meal or the hull after oil extraction (Cui, 2001; Chen et al., 2006).

While extraction of flaxseed fiber is industrially feasible, applications and functionality of flaxseed gum can vary. It has been determined that the functionality of flaxseed gum changes depending on cultivar (Cui and Mazza, 1996), extraction parameters (Cui et al., 1994), and drying methods (Wang et al., 2010a). Currently flaxseed gum has been used to stabilize emulsions in salad dressings and in meat applications (Wang et al., 2010b). With the current human fiber consumption being about half (8g of fiber/1000 calories) (Anderson et al., 2010), of the Center for Disease Control (CDC) recommendation (14g of fiber/1000 calories) (CDC, 2011), the incorporation of flaxseed fiber into bread is an opportunistic market (Wang et al., 2002). Furthermore, its water holding functionality makes flaxseed fiber an applicable ingredient in frozen bread dough systems based on observations that fibers can be a functional ingredient in frozen dough (Sharardanant and Khan, 2003a). This project will address the functionality of flaxseed fiber in frozen bread dough and the quality of bread obtained from the dough.

2.0. LITERATURE REVIEW

2.1. Overview of flaxseed

Flaxseed, *Linum usitatissimum*, is an oilseed thought to have biologically evolved from *Linum angustifolium* or *Linum bienne* (Cullis, 2007). There are three breeding programs for flaxseed in Canada and one in the United States (Daun et al., 2003; Hammond, 2011). Breeding objectives include disease resistance, improved yield and oil characteristics; however, objectives remain flexible to meet new product development needs (Cullis, 2007).

While many may prefer yellow flaxseed for baking and beverages, brown flaxseed is more common (Wilkes, 2007). Brown flaxseed was used in the following study. Physical and structural properties of flaxseed have been studied and an attempt to correlate properties to flaxseed color. However, little to no correlation between color and flaxseed properties have been made (Daun et al., 2003). Aside from color, dimension is another physical property of flaxseed. In general, flaxseeds have a length of 3.0-6.4 mm, a width of 1.8-3.4 mm and a thickness of 0.5-1.6 mm (Coskuner and Karababa, 2007). Other flaxseed physical properties can be correlated to moisture content. For example, both the true density of flaxseeds and porosity increases as the moisture content increases (Coskuner and Karababa, 2007). While physical properties dictate how flaxseeds are mechanically processed, the chemical composition of flaxseed dictates its final end use.

2.2. Composition of flaxseed

A general chemical composition of flaxseed is as follows (Figure 1): lipid- 40%, carbohydrate-30%, protein-20%, ash-4%, and moisture-6% (Daun et al., 2003). However, composition percentages range as different analytical techniques and procedures are used. A discussion of each chemical component can be found in the proceeding sections.

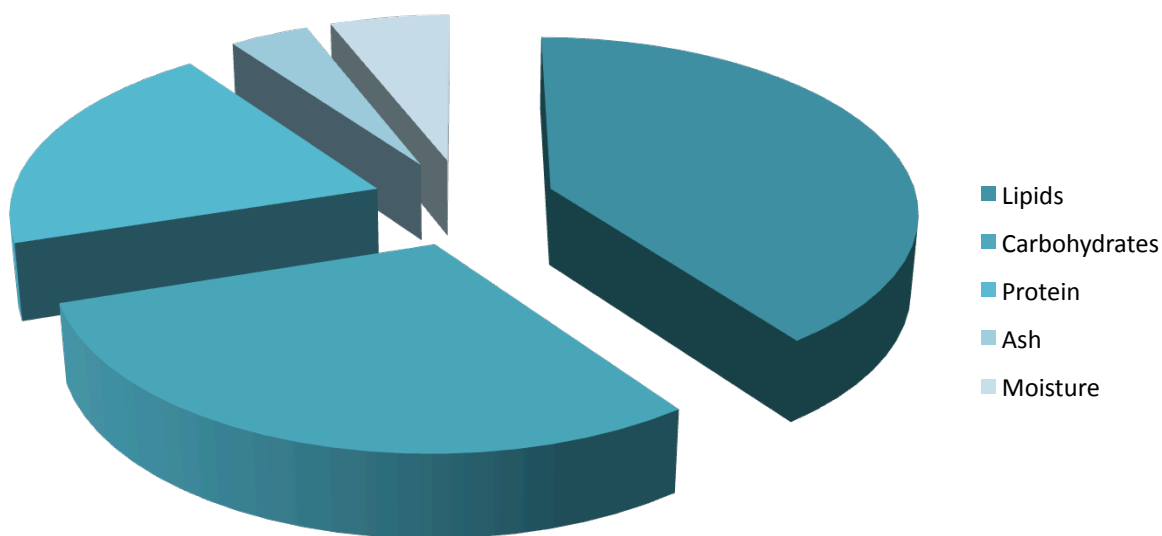


Figure 1. General proximate composition of flaxseed (lipid- 40%, carbohydrate-30%, protein-20%, ash-4%, and moisture-6%) as reported by Daun et al., 2003.

2.2.1. Lipid

A majority of the lipids found in flaxseed are neutral lipids, more specifically acylglycerols and fatty acids (Daun et al., 2003). Of the fatty acids, over 50% of them are alpha-linolenic acid (Muir and Westcott, 2003). In addition, oleic and linoleic acid make up 18% and 14% of the fatty acids, respectively (Daun et al., 2003). Thus, over 82% of the fatty acids are unsaturated. The highest degree of unsaturation is nutritionally desirable but undesirably unstable in food applications, making quality control extremely difficult.

Recent research has indicated that enhanced quality and nutritional properties could be obtained in flaxseed oil incorporated with flaxseed lignin. In addition, current studies have explored the minor fractions of flaxseed oils such as phospholipids (Herchi et al., 2011). Herchi et al. (2011)

reported phospholipids ranged from 1.8-2.5% of the total lipids. More specifically, phosphatidylethanolamine and phosphatidylinositol derivatives containing stearic and palmitic fatty acids were the main phospholipids in flaxseed oil (Herchi et al., 2011).

2.2.2. Protein

While flaxseed is approximately 20% protein, in general, as the protein content of flaxseed increases, the lipid content decreases, this inverse relationship is common in many oilseeds (Oomah and Mazza, 1993). Although there is 20% protein in flaxseed, much difficulty has been encountered during protein extraction due to water soluble mucilage (Oomah and Mazza, 1993). In general, most studies reported a salt-soluble protein (High molecular weight, HMW) and a water soluble protein (LMW) fraction (Wang et al., 2010a; Chung et al., 2003).

Functionally, in comparison to soybeans, flaxseed proteins are more lipophilic (Daun et al., 2003) and mimic the solubility of sunflower proteins (Oomah and Mazza, 1993). However, crude flaxseed proteins, containing water soluble mucilage and lipophilic proteins, have exhibited good emulsifying and foaming capabilities and stability (Wang et al., 2010b; Oomah and Mazza, 1993). Moreover, the extent of functionality is dependent on pH and presence of salts during extraction (Oomah and Mazza, 1993).

2.2.3. Carbohydrate

Carbohydrates are often classified into two groups. Flaxseed contains small amounts (1-2%) of monosaccharide, disaccharide and starch (Daun et al., 2003). In contrast, flaxseed contains a substantial amount (28%) of non-digestible carbohydrates (i.e. dietary fiber) compared to other oilseeds (Daun et al., 2003). The dietary fiber fraction includes both soluble and non-soluble components (Kristensen et al., 2011). Of interest and topic of further discussion, flaxseed soluble dietary fiber will be covered in section 2.3. Flaxseed Fiber.

2.2.4. Minor Components

Minor components that constitute flaxseed include minerals, vitamin B, phenolics and lignans (Daun et al., 2003). Significant levels of potassium (0.55%), phosphorus (0.44%), iron (3.7%), zinc (3.8%), copper (0.79%) and manganese (1.3%) (Daun et al., 2003). Phenolics and lignans in flaxseeds have become a recent research interest due to potential health benefits (Bravi et al., 2011). Of significant quantities and interest in flaxseeds is the lignan SDG and ferulic acid (Daun et al., 2003). However, the quantity of these compounds varies with cultivar, growing environment and extraction procedure (Hall et al., 2006).

2.3. Flaxseed Fiber

2.3.1. Extraction

The epidermal layer of flaxseed is a water soluble fiber (Ziolkowska, 2011) that is approximately 8% of flaxseed by weight (Cui et al., 1994). The biological function of flaxseed mucilage is to entrap water for seed germination (Naqvi et al., 2011). Water binding capabilities are the premise for extraction. Thus, aqueous extraction of whole seed, followed by alcohol precipitation and drying are sufficient to isolate the flaxseed mucilage (Oomah, 2003).

Various extraction yields have been reported. Yield is significantly influenced by temperature; however, rheological properties and protein content depend on pH and seed to water ratio (Cui et al., 1994). Cui et al. (1994) suggested that optimal conditions for obtaining mucilage with desired rheological behavior was a three hour extraction with 13 parts water to 1 part whole flaxseed (Cui et al., 1994). Water was at a pH of 6.7-7.0 and 85-90°C (Cui et al., 1994). Cui et al. (1994) reported a yield of 8.0% and less than 80 g protein/kg mucliage. However, recent research suggests that for commercial production of flaxseed mucilage, different parameters are needed (Ziolkowska, 2011). Ziolkowska (2011) reported that flaxseed hulls exposed to a 30 minute

countercurrent extraction at a water to seed ratio of 25:1 with water at 78-82°C will result in yields upwards to 8.2%. However, no protein analysis was reported in the Ziolkovska's (2011) study. Therefore, the true carbohydrate yield may be lower than their reported value due to higher protein yields.

2.3.2. Composition

Compositional analysis on the mucilage fraction of flaxseed has been reported in several studies. However, it should be noted that composition was limited to protein and carbohydrate analysis.

2.3.2.1. Protein

Crude flaxseed mucilage may contain upwards to 20% protein after extraction (Cui, 2001). This is due to mucilage being located at the epidermis, where it can be tightly linked to protein (Cui, 2001; Izydorczyk et al., 2005). Cultivar and growing environment influence the protein content of flaxseed gum (Cui and Mazza, 1996; Oomah and Mazza, 1993). Reduction in pH and temperature during extraction can reduce the amount of protein found in the crude flaxseed mucilage (Cui et al., 1994; Zhang et al., 2009). While protein is often undesirable, no research has been completed on purified flaxseed gum. Limited research on purified flaxseed gum is most likely due to handling difficulties and product loss that occurs during the purification process.

While protein contamination seems inevitable, it may have some function in flaxseed mucilage properties. In general, flaxseed proteins are lipophilic (Daun et al., 2003); therefore, it is proposed that the emulsifying and interfacial properties may be due to proteins present in the mucilage (Cui and Mazza 1996). However, no research has been reported that isolated and characterized the protein from mucilage.

2.3.2.2. Carbohydrate

The polysaccharide portion of flaxseed gum, observed by Cui (2001), varies widely in monosaccharide composition, mostly due to cultivar and growing condition (Cui and Mazza 1996). Structural determination of flaxseed gum polysaccharides has been conducted. Izydorczyk et al. (2005) reported flaxseed mucilage had two fractions. A backbone of (1→4)-linked β -D-xylopyranosyl residues was observed for the neutral fraction. This fraction contained O-2 and/or O-3 arabinose and galactose side chains. The second fraction is acidic and composed of (1→4)-linked α -D-galacturonopyranosyl and (1→2)-linked α -L-rhamnopyranosyl backbone. This backbone contains L-fucose and D-galactose chains (Izydorczyk et al., 2005). The galacturonopyranosyl backbone provides carboxylic acid groups which cause the backbone to be acidic (Izydorczyk et al., 2005).

