# Addition of Soybean Lipoxygenase to All-Purpose Flour and its Effects on Dough Gluten Strength and Bread Quality

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# The Effects of Added Soy Lipoxygenase to Wheat Flour on Dough Gluten Strength and Bread Volume

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## ABSTRACT

The goal of this research is to determine the effects of added soybean lipoxygenase (LOX) on bread dough rheological properties and physical properties of bread loaves compared to controls, and to determine sensory attributes of bread loaves using quantitative descriptive analysis (QDA). Protein fractions were obtained through the use of isoelectric precipitation. The pH 4.8 precipitate was found to yield the greatest LOX activity when compared with other fractions ($p<0.05$). The addition of pH 4.8 precipitate improved rheological properties of bread dough, examined in a farinograph, when compared to the all-purpose control ($p<0.05$). Addition of soy flour also increased the gluten strength of all-purpose flour ($p<0.05$). The addition of pH 4.8 precipitate to all-purpose flour did not improve bread loaf volume or texture. Sensory panelists described pH 4.8 supplemented bread as having firmer crumb when compared with controls ($p<0.05$). There were slight color differences among the loaves. The crust and crumb of bread flour loaves was lighter in color than any other sample. It was concluded that the addition of pH 4.8 precipitate to all-purpose flour greatly improved the rheological properties when compared with all-purpose flour alone.

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# Chapter 1: Introduction

Wheat flour is the ingredient added in the largest proportion to dough and bread formulations (Cauvain, 2003b). In particular, wheat flour proteins have special properties to allow formation of gluten after hydration and during mixing. The mixing process allows dough aeration and gluten formation, which forms a network to trap air bubbles for inflation by carbon dioxide gas from yeast fermentation (Cauvain, 2003b). This helps form the bread structure upon baking. Due to the special properties of wheat proteins, much research has been dedicated to them (Cauvain, 2003b).

There are three major types of wheat grown in North America, which are divided into classes of wheat that refer to the season during which the crop is grown (Curtis, 2002). This classification is usually either spring or winter wheat. For winter wheat, the plant experiences a period of cold winter temperatures (0°- 5°C) (Curtis, 2002). It is typically planted in the fall season to germinate and develop into young plants that remain in the vegetative phase during winter and resume growth in early spring (Curtis, 2002). Spring wheat is planted in the spring so that it matures by late summer (Curtis, 2002). There are large differences in grain composition and processing quality among wheat cultivars within a particular species (Pena, 2002). Almost 95 percent of all the wheat produced belongs to the *Triticum aestivum* L species and is better known as hard or soft wheat, depending on grain hardness (Gill and Friebe, 2002; Pena, 2003). Grain hardness refers to the resistance the grain opposes to being fractured and to being reduced to fine whole meal flour or to fine endosperm particles (semolina or refined flour) and is determined by the way different components are packed in the endosperm cells (Pena,

2003). Hard red winter and spring wheat of the *Triticum aestivum* L species is most often used in baking bread due to the production of high gluten dough (Pena, 2003).

# Chapter 2: Review of Literature

## 2.1 Lipoxygenase

Lipoxygenase (LOX) is an enzyme that catalyzes the oxidation of specific polyunsaturated fatty acids, which results in the production of conjugated unsaturated fatty acid hydroperoxides (Liu, 1997; Faubion and Hoseney, 1981; Nicolas et al., 1982; Wolf and Cowan, 1975). The enzyme is widely distributed in plant tissues, being particularly abundant in legume seeds (Hildebrand et al., 1991). LOX is a naturally occurring enzyme in wheat flour (Rakotozafy et al., 1999). This enzyme has been found to have many functions in bread making. It has been shown that during mixing, LOX promotes the destruction of free and esterified linoleic and linolenic acids, as well as the bleaching of carotenoid pigments to give bread a whiter crumb (Nicolas et al., 1982; Gelinas et al., 1998). In addition, LOX is credited with increasing mixing tolerance and relaxation times, which results in enhanced loaf volume (Nicolas et al., 1982). However, according to Rakotozafy et al. (1999), LOX is found to have a loss in activity as mixing continues. Thus, to overcome this inhibition, lipoxygenase from soybean flour (SLOX) has been used increasingly over the decades as a dough improver (Rakotozafy et al., 1999).

### 2.1.1 Soybean Lipoxygenase

Lipoxygenase is found in several sources including corn, alfalfa, peas, and wheat (Faubion and Hoseney, 1981). However, soybean seeds were found to be the richest known source of lipoxygenases (Liu, 1997; Faubion and Hoseney, 1981; Gelinas et al., 1998). Despite the abundance of LOX enzymes, there are indications that they contribute

to the primary cause of the undesirable flavors in soybean products. These flavors are commonly known as "greeny" or "beany" flavors (Liu, 1997; Gelinas et al., 1998). Due to the adverse effects (i.e. off-flavors), enzyme active soy flour is only used commercially up to 0.5% in wheat-based breads (Cumbee et al., 1997; Gelinas et al., 1998).

### 2.1.2 Soybean Lipoxygenase Isozymes

Four different soybean SLOX isozymes have been isolated and are known as L-1, L-2, L-3a, and L-3b (Liu 1997; Cumbee et al., 1997; Faubion and Hoseney, 1981). Often the last two isozymes are so similar in composition and behavior they are considered a single type, L-3. All of the SLOX isozymes are monomeric proteins with molecular weights ranging between 94 and 97 kDa and containing one atom of tightly bound nonheme iron per molecule (Liu, 1997; Hildebrand et al., 1991). L-1 is unique from the other isozymes in that it is heat stable. Unlike L-2 and L-3, which are not as heat stable, L-1 has an optimum pH of 9 and prefers anionic substrates, such as linoleic and linolenic acids, while L-2 and L-3 activity are optimal at neutral pH and these isoenzymes prefer esterified substrates (Liu, 1997). Research suggests that L-3 is the most abundant isozyme in mature soybean seeds, followed by L-1 and L-2 (Hildebrand et al., 1991; Liu, 1997).

These isozymes have been isolated and characterized in seeds of commercial soybean cultivars; however, only a few attempts have been made to isolate the individual isozymes and determine rheological and baking properties of wheat flours fortified with them (Cumbee et al., 1997).

## 2.2 Characteristics of Lipoxygenase

As mentioned previously, the lipoxygenase enzyme exists in multiple forms known as isoenzymes: four in soy, wheat and peas and two in corn (Faubion and Hoseney, 1981). These different isoenzymes are found to differ in properties such as optimum pH, bleaching capacity, substrate specificity and products produced. The enzyme was originally thought to be unique among the oxygenases in that it lacked prosthetic groups; however, it has since been reported that soybean lipoxygenases contain one mole of iron per mole of enzyme (Faubion and Hoseney, 1981).

All lipoxygenases have one common feature. They catalyze the oxidation of fatty acids possessing *cis, cis*-1-4-pentadiene unsaturated systems (Faubion and Hoseney, 1981; Nicolas et al., 1982). Beyond this fact, there are large differences in enzyme and substrate solubility. This makes the actual amount of substrate available to the enzyme uncertain.

One of the oldest known characteristics of the enzyme is the ability to bleach or decolorize several pigments (Faubion and Hoseney, 1981; Gelinas et al., 1998). However, this is the least understood characteristic of LOX. It has been found that the substrates for bleaching vary from carotene, xanthophylls, bixin, chlorophyll, cholesterol, crocin, lutein and various dyes (Faubion and Hoseney 1981). Despite these known substrates, there is little information known about the actual mechanism to explain the bleaching ability of LOX.

### 2.2.1 Thermal Stability of Lipoxygenase

Thermal stability of the different isoenzymes was measured in a study performed by Nicolas et al (1982). Isozyme stabilities were measured at both ambient temperature and at 4°C in a phosphate buffer at pH 7 (Nicolas et al., 1982). It was found that active fractions were very stable at 4°C, but lost half of their activity after 10 days of storage at laboratory temperature. According to Shiiba et al. (1991), the three major LOX isoenzymes (L-1, L-2 and L-3) had similar thermal sensitivities, with optimum activity at approximately 45°C and traces of activity at 65°C.

## 2.3 Wheat

Wheat is the most widely grown crop in the world (Stolh, 2002). It is grown from temperate, irrigated to dry and high-rain-fall areas and from warm, humid to dry, cold environments (Acevedo et al., 2002). This adaptation is attributed to the complex nature of the plant's genome. Wheat is divided into different types, including *Triticum aestivum* L, which accounts for approximately 90-95 percent of wheat produced in the world (Pena, 2002). Wheat is utilized mainly as flour, either whole grain or refined (Pena, 2002). It is used for the production of a variety of leavened and flat breads, as well as other baked products. Another type of wheat, *Triticum durum*, produces semolina (coarse flour), which is the main ingredient in pasta making.

The protein content of wheat grain varies between 8 and 17 percent (Pena, 2002). These differences depend on genetic make-up and external factors associated with the crop. A unique property of wheat is that it contains insoluble proteins, which react with water to form a viscoelastic protein mass known as gluten (Pena, 2002). Gluten is a very

large complex composed of polymeric and monomeric proteins, known as glutenins and gliadins. Furthermore, gluten viscoelasticity accounts for a large part of a flour's dough strength. Different variations in grain protein content can significantly influence the gluten strength of wheat varieties (Pena, 2002). Wheat flour contains about the same amounts of glutenins and gliadins, and an imbalance of the ratio of these may alter its viscoelastic properties (Pena, 2002).

### 2.3.1 Wheat Proteins

Wheat contains hundreds of proteins, which contain sequences of amino acids in specific arrangements to help form their three dimensional structures (Cornell, 2003). Glutenins and gliadins form the wheat gluten complex, which is about 80 percent protein, 10 percent starch and 5 percent lipids plus other minerals and impurities (Cornell, 2003). Wheat proteins contribute functional properties in bread.

Glutenins form an extensive three-dimensional network of molecules through disulfide bonding, hydrogen bonding and hydrophobic interactions (Cornell, 2003). These properties contribute to the cohesive elasticity of the dough. Gliadin is also an important component to this network. Gliadins contribute to the viscous nature, or extensibility, of the dough, while glutenins contribute to the elastic nature of the dough (Belton, 2003). The proteins in the gluten are denatured (conformational changes) through heat and changes in pH. One reaction, the Maillard reaction, is typical of the properties of proteins. This reaction occurs during the baking of bread through the interaction of flour proteins and sugar, which results in the browning of the crust (Cornell, 2003).

Glutenins are responsible for the elasticity of the gluten complex. The glutenins have both high molecular weight (HMW) proteins, ranging between 80 and 100 kDa, and low molecular weight (LMW) proteins ranging between 30 and 40 kDa (Shimoni and others, 1997). The HMW glutenins are most important, even though they only constitute 12% of the total flour proteins (Belton, 2003). The degree of cross-linking by disulfide bonds varies in the glutenins, giving these proteins a range of molecular weight. The glutenins make up about 30-40 percent of the total protein of flour.

The characteristics of the glutenins are summarized in Table 1 (Cornell, 2003). Differences have been found in the amino acid composition of LMW and HMW proteins or subunits. LMW subunits are lower in glycine, but higher in valine, isoleucine, leucine and phenylalanine compared to subunits with HMW (Cornell, 2003). Furthermore, the intermolecular disulfide bonds involve mostly glutenins and contribute significantly to the viscoelastic properties of wheat gluten. This involves the oxidation of sulfhydryl groups to disulfide groups, which is an important reaction during dough formation and baking (Cornell, 2003). Also, intramolecular disulfide bonds should be noted as important bonds, which are formed between cysteine side chains in the same protein molecule.

Gliadins account for about 40-50 percent of the total protein content of wheat and are termed a-, b-, or w-gliadins in order of their electrophoretic mobility and are all monomeric (Cornell, 2003). Characteristics of gliadins are outlined in Table 2. It is found that gliadins have a higher content of proline, which is believed to be responsible for the b-turns, but yet do not display strong viscoelastic behavior. This indicates that

disulfide bonds contribute more significantly than the b-turns to viscoelasticity (Cornell, 2003).

**Table 1: Properties of the Glutenins**

| *Property* | *Behavior/Characteristics* |
|---|---|
| Solubility | Low solubility in water and neutral buffers. Products are viscoelastic materials. Small amount of material soluble in 70% (v/v) ethanol. |
| Molecular Structure | Protein chains cross-linked by intermolecular disulfide bonds, and insoluble in 70% (v/v) ethanol. High molecular weight and low molecular weight subunits present. Regions of α-helix, β-structure and random coil structure. |
| Amount in wheat | 30-45% of total protein. |
| Amino acid content | High in glutamine (about 30%) and proline (about 13%) Lower amounts of all the other amino acids. (Cysteine content about 2.4%) |

*Cornell (2003)

**Table 2: Properties of the Gliadins**

| *Property* | *Behavior/Characteristics* |
|---|---|
| Solubility | Extremely low solubility in water and neutral buffers. Products are of sticky texture. Dry products are mostly soluble in 70% (v/v) ethanol. |
| Molecular Structure | Single polypeptide chains capable of some intramolecular disulfide bonding. Considerable amount of α-helical and random coil structure and high incidence of β-turns. |
| Molecular weight | 30,000-50,000 |
| Amount in wheat | 40-50% of total protein |
| Amino acid content | Very high in glutamine (about 35%)<br>Proline high (about 20%)<br>Low levels of arginine, lysine and histidine, as well as aspartic acid and glutamic acid<br>Cysteine content 3% |

*Cornell (2003)

## 2.4 Bread Making, an Overview:

There are a wide range of products with an assortment of shapes, sizes, textures, colors, softness and flavors that can be described by the term "bread" (Cauvain, 2003a). The character of bread depends on the formation of the gluten network, which traps gas from yeast fermentation and contributes to the cellular crumb structure. There are many different bread making processes, but the aim is to convert wheat flour and other ingredients into a light, aerated and palatable food (Cauvain, 2003a).

The basis of bread making involves the mixing of wheat flour and water, as well as yeast and salt and other specified ingredients in the appropriate ratios (Cauvain, 2003a). The formation of gluten must also be achieved through the application of energy during mixing. In addition, there must be an incorporation of air bubbles during mixing. The dough development is associated with the formation of the gluten, which requires the hydration of the proteins in the flour, as well as energy input in the form of kneading. The energy requirement is a significant contributor to the bread making process (Cauvain, 2003a). However, there is more to dough development than the kneading process. One of the most important aspects of bread making includes physical changes, in particular the improvement in the ability to retain carbon dioxide gas. Gas retention contributes to the loaf volume and crumb structure.

## 2.5 Functional Ingredients in Bread Making:

Bread quality is determined by the complex interactions of the ingredients used, as well as their qualities and quantities used in the dough processing method (Cauvain, 2003a).

### 2.5.1 Flour

The formation of gluten is a critical component of bread formation and wheat is the contributor of the necessary proteins needed for this formation. Thus, a significant factor that determines final bread quality comes from the wheat and the flour from the mill (Cauvain, 2003a). The wheat variety, agricultural practices and environmental contributors determine both the level and quality of the gluten forming proteins. The protein content of the flour varies, but in general, the higher the protein content in the wheat, the higher the protein content in the flour produced from it. Usually, the higher the protein content of a flour, the better its ability to trap and retain carbon dioxide gas, which allows for a larger and more desirable bread volume (Cauvain, 2003a). In addition, the protein quality influences the final product quality, and it is often tested by measuring the rheological properties of the dough.

### 2.5.2 Yeast

*Saccharomyces cerevisiae* is baker's yeast and comes in different forms. The yeast produces carbon dioxide gas to expand the dough at its different processing stages, especially during proofing and the early stages of the baking process (Cauvain, 2003a).

### 2.5.3 Sugar

Since high levels of sugar inhibit yeast activity, even though it is fermentable, products may have up to 15 percent sugar (Cauvain, 2003a). Sugars provide fermentable substrate, glucose, for yeast and usually contribute to product sweetness and crust color.