While structural determination has been completed, Whistler and BeMiller (1997) noted that mucilage and heterogeneous gums (i.e. flaxseed gums) are often highly branched with a number of possible structures (Whistler and BeMiller, 1997). The wide range of monosaccharide composition (Cui, 2001), neutral to acidic monosaccharide ratio (Izydorczyk et al., 2005) and knowledge of mucilage and heterogeneous gums indicates a large number of possible structures for flaxseed gum (Whistler and BeMiller, 1997).

2.3.3. Functional Properties

While it is applicable to place flaxseed mucilage in many products due to its health benefits (Muir and Westcott, 2003), flaxseed mucilage in products causes changes in sensory attributes of the final product (Pohjanheimo et al., 2006). Furthermore, variation can arise between batches of flaxseed mucilage depending on flaxseed cultivar and component extraction methods (Cui et al.,

1994; Cui and Mazza, 1996). Thus it poses sensory, quality, and consistency challenges for product development (Pohjanheimo et al., 2006; Cui et al., 1994; Cui and Mazza, 1996).

The chemical composition of flaxseed mucilage contributes to its functional properties (Cui et al., 1994; Cui and Mazza, 1996; Chen et al., 2006). While functionality information about mucilage proteins is minimal, in general it is reported that mucilage proteins contribute to the emulsifying properties of mucilage via lipophilic interactions (Oomah and Mazza, 1993; Cui and Mazza, 1996). In addition to protein, carbohydrates also possess functional properties. It was observed by Cui (2001) that the neutral and acidic carbohydrate fraction of the flaxseed mucilage polysaccharides exhibit individual flow properties. Cui and Mazza (1996) report that the smaller molecular weight acidic carbohydrate fraction exhibits Newtonian flow with weak rheological properties. In contrast, the larger molecular weight neutral fraction exhibits shear thinning and weak gel properties (Cui and Mazza, 1996). Differences in flow properties could be a result of molecular size differences and ability to hold water (Cui and Mazza, 1996).

While flaxseed is generally recognized as a non-gelling hydrocolloid (Cui et al., 1994), its weak gel properties have been reported and studied. Chen et al. (2006) reported flaxseed mucilage was a thermoreversible gel (melting upon heating and gelling upon cooling). However, gel strength was dependent on source, concentration and additives (Chen et al., 2006). Cui and Mazza (1996) observed weak gel properties at 1 and 2% (w/w) concentrations. However, as concentration (upwards to 4%) increased the gel strength also increased (Chen et al., 2006). They proposed that increasing the concentration resulted in more interactions (i.e. junction zones) between the polysaccharide molecules. Chen et al. (2006) also reported that it was possible to alter the functionality of flaxseed mucilage by controlling the number of junction zones with salts and altering the pH. A pH of 3 to 4 resulted in the firmest gel. Sodium chloride (NaCl) reduced the strength of

the gel due to a reduction of junction zones via repulsive forces. In contrast, CaCl_2 promoted gel strength by bringing two polysaccharide molecules together (Chen et al., 2006).

In addition to chemical composition, different drying methods of the aqueous flaxseed mucilage altered the functionality (Wang et al., 2010a). Wang et al. (2010a) reported that in comparison to untreated samples, ethanol precipitated gum had the best emulsion stability. Gum prepared by spray drying had the least emulsion stability, but had an increased foam stability. Oven drying at 105°C increased foam capacity. In general, all drying methods reduced gel strength. Research suggest that in addition to cultivar, a specific drying method should be considered for desired functionality (Wang et al., 2010a).

2.3.4. Applications

Multiple researchers have reported several uses for flaxseed mucilage. Adjusting the pH of flaxseed mucilage solutions can change the charge of flaxseed mucilage particles, allowing attraction or repulsion to other compounds (Chen et al. 2006). Recently this technology has been applied to stabilizing commercial drink products containing whey protein isolates (Khalloufi et al., 2009; Kuhn et al., 2011). Qin et al. (2005) explained that flaxseed mucilage can stabilize cloudy carrot juice via steric repulsion and reduce the creaming of carrot juice due to its emulsion capacity. Moreover, Whistler and BeMiller (1997) suggested flaxseed mucilage could replace gum arabic in emulsion systems. Flaxseed mucilage has numerous emulsion applications. Cui and Mazza (1996) reported that flaxseed mucilage has the potential to replace all non-gelling gums.

Previous results suggest flaxseed mucilage can be successfully incorporated in a baked bread product (Garden-Robinson, 1993; Unpublished data, 2007). Garden-Robinson (1993) reported that flaxseed fiber increased loaf volume of fresh bread in comparison to bread with no fiber. However, various effects of flaxseed fiber on texture have been reported. At storage day 0, 1 and 4, 3%

flaxseed fiber greatly increased the firmness of the fresh bread (Unpublished data, 2007). However, Garden-Robinson (1993) reported less firm texture in bread with flaxseed gum in comparison to bread containing xanthan and bread without gum.

It has been reported that flaxseed mucilage has the potential to be incorporated into food systems; however, currently there are no large commercial applications of flaxseed mucilage (Warrand et al., 2005), with the exception of a texturizing agent for the cosmetic industry (Khalloufi et al., 2009). Moreover, it should be noted that flaxseed mucilage has a potential to be used in a variety of applications with appropriate processing (extraction and drying methods) and cultivar selection (Cui and Mazza, 1996; Cui et al., 1994).

2.4. Frozen bread dough

2.4.1. Applications

Currently, 7% of bread and pastries in Europe are frozen products (Ribotta et al., 2006). These frozen goods include products such as: bread, prepared pizzas, pastries and other wheat dough products (Ribotta et al., 2006). For individual consumers, restaurants and grocery stores, frozen bread offers the convenience of fresh baked bread without the early mornings and laborious work involved in making bread dough (Lorenz and Kulp, 1995; Minervini et al., 2011). However, increased leavening time, small loaf volume and poor bread quality in breads baked from frozen dough are common problems observed by users (Minervini et al., 2011). To increase overall quality of breads made from frozen bread dough, researchers have explored processing and ingredient factors to stabilize dough during frozen storage.

2.4.2. Processing

While researchers have discovered that ingredients can be added to frozen bread dough to improve the quality, much research has explored how processing effects play a key role in final

quality of bread made from frozen dough. Key processing operations in frozen bread dough production are mixing, pre-fermentation and freezing.

2.4.2.1. Mixing

The objectives of mixing are straight forward: combine all ingredients uniformly, hydrate the ingredients, develop gluten and incorporate air (Ribotta et al., 2006). However, a number of variables during mixing makes the process less straight forward. In frozen bread dough, Lorenz and Kulp (1995) and Huang et al. (2008) both agree that the dough temperature during mixing can have detrimental effects on bread quality. Due to the high amount of energy being introduced to the dough system during mixing, the temperature of the dough begins to rise allowing for fermentation to begin (Lorenz and Kulp, 1995). Fermentation prior to freezing can result in bread with lower quality (i.e. proof time and loaf volume) in comparison to bread baked from frozen bread dough with no fermentation prior to freezing (Hsu et al., 1979).

The temperature rise in dough during mixing is a result of friction between the dough, the mixing bowl and the hook/pin/paddle (Ribotta et al., 2006). The longer the mixing time the more heat will be generated; however cutting the mixing time short can also produce low quality product. Mixing the dough to full development is critical to both fresh and frozen bread dough quality (Lorenz and Kulp, 1995).

2.4.2.2. Fermentation

Although fermentation is necessary to leaven dough, fermentation prior to freezing can be detrimental to bread quality (Gabric et al., 2011). As the fermentation time prior to freezing increases, the reduction in loaf volume becomes increasingly significant (Nemeth et al., 1996). Due to increased proof times required, researchers suggest that the reduction in loaf volume, as a result

of pre-fermentation, is often due to a loss of yeast viability caused by the freezing process (Nemeth et al., 1996).

2.4.2.3. Freezing

The process of freezing converts a portion of the dough (water) from an amorphous structure to a crystalline structure (ice) (Cauvain, 2003). The process of converting water into ice requires nucleation (Kiani and Sun, 2011). In dough systems, when dough has been super-cooled (Kiani and Sun, 2011), nucleation occurs at the gas pore interface and within the dough matrix (Baier-Schenk et al., 2005). Either simultaneously or immediately after nucleation, crystal growth begins (Kiani and Sun, 2011). Ice crystal growth results when water, within the dough, is redistributed from dough constituents (i.e. protein, starch, etc) to ice crystal nucleation sites to minimize the surface to volume ratio of ice (Leray et al., 2010; Baier-Schenk et al., 2005).

In bread dough, it has been reported that ice crystals at the gas pore interface are much larger than the ice crystals within the dough matrix (Baier-Schenk et al., 2005). Large ice crystals at the pore interface are a result of nucleation preference (Baier-Schenk et al., 2005). The gas pore interface provides a solid surface with low interfacial energy, surface irregularities for promoting nucleation (Baier-Schenk et al., 2005). Therefore, water is more readily redistributed there to minimize the surface to volume ratio (Baier-Schenk et al., 2005).

Processing factors such as rate of cooling, the temperature and the length of time for which dough is frozen, controls the ice crystal size. These parameters have been correlated to final bread characteristics and are significantly important for final bread quality (Matuda et al., 2011). Research has reported that at slower freezing rates yeast retains its gassing power or ability to produce CO₂ (Yi and Kerr, 2009). However, at slower freezing rates ice crystal sizes are much larger and more

damaging to the gluten network (Kiani and Sun, 2011; Yi and Kerr, 2009). In addition to freezing rate, temperature of storage also has an effect of yeast and gluten retention. According to Yi and Kerr (2009), lower freezing temperatures are beneficial for both yeast vitality and retention of the gluten structure. While freezing rate and freezing temperature are critical processing factors, the most damaging to the gluten network and frozen bread dough quality was the amount of time dough spends in frozen storage (Yi and Kerr, 2009).

2.4.3. Ingredients

Bread made from frozen dough has similar quality to bread made from fresh dough during the first few weeks of storage. However, small loaf volume and lack of carbon dioxide production by yeast has been reported after a few weeks of storage (Lorenz and Kulp, 1995). In frozen dough systems, current research is being conducted to determine processing or ingredient treatments that increase yeast viability (Nemeth et al., 1996). Most commonly, additional ingredients are used to increase the viability of yeast (Lorenz and Kulp, 1995). Therefore, the following section will focus on ingredients that can potentially increase the shelf life of frozen bread dough.

2.4.3.1. Flour

Flour is the source of protein and starch for bread's structure. The type of flour used plays a critical role in final bread quality (Giannou et al., 2003). Although, suppliers offer a wide array of flours for bakers, there are a few key specifications that should be noted when purchasing flour to be used for frozen bread dough.

While protein content generally increases water absorption, protein quality, more specifically, gluten quality is generally more important than total protein in bread making (Hoseney, 1994). Gluten is a viscoelastic mass formed when wheat flour proteins, glutenin and gliadin are hydrated

and mixed (Giannou et al., 2003). Gluten is extensible and able to stretch bi-axially facilitating entrapment of carbon dioxide produced from yeast (Hoseney, 1994). However, it is also resistant to extension; allowing for the formation of many bubbles instead of a few large bubbles (Hoseney, 1994). In bread dough, gluten forms a continuous network in contrast to starch, which is discontinuous within the dough system (Schiraldi and Fessas, 2003).