### 2.5.4 Fat

Fat is incorporated to improve the gas retention of dough and subsequently increase loaf volume and tenderness. The amount of fat used varies according to the type of flour. Whole meal flours require more fat than white flour, often two or three times more (Cauvain, 2003a). The typical amount of fat used in bread making ranges from about 2-6% (McWilliams, 2001). A proportion of fat should remain solid in the dough at the end of the final proof (Cauvain, 2003a).

### 2.5.5 Water

The amount of water added affects the properties of dough. If only a small amount of water is added, then the dough will be firm and produce poor volume and appearance, which is undesirable. If too much water is added, the dough will be soft and be difficult to mould and result in poor quality bread. The optimum amount of water is the maximum quantity that can be incorporated into the dough and still allow it to be molded to give the bread acceptable quality (Cauvain, 2003a). The amount of water depends on the flour qualities.

### 2.5.6 Improvers

A bread improver is an ingredient added to improve the bread making potential of a flour. Oxidizing agents, such as potassium bromate, ascorbic acid and potassium iodate, are added to improve gas retention properties of the dough, and their function is related to cross-linking of proteins (Cauvain, 2003a; Indrani and Rao, 2006). Reducing

agents can also be added, such as L-cysteine and potassium metabisulfite, at low levels to help in molding and shape forming (Indrani and Rao, 2006). Emulsifiers, such as lecithins, are often added to improve bread quality as well. In addition, full-fat, enzyme-active soy flour is sometimes used as a functional dough ingredient (Cauvain, 2003a). This addition has two functions arising from its lipoxygenase enzyme system, which helps bleach the flour and assists in dough oxidation (Cauvain, 2003a).

### 2.6 Bonds

During the bread making process, different types of bonds are formed to allow for protein structure development. These bonds include: hydrogen, hydrophobic, ionic, disulfide and possibly dityrosine cross-links. The bonds formed in the proteins have a direct affect on dough formation and bread making quality (Tilley et al., 2001). Upon the addition of water, dough formation and manipulation, the glutenin will form cross-links around gliadin to form the gluten complex (McWilliams, 2001). The mixing of the hydrated protein causes the disruption and breakage of intermolecular secondary protein bonds and forms new bonds, which results in the development of gluten (McWilliams, 2001). Gluten is an important part of dough formation for bread making.

Both intramolecular and intermolecular disulfide bonds are of particular interest and importance in bread making. The glutenin cross-links formed during gluten formation are thought to occur through disulfide interactions. Sulfhydryl-disulfide interchange reactions involve the breaking and reforming of new disulfide bonds during dough manipulation. This particular mechanism describes the changes in intermolecular or intramolecular disulfide bonds. New research shows that there are also dityrosine and

isodityrosine cross links involved in gluten formation (Tilley et al., 2001). These cross links are thought to form between the central domain of glutenin and other glutenin molecules, while intermolecular disulfide bonds form at the C and N-terminus of glutenin molecules (Tilley et al., 2001). It is thought that peroxidase contributes to the formation of dityrosine cross-links and perhaps lipoxygenase plays a role as well. These cross-links are important for gluten formation because they help form a network around gliadin proteins.

### 2.7 Farinograph Interpretation

Farinograph studies are used to measure functional properties of flour, such as water absorption and to differentiate wheat flours of good and poor baking quality (Ram et al., 2005). The dough is developed by a pack-squeeze type of gentle kneading and shearing action in the Farinograph (Ram et al., 2005). Parameters measured include: arrival time, dough development time, stability, departure time (DEP), twenty-minute drop (TMD) and mixing tolerance index (MTI).

Arrival time is the time required for the top of the curve to reach the 500 line after the mixer has been started and is a measurement of the rate at which the water is taken up by the flour (Shuey et al., 1972). The dough development time is the time to the nearest half-minute from the first addition of water to the development of the dough's maximum consistency before the first indication of weakening (Shuey et al., 1972). This time is also referred to as peak time. The top of the curve on the graph is nearly flat for several minutes such that the peak time is determined by taking the mean between the mid-point

of the flat portion of the top of the curve and the top of the arch at the bottom of the curve (Shuey et al., 1972).

Stability is the difference in time between the point where the top of the curve first intercepts the 500 BU line (arrival time) and the point where the top of the curve leaves the 500 BU line (departure time). This value gives some indication of the tolerance to mixing a flour will have (Shuey et al., 1972). Departure time is the time from the first addition of water until the top of the curve leaves the 500 BU line, therefore, the longer the departure time, the stronger the gluten in the flour.

The twenty minute drop is the change in the height of the center of the curve at the peak and the center of the curve 20 minutes after the first addition of water, expressed to the nearest 5 BU units (Shuey et al., 1972). It gives the rate of breakdown and strength of a flour such that the higher the value, the weaker the gluten strength of the dough (Shuey et al., 1972). The tolerance index is the difference in Brabender units from the top of the curve at the peak to the top of the curve measured 5 minutes after the peak is reached (Shuey et al., 1972). Flours with good tolerance to mixing have low MTI's, such that the higher the MTI value, the weaker the gluten in the flour (Shuey et al., 1972).

### 2.8 Isoelectric Precipitation of Soy Flour

Isoelectric precipitation is a method used to separate different protein fractions by adjusting the pH of a dilute buffer containing the sample. It is a way to separate the two major soybean proteins, the 7S and 11S (Thanh and Shibasaki, 1976). At different pH's, certain protein fractions will precipitate out of solution, depending on the isoelectric point of the particular protein. According to Thanh and Shibasaki (1976), adjusting the pH to

4.8 of a dilute tris(hydroxymethyl)aminomethane buffer extract of soybean flour causes precipitation of the 7S globulin. The pH 4.8 precipitate of soy flour is thought to also contain the enzyme of interest, lipoxygenase. The 11S globulin is found to precipitate out of solution at a pH of 6.4. Determination of lipoxygenase activity in the different protein fractions is done by a spectrophotometric method, which measures the increase in absorbance at 234 nm (Wu et al., 1997).

### 2.9 Sensory Evaluation: Quantitative Descriptive Analysis (QDA)

Sensory evaluation is an important aspect of the food industry and food product development. It comprises a set of techniques for measurement of human response to foods (Lawless and Heymann, 1998). Sensory evaluation also helps to minimize the biasing effects of brand identity and consumer perception. Essentially, it attempts to determine isolate sensory properties of foods and provides important and useful information to product developers, food scientists and managers about the sensory characteristics of their products (Lawless and Heymann, 1998).

Quantitative descriptive analysis, QDA, is a sensory method that was developed by the Tragon Corporation in the mid-1970s to address the problem of quantifying sensory descriptive data (Gacula, 1997). Trained panelists are a key aspect of the characterization of the perceived flavor of a food since it is a very difficult task. The technique involves the training of individuals to identify and quantify the sensory properties of a food product or ingredient (Stone et al., 1974). The basics of the method involve the screening of prospective judges, selection of the most discriminating judges and training (10-20 hrs) of the selected judges (Zook and Wessman, 1977). In addition,

the training period involves the development of terminology to describe the appearance, flavor, and texture of food products.

A QDA graph is constructed to describe the products being tested. A typical QDA graph consists of lines radiating outward from a central point (Zook and Wessman, 1977). The lines each represent a descriptive term, and the average intensity for that term is plotted on the line (Zook and Wessman, 1977). Upon analysis, sensory characteristics of standard products and experimental products are compared to help aid in the development of a new food product. QDA has been an important tool of sensory evaluation over the years, especially as a benefit to research and development (Stone et al., 1980).

# Chapter 3: Justification of Research and Objectives

Improving the quality of low protein wheat flour is a goal in the baking industry. Research on dough strengtheners and the advantages of both natural and chemical improvers must be thoroughly examined. Due to reported potential hazards of chemical dough conditioners, such as potassium bromate, the baking industry is involved in determining alternate conditioners (Rakotozafy et al., 1999). Thus, there is a need to examine the potential benefits of maximum utilization of naturally occurring strengtheners in wheat, such as oxidoreductases. Oxidoreductases are enzymes that include lipoxygenase, peroxidase and catalase and are naturally present in wheat flour (Delcros et al., 1998; Rakotozafy et al., 1999). These enzymes catalyze oxidative reactions, which are of great importance to the rheological properties of dough (Rakotozafy et al., 1999). There is supporting research that exogenous, as well as endogenous, enzymes might help improve the gluten strength of wheat bread (Rakotozafy et al., 1999).

The goal of this study was to increase the bread making ability of low protein (all-purpose) flours by adding soybean lipoxygenase to increase gluten formation. The soybean lipoxygenase was isolated from soy flour. The rheological properties, as well as bread loaf volume, color and texture, was measured and compared with control loaves that lack the added enzyme. Significant differences between the experimental and control loaves were determined through ANOVA and Tukey's post hoc test. Therefore, the objectives of this study were:

1. To determine the protein fraction of soy flour with the greatest lipoxygenase activity via isoelectric precipitation and enzyme assay

2. To determine the effects of added soybean lipoxygenase on bread dough rheological properties and physical properties of bread loaves, such as loaf volume, color and texture, compared to controls
3. To determine, through QDA, effects on the sensory attributes of bread due to the addition of soy flour and soy protein fractions

# Chapter 4: Materials and Methods

## 4.1 Determination of Lipoxygenase in Soy Flour

The protein fractions of soy flour were separated via isoelectric precipitation at pH 6.4, 4.8 and 4.1 using a method from Thanh and Shibasaki (1976). Tris(hydroxymethyl)-aminomethane buffer, at pH 8.0, was mixed with soy flour in the ratio of 15:1 (*w/v*). The mixture was allowed to stir overnight at 4°C. Afterward, the slurry was centrifuged for 40 minutes at 5000 x g in a refrigerated (4°C) Sorvall centrifuge. The supernatant was then removed and 6N HCl was added drop-wise until a pH of 6.4 was achieved. The mixture was then centrifuged again using the same conditions. This procedure was repeated at pH 4.8 and 4.1. This was done sequentially. The lipoxygenase activity of the supernatant at each pH was determined based on a rapid spectrophotometric assay according to Wu et al. (1997). This assay measures LOX activity, using linoleic acid as the substrate, by monitoring the increase in absorbance at 234 nm due to the formation of the conjugated diene (Wu et al., 1997).

Enzyme activity was quantified by multiplying the difference in absorbance between the substrate with buffer versus the substrate, enzyme and buffer times the dilution factor of 20. The activity was expressed as a change in absorbance per minute (DA/min). The activity was then compared with the amount of protein present in the solution. Protein concentration was determined using the AACC 46-15 method for the Biuret protein assay with bovine serum albumin as the primary reference standard (AACC 2000). The final activity was determined by calculating the ratio of average activity to protein concentration. The fraction with the greatest activity was determined to contain the greatest amount of LOX enzyme activity.

### 4.2 Gluten Strength Determination

Table 3 shows the different variations of flour and treatments placed in the farinograph (C.W. Brabender Instruments, South Hackensack, NJ) using AACC method 54-21 constant flour weight procedure for 50 g sample (AACC 2000). Fifty grams of Wingold H&R (hotel and restaurant) bleached enriched all-purpose flour (Bay State Milling, Quincy MA), All Trump's baker's high gluten bleached enriched brominated bread flour (General Mills, Minneapolis, MN), all-purpose flour plus Bob's Red Mill stone-ground whole grain soybean flour, 35% protein, (Bob's Red Mill Natural Foods, Milwaukie, OR), all-purpose flour plus isolated soybean precipitate (both pH 6.4 and pH 4.8) as well as all-purpose flour plus enzyme inhibitor were placed in the farinograph. The precipitate of the protein fractions that were determined to have the greatest lipoxygenase activity were freeze-dried in a Virtis freeze dryer (SP Industries, Inc., Gardiner, NY) for 72 hours, crushed via mortar and pestle and were then added to all-purpose flour (2.81 g of precipitate to 47.19 g of flour). The mix of the flour and precipitate was then placed in the farinograph and allowed to run for at least 20 minutes or until the curve left the 500 Brabender units (BU) line. Three trials of each sample were run. These samples were compared with farinograph measurements of all-purpose flour, Trump's bread flour and all-purpose flour with soy flour added. In addition, purified lipoxidase enzyme (from *Glycine max* soybean, lyophilized, powder obtained from Biochemika Fluka) was also added to all-purpose flour (25mg) for comparison. Esculetin (98%, obtained from Aldrich) was added to all-purpose + soy flour as an enzyme inhibitor (15 mg) to the farinograph, as well.

Approximately 62% water was added to 50 g of flour or mixture of flour and precipitate, such that at the peak time of the initial dough consistency was 500 BU. Differences in gluten strength between control and experimental loaves were determined by comparison of arrival time, dough development time, stability, departure time (DEP), twenty minute drop (TMD) and tolerance index (MTI) of the different samples.

### 4.3 Bread Making

Bread loaves were baked based on the results of the farinograph experiment. Two different types of control loaves were included. The negative control consisted entirely of all-purpose flour (Wingold H&R, Bay State Milling, Quincy, MA), along with tap water, salt (Morton's, Rohm and Haas, Chicago, IL), active dry yeast (Fleischmann's, AB Mauri Food, Inc., St. Louis, MO), shortening (Crisco, J.M. Smucker Co., Orville, OH) and granulated sugar. A detailed description of the formulation is listed in Table 4. The positive control used higher protein quality flour, all Trump's bread flour (General Mills, Minneapolis, MN), to replace the all-purpose flour in the formulation. The formulation used was according to the AACC 10-10 method (AACC 2000). The experimental loaves were made according to the same method; however, the flour used was in the same ratio as those used in the farinograph. Soy flour (Bob's Red Mill Natural Foods, Milwaukie, OR) and 4.8 precipitate were added to the formulation (see Table 4 for details) to determine the differences of both physical and sensory attributes of the bread compared with the controls.

Yeast (10.6 g) was mixed with 40 ml warm water (105°F) and 6 g of sugar and allowed to sit for 10 minutes. Meanwhile, flour (200 g), shortening (6 g), salt (3 g) and

sugar (6 g) were combined in a large mixing bowl. After the yeast was activated, it was poured into the mixing bowl with 100 ml cold tap water and the Kitchen-Aide mixer kneaded the dough (dough hook attachment) for 5 minutes. The dough was formed into a ball and allowed to rise for 52 minutes. It was then rolled out, folded into thirds and allowed to rise for 24 minutes. Finally, the dough was divided in two parts (weighed equally) and rolled out again. The dough was folded in thirds, flipped, rolled and folded in thirds again. These were placed into two pup loaf pans covered and allowed to rise for 33 minutes before being placed in a preheated oven (425°F). The loaves were baked for 23 minutes. They loaves were immediately removed from the pans and were allowed to cool for 30 minutes before being wrapped in plastic. The loaves were stored for 24 hours before volume, texture and color was determined. Each batch of dough, which was divided into two equal parts, yielded two pup loaves and the average of these was used per trial.