Much of the reduced quality reported from frozen bread dough is due to stress and damage on the gluten network (Ribotta et al., 2006). The process of freezing on bread dough is complex. In general, ice crystals increase the amount of pores and reduce the thickness and uniformity of the gluten strands (Ribotta et al., 2004). Lorenz and Kulp (1995) suggest flour with strong protein quality is desirable for frozen dough to compensate for loss of strength during freezing; however, extra strong flour can be undesirable for frozen dough (Bhattacharya et al., 2003). Bhattacharya et al. (2003) and Lu and Grant (1999) reported that extra strong flour increased the amount of water absorbed; therefore, increasing the amount of water available for redistribution during crystal growth (i.e. freezable water). An increase in freezable water correlates to increased damage to the protein network (Lu and Grant, 1999; Bhattacharya et al., 2003).

While protein plays a significant role in bread quality, starch has also been shown to play an important role in bread quality made from frozen dough. When purchasing flour for frozen bread dough it is suggested that starch granule damage and enzyme activities should be low to reduce premature fermentation (Lorenz and Kulp, 1995). Ribotta et al. (2003) reported the freezing process does have an effect on starch gelatinization properties. More specifically, Ribotta et al. (2003) reported a more pronounced retrogradation with an increase in frozen storage time. It was hypothesized that the redistribution of water could affect the arrangement of amylose and

amylopectin within the starch granule causing changes in the gelatinization and retrogradation profile (Ribotta et al., 2003).

In addition, to the starch granule structure, the starch granule composition (amylose to amylopectin ratio) can also have an effect on bread quality (Yi et al., 2009). Yi et al. (2009) discovered that adding waxy wheat (high amylopectin) retained softness and loaf volume through 90 days of frozen storage. The increase in bread quality, due to waxy wheat was thought to be caused by a reduction in water redistribution, thus reducing the ice crystal damage to the gluten network (Yi et al., 2009).

Protein and starch are two main components in wheat flour affecting bread quality. Complete removal of the starch or protein would not produce bread. However, mills often add additional ingredients into flour such as bromate (Lorenz and Kulp, 1995). Hsu et al. (1979) suggests that bromate have a negative effect on yeast activity, while other studies indicate that bromate improves loaf volume (Lorenz and Kulp, 1995). Currently, bromate is allowed in baked products at 0.0075 part for each 100 parts flour (w/w) in the United States (21 CFR §136.110); however, the European Union and other countries do not allow bromate in food products (Oreofero, 2011).

Water absorption is another important flour property. Dough is largely amorphous (Cauvain, 2003); however, during the freezing process water will reach a critical zone in which ice crystal formation occurs (Kiani and Sun, 2011). The time it takes to pass the critical zone (rate of heat transfer) is important for ice crystal size, shape and location (Kiani and Sun, 2011). The slower the freezing rate the more time the product will stay in the critical zone, resulting in a large ice crystals. In contrast, the faster the freezing rate the less time the product will stay in the critical zone, resulting in a large amount of small ice crystals (Fellows, 2000).

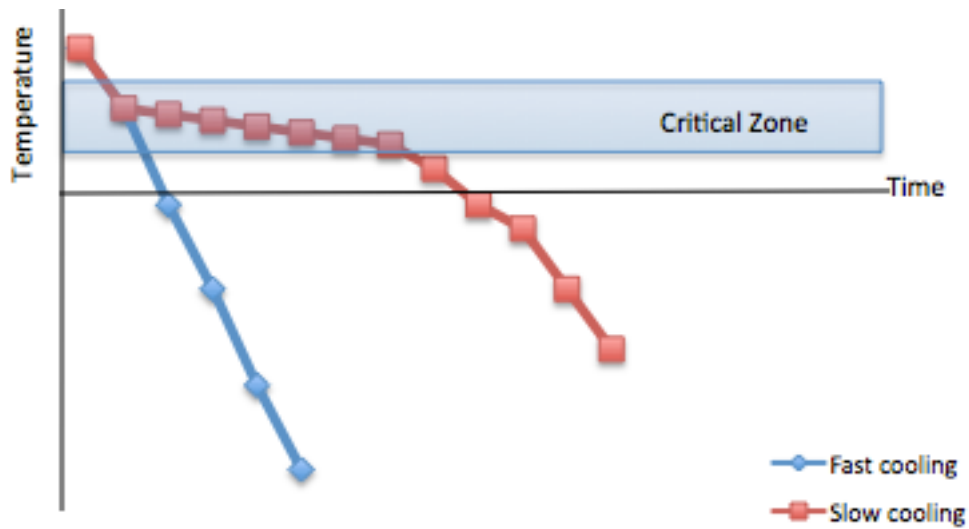


Figure 2. Affect of freezing rate on the amount of time foods spend in the critical zone during ice crystal formation (adopted from Fellows, 2000).

According to Matuda et al. (2011), the critical zone begins at -3.4°C for bread dough. However, this value can change based on the composition of the bread dough (Matuda et al., 2011). For example, the amount and type of solutes can depress the freezing point of water to various extents (Fellows, 2000). However, the addition of excess water to counter act the effects of solutes is detrimental to both gluten structure and yeast viability (Lorenz and Kulp, 1995). As water turns to ice the volume increases by approximately 9% (Fellows, 2000) resulting in damage to yeast cells and gluten structure. Moreover, limiting water is not appropriate as complete hydration is required for gluten development and starch gelatinization (Lorenz and Kulp, 1995).

2.4.3.2. Yeast

Similar to the gluten network, freezing for extended periods of time can damage the yeast resulting in a lack of CO_2 production and flavor development (Hino et al., 1987). Currently there is an array of yeast types, i.e. compressed yeast, instant dry yeast, active dry yeast, (Lorenz and Kulp, 1995) and developments of new strains have given frozen dough producers more flexibility. In

contrast to yeast types and developments, there are several processing factors that can be controlled to retain yeast vitality. A slow freezing rate can help retain yeast vitality and reduce the amount of fermentation prior to freezing has also been reported to retain yeast vitality (Hino et al., 1987; Yi and Kerr, 2009).

2.4.3.3. Dough additives

2.4.3.3.1. Oxidants. In frozen bread dough, the oxidant and the yeast contribute greatly to dough and bread characteristics (De Stefanis, 1995). A variety of oxidants are available for bakers to use; however, each has a different functionality and interchanging is not easy. Therefore, the baker should be aware, not only of functionality, but of acceptable concentrations for each type of oxidant set forth by the regulatory government. For example, the United States Food and Drug Administration (FDA) requires that no more than 75 parts per million (ppm) for potassium bromate; while, no more than 45 ppm for azodicarbonamide is acceptable (21 CFR §136.110).

While some countries have acceptable limits, others do not accept bromate as an oxidant (De Stefanis, 1995). Formulation challenges arise because bromate (in the form of potassium bromate) reacts at a slower rate than other available oxidants (De Stefanis, 1995). Therefore, finding a replacement is difficult. Research suggests that ascorbic acid and extended mixing time (more incorporation of air) can act as an effective bromate replacer (Chamberlin and Collins, 1979).

2.4.3.3.2. Non-fat dry milk (NFDM). NFDM, like other bread ingredients, has dual functions. The first function is to improve crust color. NFDM is a source of lysine (United States Dairy Export Council, 2011), which is an amino acid with an amine side group, which is limiting in wheat. The amine side group is important as it is reactive in the Maillard browning reaction, which involves reducing sugars and amine groups to form brown pigments (BeMiller and Huber 2008). Buffering the effects of fermentation acids is a second function of NFDM (Lorenz and Kulp, 1995).

2.4.3.3.3. Vital wheat gluten (VWG). VWG can be added to flour to enhance strength, nutrition and functionality. Most importantly, VWG provides additional strength to withstand processing stressor such as mixing or in frozen dough the freezing process (Ribotta et al., 2006). However, VWG should be used cautiously. Breads with extra strong gluten have been reported to have poor quality in comparison with breads with strong gluten (Lu and Grant, 1999). Lu and Grant (1999) report that dough with extra strong gluten is undesirable for frozen dough, as it has a higher water-binding capacity.

2.4.3.4. Hydrocolloids

As discussed earlier, free water is detrimental to both the gluten network and yeast. Therefore, the ability of hydrocolloids to hold water is desirable when trying to minimize ice crystal formation and water mobility (Ribotta et al., 2006). Although, hydrocolloids do not completely counteract the effects of the freezing process on dough, compared to control breads (no hydrocolloids), hydrocolloids improve loaf volume (Sharadanant and Khan, 2003a,b). Sharadanant and Khan (2003b) reported hydrocolloids better retained the continuous nature of the gluten network more similarly to bread dough without frozen storage; thus allowing for better gas retention (Sharadanant and Khan, 2003b). In addition, the amount of residual proteins found in dough's made with hydrocolloids was significantly more than the amount of residual proteins found in dough without hydrocolloids (Sharadanant and Khan, 2003b). Increasing the amount of residual proteins was positively correlated to increase in loaf volume (Sharadanant and Khan, 2003b).

Furthermore, the functionality of hydrocolloids is as numerous as the number of available hydrocolloids (Ribotta et al., 2006). Some hydrocolloids, such as hydroxypropylmethylcellulose, significantly affect the hydration properties of gluten. However, some hydrocolloids, such as gum arabic, do not affect the hydration properties of gluten (Barcenas et al., 2009). In addition, each

hydrocolloid has a unique effect on the viscosity profiles of starch (Barcenas et al., 2009). Viscosity profiles of starch have been correlated to the rate of bread firming (Barcenas et al., 2009). Therefore, some hydrocolloids can increase or decrease the rate of bread firming.

2.4.3.5. Shortening/lipids

While an excessive amount of shortening can be detrimental, at optimal levels, shortening can improve the processing and sensory characteristics of bread (De Stefanis, 1995). Shortening provides flavor, structure, good crumb grain, anti-firming and many other desirable attributes to bread in contrast to breads made without shortening (Pareyt et al., 2011). In addition, shortening aids in the incorporation of air during mixing, lubricates dough, and plasticizes to increase final loaf volume (Pareyt et al., 2011).

2.4.3.6. Sugar

The primary purpose of sugar is to provide an initial energy source for yeast (Ribotta et al., 2006). However, an excessive quantity of sugar can actually reduce yeast cells via osmotic pressure (Ribotta et al., 2006). In addition, only small amounts of sugar are needed because after enzymatic activity of α - and β -amylase on starch, starch become yeast primary energy source (Selomulyo and Zhou, 2007). Sugar is not only important for yeast survival, after invertase breaks it into glucose and fructose it aids in crust color formation (BeMiller and Huber, 2008). Therefore, without sugar the crust color would be light in color.

2.4.3.7. Sodium chloride (salt)

While salt provides desirable flavor, it also plays a biological and chemical role in bread making (Day et al., 2009). Salt creates an osmotically stressful environment that delays yeast activity, thereby preventing over fermentation and the biological activity of protease to retain gluten structure (Kulp and Lorenz, 1995; Ribotta et al., 2006). Chemically, salt can create ionic links

(bridges) between gluten strands via ionic interactions. Salt links result in an increase in gluten strength, most likely due to increase polymer size (Hammer et al., 2000).

2.5 Sensory analysis

While there are several instruments to describe characteristics of food, such as texture analyzers and gas chromatographs, only sensory analysis takes into consideration predominate characteristics perceived by humans. Furthermore, sensory analysis can give an indication of the acceptance of a food product; ultimately, it can provide direction for the food industry (Stone and Sidel, 1993). The consumer's satisfaction of a product is important for a successful business (Lyon et al., 1992). Determining how long a product will last on the shelf helps food manufacturing companies maintain and expand business (Lyon et al., 1992). Although there are several sensory tests available depending on the objective of the study (Lawless and Heymann, 1998), in this study a trained panel was used to detect flavor differences in bread made from dough stored up to 20 weeks.