**Table 3: Farinograph Trials**

| *Trial* | *Flour* | *Additional Treatments* |
|---|---|---|
| 1 | All-Purpose Flour (50 g) | None |
| 2 | Trump's Bread Flour (50 g) | None |
| 3 | All-Purpose Flour (47.19 g) | Soy Flour (2.81 g) |
| 4 | All-Purpose Flour (47.19 g) | pH 4.8 Precipitate (2.81 g) |
| 5 | All-Purpose Flour (47.19 g) | pH 6.4 Precipitate (2.81 g) |
| 6 | All-Purpose Flour (50 g) | Lipoxidase (25 mg) |
| 7 | All-Purpose Flour (47.19 g) | Soy Flour (2.81 g) Esculetin (15 mg) |

**Table 4: Bread Formulation**

| *Bread Sample* | *All-purpose flour* | *Bread Flour* | *Soy Flour* | *4.8 Precipitate* | *Yeast* | *Sugar* | *Salt* | *Shortening* | *Water* |
|---|---|---|---|---|---|---|---|---|---|
| **All-purpose** | 200 g | 0 g | 0 g | 0 g | 10.6 g | 6 g | 3 g | 6 g | 140 g |
| **Trump's Bread flour** | 0 g | 200 g | 0 g | 0 g | 10.6 g | 6 g | 3 g | 6 g | 140 g |
| **All-purpose and soy flour** | 188.76 g | 0 g | 11.24 g | 0 g | 10.6 g | 6 g | 3 g | 6 g | 140 g |
| **All-purpose flour and pH 4.8 precipitate** | 188.76 g | 0 g | 0 g | 11.24 g | 10.6 g | 6 g | 3 g | 6 g | 140 g |

### 4.4 Loaf Volume

The loaf volume of the baked breads was determined by rapeseed displacement. Initially, the empty chamber was filled with rapeseeds and the volume was recorded in cubic centimeters. The cooled loaves, still wrapped in plastic, were then individually placed in the chamber. The chamber was then filled again with rapeseeds and the difference of volume containing the loaves and the empty chamber was calculated and used as an estimate of the loaf volume. Since each replication of bread making yielded two small pup loaves, the volumes of these were averaged and only one volume was used per replication.

### 4.5 Color Determination of Bread Loaves

Immediately after loaf volume determination, color of both bread crust and crumb was determined. A Minolta chromameter was used to determine the Hunter L, a, and b values of the bread. L values indicate black or white (L=0, Black and L=100, White). A greater L value indicates a lighter sample color. Hunter a values indicate whether the sample contains red of green hues, depending on if the value is positive or negative, respectively. Hunter b values describe either yellow or blue values depending on if the value is positive or negative, respectfully. Crust measurements were taken on the center of the top of the bread, while crumb measurements were taken from the middle of the loaf, which was cut in a one-inch thick slice. Each pup loaf had one crust and one crumb measurement taken and the average of these was used per trial.

### 4.6 Texture Determination of Bread Loaves

Texture was determined in conjunction with color determination. An EZ-Test Texture Analyzer (Rheology Solutions Pty Ltd., Bacchus Marsh, Victoria) was used. The bread compression jig probe was used for both crust and crumb. The texture of the crust was measured on the top, center of the loaf. The probe was allowed to compress into the bread for 1 cm before it regressed back. The compression cake test was used on the software. The crumb texture was measured in the center of a 1-inch thick slice of bread cut from the center of the loaf. Measurements were recorded in grams of force needed to compress the bread 1 cm. Since each replication of bread making yielded two small pup loaves, the texture measurements of these were averaged and only one measurement was used per replication.

### 4.7 Sensory Analysis

Quantitative descriptive analysis (QDA) was performed to determine if there were significant differences of certain bread attributes among the treatments (Gacula, 1997; Stone et al., 1980; Zook and Wessman, 1977). Panelists underwent a two-week training period before the trials were conducted. During the training period, the panelists' defined bread attributes to test. These attributes included: outside color, uniformity of outside (crust), porosity of inside (crumb), chewiness of inside, toughness of outside, yeastiness, sourness, and overall flavor (bland versus intense). All-purpose flour loaves, Trump's bread flour loaves and 4.8 precipitate supplemented loaves were involved in the sensory analysis that was performed over the course of two days.

Ten panelists between the ages of 20-60 years of age were recruited for QDA. Two panelists were let go, leaving 8 female panelists for a 10hr, two-week, training period followed by two 20 minute testing periods. The panelists were to score each attribute on a horizontal line measuring 15 cm. The left end of the line was marked as less intensity of the attribute while the right end of the line was marked as more intensity of the attribute. An example of a scorecard is shown in Appendix D. The scorecard line scales were not presented all at once. They were separated into 3 groups: 1) color & uniformity; 2) porosity, chewiness and toughness; and 3) yeasty, sourness and flavor More intensity of the specific attribute was to be scored further from the left or zero. The values were measured in centimeters with 0 cm starting at the left end of the line. Greater values associated with more intensity of each attribute. The spectrum used for each attribute is presented in Appendix E.

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**4.8 Statistical Analysis**

All data were analyzed using JMP IN 5.1 (SAS Institute, Cary NC). One-way analysis of variance (ANOVA) was initially performed to determine differences between the control and the treatments. If a significant difference ($p<0.05$) was determined, a Post-hoc test, Tukey's Honestly Significant Difference test (HSD), was performed to determine which loaves are significantly different from each other.

# Chapter 5: Results

## 5.1 Results of LOX Assay

The results of the LOX assay are shown in Table 5. It was determined that the greatest lipoxygenase activity was obtained in the pH 4.8 precipitate based on the amount of protein present. As the pH varied, differing amounts of protein were present. A protein assay was carried out to determine the protein concentrations at different pHs. The activity of the enzyme at different pHs was compared to the protein concentrations at each of those pHs.

The LOX activity of all-purpose flour was significantly less than any of the other samples ($p<0.05$). The activity of the pH 4.8 precipitate was significantly greater than the activity of pH 4.1 ($p<0.05$). Soy flour and pH 8.0 were not significantly different from each other, which makes sense because protein has not precipitated out of the soy flour at pH 8.0. Bread flour was found to have a significantly greater LOX activity when compared with all-purpose flour ($p<0.05$). However, it was less than soy flour, but this difference was not significant.

Protein concentrations of soy flour fractions at different pHs were determined using the Biuret assay. The concentrations were compared to standards made from bovine serum albumin (BSA) stock solution. A standard curve was produced, and the linear equation derived from the curve was used to calculate the final protein concentrations, which are shown in Table 6.

Protein concentrations at different pHs varied. At pH 8.0 the protein concentration was $34.92 \pm 4.45$ mg/ml, which was significantly greater than any of the fractions ($p<0.05$). While the pH 6.4 fraction had a significantly greater protein

concentration than both pH 4.8 and 4.1 fractions ($p<0.05$), neither the 4.8 nor the 4.1 fractions differed from each other. These concentrations were then compared with the previously calculated LOX activities to determine the activity based on the protein concentration. Table 7 shows relative LOX activity per mg of protein in each fraction. These were determined by dividing the average LOX activity and the protein concentration at the same pH.

All LOX activities per protein concentration were significantly different from each other ($p<0.05$). LOX activity at pH 4.8 (0.160) was significantly greater than at any other pH ($p<0.05$). The pH 6.4 fraction had the next greatest activity followed by pH 4.1 and finally pH 8.0. The LOX purification at pH 4.8 was much greater than at pH 6.4 ($p<0.001$) and pH 4.1 ($p<0.001$). Therefore, the protein fraction obtained at pH 4.8 was used in the farinograph trials, as well as the bread making and sensory trials, and compared to the controls. The pH 6.4 precipitate was added in the farinograph trials to determine its effects on dough rheological properties since it contained the second greatest enzyme activity.

**Table 5: LOX Activity in Different Flour Samples**

| ***Sample*** | ***Average LOX Activity (ΔA/min)*** |
|---|---|
| Soy Flour | $2.15 \pm 0.57^{a}$ |
| All-Purpose Flour | $-0.35 \pm 0.51^{c}$ |
| Trump's Bread Flour | $1.65 \pm 0.73^{a,b}$ |
| pH 8.0 | $2.37 \pm 0.93^{a}$ |
| pH 6.4 | $1.95 \pm 0.30^{a}$ |
| pH 4.8 | $1.75 \pm 0.43^{a,b}$ |
| pH 4.1 | $0.69 \pm 0.93^{b,c}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 6: Average Protein Concentrations (mg/ml)**

| *Protein Fraction at Different pHs (1:5 dilution factor)* | *Average Protein Concentration (mg/ml)* |
|---|---|
| pH 8.0 | $34.92 \pm 4.45^{a}$ |
| pH 6.4 | $21.16 \pm 1.93^{b}$ |
| pH 4.8 | $10.95 \pm 1.96^{c}$ |
| pH 4.1 | $9.73 \pm 2.07^{c}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 7: Relative LOX Activity in Original Extract and Isoelectric Precipitation Fractions**

| *Protein Fraction* | *Relative LOX Activity* |
|---|---|
| pH 8.0 | $0.068^{d}$ |
| pH 6.4 | $0.092^{b}$ |
| pH 4.8 | $0.160^{a}$ |
| pH 4.1 | $0.071^{c}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

### 5.2 Results of Farinograph Trials

Results of the farinograph trials are presented in Appendix A. The different parameters determined from the farinograph are shown for each trial performed. A total of three trials were done for each different sample. One example farinograph from each of the different samples is shown in Appendix B, Figures 2-8.

The absorption (Table 8), or amount of water added, was found to be significantly different among the samples ($p<0.01$). Upon a post hoc statistical analysis, it was determined that the mean absorption of all-purpose and bread flour samples were not statistically different from each other; however, the two samples were statistically different from all other samples ($p<0.05$). The all-purpose and bread flour samples were found to have greater percent water absorption than the other samples.

Arrival time (Table 9) of the samples exhibited a similar trend to that of the absorption percentage. Mean arrival times were determined to be significantly different ($p<0.01$). The mean arrival time of both of the control samples were not statistically different. The mean arrival time was significantly greater for the bread flour samples compared with other samples ($p<0.01$). The mean arrival time was greatest for the bread flour samples and the all-purpose samples with soy flour and inhibitor yielded the shortest mean arrival time.

The mean peak time (Table 10) was also found to be significantly different among the different samples of bread ($p<0.01$). The mean peak time of the bread with soy flour and inhibitor was significantly greater ($11.67 \pm 0.29$ min) than the other samples ($p<0.01$). The mean bread flour peak time was not significantly different from that of all-purpose with added soy flour; however, these two samples were significantly different

from the samples with 4.8 and 6.4 precipitate ($p<0.001$). The mean peak time was significantly greater in the 4.8 precipitate samples when compared with the 6.4 precipitate samples ($p<0.05$). The mean peak time of the all-purpose control samples was significantly lower ($1.83 \pm 0.29$) than all other samples, with the exception of the sample with added lipoxidase ($p<0.05$).

**Table 8: Mean % Water Absorption of Different Flour Samples (%)**

| *Flour Sample* | *Mean Absorption (%)* |
|---|---|
| All-purpose flour | $63.00 \pm 0.20^{a}$ |
| Trump's Bread flour | $63.67 \pm 0.58^{a}$ |
| All-purpose + Soy flour | $61.93 \pm 0.23^{b}$ |
| All-purpose + 4.8 precipitate | $62.03 \pm 0.75^{b}$ |
| All-purpose + 6.4 precipitate | $61.93 \pm 0.06^{b}$ |
| All-purpose + Lipoxidase | $62.30 \pm 0.17^{b}$ |
| All-purpose + soy flour + esculetin | $62.10 \pm 0.00^{b}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 9: Mean Arrival Time (min) of Different Flour Samples**

| *Flour Sample* | *Mean Arrival Time(min)* |
|---|---|
| All-purpose flour | $1.17 \pm 0.29^{b}$ |
| Trump's Bread flour | $2.00 \pm 0.00^{a}$ |
| All-purpose + Soy flour | $1.17 \pm 0.14^{b}$ |
| All-purpose + 4.8 precipitate | $1.25 \pm 0.25^{b}$ |
| All-purpose + 6.4 precipitate | $1.08 \pm 0.14^{b}$ |
| All-purpose + Lipoxidase | $1.75 \pm 0.00^{a}$ |
| All-purpose + soy flour + esculetin | $1.00 \pm 0.00^{b}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 10: Mean Peak Time (min) of Different Flour Samples**

| *Flour Sample* | *Mean Peak Time (min)* |
|---|---|
| All-purpose flour | $1.83 \pm 0.29^{e}$ |
| Trump's Bread flour | $10.17 \pm 0.76^{b}$ |
| All-purpose + Soy flour | $10.67 \pm 0.29^{b}$ |
| All-purpose + 4.8 precipitate | $9.83 \pm 0.29^{c}$ |
| All-purpose + 6.4 precipitate | $2.00 \pm 0.00^{de}$ |
| All-purpose + Lipoxidase | $2.50 \pm 0.00^{d}$ |
| All-purpose + soy flour + esculetin | $11.67 \pm 0.29^{a}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

The mean stability time (Table 11) was also found to be significantly different between the samples ($p<0.01$). It was found that the mean stability time of the samples containing pH 6.4 precipitate, lipoxidase, and soy flour plus inhibitor were significantly lower than all other samples ($p<0.05$). The mean stability of the bread flour samples and those containing the pH 4.8 precipitate were not significantly different from each other ($23.67 \pm 2.31$ min and $24.75 \pm 1.15$ min, respectively); however, they were significantly higher than any other sample ($p<0.05$). The mean stability time of the all-purpose plus soy flour samples was not as great as that of the bread flour and 4.8 precipitate supplemented sample, but it was significantly greater than the all-purpose control ($p=0.004$), the sample with pH 6.4 precipitate ($p=0.04$), the sample with added lipoxidase ($p=0.003$), and the sample containing the inhibitor ($p=0.003$).

The mean departure times are presented in Table 12. The mean departure time of the samples was found to be significantly different ($p<0.01$). The mean departure time of the bread flour samples and the pH 4.8 precipitate supplemented samples were not statistically different from each other; however, they were significantly higher than the mean departure time of all other samples ($p<0.05$). It was found that the all-purpose samples, pH 6.4 precipitate supplemented samples and lipoxidase supplemented samples had significantly lower departure times than the samples containing soy flour or the pH 4.8 precipitate ($p<0.05$).

Table 13 illustrates the mean twenty minute drop (TMD) values. The mean TMD values (BU) were found to be significantly different between the samples ($p<0.01$). The samples containing the pH 4.8 precipitate were found to have a mean TMD of 0 BU, which was significantly lower than any other sample ($p<0.001$). This meant that the

curve did not leave the 500 BU line after 20 minutes of run time, indicating no breakdown in the gluten. The all-purpose control samples and then pH 6.4 supplemented samples had a significantly higher mean TMD than the other samples ($p<0.001$).

The mean mixing tolerance index (Table 14) was found to be significantly different among the samples ($p<0.01$). The mean mixing tolerance index was significantly lower in the all-purpose control samples when compared with the pH 4.8 precipitate supplemented samples ($p<0.001$). The all-purpose control samples were not significantly different from the pH 6.4 precipitate supplemented samples or lipoxidase supplemented samples. In addition, no significant differences were found between the bread flour controls and the samples supplemented with soy flour.

**Table 11: Mean Stability (min) of Different Flour Samples**

| *Flour Sample* | *Mean Stability (min)* |
|---|---|
| All-purpose flour | $4.17 \pm 0.29^{d}$ |
| Trump's Bread flour | $23.67 \pm 2.31^{a}$ |
| All-purpose + Soy flour | $16.67 \pm 0.52^{b}$ |
| All-purpose + 4.8 precipitate | $24.75 \pm 1.15^{a}$ |
| All-purpose + 6.4 precipitate | $10.25 \pm 8.29^{c}$ |
| All-purpose + Lipoxidase | $6.92 \pm 2.08^{cd}$ |
| All-purpose + soy flour + inhibitor | $6.67 \pm 0.29^{cd}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 12: Mean Departure Time (min) of Different Flour Samples**

| *Flour Sample* | *Mean Departure Time (min)* |
|---|---|
| All-purpose flour | $5.33 \pm 0.58^{d}$ |
| Trump's Bread flour | $25.67 \pm 2.31^{a}$ |
| All-purpose + Soy flour | $17.83 \pm 0.58^{b}$ |
| All-purpose + 4.8 precipitate | $26.00 \pm 1.32^{a}$ |
| All-purpose + 6.4 precipitate | $11.67 \pm 7.77^{c}$ |
| All-purpose + Lipoxidase | $8.67 \pm 2.08^{c}$ |
| All-purpose + soy flour + inhibitor | $18.33 \pm 0.29^{b}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 13: Mean Twenty Minute Drop (BU) of Different Flour Samples**

| *Flour Sample* | *Mean Twenty Minute Drop (BU)* |
|---|---|
| All-purpose flour | $76.67 \pm 11.55^{a}$ |
| Trump's Bread flour | $36.67 \pm 5.77^{bc}$ |
| All-purpose + Soy flour | $20.00 \pm 0.00^{d}$ |
| All-purpose + 4.8 precipitate | $0.00 \pm 0.00^{e}$ |
| All-purpose + 6.4 precipitate | $73.33 \pm 5.77^{a}$ |
| All-purpose + Lipoxidase | $43.33 \pm 5.77^{b}$ |
| All-purpose + soy flour + inhibitor | $31.67 \pm 2.87^{c}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 14: Mean Tolerance Index of Different Flour Samples (BU)**

| ***Flour Sample*** | ***Mean Tolerance Index (BU)*** |
|---|---|
| All-purpose flour | $60.00 \pm 10.00^{a}$ |
| Trump's Bread flour | $26.67 \pm 11.55^{cd}$ |
| All-purpose + Soy flour | $20.00 \pm 0.00^{cd}$ |
| All-purpose + 4.8 precipitate | $13.33 \pm 5.77^{d}$ |
| All-purpose + 6.4 precipitate | $56.67 \pm 11.55^{a}$ |
| All-purpose + Lipoxidase | $46.67 \pm 11.55^{b}$ |
| All-purpose + soy flour + inhibitor | $33.33 \pm 5.77^{bc}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

### 5.3 Results of Bread making Experiments

Four different types of bread loaves were baked in the lab. There were two controls: one type baked with all-purpose flour and the other with Trump's bread flour. Two different experimental loaf trials were also done, which included pH 4.8 soy flour precipitate with all-purpose flour and soy flour with all-purpose flour, in the sample ratios. Volume ($cm^3$), Hunter L, a, and b values for crust and crumb, as well as crust and crumb texture were analyzed. Appendix C displays the results of the analyses.

Three trials of each sample were baked and the average data of each trial (one trial produced two pup loaves, which were averaged) was statistically analyzed. It was found that the mean volume ($cm^3$), Table 15, of the samples were statistically different from each other ($p<0.001$). The mean volume of the bread flour loaves was significantly greater ($745.83 \pm 7.22\ cm^3$) than the other loaves ($p<0.01$). Loaf volume of the all-purpose control and the pH 4.8 supplemented loaves was not significantly different ($629.17 \pm 7.22\ cm^3$ and $600.00 \pm 54.49\ cm^3$, respectively; $p=0.27$). The loaves containing soy flour were found to be significantly smaller than other loaves ($p<0.05$), with the exception of the 4.8 precipitate loaves.

The average L values of the crust color are shown in Table 16. The mean values were found to be significantly different between the samples ($p<0.01$). Loaves prepared with bread flour were found to be significantly lighter in color when compared with the other loaves ($p<0.05$). No significant difference was found between the all-purpose control and the pH 4.8 precipitate loaves ($56.13 \pm 0.65$ and $55.57 \pm 0.45$, respectively) when L values were considered. The loaves prepared with soy flour were found to be significantly lighter in crust color when compared with the all-purpose controls ($p<0.05$);

however, there was no difference found in crust color when compared with the pH 4.8 precipitate loaves.

The average a values obtained from the crust of the bread loaves are presented in Table 17. There were no significant differences found between the loaves for the a color value ($p=0.71$). In addition, there were no significant differences found between the loaves for the b color ($p=0.14$) with the exception of the bread flour control and the pH 4.8 precipitate loaves ($p<0.05$). Bread flour loaves were found to have a slightly greater b value when compared with loaves containing the 4.8 precipitate. The average b color values for the loaf crust are found in Table 18.

The average L values of the crumb color (Table 19) of the loaves were found to be significantly different ($p<0.01$). Bread flour loaf crumb was found to be significantly lighter than any other loaf crumb ($p<0.01$). All-purpose loaf crumb color was not significantly different from the crumb color of the soy flour experimental loaves ($76.49 \pm 0.84$ and $75.59 \pm 0.56$, respectively). However, the average L values of the crumb color of both controls were found to be significantly lighter than the pH 4.8 precipitate bread loaves ($p<0.001$).

The average a value of crumb color of bread loaves (Table 20) was found to be significantly different ($p<0.001$). The average crumb color for loaves with pH 4.8 precipitate was $0.78 \pm 0.02$, which was significantly more positive (red) than any other loaves ($p<0.001$). The all-purpose loaves were not significantly different than those with soy flour added, with respect to the a values for crumb color. Bread flour loaves were found to be significantly different ($p<0.001$) than the rest of the loaf samples. The bread

flour loaves were found to have negative a values, indicating a slight green hue as opposed to red.

The average b values of the bread loaf crumb color are found in Table 21. There was a significant difference between all the loaf samples in terms of b color values ($p<0.001$). All average b color values of bread loaf crumb were positive, indicating a more yellow color. Bread loaves with soy flour were significantly more yellow than the other samples ($p<0.001$). Loaves made of bread flour were significantly less yellow than any other sample ($p<0.05$). Mean b color values of the all-purpose control and the pH 4.8 precipitate were $19.39 \pm 0.24$ and $19.81 \pm 0.18$, respectively, but were significantly different ($p<0.05$).

It was found that there were no significant differences between the crust textures among the different samples; however, large standard deviations were observed. Average crust texture values are found in Table 22. There were significant differences in the crumb texture of the different loaf samples. The average crumb texture values are found in Table 23. A significant difference in crumb texture was found between the bread flour loaves and the loaves with added soy flour ($p<0.05$). The crumb of the loaves prepared with bread flour was significantly softer than the loaves prepared with soy flour, but was not found to be different than the other samples. No differences were found between the all-purpose control and the pH 4.8 precipitate loaf samples with respect to the crumb texture.

**Table 15: Average Bread Loaf Volume ($cm^3$)**

| *Bread Sample* | *Average Loaf Volume ($cm^3$)* |
|---|---|
| All-purpose flour | $629.17 \pm 7.22^{b}$ |
| Trump's Bread flour | $745.83 \pm 7.22^{a}$ |
| All-purpose + Soy flour | $562.50 \pm 25.00^{c}$ |
| All-purpose + 4.8 precipitate | $600.00 \pm 54.47^{bc}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 16: Average L Values of Bread Loaf Crust**

| *Bread Sample* | *Average L value* |
|---|---|
| All-purpose flour | $56.13 \pm 0.65^{b}$ |
| Trump's Bread flour | $58.44 \pm 1.47^{a}$ |
| All-purpose + Soy flour | $53.86 \pm 1.54^{c}$ |
| All-purpose + 4.8 precipitate | $55.57 \pm 0.45^{bc}$ |

0=Black; 100=White

* Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 17: Average a Values of Bread Loaf Crust**

| *Bread Sample* | *Average a Value* |
|---|---|
| All-purpose flour | $13.35 \pm 0.36^{a}$ |
| Trump's Bread flour | $13.26 \pm 1.03^{a}$ |
| All-purpose + Soy flour | $13.68 \pm 0.36^{a}$ |
| All-purpose + 4.8 precipitate | $13.08 \pm 0.56^{a}$ |

+a=Red; -a=Green

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 18: Average b Values of Bread Loaf Crust**

| *Bread Sample* | *Average b Value* |
|---|---|
| All-purpose flour | $29.82 \pm 0.50^{ab}$ |
| Trump's Bread flour | $30.16 \pm 2.01^{a}$ |
| All-purpose + Soy flour | $29.51 \pm 0.31^{ab}$ |
| All-purpose + 4.8 precipitate | $27.81 \pm 1.00^{b}$ |

+b=Yellow; -b=Blue

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 19: Average L Values of Bread Loaf Crumb**

| ***Bread Sample*** | ***Average L Value*** |
|---|---|
| All-purpose flour | $76.49 \pm 0.84^{b}$ |
| Trump's Bread flour | $78.89 \pm 0.66^{a}$ |
| All-purpose + Soy flour | $75.59 \pm 0.56^{bc}$ |
| All-purpose + 4.8 precipitate | $74.55 \pm 0.99^{c}$ |

0=Black; 100=White

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 20: Average a Values of Bread Loaf Crumb**

| ***Bread Sample*** | ***Average a Value*** |
|---|---|
| All-purpose flour | $0.36 \pm 0.07^{b}$ |
| Trump's Bread flour | $-0.10 \pm 0.05^{c}$ |
| All-purpose + Soy flour | $0.34 \pm 0.11^{b}$ |
| All-purpose + 4.8 precipitate | $0.78 \pm 0.02^{a}$ |

+a=Red; -a=Green

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 21: Average b Values of Bread Loaf Crumb**

| ***Bread Sample*** | ***Average b Value*** |
|---|---|
| All-purpose flour | $19.39 \pm 0.24^{c}$ |
| Trump's Bread flour | $18.88 \pm 0.24^{d}$ |
| All-purpose + Soy flour | $21.26 \pm 0.17^{a}$ |
| All-purpose + 4.8 precipitate | $19.81 \pm 0.18^{b}$ |

+b=Yellow; -b=Blue

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 22: Average Texture Values of Bread Loaf Crust**

| ***Bread Sample*** | ***Average Texture Value (g)*** |
|---|---|
| All-purpose flour | $445.94 \pm 54.33^{a}$ |
| Trump's Bread flour | $395.44 \pm 79.64^{a}$ |
| All-purpose + Soy flour | $456.48 \pm 120.7^{a}$ |
| All-purpose + 4.8 precipitate | $375.25 \pm 2.14^{a}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

**Table 23: Average Texture Values of Bread Loaf Crumb**

| ***Bread Sample*** | ***Average Texture Value (g)*** |
|---|---|
| All-purpose flour | $223.58 \pm 30.16^{ab}$ |
| Trump's Bread flour | $176.79 \pm 10.55^{b}$ |
| All-purpose + Soy flour | $278.51 \pm 67.76^{a}$ |
| All-purpose + 4.8 precipitate | $232.65 \pm 23.79^{ab}$ |

*Means and standard deviations followed by different superscripts are significantly different at the $p<0.05$ level.

### 5.4 Results of QDA Sensory Evaluation

QDA sensory evaluation was performed to determine differences in different sensory attributes of bread samples. Appendix E describes the definitions of the sensory attributes for bread as defined by the trained panelists. Appendix F describes the standards (products) used for the training sensory panel. The sensory panel was comprised of 8 students who went through a two-week training period prior to final evaluations. Final evaluations consisted of two replications each of the bread flour and all-purpose controls, as well as the 4.8 precipitate treated loaves.

The mean scores of each sensory attribute are shown in Table 24. The number values equate to the anchors, going from less to more. It was scored on an unstructured line scale of 15 cm. Therefore, a larger number is consistent with more or stronger attributes (15 cm = very), whereas a small number is consistent with little or none of the specific attribute (0 cm = none). Two sensory QDA replications of the loaves were performed from the same bread sample to compare consistency of the results. These values were averaged and presented in the data table. Additionally, Figure 1 shows the different bread treatment samples in a spider plot. This plot shows the differences between the samples.

According to panelists, all-purpose flour prepared loaves had a significantly lighter crust color when compared with the bread flour loaves and the experimental loaves ($p=0.0009$). However, there was no significant difference in the uniformity of the crust among all-purpose and bread flour loaves. The samples containing the 4.8 precipitate were found have significantly more unevenness, or marbling, on the outside crust ($p=0.002$). There were no significant differences found in the toughness of the crust

between the different samples; however, values indicate that the 4.8 precipitate loaves might have been slightly tougher than the control loaves.

No significant differences in the porosity of the crumb were found among the groups. Values indicate that the bread flour loaves yielded slightly greater, or more dense, crumb structures. A significant difference in chewiness was found between the all-purpose loaves and the 4.8 precipitate loaves ($p=0.0063$). The 4.8 precipitate loaves were found to be much firmer when compared with the all-purpose control loaf. The firmness of the bread flour loaves was not significantly different from the other two samples.

There was no difference in yeasty taste found among the groups. Differences in sourness were not found to be significant; however, all-purpose loaves yielded a slightly greater value, meaning they were found to have a slightly greater sourness taste. There were no significant differences found in flavor among the three different samples.

**Table 24: Mean Scores of Bread Attributes from QDA Sensory Analysis (Average of Rep 1 and 2)**

| *Sample* | *Outside Color*[1] | *Uniformity of outside*[2] | *Porosity of inside*[3] | *Chewiness of inside*[4] | *Toughness of outside*[5] | *Yeasty*[6] | *Sourness*[7] | *Flavor*[8] |
|---|---|---|---|---|---|---|---|---|
| Bread Flour | 3.29[a] | 11.65[a] | 3.93[a] | 5.21[ab] | 3.94[a] | 5.63[a] | 4.89[a] | 3.56[a] |
| All-purpose flour | 1.93[b] | 10.57[a] | 2.64[a] | 3.62[b] | 3.15[a] | 5.61[a] | 5.63[a] | 3.38[a] |
| 4.8 Precipitate | 3.87[a] | 7.15[b] | 2.72[a] | 6.90[a] | 4.29[a] | 5.63[a] | 4.34[a] | 3.81[a] |

[1]Degree of darkness (from baking or type of dough) of the crust on side of bread sample (inside bread pan portion); Light (0) → Dark (15)
[2]Degree of evenness of color of crust on side of bread sample; Uneven (0) → Even (15)
[3]Size of air cells/air pockets inside of bread; Large or Airy (0) → Small or Dense (15)
[4]Degree of density of inside of bread; Soft (0) → Hard (15)
[5]Degree of toughness of outside of bread; Soft (0) → Hard (15)
[6]Degree of fermented yeast scent, taste; Less (0) → More (15)
[7]Degree of tanginess, lingering aftertaste; Less (0) → More (15)
[8]Degree of flavor when sample is tasted; Bland (0) → Strong (15)
*Means followed by different superscripts are significantly different at the $p<0.05$ level.

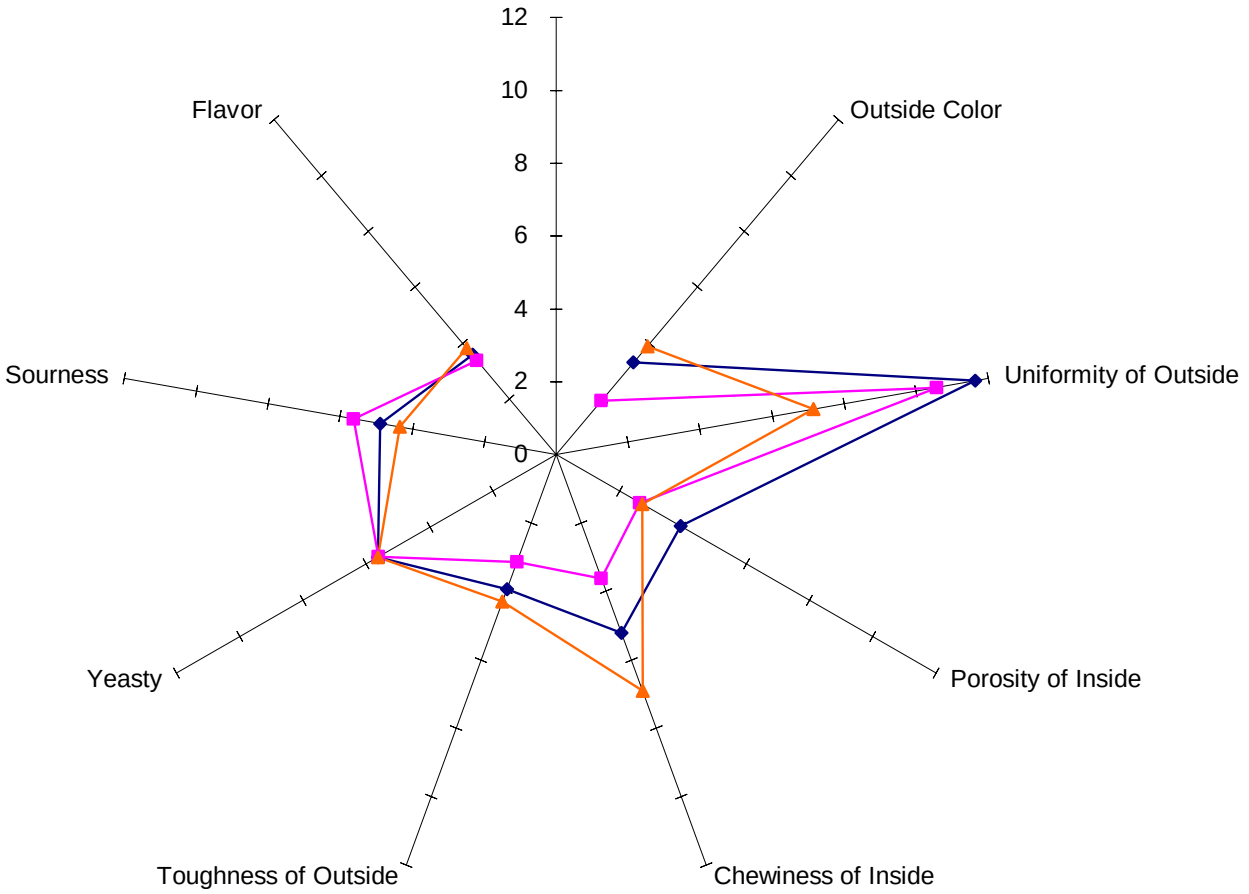


**Figure 1: Sensory Spider Plot of Bread Attributes**

# Chapter 6: Discussion of Results

## 6.1 LOX Assay

The LOX assay performed was derived from the methods of Wu et al. (1997). The enzyme was extracted from full-fat soy flour with potassium phosphate. The mixture was centrifuged, and the supernatant was used in the assay to determine the activity. The LOX activity was determined based on the increase in absorbance at 234 nm due to a formation of a conjugated diene (Wu et al, 1997). The activity was monitored at different pH environments of defatted soy flour to determine the greatest amount of activity. The protein concentration differed among the different precipitates; therefore, the LOX activity was compared with the protein concentration at each pH environment.