While the maintenance of a trained panel can be overwhelming, accurate results can provide good insight to when an ingredient, processing, or time lapse causes a product to become recognizable different (Meilgaard et al., 2007). Often, quantitative descriptive analysis is used to determine these changes. However, it should be noted that these changes do not determine the likeness of the product but rather the intensity of specific characteristics (Lawless and Heymann, 1998).

In bread, over 300 volatile compounds have been identified that correlate to bread flavor (Jensen et al., 2011). However, chemical and physical changes occur over time that alters the flavor and texture of bread (Selomulyo and Zhou, 2007). These changes are grouped together to form a process called staling (Selomulyo and Zhou, 2007). In general, as bread undergoes staling the firmness of the crumb increases (Selomulyo and Zhou, 2007), yeast flavor intensity declines, while

dusty and chemical acid flavors increase in intensity (Jensen et al., 2011). It should be noted that differences in bread formulations between research studies make data comparison difficult due to different flavor profiles. However, it is the changes in flavor and texture intensities detected by trained sensory panel that ultimately give insight into the stability and acceptability of the bread.

3.0. HYPOTHESIS

The hypothesis of this research is that flaxseed fiber present in frozen bread dough will improve the overall quality (i.e. loaf volume, texture and flavor) of bread in comparison to bread baked from frozen dough without flaxseed fiber. In addition, the incorporation of flaxseed fiber will help retain overall bread quality (i.e. loaf volume, texture and flavor) as dough frozen storage time increases in comparison to bread baked from frozen dough without flaxseed fiber.

4.0. OBJECTIVE

Previous studies have incorporated hydrophilic gums in frozen bread dough (Sharardanant and Khan, 2003a,b) and flaxseed gum into fresh bread dough with good results (Garden-Robinson, 1994). However, no research has been published on incorporate flaxseed gum into frozen bread dough. The objective of this research was to assess quality changes in breads baked from frozen bread dough with different frozen storage times, sources and concentrations. Quality changes will be assessed by determining loaf volume, firmness, C-cell imaging, water activity and sensory profile of the breads with different flaxseed fiber different sources, concentrations and frozen storage times.

5.0. MATERIALS AND METHODS

5.1. Materials

5.1.1. Flaxseed

Brown flaxseed was obtained from two separate sources. The first source was grown in North Dakota, obtained from Heartland Flax: Valley City, North Dakota. Lot number 3C197-020410-100. The second source of brown flaxseed was a product of Canada, obtained from Specialty Commodities Inc: Fargo, North Dakota. Lot number 1368-G-644-BF10.

5.1.2 Reagents

Ascorbic Acid (fine granular); Lorann Gourmet: Lansing, MI.

Baker's Emplex Supreme (Caravan Ingredients, Lenexa, KS).

Canola Oil (J.M. Smucker Company, Orville, OH).

Ethanol (190 Proof: ACS/USP grade). Pharmco-AAPER, Shelbyville, KY.

Hydrochloric acid (ACS grade). VWR International, West Chester, PA.

Iodized sodium chloride (Morton Salt, Chicago, IL).

Non-fat dry milk (Premium Sanalac). ConAgra Foods Inc., Omaha, NE.

Sugar (granulated sugar). Distributed by Supervalu, Eden Prairie, MN

Vital Wheat Gluten (Bob's Red Mill, Milwaukee, OR).

Wheat Flour (High Gluten Spring Wheat Flour. Unbleached-Bromated-Unenriched). North Dakota Mill, Grand Forks, ND.

Baker's Select Yeast (Fleischmann's). Distributed by Food Service of America, Fargo, ND.

5.2. Methods

5.2.1. Flaxseed mucilage extraction

Thirteen parts water (with pH adjusted to between 6.0 and 7.0 with hydrochloric acid) was heated in a steam-jacketed stainless steel tank reactor (30 D9MT, Lee Industries, Philipsburg, PA) to 80° C. One part whole brown flaxseed was added to the hot water. The 1:13 flaxseed to water ratio was continuously stirred at nine revolutions per minute (rpm) for three hours while the temperature was maintained between 70 and 80°C. After three hours, the stirring agitator was turned off and mixture was allowed to sit for 15 minutes to promote settling. After 15 minutes, an under kettle valve was opened and flaxseeds were collected and vacuum dried overnight (dried degummed flaxseeds were not used in this study). After flaxseed had been removed, aqueous gum could then be collected via the under kettle valve into 10 gallon pails. Residual flaxseeds found in the aqueous gum were removed using a kitchen strainer. Aqueous gum was stored at 4°C for no longer than two weeks until the mucilage could be precipitated. Three extraction batches from each variety of flaxseed were conducted to obtain adequate amount of flaxseed gum.

Aqueous flaxseed mucilage was precipitated using 95% food grade ethanol. Approximately 2 gallons of aqueous flaxseed mucilage was placed in a 10-gallon pail, then approximately 6 gallons of food grade ethanol was added to the pail. The mixture was stirred with a plastic spaghetti spoon for approximately 1 minute. The precipitate was collected via the spaghetti spoon. Precipitated flaxseed mucilage was stored at 4° C until sufficient material was collected before drying. Precipitated flaxseed mucilage was frozen prior to freeze-drying using a Freezone 4.5 Labconco (Kansas City, MO) Freeze Dry System. Freeze dried samples were stored at 4° C until further procedures could be carried out.

Freeze dried flaxseed mucilage was milled in a Retsch (Germany) ZM 100 Mill. Initial particle size was reduced using a 1.00 mm screen. A final particle size reduction was completed with a screen size of 0.25 mm. Final weight of dried ground flaxseed mucilage was used to determine extraction yield (Equation 1). Milled samples were stored at 4° C until further chemical analysis procedures could be carried out. Flaxseed fiber yield was calculated following the effective mass yield calculation, using the following equation

$$\text{Yield} = \frac{DFM_m}{WF_m} * 100\%$$

Note: where DFM_m is the mass of freeze dried flaxseed mucilage and WF_m is the mass of whole flaxseed used.

5.2.2. Dough formation

Before dough formation a tentative water absorption or the percent of water required to produce a torque of 1.1 Nm was determined by Mixolab (Chopin type: Mixolab) using the 95 minute Chopin + protocol (Table 1). The amount of flour-gum mixture used in the Mixolab analysis was determined using the following equation:

$$\text{Flour mass (g)} = \frac{75g * TA}{1.00 - moisture_f}$$

Note: where 75 g is the desired final dough weight, TA is the trial absorption, and $moisture_f$ is the moisture of the flour.

In addition to water absorption, torque, temperature and time at the following points was recorded: gluten/dough development, gluten weakening, starch gelatinization/peak viscosity, amylose activity/breakdown and retrogradation/final viscosity. Interpretation of the Mixolab profile can be found in Figure 3.

Table 1. Mixolab Chopin + protocol for determining water absorption.

Parameter	Setting
Mix Speed	80 rpm
Target Torque (C1)	1.1 Nm
Dough Weight	75.0 g
Tank Temperature	30°C
Temperature (1st step)	30°C
1st Gradient	Fix time
Duration (1st step)	8 minutes
Duration (1st step)	90°C
1st Temperature Gradient	15 minutes 4°C/minute
Duration (2nd step)	7 minutes
2nd Temperature Gradient	10 minutes 4°C/minutes
Duration (3rd step)	5 minutes
Temperature (3rd step)	50°C

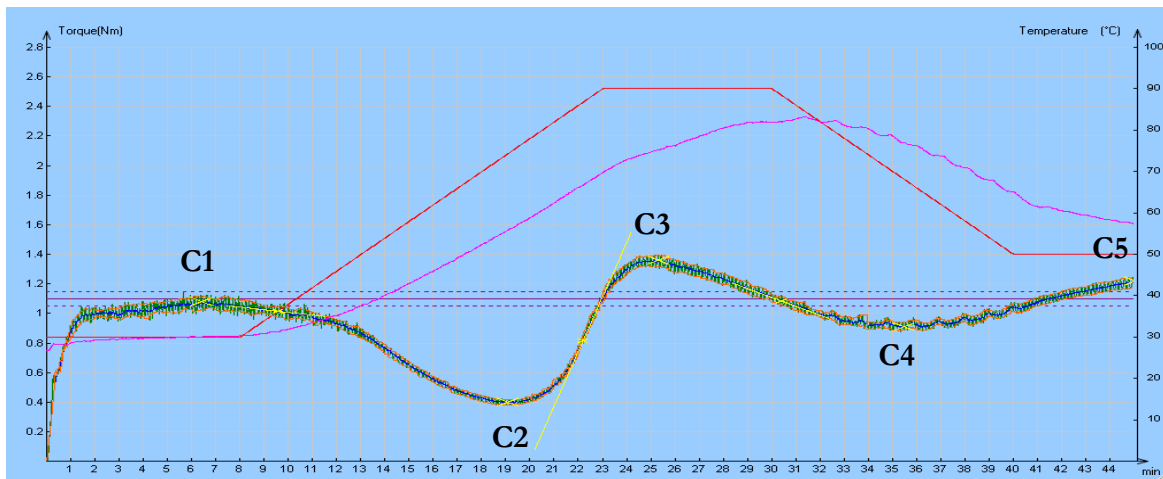


Figure 3. Mixolab profile of the control flour (C1= Gluten development; C2= gluten weakening due to over mixing and temperature begins to rise; C3= starch gelatinizes/peak viscosity; C4= amylose activity/break down; C5= starch retrogrades/final viscosity).

Five batches of dough were made containing 0% flaxseed fiber, 1% heartland flaxseed fiber (H1), 1% specialty flaxseed fiber (S1), 3% heartland flaxseed fiber (H3) and 3% specialty flaxseed fiber (S3) were formed with several adjustments to the American Association of Cereal Chemist (AACCC) method 10-10.03 (1995). Batches were individually mixed in a 20 pound Hobart spiral mixer (model H-600). At speed one, dry ingredients including dry flaxseed fiber were added based on flour weight (Table 2). Dry ingredients were mixed for two minutes prior to adding the oil, sugar-salt solution, ascorbic acid solution and ice water. The amount of water added was based on mixolab profile for the respective formula. However, the water absorption provided by the mixolab profile was a tentative guideline; additional water was added to reach an optimal visco-elastic character of bread dough as determined by mixing operator. Three minutes after adding liquid ingredients (oil, salt-sugar solution, ice water) the mixer speed was increased to speed two. The dough was mixed until optimum; i.e. dough could be stretched thin enough to see through but not break. The temperature of the dough was recorded after mixing was completed. Dough was divided into one-pound dough balls that were rounded by hand. After 15 minutes of rest, the dough balls were sent through a Moline molder (model PV1151) with settings 1.5", 1.5", 2.85" front and 3.05" back. Molded loaves were blast frozen (Convochill by Delfielol) to -21°C. It took approximately 30-35 minutes to blast freeze 16 loaves. The dough was placed in a -20°C freezer until appropriate baking time.

5.2.3. Proofing and bread baking

Frozen loaves were thawed for 14-16 hours in a 4° C refrigerator, prior to proofing and baking. Bread loaves were proofed to time (103 minutes or 133 minutes after week 8) at 40.5 °C and 70% humidity. Proof time was selected based on the time 0, which was proofed to 1 inch above pan height. After proofing, dough was baked in a 200° C oven for 20 minutes. Bread was immediately

removed from the pan and allowed to cool for two hours on a metal rack before being sealed in a clear polyethylene bag. Quality analysis was conducted the following day as determined below.

Table 2. Formulas for 0%, 1% and 3% flaxseed fiber bread dough based on baker's percent.