It was found that a pH of 4.8 yielded the greatest activity when compared with the other precipitates ($p<0.05$). According to Thanh and Shibasaki (1976), two major soybean proteins are the 7S and 11S globulins, which can be fractionated through the utilization of isoelectric precipitation. Thanh and Shibasaki (1976) successfully separated the 7S globulin, or beta conglycinin, containing fraction, which is also thought to contain the lipoxygenase enzyme, via isoelectric precipitation with a Tris buffer and concentrated HCl. They found that the 11S globulin is collected by centrifugation when the pH is adjusted to 6.4, whereas the 7S globulin can be separated at a pH of 4.8 (Thanh and Shibasaki, 1976). Since the 7S fraction contains lipoxygenase, the pH 4.8 precipitate should have had the greatest LOX activity, which was indeed the case according the results (see Table 7). The fact that the pH 4.8 precipitate yielded the greatest LOX activity allowed for it to be the fraction of interest to examine whether or not its addition

to all-purpose flour would increase the rheological and physical properties of bread dough and loaves.

### 6.2 Farinograph Interpretation

Water absorption is one of the most commonly used parameters of farinographs (Shuey et al., 1972). It is defined as the amount of water required to center the farinograph curve on the 500 BU line for a flour-water dough (Shuey et al., 1972). Shuey et al. (1972) stated that there is an approximate absorption increase of 1.5% for each 1% increase in the protein content of the flour. Therefore, when there is a greater amount of protein in flour, there is greater water absorption during dough formation.

It was found that there was no difference in water absorption for the two control samples (Table 8). These two samples yielded a greater amount of water absorption than the other samples, which meant more moisture was needed for dough formation. According to Eliasson and Larsson (1993), the rheological properties of wheat flour dough are sensitive to water content. Soy flour supplemented flour dough was much more moist than all-purpose or bread flour because less absorption was needed for the same effect. It was seen that soy flour absorbs a greater mass of water than the same quantity of wheat flour (Cauvain and Young, 2006). Soy proteins have been seen to have greater water retention (Zayas, 1997). Bread flour contains more protein than all-purpose flour; however the farinograph results did not conclude there was a difference in water absorption between the two flours. While there was no significant difference found in water absorption between the bread flour and all-purpose flour, the average absorption of the bread flour was slightly greater than that of the all-purpose flour.

The arrival time (Table 9) is the time required for the top of the curve to reach the 500 line after the mixer has been started and the water has been added (Shuey et al., 1972). It is a measurement of the rate at which the water is taken up by the flour. In general, it is found that as protein content increases, more time is needed for gluten development; therefore, the arrival time also increases (Shuey et al., 1972). This is consistent with the water absorption of the flours, as well. The arrival times (Table 9) of the samples were found to exhibit a similar trend to that of the absorption percentages, which is in accordance with the literature. Bread flour, containing the greatest amount of protein, was found to have a longer arrival time because more time was needed for gluten development. It was seen that samples with less protein also had less gluten development so that they tended to have a shorter arrival time.

The dough development time, also referred to as peak time, is the time that elapses when the curve is at its highest point. In the case that there is more than one "peak" observed, the second peak is when that time occurs. Second peaks were observed in the farinograph with added soy flour (Appendix B, Figure 4), the farinograph with the added 4.8 precipitate (Appendix B, Figure 5) and the farinograph with added soy flour plus inhibitor (Figure 8). According to Eliasson and Larsson (1993), the development is the time it takes to stretch and extend the glutenins; therefore, the more glutenins and the higher their molecular weight, the longer the development time will be. Longer peak times should be associated with greater protein content in a flour, meaning the samples with the longest peak times should be the samples with more protein in comparison, which was seen. The addition of the soy flour and pH 4.8 precipitate resulted in a longer peak time, as well as the high protein bread flour. Shuey et al. (1972) mentioned that

there was a curvilinear relationship between peak time and the percent water absorption such that increased absorption usually resulted in an increase in the peak time. This was only shown to be true with the bread flour sample. It yielded greater water absorption and a longer peak time. All-purpose flour samples resulted in a very short peak time in comparison. Soy flour and 4.8 precipitate, when added to all-purpose flour, greatly improved the peak time when compared with the control sample of all-purpose flour. The added soy flour and 4.8 precipitate allowed for similar results as the bread flour samples when peak time was concerned.

Stability is the difference in time between the point where the top of the curve first intercepts the 500 BU line (arrival time) and the point where the top of the curve leaves the 500 BU line (departure time) (Shuey et al., 1972). In general, this value gives some indication of the tolerance to mixing, or strength of the flour and gluten breakdown a flour will have (Shuey et al., 1972). Bread flour exhibited a significantly greater mean stability time when compared with the all-purpose controls ($p<0.05$) (Table 11). The samples of all-purpose flour with added 4.8 precipitate achieved the greatest mean stability time ($24.75 \pm 1.15$ min). This value was similar to that of the bread flour control samples ($23.67 \pm 2.31$ min). Soy flour was found to have some effect on the stability when compared to the all-purpose control, but did not have as large of an effect as the 4.8 precipitate did. Adding soy products to all-purpose flour seemed to increase the stability, unlike the addition of pure lipoxidase. Therefore, it seemed soy products improved the gluten strength of all-purpose flour; however, the 4.8 precipitate had the greatest effect. This large effect could be attributed, at least in part, to the LOX enzyme, since it was

found to exhibit the most activity at pH 4.8. In a study by Hoseney et al. (1980), soy flour lipoxygenase was seen to improve the rheological properties of wheat flour dough.

Departure time is the time elapsed from the first addition of the water until the top of the curve leaves the 500 BU line (Shuey et al., 1972). Essentially, it is the sum of the arrival time and the mixing stability. Stronger flours will exhibit a longer departure time. The bread flour samples and the all-purpose with 4.8 precipitate were found to exhibit the longest departure times, therefore, these two samples were the strongest flours compared with the other samples. Bread flour and all purpose with 4.8 precipitate did not have significantly different departure times; however, the sample containing the 4.8 precipitate had a slightly longer departure time (Table 12). The all-purpose control sample had the shortest departure time so that it was an extremely weak flour compared with other samples. This was interesting in that when the all-purpose flour was supplemented with 4.8 precipitate there was a great improvement in the strength of the flour. According to Rakotozafy et al. (1999), lipoxygenase from soy flour has been used for decades as an improver in bread making. This effect was seen in the departure time of the farinographs in the samples containing the all-purpose flour with added 4.8 precipitate. Soy flour also improved the departure time when compared with the all-purpose control; however this increase was not as great as the increase with the added precipitate. This could indicate that the precipitate contains a more concentrated form of the enzyme or that there is something in soy flour that interferes with the lipoxygenase, or other enzyme, activity.

The twenty minute drop gives the rate of breakdown and strength of a flour (Shuey et al., 1972). A higher value indicates a weaker flour since it is the change in height from the center of the curve at the peak and the center of the curve twenty minutes

after the first addition of water (Shuey et al., 1972). A large drop would indicate that the flour has weakened. The all-purpose control and the all-purpose with 6.4 precipitate yielded the largest values, significantly larger, than the other samples (Table 13). These samples had almost 50% greater drop than that of the bread flour samples. The samples with added soy flour were stronger than the bread flour samples since the TMD of these samples was about 16 BU less than that of the bread flour samples. Interestingly enough, the samples with added 4.8 precipitate did not yield any drop after 20 minutes. In fact, according to the farinograph (Figure 5), the samples supplemented with the 4.8 precipitate did not see a drop until the 25-27 minute range. This was also an indication that 4.8 precipitate has a positive rheological effect when added to all-purpose flour.

The mean mixing tolerance index is the difference in BUs from the top of the curve at the peak to the top of the curve measured five minutes after the peak has been reached (Shuey et al., 1972). Flours that have a low mixing tolerance index tend to have a good tolerance to mixing; whereas, the higher the tolerance index, the weaker the flour (Shuey et al., 1972). The all-purpose control samples yielded a very large tolerance index when compared with the other samples; however, when it was supplemented with 4.8 precipitate, the sample yielded the lowest tolerance index (Table 14). Therefore, the addition of the concentrated precipitate increased the mixing tolerance and improved the rheological aspects of the all-purpose flour, which is in accordance to Hoseney et al. (1980). They examined that soy flour lipoxygenase increased the mixing tolerance of wheat flour dough (Hoseney et al., 1980). The tolerance index of the 4.8 precipitate samples was much lower than that of the bread flour samples and the samples supplemented with soy flour, though this difference was not found to be significant,

probably due to the large standard deviations. The samples with 6.4 precipitate were not different from the all-purpose flour samples, indicating that there was really no effect of adding the 6.4 precipitate.

### 6.3 Physical and Sensory Bread Attributes

The bread loaves baked with Trump's bread flour had the greatest loaf volume, which was significantly greater than the other loaves (Table 15). All-purpose control loaves were not different from those with added 4.8 precipitate. Therefore, the precipitate did not seem to have any effect on loaf volume, which contradicts the results of the rheological studies. In addition, there was no difference in loaf volume between the 4.8 precipitate loaves and those supplemented with soy flour. Neither soy flour, nor 4.8 precipitate had any effect on loaf volume. An increase in protein content, which occurred when soy bean lipoxygenase was added to all-purpose flour, should have allowed for an increase in loaf volume; however, this was not seen, which could have been due to the inactivation of the enzyme due to heat. In a previous study, no improvements were seen when lipoxygenase was inactivated by heat (Hoseney et al., 1980).

The Hunter color scale was used when determining the difference in color between the bread loaf crust and crumb samples. The L value is an indicator of black and white. A large L value indicates more whiteness in color in a sample (White is when L = 100). Subsequently, a small L value indicates more black (Black is when L = 0). Therefore, the darker a sample, the lower the L value and vice versa. Lipoxygenase has been used for decades as a bleaching agent in bread dough (Gelinas et al., 1998; Hoseney

et al., 1980; Nicolas et al., 1982). The enzyme can be attributed to bleaching carotenoid pigments, which results in a whiter crumb (Gelinas et al., 1998; Nicolas et al., 1982). Since the addition of soy flour or pH 4.8 precipitate did not result in a significantly lighter crumb color, the bleaching effect of the added enzyme was not necessarily seen.

Bread flour loaf crusts were significantly lighter than the other samples. The interaction of proteins and carbohydrates, with the addition of heat, results in the Maillard reaction, or browning of foods (Hegarty, 1982). This reaction can be attributed to differences in crust color. Sensory panelists described the all-purpose control loaves as being significantly lighter in crust color than the other samples ($p<0.05$) (Table 16, 19). The panelists did not find differences in the 4.8 precipitate bread and the bread flour loaves (Table 24). These findings may be inconclusive since the bread in each test was baked on different days; however, the same oven and temperature was used for each trial.

Hunter a values describe whether the sample contains red or green hues, depending on if the value is positive or negative, respectively. Hunter b values describe either yellow or blue values depending on if the value is positive or negative, respectfully. The difference, or $\Delta a$ or $\Delta b$, in the values is determined by calculating the difference in the experimental loaf value minus the control loaf values. If the difference is positive, then the experimental loaf is either redder (when $\Delta a$ is positive) or yellower (when $\Delta b$ is positive) than the control loaves.

For all bread crust samples, there was no difference in a value found. All of the values were positive; therefore, the bread samples contained a red hue as opposed to a green hue. When examining the actual mean a values, the all-purpose loaves with added soy flour were found to have a slightly higher value indicating these samples were redder

than the controls. The two control loaves did not have much of a difference in a value. The all-purpose plus 4.8 precipitate was ever so slightly less red than the other samples.

The two control crust samples were not different from each other when considering the b values (Table 18). The b values of these samples were also positive indicating they were yellower rather than bluer. The difference between the controls and the experimental crust of the loaves is slight, but both experimental loaves had smaller values. The difference between the experimental loaves and the control loaves were negative indicating that the experimental loaves were bluer than the control loaves; however, all samples yielded positive values.

The loaf crumb a values were found to have significant differences ($p<0.05$) (Table 20). The all-purpose crumb color was not different from that of the all-purpose plus soy flour. These values were positive and indicate a redder color rather than greener color. The $\Delta a$ value of the all-purpose plus 4.8 precipitate experimental loaf and the all-purpose control was positive, which indicated that the experimental loaf was redder than the control loaf. The $\Delta a$ value between the 4.8 precipitate loaves and the bread flour control loaf was also positive. Therefore, the experimental loaf crumb was redder than the control loaf crumb. It should be noted that the bread flour crumb yielded a negative mean value. This meant that the bread loaf crumb was greener than any other sample.

Average b values of the bread loaf crumb were found to be significantly different from each other (Table 21). The all-purpose control bread loaf crumb was yellower than that of the bread flour control loaf crumb. Both experimental loaves were also found to be yellower than either of the controls. All of the b values of the bread crumb were positive, indicating that they were all yellower rather than bluer. Differences in the color

between the loaves can probably be attributed to the difference in color of the soy flour versus the all-purpose and bread flour, as well as the color of the 4.8 precipitate.

Crust texture did not differ among the samples, which could have been due to the large standard deviations of the values. Based on the average values, the all-purpose control and the all-purpose plus soy flour sample could indicate a slightly tougher texture because the values were slightly greater than the other samples. A larger texture value indicates more toughness in that more grams of force were required to compress the sample 1 cm.

The results of the QDA sensory panel (Table 24) were in accordance with the results of the texture analyzer in that there were no significant differences in outside toughness found. The value of the 4.8 precipitate loaves was slightly greater than that of the other loaves, which indicated that the outside toughness was harder. This was not the case with the texture analyzer; however, the difference might be attributed to the fact that there were large standard deviations associated with both the values of texture analysis and the QDA tests.

Significant differences in crumb texture were observed in the texture analysis (Table 23). The crumb texture was toughest for the all-purpose plus soy flour loaves, which was significantly tougher than the crumb of the bread flour control loaves ($p<0.05$). The bread flour loaves yielded the softest crumb texture, probably due to the greater volume of the loaves. The other loaf samples had no significant difference in crumb texture. The texture values of the crumb were smaller than those of the crust, which was desired.