Ingredient	Control	1% Flaxseed Fiber	3% Flaxseed Fiber
Flour	100%	100%	100%
Sugar	6.0%	6.0%	6.0%
Yeast	3.0%	3.0%	3.0%
Salt	1.5%	1.5%	1.5%
Flaxseed Fiber ¹	0%	1%	3%
Non-Fat Dry Milk	1.0%	1.0%	1.0%
Vital Wheat Gluten	1.0%	1.0%	1.0%
Bakers Emplex	0.5%	0.5%	0.5%
Canola Oil	1.5%	1.5%	1.5%
Ascorbic Acid	0.2%	0.2%	0.2%
Water	Optimum*	Optimum*	Optimum*

¹Note: Two batches of 1% flaxseed fiber and 3% flaxseed fiber formulas were made. One batch used Specialty flaxseed fiber while the other used Heartland flaxseed fiber.

*Note: The amount of water added varied to obtain optimal dough characteristics.

5.2.4. Bread quality

Bread loaves were analyzed for comparative quality. Texture, water activity and C-cell imaging was conducted on center slices of bread as described below. In addition, center slices were used for sensory analysis.

5.2.4.1. Loaf volume

Loaf volume was determined by canola seed displacement according to the AACC method number 10-05.01 (AACC International, 1995).

5.2.4.2. Texture analysis

Peak firmness was determined by a CT3 texture analyzer (Brookfield Model CT3 10K). A clear acrylic 9 mm diameter cylinder probe with the parameters found in Table 3, was used to test a center slice of bread for firmness.

Table 3. CT3 texture analyzer procedure to determine peak firmness.

Parameter	Target Value
Test type	Compression
Target type	Distance
Target Value	4.0 mm
Hold time	0 seconds
Trigger Load	7.0g
Test speed	0.50 mm/s
Probe	TA3/100
Fixture	TA-RT-KIT

5.2.4.3. C-Cell imaging

Bread slice characteristics from a center slice of bread including: number of cells, number of holes, slice area, wrapper length, hole volume and cell wall thickness were analyzed by using a C-cell imaging instrument by CCFRA Technology LTD (CC.200.05). The C-cell was calibrated before testing using a grey card to standardize reflectance. Measurements were taken in units of pixels.

5.2.4.4. Water activity

Water activity was determined using an AquaLab (Series 3TE) Decagon meter from Devices Inc. (Pullman, Washington). A center slice of bread from each loaf was selected and the center of that slice was sampled for water activity.

5.2.4.5. Sensory analysis

Sensory analysis with nine-trained panelist was used to determine the staleness, firmness and overall acceptability of the bread over time. Training of sensory panelists took place on three consecutive days and involved two discussion sessions and one independent testing session. Discussion sessions required panelists to taste, smell and touch fresh to stale bread at various intensities. In the first discussion session, panelists were introduced to the continuous line scale they would be using throughout the study (Figure 4). Panelists then touched, smelled and tasted the fresh bread and identified flavors associated with fresh bread. Panelists then touched, smelled and

tasted stale bread and identified flavors associated with stale bread. The stale bread sample was produced by storing fresh bread at 2.0°C for 7 days. A discussion of the two flavors, smells and textures was conducted until a consensus of flavors identified could be reached.

In the second discussion session, panelists were refreshed with a written and verbal description of aroma, flavors and textures discussed in the previous discussion session. Panelists were then asked to touch, smell and taste three samples of bread of various staleness. Samples with different intensities of staleness were produced by placing baked bread at 2.0°C for 3 (medium stale) or 7 (high stale) days. Panelists were asked to identify the intensity of the stale flavor and texture on a continuous scale. In addition, panelists identified aromas by placing a check mark in a check box next to an aroma descriptor. Aroma descriptors included yeasty, sweet, rancid, sour, cardboard or other. After discussion and combining of results, average values for low, medium and high stale flavor intensities and textures were determined. In the last training session, panelists were asked to independently touch, smell and taste bread samples and rate the intensity of the stale flavor. These results were analyzed to determine training effectiveness and reliability of panelists. Of the nine panelists, seven were selected for their availability.

To determine detected intensity of stale flavor, crumb firmness and overall acceptability a ruler with centimeter (cm) markings was used. The 0 cm marking was placed at the left most side of the continuous line. The distance, in cm, from the left most point of the line to the point indicated by the panelist with a vertical line was recorded as the intensity of the bread attribute.

###

Aroma

<input type="checkbox"/> Rancidity	<input type="checkbox"/> Yeasty	<input type="checkbox"/> Cardboard
<input type="checkbox"/> Sour	<input type="checkbox"/> Sweet	<input type="checkbox"/> Other _____

Crumb firmness

Barely Detectable firmness	Weak	Moderate	Strong	Very Strong	Strongest Imaginable firmness
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Stale Flavor Intensity

Barely Detectable stale flavor	Weak	Moderate	Strong	Very Strong	Strongest Imaginable stale flavor
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Overall Acceptability

Not at all acceptable	Slightly Acceptable	Moderately acceptable	Acceptable	Very Acceptable	Extremely Acceptable
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Figure 4. Sensory scale used by trained sensory panelist to determine the characteristics of flaxseed fiber bread over 20 weeks.

After training was completed, bread samples, with random three digit codes, were tested every two or four weeks one day after baking. Samples were served to panelists according to the randomized block design (Table 4). In addition to samples, panelists received standards every other week to determine if extra training was needed. Standard of high or medium stale bread were prepared in the same manner as high and medium stale bread was for training.

Table 4. Serving order of samples 1-6 for nine panelists.

	Served 1 st	Served 2 nd	Served 3rd	Served 4th	Served 5th	Served 6th
Panelist 1	1	2	4	5	6	3
Panelist 2	5	1	3	6	4	2
Panelist 3	2	6	1	4	3	5
Panelist 4	6	3	2	1	5	4
Panelist 5	4	2	5	3	1	6
Panelist 6	3	4	6	5	2	1
Panelist 7	4	1	6	2	3	5
Panelist 8	1	3	4	6	5	2
Panelist 9	2	5	3	1	6	4

5.2.5. Crude flaxseed fiber analysis

Extracted flaxseed mucilage was subjected to LECO combustion nitrogen analysis method 46-30 (AACC International, 1995.) using a conversion factor of 5.41 to determine protein content (Tkachuk, 1969). Total Dietary fiber, insoluble fiber and soluble fiber was determined using AOAC dietary fiber assay according to AOAC method number 991.43 (AOAC, 1995).

5.2.6. Statistical analysis

Duplicate loaves of one replicate of the following five formula treatments were produced: no flaxseed fiber (Control), 1% heartland flaxseed fiber (H1), 1% Specialty flaxseed fiber (S1), 3% Heartland flaxseed fiber (H3), 3% Specialty flaxseed fiber (S3). Results were analyzed using an unpaired t-test with a two-tailed 95% confidence interval.

6.0. RESULTS AND DISCUSSION

6.1. Crude Flaxseed Fiber Analysis

6.1.1. Extraction yield

Crude fiber was extracted from Heartland and Specialty flaxseed at 2.02% and 2.61% of the seed weight, respectively. Theoretically, an 8.0-9.0% seed weight extraction yield for flaxseed mucilage is possible (Cui et al., 1994; Ziolkovska, 2011). Ziolkovska (2011) discussed the need for a commercially optimized process of flaxseed fiber extraction. Previously much of the research completed had been on a laboratory scale (Ziolkovska, 2011). However, in this study the low yield is partially attributed to the large pilot scale equipment and lack of a better optimized process. The equipment had many limitations. The first limitation would be the lack of a filter to separate degummed flaxseeds from aqueous mucilage. Some aqueous gum was lost due to the inability to remove sufficient amounts of flaxseed. A second limitation would be the surface area and crevices present on the equipment. Large stirring paddles and unusual outlets on the interior of the steam jacketed kettle caused a large amount of flaxseed mucilage to stick to the surface making it uncollectable. Lastly, the amount of container transfers required to extract and precipitate fiber was a limiting factor in extraction yield. A minimum of three transfers was needed before precipitated flaxseed mucilage was obtained for freeze drying. In general, large amounts of aqueous flaxseed mucilage was lost due to limitations of equipment.

Although large amounts of aqueous flaxseed mucilage were lost due to processing, the extraction process was repeatable. Extraction yields from Heartland and Specialty flaxseed at 2.02% and 2.61% of the seed weight, respectively, are very similar. Therefore, it may be possible to get approximately 2.0-3.0% flaxseed fiber using another source of whole brown flaxseed.

6.1.2. Proximate analysis

6.1.2.1. Protein

Heartland and Specialty flaxseed fiber contained 14.7% and 12.6% protein, respectively. Theoretically, protein content of flaxseed fiber has ranged from 0.0% to 20.0% (Cui et al., 1994). Cui et al. (1994) reported that protein content in flaxseed fiber is inversely related to extraction pH. However, a similar pH was used for the extraction of Heartland and Specialty flaxseed. In addition, extraction-processing parameters were similar as indicated by similar extraction yields for Heartland and Specialty sources. Moreover, variety and growing conditions can have a significant effect on protein content (Cui and Mazza, 1996) and protein solubility (Mueller et al., 2010). For example, while brown and yellow flaxseeds have similar protein contents, the solubility of brown flaxseed protein is significantly more soluble than yellow flaxseed (Mueller et al., 2010).

Although it is possible that mucilage protein content is different due to processing parameters, it is more likely that differences in protein content are due to source. Cui and Mazza (1996) determined the protein content of four sources of flaxseed mucilage. It was reported that the mucilage protein content ranged from 8.6%-16.8% with three of the four sources ranging between 13.8-16.8% (Cui and Mazza, 1996). Therefore, differences in protein content of the two sources of flaxseed mucilage obtained are likely due the growing conditions, variety of flaxseed used and to a lesser extent extraction protocols.

6.1.2.2. Dietary fiber

Total Dietary fiber for Heartland and Specialty flaxseed fiber was 73.6 % and 70.1%, respectively. Singer et al. (2011) reported total dietary fiber of flaxseed mucilage to range from 20.8 - 86.1% depending on the extraction method. However, more recently using AOAC method number 985.29, Ibrügger et al. (2012) reported a dietary fiber of 70% in flaxseed fiber extracts. Therefore,

Heartland and Specialty flaxseed mucilage have similar dietary fiber as previously reported for other flaxseed sources.

Insoluble fiber of Heartland and Specialty flaxseed mucilage was 10.3% and 9.8% of the total fiber, respectively. In addition, soluble fiber for Heartland and Specialty flaxseed mucilage was 66.0% and 63.2% of the total fiber, respectively. No soluble and insoluble fiber values for flaxseed mucilage have been reported. However, Carter (2001) reported whole flaxseed had 10% soluble fiber and 30% insoluble fiber. Furthermore, in cereal grains, insoluble fiber ranges from 8.6% to 16.0% and soluble fiber ranges from 0.49% to 4.52% (Silva and Ciocca, 2005). High soluble fiber content in flaxseed mucilage was due to the aqueous method used to extract flaxseed mucilage. Insoluble fiber has limited water solubility due to the composition of the fiber being made up of lignin, cellulose and hemicellulose (Bauer, 2005). In contrast, soluble fiber is soluble in water, usually containing pectin's, gums and mucilage (Bauer, 2005). Therefore, due to the aqueous method of extraction flaxseed mucilage has a high amount of soluble fiber in comparison to the whole seed.