The crumb texture analysis of the loaves was not in accordance with the QDA sensory tests. The panelists indicated that the all-purpose control loaves were significantly more chewy (less firm) than the bread flour control loaves or the loaves with 4.8 precipitate ($p<0.05$). The panelists indicated that the 4.8 precipitate loaves were hardest when considering the crumb. The 4.8 loaves were probably harder because the precipitate increased the strength of the dough, as indicated by the farinograph results.

Porosity of the crumb (Table 24) was also described by the sensory panelists. Samples were described as either having dense, small sized air cells or large, airy air cells. Larger air cells would indicate perhaps a greater loaf volume, as well as a softer crumb texture. Whereas small, dense cells would indicate a harder, dense crumb texture and smaller loaf volume might correlate. The all-purpose loaves were described as having large air cells; however, no significant difference was found among the samples. The all-purpose loaves were fluffier; therefore, the fact that they also had the largest air cells makes sense. The other samples were found to have more dense cells, which corresponded with the fact that they were described as harder. Bread flour loaves were found to have the greatest loaf volume, which did not correspond to their dense, hard crumb texture and less porous air cells. All-purpose loaves had smaller volumes than the bread flour loaves, which also did not correspond to the more chewy and airy crumb structure of the all-purpose flour loaves. The all-purpose with added 4.8 precipitate loaves did not have large loaf volumes, and these loaves did show indication of a harder, denser crumb. The porosity of the 4.8 loaves was not found to be as airy as the all-purpose control loaves, but the 4.8 loaves were seemingly similar to the bread flour controls when chewiness was considered. This might have indicated that the pH 4.8

precipitate had an effect on crumb structure as it was very similar to that of the bread flour controls.

### 6.4 Flavor of Bread

QDA sensory analysis included the description of yeastiness, sourness and general flavor (bland or more intense) of the bread samples (Table 24). Yeastiness was defined as the degree of fermented yeast smelled and/or tasted. A small value would indicate less yeastiness versus a large value that would indicate a stronger, more intense yeastiness of the bread samples. Among the samples, no difference was seen when considering yeastiness, which was expected since the same amount of yeast was used in each loaf. The values obtained from the QDA test were basically the same for each sample; bread flour and all-purpose plus 4.8 were 5.63, while the all-purpose control was 5.61. All samples contained the same amount of yeast, and therefore, no difference in yeastiness was desired.

Sourness was described as either less (bland) or more intense (sourdough-like) (Table 24). No significant differences were found among the samples in sourness. All-purpose loaves indicated a slightly higher value than the other two samples, which meant panelists thought those loaves (all-purpose) were more sour, but not significantly more sour. A difference in sourness was not desired because loaves were meant to have no taste difference.

Finally, the overall intensity of flavor was assessed by panelists. No significant difference was determined from the tests, which also was desired. Small values were seen, indicating the bread did not have intense flavor. Loaves were made exactly the

same, with the exact same proportions of ingredients, with the exception of the type of flour. The experimental loaves contained less all-purpose flour and had added soy flour or precipitate in the same amount; the total amount of all-purpose flour plus either precipitate or soy flour equaled the proportion of flour used in the control loaves. Since part of the purpose of the research was to see if adding soy products to bread would affect the flavor, the fact that no significant difference was seen between the experimental loaves and the controls was very well desired. Therefore, the addition of 4.8 precipitate did not seem to have an effect on flavor when added to bread.

# Chapter 7: Conclusions of Research

There were three research objectives of this study. They included: determining the protein fraction of soy flour with the greatest lipoxygenase activity, determining rheological and physical attributes of bread samples, and conducting QDA sensory analysis of bread samples. It was determined that soy protein fractions at pH 4.8 yielded the greatest lipoxygenase activity. This made sense because the 7S globulin fraction, which is thought to contain the enzyme lipoxygenase, precipitates at a pH of 4.8; therefore, the activity was greatest in that fraction. Since the 4.8 fraction had the greatest activity, it was utilized in further tests as an experimental adjunct. In addition, soy flour was also used as an experimental adjunct to examine differences in the protein fraction and the product as a whole.

The farinograph results indicated that the addition of the 4.8 soy flour precipitate increased the strength of all-purpose flour. The farinographs of the 4.8 precipitate trials yielded longer stability times than any other sample, as well as almost no drop after twenty minutes. The farinograph results indicated that the addition of the 4.8 precipitate to all-purpose flour yielded similar results of that of the bread flour trials, with even more strength than the bread flour samples. The addition of the concentrated precipitate increased the mixing tolerance and improved the rheological aspects of the all-purpose flour, which is in accordance to Hoseney et al. (1980). Overall, the addition of soy flour had some effect in increasing the strength of all-purpose flour but not to the same degree as the added 4.8 precipitate.

Bread flour control loaves had the greatest loaf volume, while the soy flour supplemented bread had the smallest average loaf volume. The addition of 4.8

precipitate to all-purpose bread loaves did not improve the loaf volume. The average volumes of the all-purpose control breads were greater, but not significantly, than the pH 4.8 precipitate supplemented bread. The color of the crust and crumb of the bread measured was mostly in accordance with the sensory panel. All-purpose flour bread loaves had a lighter crust than bread flour loaves; however, differences in crust color could be attributed to the fact that the different bread loaves were baked separately. Crumb color, while there were slight differences between the different samples, did not differ greatly.

Bread loaf texture was measured through the use of a texture analyzer, and sensory panelists described different bread texture attributes. Both the texture analyzer and the panelists saw no differences between the samples when the crust texture was considered. Additionally, panelists found that the all-purpose flour bread loaf crumb was softer, while the texture analyzer determined that bread flour loaves had the softer crumb. The addition of 4.8 precipitate to bread did not result in a softer crumb according to the texture analysis. However, according to the sensory results, panelists found the crumb to be significantly harder than that of the all-purpose loaves. In other words, the 4.8 precipitate might have caused the crumb of the bread to increase in toughness.

Overall, the addition of pH 4.8 soy flour precipitate obtained through isoelectric precipitation was seen to have some effects on dough rheological properties and bread properties. Rheologically, the addition of pH 4.8 precipitate to all-purpose flour increased the strength of the dough to mimic, at the very least, the strength of bread flour. Soy flour, as a whole, also had similar effects, but not to the same degree. However, this increase in strength did not mimic the physical properties that bread flour exhibited.

Bread flour loaves were found to have greater volume and a softer crumb. The addition of pH 4.8 precipitate caused a slight decrease in volume, which translated to a firmer crumb. Bread flavor among all samples was not found to be affected by the different treatments. While pH 4.8 precipitate seemed to improve bread rheological properties, no improvements on the physical aspects of bread were observed. Heat causes proteins to denature, or undergo conformational changes, which can affect the functionality of the enzyme (Zayas, 1997). As previously mentioned, the most abundant form of soy bean lipoxygenase is not heat stable; therefore, it might be assumed that the enzyme was inactivated due to heat during bread making. In addition, pH plays a role in influencing physical properties of dough and bread (Eliasson and Larsson, 1993). The pH 4.8 precipitate added to the dough could have contributed to a change (or lowering) in overall pH, therefore, affecting physical aspects of bread. Heat inactivation of the enzyme or the lowered pH of the bread due to the added precipitate might have caused there to be no effect seen. More in-depth research should be performed to confirm these findings as there are limited publications thus far on the subject matter.

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# Appendix A: Farinograph Parameters Measured on Different Flour Samples

| Sample | Absorption (%) | Arrival Time (min) | Peak Time (min) | Stability (min) | Departure Time (min) | Twenty Minute Drop (BU) |
|---|---|---|---|---|---|---|
| | | | | | | |
| **All-Purpose Flour** | | | | | | |
| Rep 1 | 63.00% | 1.00 | 2.00 | 4.00 | 5.00 | 70.00 |
| Rep 2 | 62.80% | 1.00 | 1.50 | 4.00 | 5.00 | 90.00 |
| Rep 3 | 63.20% | 1.50 | 2.00 | 4.50 | 6.00 | 70.00 |
| | | | | | | |
| **Trump's Bread Flour** | | | | | | |
| Rep 1 | 63.00% | 2.00 | 10.00 | 21.00 | 23.00 | 40.00 |
| Rep 2 | 64.00% | 2.00 | 9.50 | 25.00 | 27.00 | 40.00 |
| Rep 3 | 64.00% | 2.00 | 11.00 | 25.00 | 27.00 | 30.00 |
| | | | | | | |
| **All-Purpose + Soy Flour** | | | | | | |
| Rep 1 | 61.80% | 1.25 | 11.00 | 16.25 | 17.50 | 20.00 |
| Rep 2 | 61.80% | 1.25 | 10.50 | 17.25 | 18.50 | 20.00 |
| Rep 3 | 62.20% | 1.00 | 10.50 | 16.50 | 17.50 | 20.00 |
| | | | | | | |
| **All-Purpose + 4.8 Precipitate** | | | | | | |
| Rep 1 | 62.80% | 1.00 | 9.50 | 23.50 | 24.50 | 0.00 |
| Rep 2 | 62.00% | 1.50 | 10.00 | 25.00 | 26.50 | 0.00 |
| Rep 3 | 61.30% | 1.25 | 10.00 | 25.75 | 27.00 | 0.00 |
| | | | | | | |
| **All-Purpose + 6.4 Precipitate** | | | | | | |
| Rep 1 | 61.90% | 1.25 | 2.00 | 12.75 | 14.00 | 70.00 |
| Rep 2 | 61.90% | 1.00 | 2.00 | 17.00 | 18.00 | 80.00 |
| Rep 3 | 62.00% | 1.00 | 2.00 | 1.00 | 3.00 | 70.00 |
| | | | | | | |
| **All-Purpose + Lipoxidase** | | | | | | |
| Rep 1 | 62.50% | 1.75 | 2.50 | 6.25 | 8.00 | 40.00 |
| Rep 2 | 62.20% | 1.75 | 2.50 | 5.25 | 7.00 | 50.00 |
| Rep 3 | 62.20% | 1.75 | 2.50 | 9.25 | 11.00 | 40.00 |
| | | | | | | |
| **All-Purpose + Soy Flour + Inhibitor** | | | | | | |
| Rep 1 | 62.10% | 1.00 | 11.50 | 7.00 | 18.50 | 30.00 |
| Rep 2 | 62.10% | 1.00 | 12.00 | 6.50 | 18.50 | 30.00 |
| Rep 3 | 62.10% | 1.00 | 11.50 | 6.50 | 18.00 | 35.00 |