6.2. Objective Flaxseed Fiber Bread Quality

6.2.1. Loaf Volume

6.2.1.1. Treatment effect

In general, flaxseed fiber maintained the largest loaf volume percent retention in comparison to breads without flaxseed fiber at a specific frozen storage time (Figure 5). For example, after two weeks of frozen storage bread without flaxseed fiber loss 18% of it's original loaf volume; however, breads with flaxseed fiber lost less than 6% of their original loaf volume. With the exception of week 20, throughout the study bread without flaxseed fiber retained 5-12% more of its loaf volume than bread with out flaxseed fiber. These results coincide with preliminary studies completed at North Dakota State University (NDSU), where after 12 weeks of frozen storage breads containing flaxseed

hull had the largest loaf volumes and largest loaf volume percent retention (Hall et al., 2010). The retention of loaf volume with the addition of flaxseed fiber was possibly due to additional structural support provided to the gluten network. In fact, some hydrocolloids have been used in gluten free breads as gluten replacers to provide structure to the bread (Guarda et al., 2004).

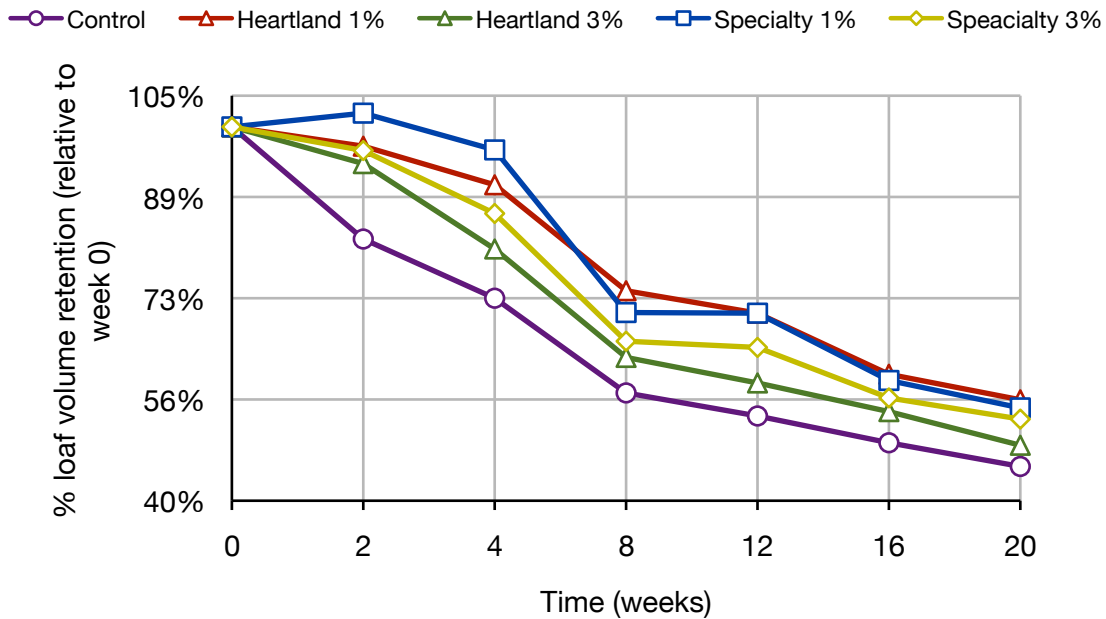


Figure 5. Percent loaf volume reduction of breads made from frozen dough with and without flaxseed fiber stored frozen over 20 weeks.

Scanning electron microscopy completed by Sharadanant and Khan (2006) indicates that hydrocolloids retain the continuous nature of the gluten network of dough in frozen storage; therefore, resulting in loaf volume retention. Although loaf volume was retained with flaxseed fiber after extended storage time, an increased amount of holes in the bread slice was observed in all treatments except the 1% flaxseed fiber samples (Figure 6). After 20 weeks of frozen storage control, S3% and H3% had a significantly larger amount of holes in the center slice of bread.

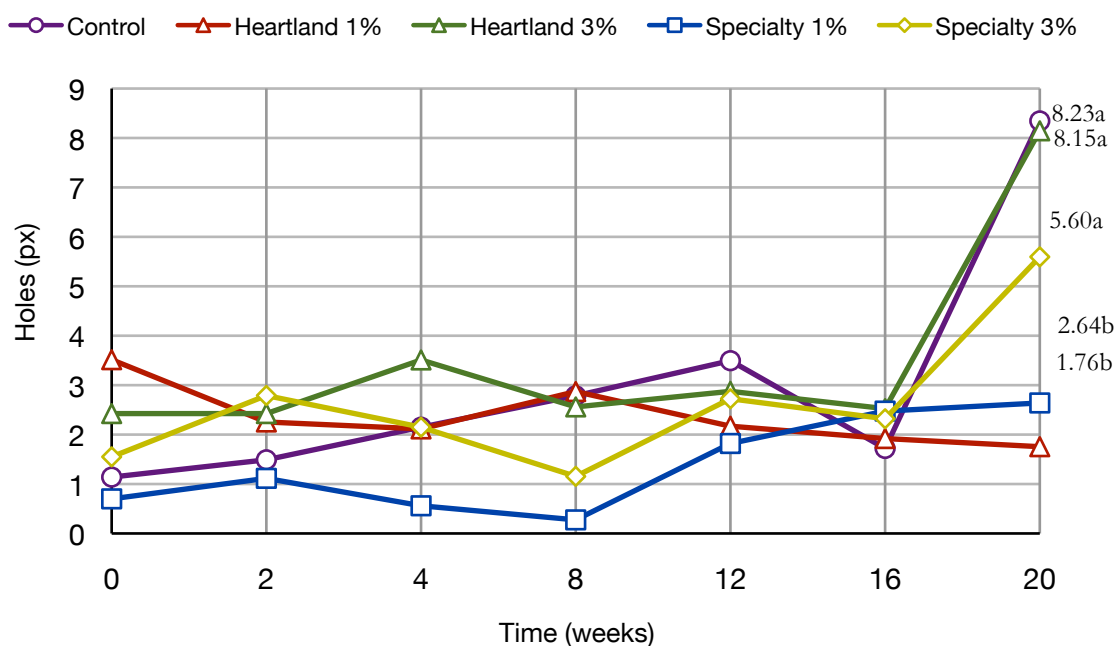


Figure 6. Number of holes (px) in breads made from frozen dough with and without flaxseed stored frozen over 20 weeks. Values indicate number of holes after 20 weeks of frozen storage, values with different letters indicate significant differences at ($p < 0.05$).

Holes in bread indicate coalescence of gas cells (Scanlon and Zghal, 2001) due to a loss of gluten properties (Liu and Scanon, 2003). Loss of gluten properties in the control bread was expected as the loss of gluten properties of dough that has been stored frozen for a period of time has been previously reported (Lorenz and Kulp, 1995). However, hydrocolloids are able to improve loaf volume by providing support to the gluten structure and gas cell interface (Mandala et al., 2007), it is possible that the increased amount of flaxseed fiber held large amounts of water close to the gluten network causing larger amounts of ice damage in comparison to other treatments. Sharadanant and Khan (2006) observed that different hydrocolloids had various amounts of dough protein depolymerization after frozen storage. It was hypothesized that each hydrocolloid caused different amounts and sizes of ice crystals to form (Sharadanant and Khan, 2006). Therefore, hydrocolloids that formed larger amounts of ice crystals that were large in size depolymerized the

gluten network. In addition, an increase in concentration of a hydrocolloid that forms large amounts of large ice crystals would also result in increased depolymerized network. The increase in amount of water held by the Heartland 3% and Specialty 3% flaxseed fiber could attract redistributing water and hold the water close to the gluten network causing large holes in bread baked, (Figure 6).

6.2.1.2. Time effect

Loaf volumes significantly decreased over time (Figure 7). The reduction in loaf volume with prolonged frozen storage in bread dough is common (Sharardanant, and Khan, 2003b). In this study it is likely that the reduction in loaf volume is caused by both the loss of yeast vitality and damage to the gluten network. The loss of yeast vitality cannot be confirmed; however, damage to the gluten network is confirmed through C-Cell imaging (Figure 8).

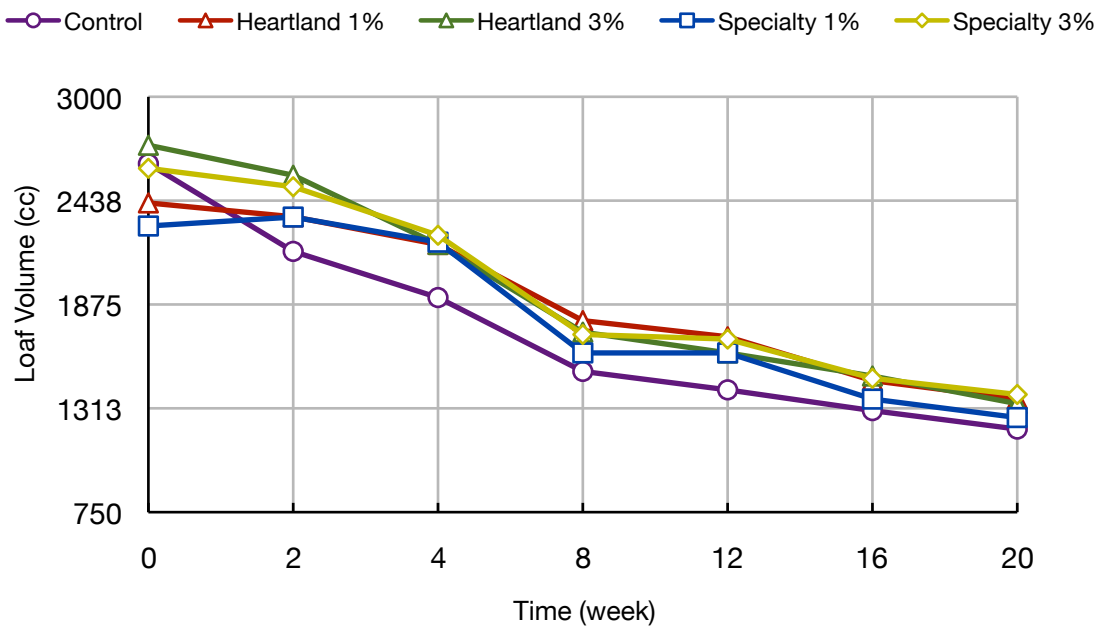


Figure 7. Loaf volumes of bread made with and without flaxseed fiber from frozen dough stored over 20 weeks.