## Appendix B: Farinographs of Rheological Studies

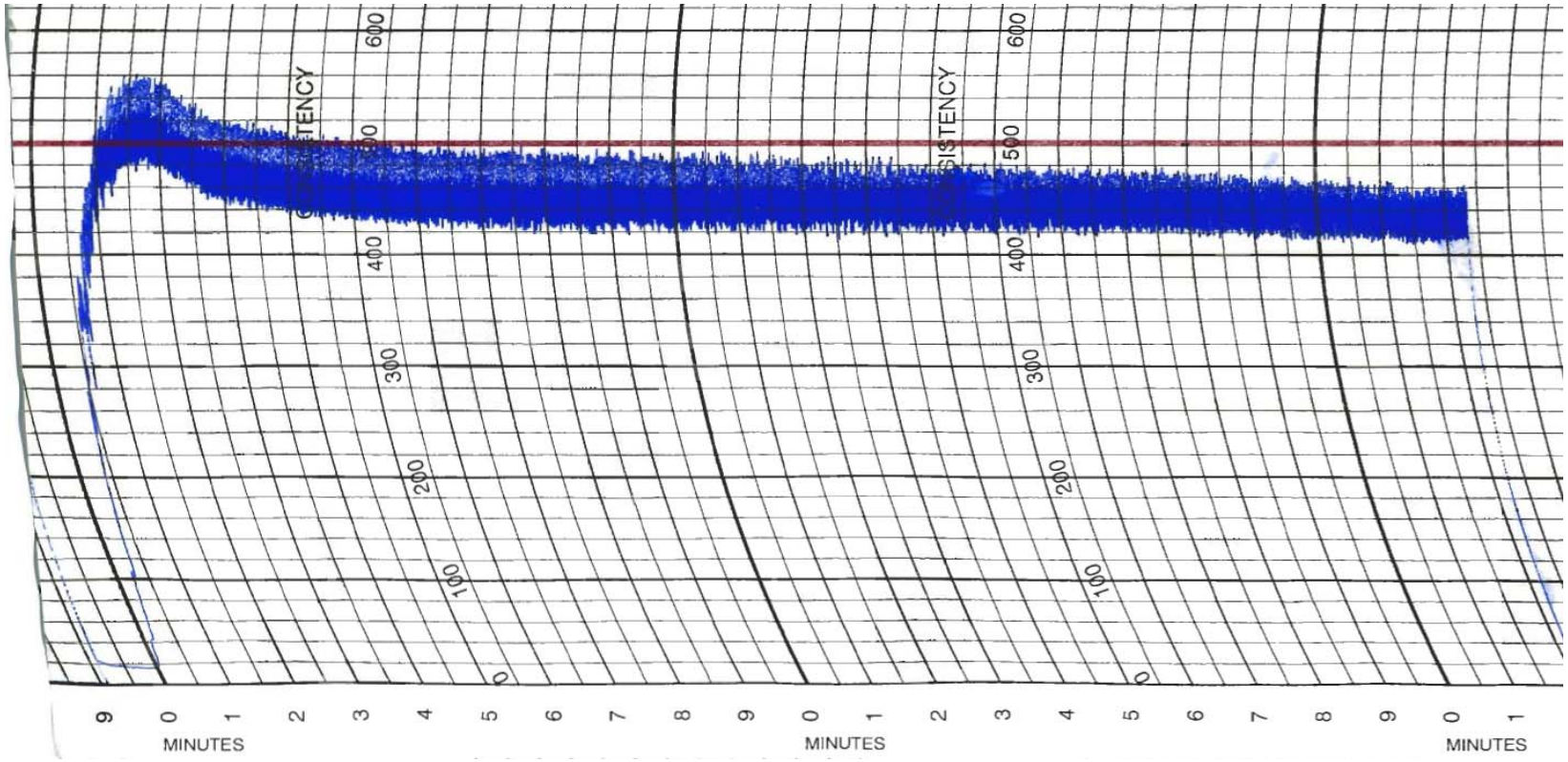


Figure 2: Farinograph of All-Purpose Flour (50g) and 63% Water

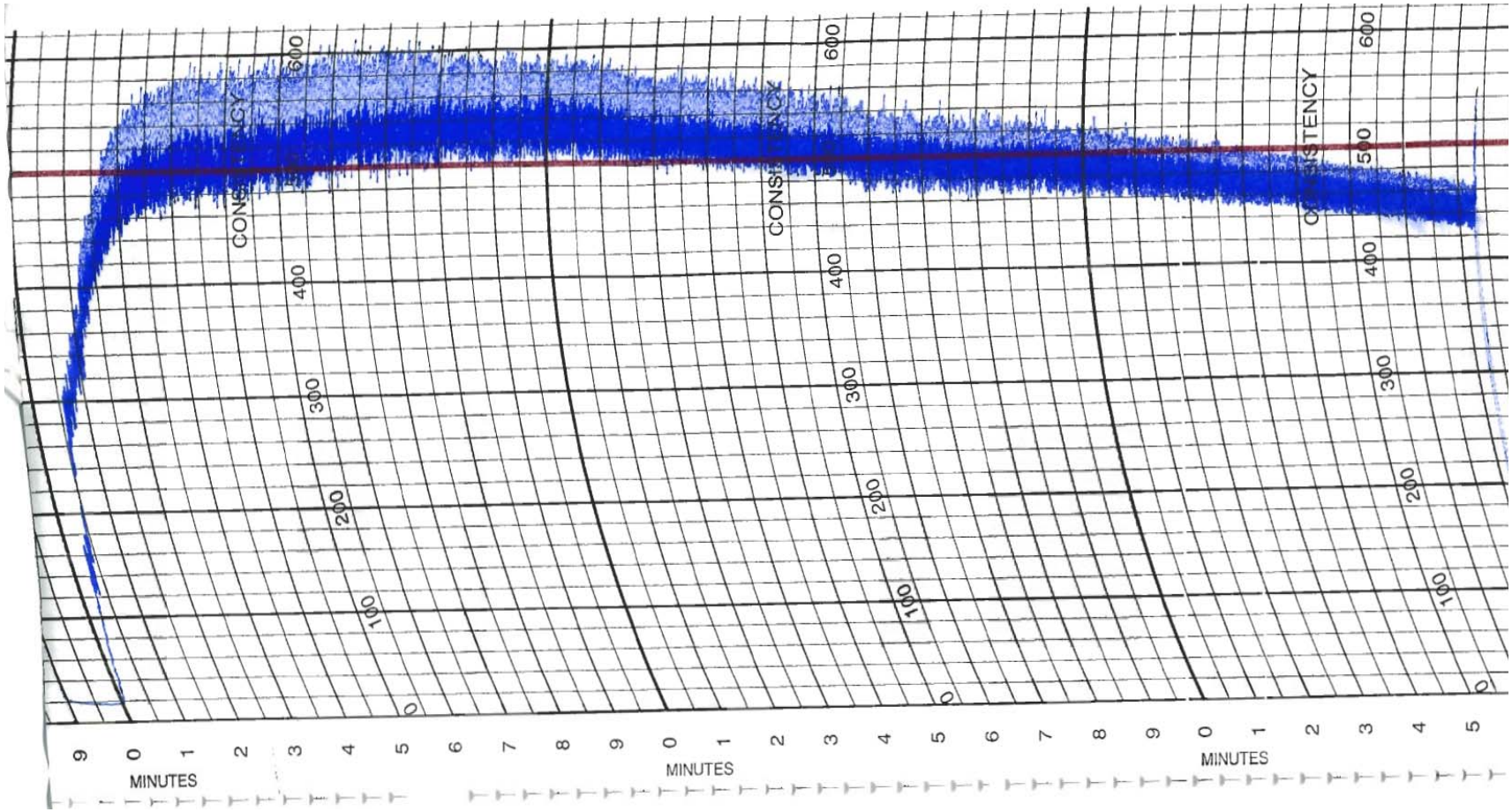


Figure 3: Farinograph of Trump's Bread Flour (50g) and 63% Water

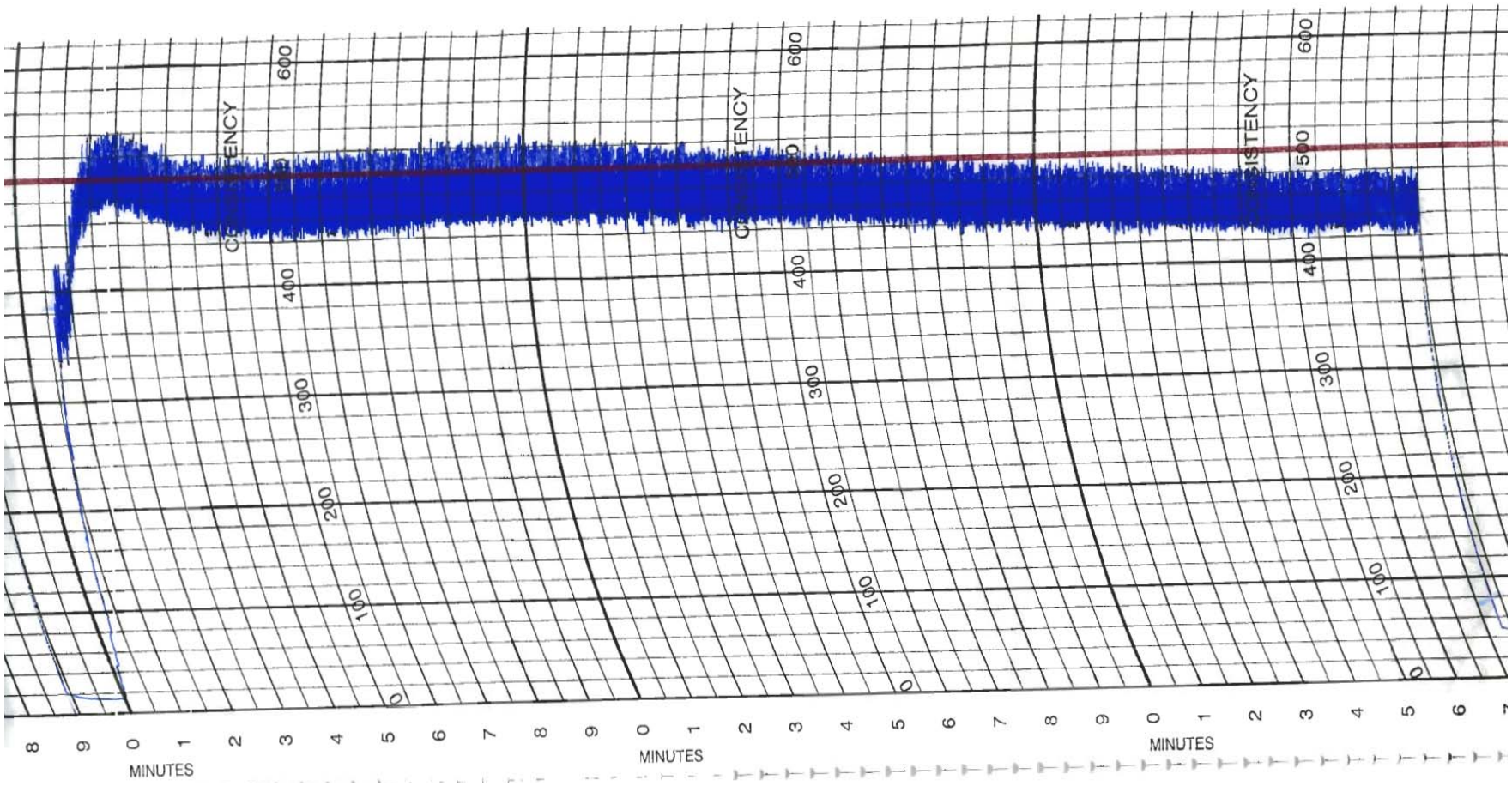


Figure 4: Farinograph of All-Purpose Flour (47.19g), Soy Flour (2.81g) and 61.8% Water

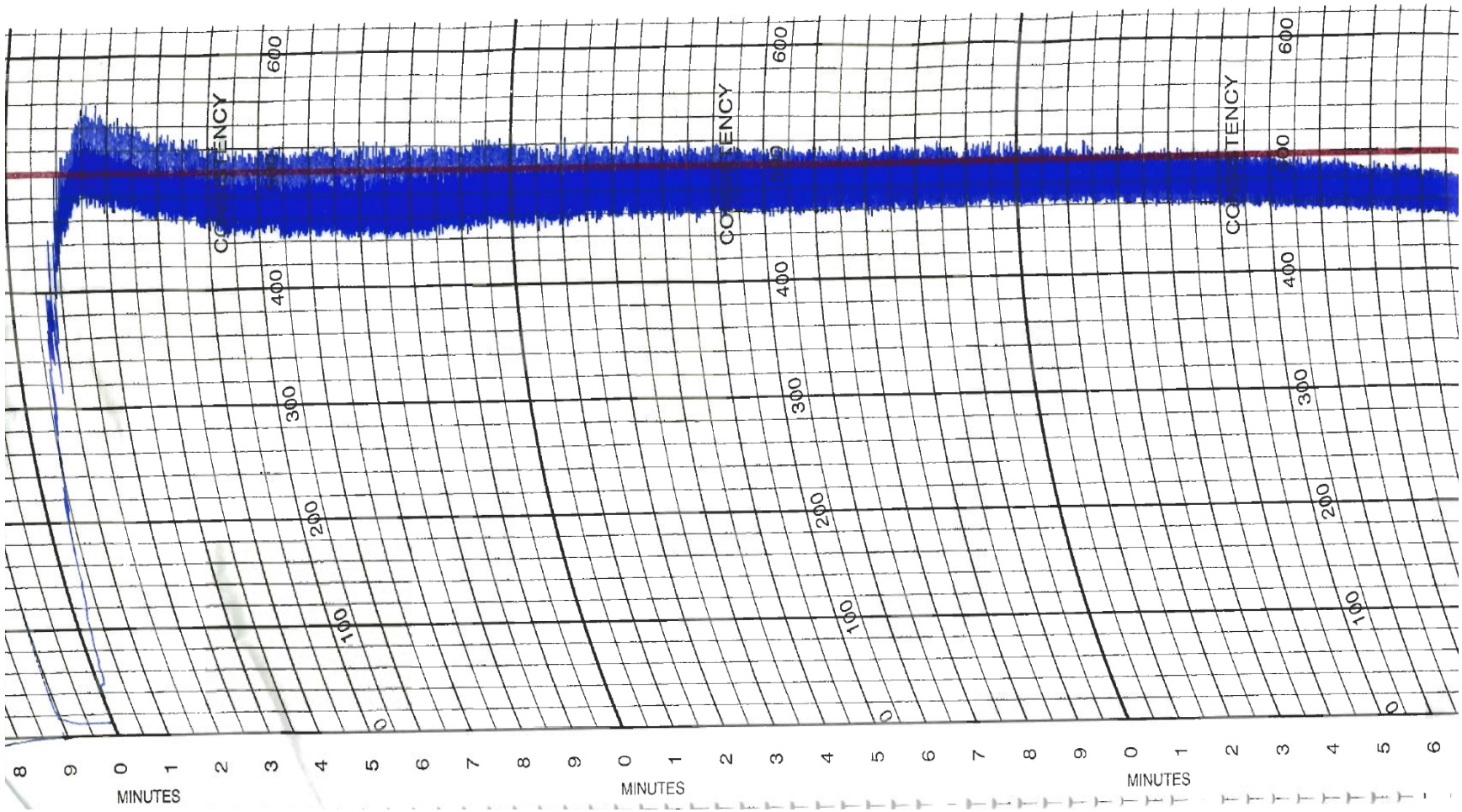


Figure 5: Farinograph of All-Purpose Flour (47.19g), pH 4.8 Precipitate (2.81g) and 62.8% Water

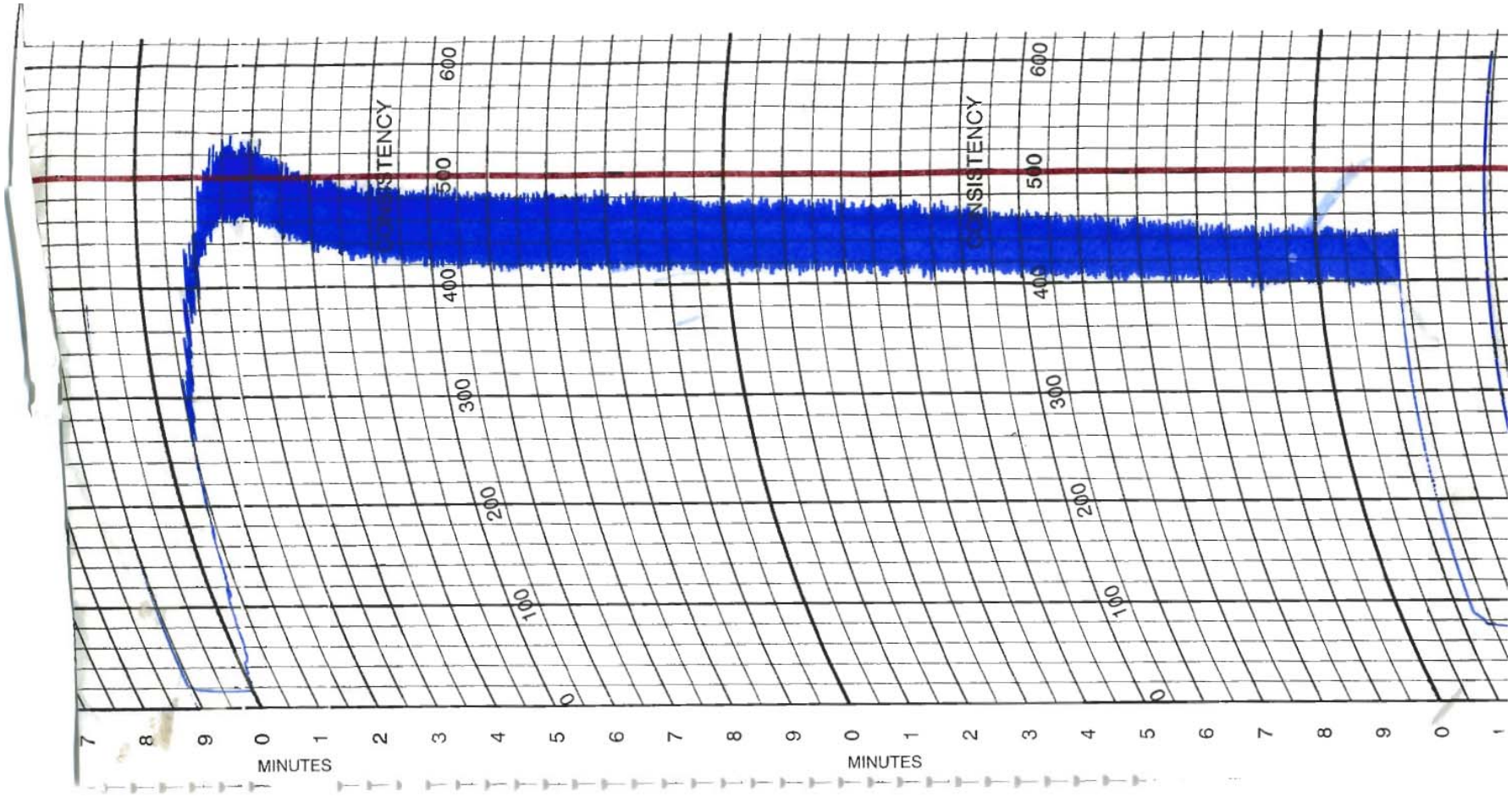


Figure 6: Farinograph of All-Purpose Flour (47.19g), pH 6.4 Precipitate (2.81g) and 62% Water