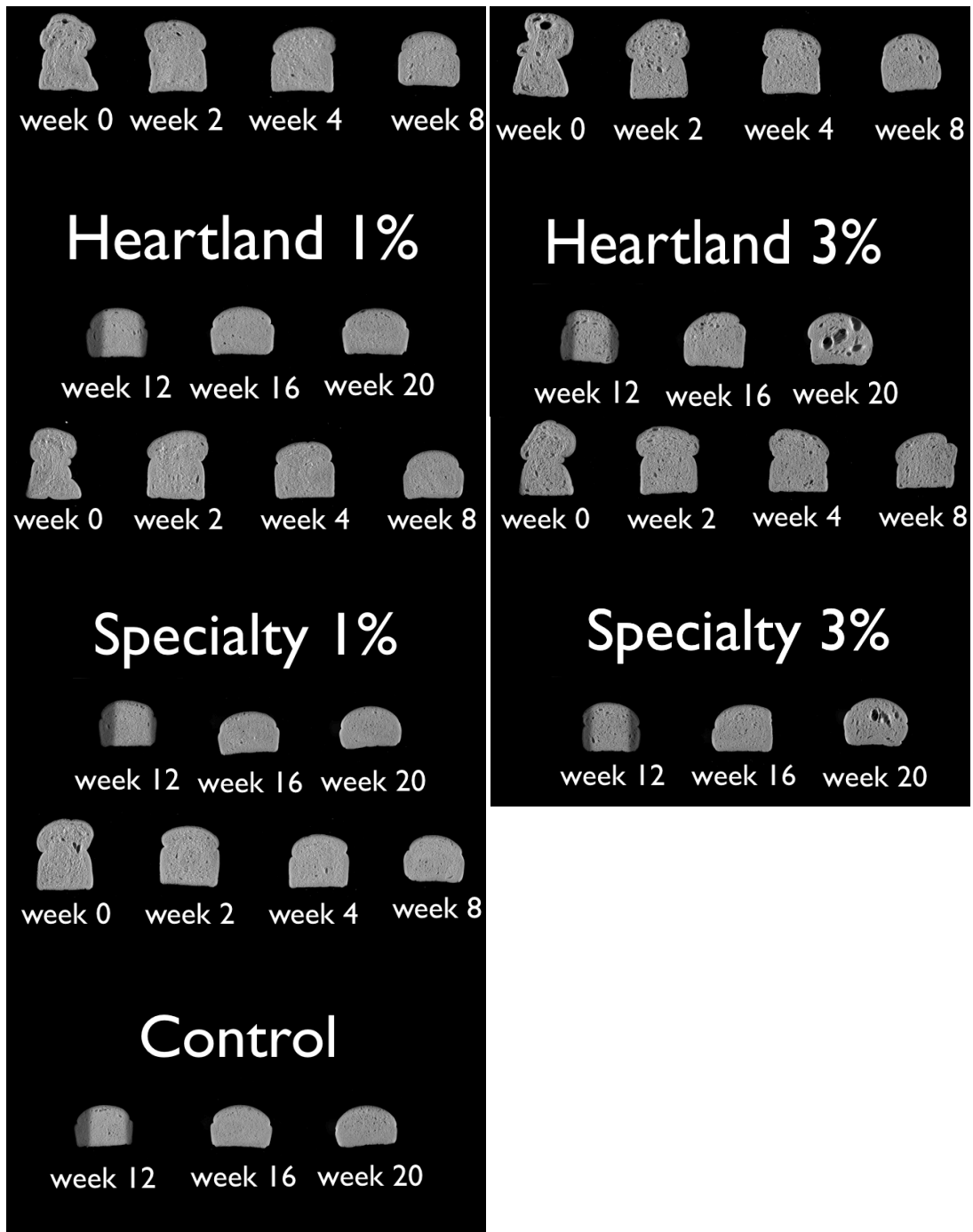


Figure 8. C-Cell images of breads with or without flaxseed fiber made from frozen dough stored over 20 weeks.

The amount of holes in the bread slice crumb increased with frozen storage time (Figure 6). Holes in bread indicate coalescence of gas cells (Scanlon and Zghal, 2001), due to loss of gluten properties (Liu and Scanon, 2003). Therefore, it is suggested that the loss of loaf volume retention is likely due to the break down of gluten network structure.

6.2.2. Firmness

6.2.2.1. Treatment effect

Use of other hydrocolloids to bread systems have had various influences on the firmness of bread (Sharardanant, and Khan, 2003b; Zhou et al., 2008). At week 0, breads containing flaxseed fiber were firmer than bread without flaxseed fiber. This trend was observed in preliminary studies at NDSU. In fresh dough the addition of flaxseed fiber increased the firmness of the bread crumb (Unpublished data, 2007). Furthermore, the addition of flaxseed hull to frozen dough increased the firmness of bread crumb at week 0. However, after week 2 breads containing flaxseed hull were less firm than breads containing oat, soy or no fiber at similar frozen storage times (Hall et al., 2010). Similar results were observed in this study. The addition of flaxseed fiber retained the lowest percent increase in firmness over time (Figure 9.) After 20 weeks of storage, the control had a firmness value of 192 g. This value is significantly higher than all other treatments containing flaxseed fiber which had firmness values ranging from 107 to 132 g after 20 weeks of frozen storage. Hall et al. (2010) reported bread containing flaxseed hull baked from frozen dough produced the softest slices of bread over 20 weeks in comparison to breads baked from frozen dough containing soy, oat or no fiber.

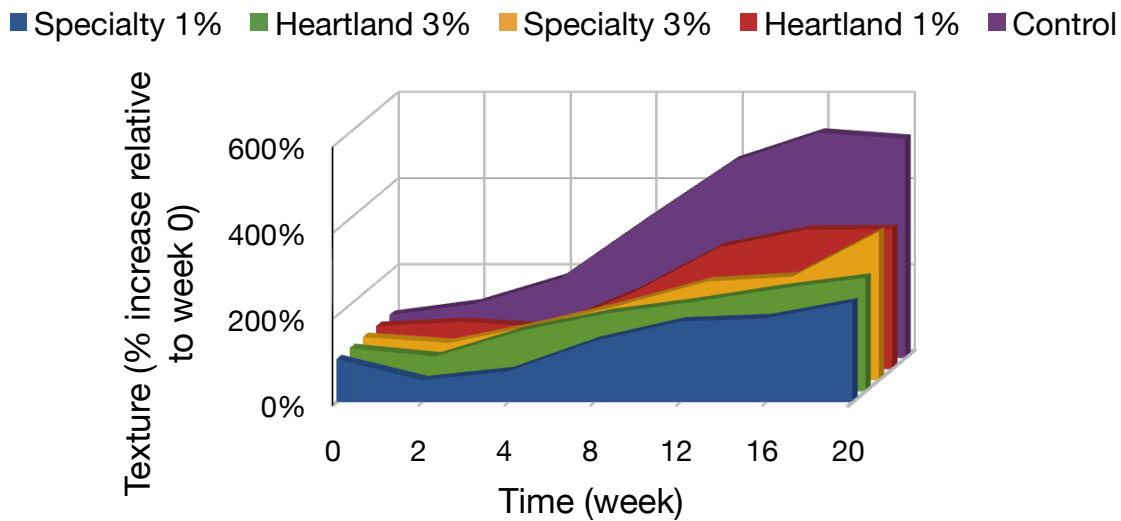


Figure 9. Percent increase in firmness (g) of breads made from frozen dough with and without flaxseed fiber stored frozen for various periods of time over 20 weeks.

Firmness of bread is often attributed to starch retrogradation characteristics (Hoseney, 1994). According to Mixolab results (Figure 10), flour mixtures with 3% Heartland or 3% Specialty flaxseed fiber had the highest retrogradation torque. This could be due to the cold set gel properties flaxseed mucilage exhibits (Chen et al., 2006). Increasing the concentration in flaxseed fiber increased the number of interactions between the mucilage molecules. Thus, a gel network was built and this increased the torque required by the mixolab to mix the retrograded sample.

A firmer crumb at week 0 in breads containing flaxseed fiber could be a result of flaxseeds gel properties. However, the retention of a soft crumb throughout frozen storage in breads containing flaxseed fiber may be due to effects of the freezing process. During the freezing process it is possible that release of compounds such as glutathione from yeast cells (Meziani et al., 2012) changed the pH resulting in gels that are weaker than in the mixolab analysis (Chen et al., 2006). It has been reported that hydrocolloids can influence amylose-lipid complexes (Rojas et al., 1999) and

interfere with gluten-starch interactions; thus reducing firmness of bread (Davidou et al., 1996). In addition, during bread making other ingredients such as sodium chloride, was added. Sodium chloride reduces the gel strength of flaxseed mucilage (Chen et al., 2006). In general, hydrocolloids intensified retrogradation, which is often associated with increased crumb firmness (Zhou et al., 2008), during mixolab analysis. However, breads containing hydrocolloids had the softest crumb, possibly due to gel-weakening ingredients and chemical changes during the freezing and baking processes.

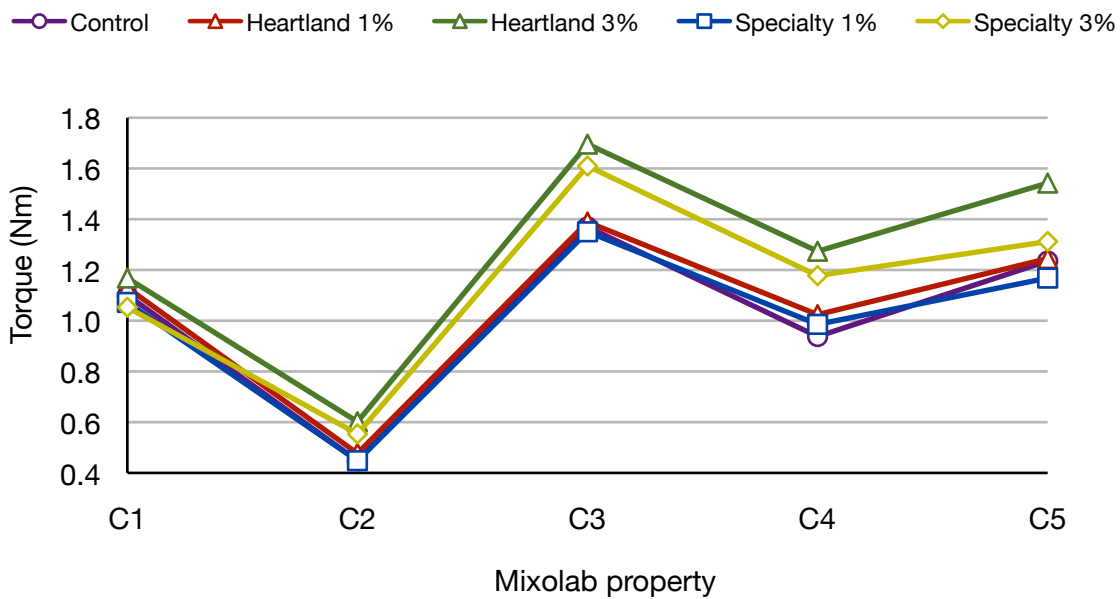


Figure 10. Mixolab torque (Nm) at points C1, C2, C3, C4 and C5 for flour with and without flaxseed fiber. See Figure 3, page 28 for description of data collection.

6.2.2.2. Time effect

As frozen storage time increased breads became more significantly firm (Figure 10).

Sharardanant and Khan (2003b) also reported an increase in crumb firmness with frozen storage

time. The increase in firmness with increased frozen storage time is likely due to changes in protein and starch properties (Sharardanant and Khan, 2006). Ribotta et al., (2003) reported an increase in frozen storage caused an increase in retrogradation of starch. The tendency for retrogradation during frozen storage of wheat starch was not affected by the addition of hydrocolloids (Zhou et al., 2008). However, the studies only analyzed wheat starch and not wheat flour (including gluten proteins). It has been reported that interactions between starch and protein have significant effects on the firming of bread (Davidou et al., 1996).

The mechanism for bread firming is highly dependent on starch retrogradation; however, it does not explain the complete firming mechanism (Goesaert et al., 2009). Moisture migration from gluten to starch also effects bread firming (Armero and Collar, 1998). As frozen storage time increases, the gluten network becomes more discontinuous (Sharardanant and Khan, 2006), resulting in increased freezable water (Lu and Grant, 1999; Bhattacharya et al., 2003). However, the addition of specific hydrocolloids can act as moisture migration buffers by aiding in the retention of the gluten network (Sharardanant and Khan, 2003b). Therefore, increasing the flaxseed fiber concentration resulted in retention of water near the gluten network and thus reduced crumb firmness.