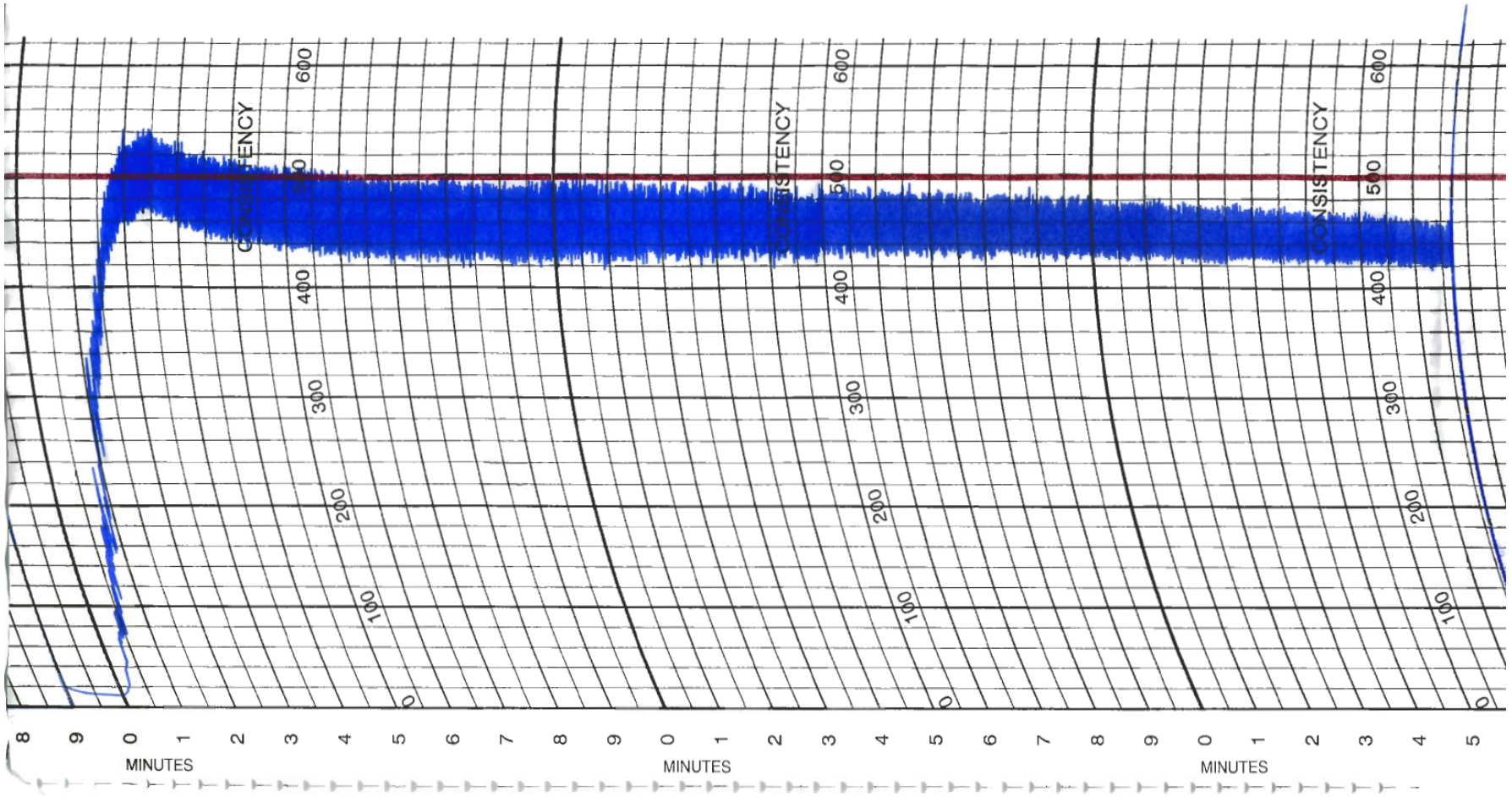


Figure 7: Farinograph of All-Purpose Flour (50g), Dissolved Lipoxidase (25mg) and 62.2% Water

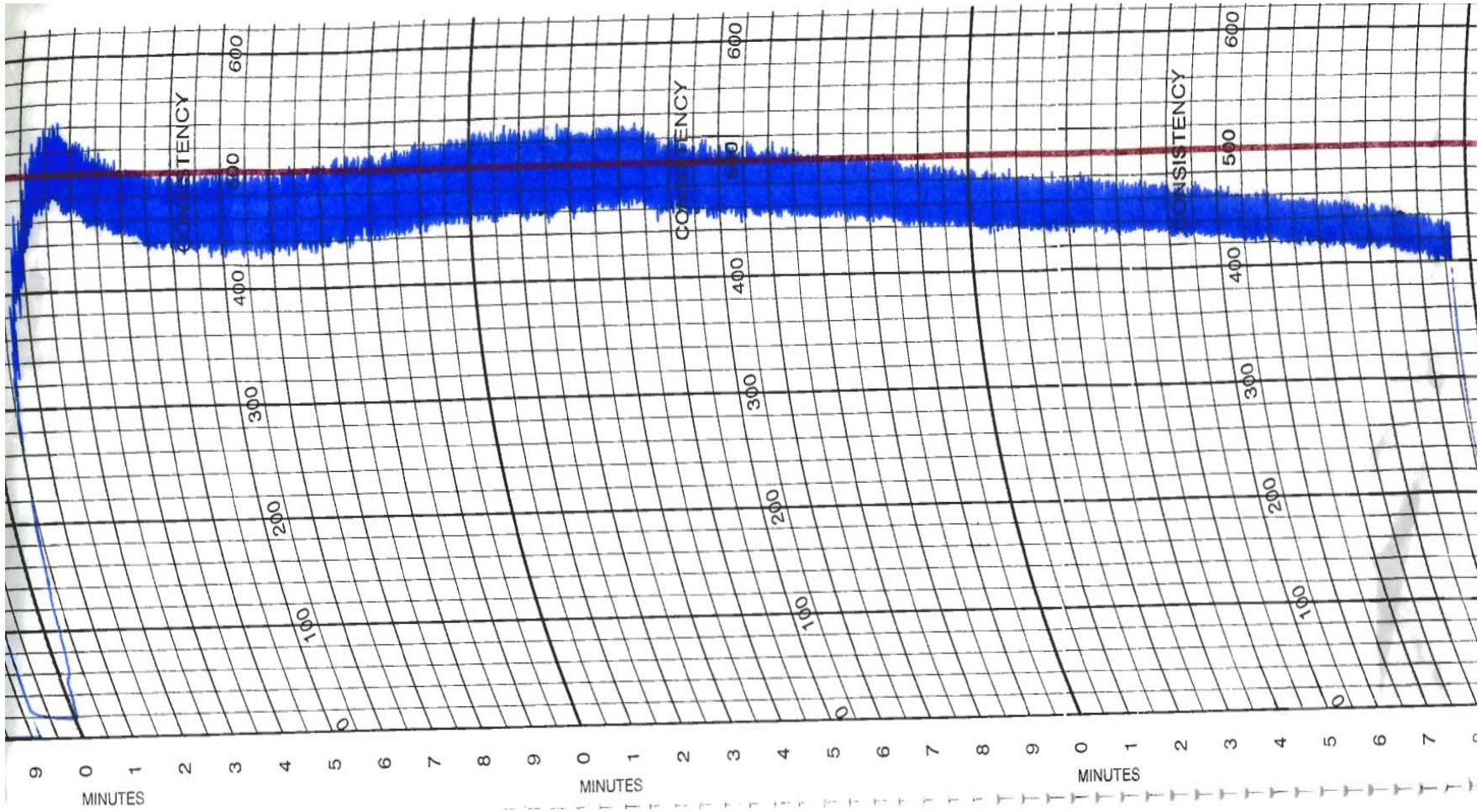


Figure 8: Farinograph of All-Purpose Flour (47.19g), Soy Flour (2.81g), Esculetin (15mg) and 62.1% Water

# Appendix C: Physical Attributes of Bread Loaves

| All-Purpose Flour | Volume (cm3) | Crust Color | | | Crumb Color | | | Crust Texture (g) | Crumb Texture (g) |
|---|---|---|---|---|---|---|---|---|---|
| | | L | a | b | L | a | b | | |
| Rep 1A | 625 | 55.73 | +14.04 | +29.02 | 76.58 | +0.34 | +19.31 | 445.9236 | 256.1027 |
| Rep 1B | 650 | 55.78 | +13.43 | +30.55 | 78.28 | +0.31 | +19.16 | 501.6003 | 245.1917 |
| **Average Rep 1** | **637.5** | **55.76** | **13.74** | **29.79** | **77.43** | **0.33** | **19.24** | **473.76195** | **250.6472** |
| Rep 2A | 625 | 57.80 | +12.55 | +30.29 | 75.58 | +0.29 | +19.21 | 536.7296 | 210.1133 |
| Rep 2B | 625 | 55.95 | +13.51 | +30.38 | 76.07 | +0.34 | +19.31 | 424.7134 | 247.9449 |
| **Average Rep 2** | **625** | **56.88** | **13.03** | **30.34** | **75.83** | **0.32** | **19.26** | **480.7215** | **229.0291** |
| Rep 3A | 625 | 55.93 | +13.53 | +29.74 | 76.70 | +0.35 | +19.33 | 374.6961 | 177.5842 |
| Rep 3B | 625 | 55.58 | +13.04 | +28.94 | 75.72 | +0.52 | +19.99 | 391.9804 | 204.5558 |
| **Average Rep 3** | **625** | **55.76** | **13.29** | **29.34** | **76.21** | **0.44** | **19.66** | **383.33825** | **191.07** |
| Trump's Bread Flour | Volume (cm3) | Crust Color | | | Crumb Color | | | Crust Texture (g) | Crumb Texture (g) |
| | | L | a | b | L | a | b | | |
| Rep 1A | 750 | 59.74 | +12.53 | +28.53 | 76.51 | +0.01 | +18.38 | 478.0283 | 201.1398 |
| Rep 1B | 750 | 59.07 | +12.80 | +29.11 | 80.76 | -0.08 | +19.28 | 477.178 | 168.0499 |
| **Average Rep 1** | **750** | **59.41** | **12.67** | **28.82** | **78.64** | **-0.04** | **18.83** | **477.60315** | **184.59485** |
| Rep 2A | 775 | 55.84 | +14.36 | +31.05 | 80.65 | -0.07 | +19.37 | 388.5643 | 175.4428 |
| Rep 2B | 725 | 57.65 | +14.53 | +33.90 | 78.63 | -0.20 | +18.93 | 391.7254 | 154.1307 |
| **Average Rep 2** | **750** | **56.75** | **14.45** | **32.48** | **79.64** | **-0.14** | **19.15** | **390.14485** | **164.78675** |
| Rep 3A | 725 | 57.55 | +13.53 | +31.04 | 77.05 | -0.08 | +18.69 | 315.9602 | 169.9873 |
| Rep 3B | 750 | 60.78 | +11.80 | +27.34 | 79.74 | -0.15 | +18.65 | 321.2118 | 191.9623 |
| **Average Rep 3** | **737.5** | **59.165** | **12.665** | **29.19** | **78.395** | **-0.115** | **18.67** | **318.586** | **180.9748** |
| All-Purpose + Soy Flour | Volume (cm3) | Crust Color | | | Crumb Color | | | Crust Texture (g) | Crumb Texture (g) |
| | | L | a | b | L | a | b | | |
| Rep 1A | 575 | 55.48 | +13.45 | +30.36 | 74.70 | +0.48 | +21.16 | 577.3145 | 365.6206 |
| Rep 1B | 550 | 52.65 | +13.93 | +29.32 | 75.42 | +0.40 | +21.08 | 611.5771 | 346.3479 |
| **Average Rep 1** | **562.5** | **54.07** | **13.69** | **29.84** | **75.06** | **0.44** | **21.12** | **594.4458** | **355.98425** |
| Rep 2A | 600 | 56.23 | +12.70 | +28.90 | 76.15 | +0.19 | +21.14 | 388.1564 | 211.5919 |
| Rep 2B | 575 | 54.33 | +13.92 | +30.05 | 76.19 | +0.25 | +21.28 | 352.9761 | 249.0156 |
| **Average Rep 2** | **587.5** | **55.28** | **13.31** | **29.48** | **76.17** | **0.22** | **21.21** | **370.56625** | **230.30375** |
| Rep 3A | 550 | 52.26 | +14.23 | +30.08 | 76.93 | +0.25 | +21.10 | 367.0992 | 276.2931 |
| Rep 3B | 525 | 52.18 | +13.85 | +28.35 | 74.16 | +0.44 | +21.78 | 441.7427 | 222.197 |
| **Average Rep 3** | **537.5** | **52.22** | **14.04** | **29.215** | **75.545** | **0.345** | **21.44** | **404.42095** | **249.24505** |
| All-Purpose + 4.8 Precipitate | Volume (cm3) | Crust Color | | | Crumb Color | | | Crust Texture (g) | Crumb Texture (g) |
| | | L | a | b | L | a | b | | |
| Rep 1A | 550 | 56.45 | +12.45 | +26.23 | 73.31 | +0.75 | +19.28 | 388.0035 | 238.0027 |
| Rep 1B | 600 | 55.53 | +12.55 | +27.25 | 74.45 | +0.79 | +19.93 | 365.6716 | 263.4446 |
| **Average Rep 1** | **575** | **55.99** | **12.50** | **26.74** | **73.88** | **0.77** | **19.61** | **376.83755** | **250.72365** |
| Rep 2A | 575 | 55.19 | +13.85 | +29.10 | 74.08 | +0.78 | +20.05 | 367.2522 | 216.9454 |
| Rep 2B | 550 | 54.98 | +13.36 | +26.82 | 74.07 | +0.76 | +19.80 | 384.9443 | 266.0959 |
| **Average Rep 2** | **562.5** | **55.09** | **13.61** | **27.96** | **74.08** | **0.77** | **19.93** | **376.09825** | **241.52065** |
| Rep 3A | 650 | 56.40 | +12.44 | +27.26 | 75.73 | +0.79 | +20.06 | 371.8409 | 193.084 |
| Rep 3B | 675 | 54.88 | +13.84 | +30.17 | 75.63 | +0.81 | +19.74 | 373.7784 | 218.3221 |
| **Average Rep 3** | **662.5** | **55.64** | **13.14** | **28.715** | **75.68** | **0.8** | **19.9** | **372.80965** | **205.70305** |

# Appendix D: Example of QDA Scorecard

Color of outside

Light Dark

Uniformity/unevenness of outside

Uneven Even

Porosity of inside

Airy Dense

Chewiness of inside

Fluffy Firm

Toughness of outside

Soft Hard

Yeasty

Less More

Sourness

Less More

Flavor

Bland Strong

# Appendix E: Definitions and Spectrum of Sensory Attributes for Bread

| *Term* | *Definition* |
|---|---|
| Color of outside | Degree of darkness (from baking or type of dough) of the crust on side of bread sample (inside bread pan portion)<br>Dark = dark brown coloring<br>Light = light golden coloring |
| Uniformity of outside | Degree of evenness of color of crust on side of bread sample<br>Even = consistent coloring<br>Uneven = speckled, blotchy appearance |
| Porosity of inside | Size of air cells/air pockets inside of bread<br>Dense = small sized air cells<br>Airy = large sized air cells |
| Chewiness of inside | Degree of density of inside of bread<br>Firm = holds shape when force applied to bread<br>Fluffy = squishes down easily when force applied to bread |
| Toughness of outside | Degree of toughness of outside of bread<br>Soft = flexible, stays together even when force applied<br>Hard = crusty, breaks off when force applied |
| Yeasty | Degree of fermented yeast smell<br>More = strong yeast scent, taste<br>Less = little to no yeast scent, taste |
| Sourness | Degree of tanginess, lingering aftertaste<br>More = sour like sourdough bread, has aftertaste<br>Less = no sour flavor, bland |
| Flavor | Degree of flavor when sample is tasted<br>Strong = intense flavor<br>Bland = less flavor |

# Appendix F: Standards (Products) Used for Training Sensory Panel

| *Term* | *Standard or Product* |
|---|---|
| Color of outside | Light: sourdough rolls<br>Dark: rye bread |
| Uniformity of outside | Uneven: Marbled cake<br>Even: Commercial sliced white bread |
| Porosity of inside | Airy: Commercial sliced white bread<br>Dense: Angel food cake |
| Chewiness of inside | Fluffy: Commercial sliced white bread<br>Firm: Stone-ground whole wheat bread |
| Toughness of outside | Soft: Hawaiian bread<br>Hard: Crusty French baguette |
| Yeasty | Less: Commercial sliced white bread<br>More: Rising bread, yeast fermented in water |
| Sourness | Less: Commercial sliced white bread<br>More: Sourdough bread |
| Flavor | Bland: Commercial sliced white bread<br>Strong: Rye bread |

# VITA

Erin M. Danielson was raised in Clifton, Virginia by her parents Ralph and Jackie. She has one brother, Jon. She graduated from Centreville High School in 2000 and began her undergraduate studies at Virginia Tech that fall. She graduated cum laude in May of 2004 with a B.S. in Human Nutrition, Foods and Exercise. Upon completion of her undergraduate studies, she made the decision to pursue her Master's degree right away. She continued her education at Virginia Tech to earn a M.S. in Human Nutrition, Foods and Exercise, focusing on Foods. She hopes to pursue a career in the food industry before considering any more school.