6.2.3. Water activity

6.2.3.1. Treatment effect

No significant differences in water activity were detected among treatments. No detected differences in water activity are possibly due to the differences in water added to specific formulas and the water hold capacity of flaxseed fiber. The water absorptions obtained from Mixolab profiles were used as a reference; however, dough was mixed to optimal consistency with various amounts of water based on the feeling of the dough within the first two minutes of mixing. In general, more

water was added to breads containing more flaxseed mucilage (Table 5). This is due to increased water holding capacity of flaxseed mucilage, as a result, no extra free water was available to increase the water activity of the baked bread.

Table 5. Water amount added to of bread dough formulas made with and without flaxseed fiber.

	Control	Heartland 1%	Specialty 1%	Heartland 3%	Specialty 3%
Water (ml)	4160	4789	4641	5058	4855
Water (% Flour)	57%	66%	64%	70%	67%

6.2.3.2. Time effect

Limited differences in water activity among frozen storage time were observed (Figure 11). Moisture migration between constituents in frozen bread dough has been reported in previous studies (Lu and Grant, 1999; Bhattacharya et al., 2003). Moisture migration between constituents could have occurred but due to extended periods of time between baking limited changes were observed.

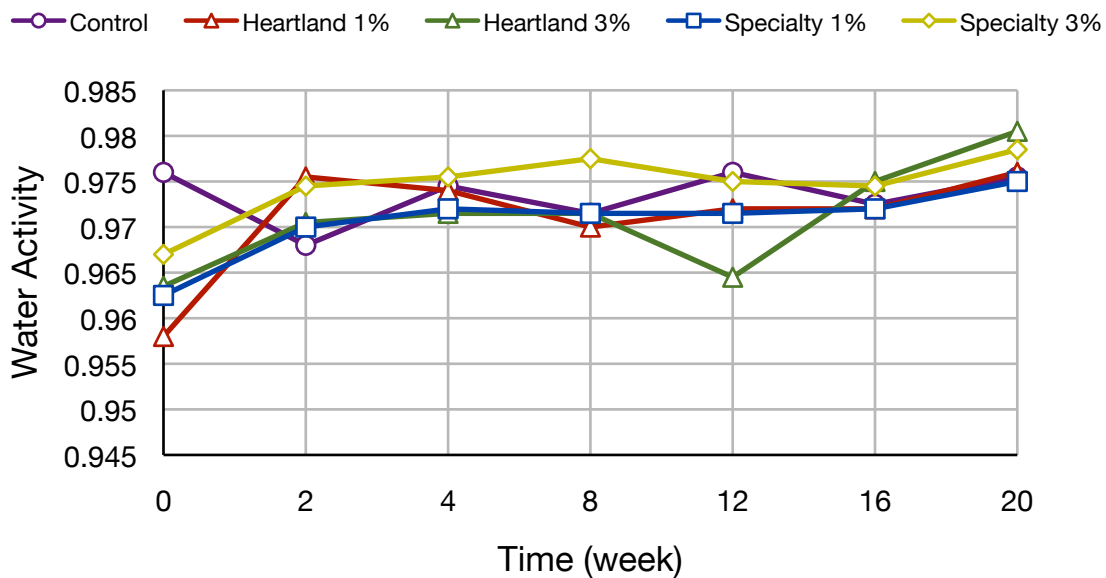


Figure 11. Water activity (A_w) of bread made from frozen dough with and without flaxseed fiber stored frozen over 20 weeks.

6.3. Subjective Flaxseed Fiber Bread Quality

6.3.1. Crumb Firmness

6.3.1.1. Treatment effect

According to a trained sensory panel, the addition of flaxseed fiber result in bread with crumb that was detected to be significantly softer than the crumb of bread made without flaxseed fiber (Table 6). However, specific trends among the flaxseed fiber treatments were not observed. It is likely that because a trained panel cannot detect the difference that consumers will also not likely be able to detect the difference among flaxseed fiber source and concentration (Meilgaard et al., 2007). Therefore, to reduce cost to bakers or users of frozen dough, 1% flaxseed fiber from Heartland or Specialty is sufficient to reduce the firmness of bread made from frozen bread dough. Table 6. Changes in firmness responses (cm) in baked bread from frozen dough with and without flaxseed fiber, as indicated by sensory panelists.

Time*	Treatment				
	0%	H1%	H3%	S1%	S3%
Week 2	4.6 ^a	2.3 ^{ab}	2.0 ^b	1.8 ^b	2.1 ^b
Week 4	4.5 ^a	1.1 ^b	2.5 ^{ab}	1.8 ^b	1.7 ^b
Week 8	6.7 ^a	1.5 ^b	3.0 ^b	2.7 ^b	2.4 ^b
Week 12	8.1 ^a	3.6 ^b	2.7 ^b	6.3 ^{ab}	4.0 ^b
Week 16	7.5 ^a	3.7 ^b	1.8 ^c	4.0 ^b	2.3 ^b
Week 20	6.1 ^a	3.3 ^b	3.5 ^{ab}	3.7 ^{ab}	3.0 ^b

*Values with the same letter within the same week (row) are not significantly ($p > 0.05$) different

6.3.1.2. Time effect

The addition of flaxseed fiber delayed the onset of the panelist's detection of bread firming in comparison to breads made without flaxseed fiber (Table 7). Bread without flaxseed fiber significantly increased the detected firmness after 12 weeks of frozen storage. However, breads with flaxseed fiber did not significantly increase the detected firmness until week 16 or 20.

Table 7. Changes in firmness responses (cm) over time for bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.

Treatment	Frozen Storage Time					
	week 2	week 4	week 8	week 12	week 16	week 20
Control	4.6 ^a	4.5 ^a	6.7 ^a	8.1 ^b	7.5 ^b	5.2 ^a
H1%	2.3 ^{ab}	1.1 ^b	1.5 ^b	3.6 ^a	3.7 ^a	3.3 ^a
H3%	2.0 ^a	2.5 ^a	3.0 ^a	2.7 ^a	1.8 ^a	3.5 ^a
S1%	1.8 ^c	1.8 ^c	2.7 ^c	6.3 ^a	4.0 ^{ab}	3.7 ^b
S3%	2.1 ^a	1.8 ^a	2.4 ^a	4.0 ^a	2.3 ^a	3.0 ^a

*Values with the same letter within the same treatment (row) are not significantly ($p > 0.05$) different

6.3.2. Stale Flavor

6.3.2.1. Treatment effect

The use of flaxseed fiber in frozen dough caused significantly less stale flavors in bread to be detected after week 4 for most treatments (Table 8). However, the panel did not detect a significant difference among breads with flaxseed fiber. Similar to crumb firmness, because a trained panel could not detect differences it is likely that the consumer will not be able to detect stale flavor differences. Therefore, to reduce cost it is suggest that Heartland or Specialty at 1% flaxseed fiber should be used.

Table 8. Changes in stale flavor responses (cm) in bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.

Time*	Treatment				
	0%	H1%	H3%	S1%	S3%
Week 2	3.5 ^a	1.4 ^a	1.6 ^a	1.7 ^a	2.3 ^a
Week 4	2.7 ^a	1.1 ^b	1.2 ^b	1.9 ^a	1.2 ^b
Week 8	5.0 ^a	1.7 ^b	1.5 ^b	1.9 ^b	2.1 ^b
Week 12	3.9 ^a	3.2 ^b	1.7 ^b	5.4 ^a	2.6 ^b
Week 16	4.3 ^a	1.9 ^b	1.6 ^b	1.9 ^b	1.7 ^b
Week 20	4.0 ^a	1.9 ^b	2.6 ^b	2.0 ^b	2.2 ^b

*Values with the same letter within the same week (row) are not significantly ($p > 0.05$) different

6.3.2.2. Time effect

Limited differences in stale flavor over frozen storage time were observed (Table 9). It is likely that because the sensory panel tasted the bread 1 day after being baked that limited stale flavors were able to develop. Limited information on the sensory quality of bread made from frozen dough is currently available. Therefore, it is difficult to determine why limited differences in stale over time were not detected.

Table 9. Changes in stale flavor responses (cm) over time for bread made from frozen bread dough with or without flaxseed fiber as indicated by sensory panelists.

	Frozen Storage Time					
	Treatment					
	Week 2	week 4	week 8	week 12	week 16	week 20
Control	3.5 ^a	3.0 ^a	5.0 ^a	3.9 ^a	4.3 ^a	4.0 ^a
H1%	1.4 ^a	1.1 ^b	1.5 ^a	3.2 ^a	1.9 ^a	1.9 ^a
H3%	1.6 ^a	1.2 ^b	1.5 ^a	1.7 ^a	1.6 ^a	2.6 ^a
S1%	1.7 ^b	1.9 ^b	1.9 ^b	5.4 ^a	1.9 ^b	2.0 ^b
S3%	2.3 ^a	1.2 ^a	2.1 ^a	2.6 ^a	1.7 ^a	2.2 ^a

*Values with the same letter within the same flaxseed fiber addition (row) are not significantly ($p >$

0.05) different

6.3.3. Overall acceptability

To determine the acceptability of a product a consumer panel should be used instead of a trained sensory panel. In this study a trained sensory panel was as an estimate to determine shelf life.. Throughout the 20 week study breads baked from frozen dough containing Heartland 1% had the highest overall acceptability, while the control had the lowest overall acceptability throughout the study. However, after 8 weeks all breads baked from frozen dough dropped below acceptable and was no longer perceived acceptable, as determined by a trained sensory panel.

7.0. CONCLUSION

As hypothesized, the addition of flaxseed fiber significantly increased the quality of bread made from frozen dough in comparison to bread baked from frozen dough without flaxseed fiber. In addition, the incorporation of flaxseed fiber retained the overall quality (i.e. loaf volume crumb structure and texture) as frozen storage time increases in comparison to bread baked from frozen dough without flaxseed fiber. However, flaxseed fiber did not completely counteract the detrimental effects of the freezing process has on frozen bread dough stored over time.

For all treatments, loaf volume decreased and the firmness increased with time. However, the addition of flaxseed fiber significantly reduced the percent increase in firmness and reduced the percent decrease loaf volume of breads baked throughout the 20-week study. Although objective testing suggests differences among the flaxseed fiber treatments, a trained sensory panel could not detect the difference. Therefore, it is suggest that Heartland or Specialty 1% flaxseed fiber be used for further formula optimization due to insignificant differences among treatments. Moreover, the use of 1% flaxseed fiber would reduce the cost of ingredients for manufacturers. In addition, a maximum shelf life of 8 weeks is suggested. Although flaxseed fiber retained the quality of bread, after 8 weeks all breads dropped below acceptable quality according to trained panelists.

Through advances in technology, the use of flaxseed and its fractions in food systems has become more practical on an industrial scale. In this study, flaxseed fiber was added to frozen bread dough systems through pilot scale processing equipment. In general, flaxseed fiber retained the overall quality of bread baked from frozen bread dough.

8.0 FUTURE WORK

In further studies an alternative experimental design should be chosen. An experimental design with additional replicates will give more power to statistical analysis. Therefore, aiding in drawing more accurate conclusions. In addition to an alternative experimental design, to understand how flaxseed fiber affects the quality of bread baked from frozen dough further studies should include tests that analyze both the dough and the final product. Dough testing should include rheological tests (i.e. alveograph and/or extensigraph) and scanning electron microscopy to observe gluten-hydrocolloid and starch-hydrocolloid interactions through out frozen storage.

Final bread quality testing should be more frequent to better determine a shelf life. In a further study, final bread quality testing should include gas-chromotography headspace analysis to determine volatile compounds. Minimal research has been completed to correlate sensory analysis of bread and volatile compounds. In addition, weight of loaves should be recorded for specific volume calculation. Specific volume will allow for better comparisons between studies.

9.0. LITERATURE CITED

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