THE EFFECT OF WHOLE GRAIN RYE FLOUR ARABINOXYLANS ON THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF A LOW MOISTURE BAKED GOOD

By

MICHELLE DENINE BEAVER

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ABSTRACT OF THE DISSERTATION

The Effect of Whole Grain Rye Flour Arabinoxylans on the Physical and Chemical Characteristics of a Low Moisture Baked Good

By MICHELLE DENINE BEAVER

Dissertation Director
Dr. Chi-Tang Ho

In recent years, greater emphasis has been placed on the health benefits of whole grains. Studies have shown that whole grains are a source of fiber, reduce the risk of cardiovascular disease, diabetes and stroke and may help achieve weight loss. Rye, a cereal typically consumed as a whole grain, possesses such benefits.

Arabinoxylans are non-starch polysaccharides comprised of a β-(1,4) linked D-xylopyranosyl backbone with α-L-arabinofuranose units attached as side residues via the α-(1,3) and/or α-(1,2) linkages. These compounds are found in whole grain and are particularly high in whole grain rye flour. These compounds are purported to contributor to the many health benefits associated with whole grain rye. While there are many health benefits attributed to arabinoxylans, they greatly impact dough rheology and baking by binding water, softening the dough and altering gluten functionality.

The removal or alteration of a portion of the arabinoxylans via water extraction or enzymatic degradation will change their functionality significantly. This has been demonstrated in wheat systems including wheat doughs and wheat breads but fewer studies have been conducted utilizing whole grain rye flour in whole grain rye doughs and low moisture baked goods such as cookies. Therefore, it is hypothesized that the chemical structure of arabinoxylans in whole grain rye flour will affect the product attributes of low moisture baked goods such as cookies.

The hypothesis can be tested by characterizing whole grain rye flour arabinoxylans which have been water extracted or enzymatically degraded with a variety of enzymes including *Bacillus subtilis, Aspergillus niger* and arabinofuranosidase. In model dough systems, it is evident that the use of the enzyme *Bacillus subtilis* produces a dough which requires less water and is less viscous vs. a control. In a model cookie system, the addition of *Bacillus subtilis* produces a cookie with a larger geometry. The *Aspergillus niger* and arabinofuranosidase show some differences vs. the control sample using these test methods as well.

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I. INTRODUCTION

In recent years, greater emphasis has been placed on the health benefits of whole grains. Studies have shown that whole grains are a source of soluble and insoluble fiber, vitamins, minerals, phenolics, sterols, etc (www.wholegrainscouncil.org - a, http://rye.vtt.fi, www.sciencedaily.com, Liu, 2007). The consumption of whole grains may lower blood cholesterol, stabilize blood glucose, increase satiety and promote the growth of beneficial bacterial in the gut. While wheat and oats are widely used as sources of whole grain in the United States, an additional cereal grain which is less popular in the United States but of great importance is rye.

Whole grain rye flour possesses the health benefits mentioned above and one of the components which contribute to its health benefits is arabinoxylans. Arabinoxylans (AX) are non-starch polysaccharides comprised of a β-(1,4) linked D-xylopyranosyl backbone with α-L-arabinofuranose units attached as side residues via α-(1,3) and/or α-(1,2) linkages (Figure 1). These compounds which are inherent to whole grains are relatively higher in whole grain rye.

As with many other health and wellness ingredients, the incorporation of whole grain rye flour into food products may be challenging. Arabinoxylans impact dough rheology and baking by interacting with other flour components such as gluten, starch and β-glucan (Izydorczyk and MacGregor, 2000).

Arabinoxylans also bind water, compete with other dough components for water, soften the dough, alter the gluten functionality and vary the intrinsic viscosity of the dough (Muralikrishna, 2007; Frederix et al., 2004a).

The removal or alteration of a portion of the arabinoxylans via water extraction or enzymatic degradation will change their functionality significantly (Verwimp et al., 2006). This has been demonstrated in wheat systems including wheat doughs and wheat breads but fewer studies have been conducted utilizing whole grain rye flour in doughs and low moisture baked goods such as cookies. Therefore, it is hypothesized that the chemical structure of arabinoxylans in whole grain rye flour will affect the product attributes of low moisture baked goods such as cookies. The hypothesis can be tested by completing the objectives discussed below which include the characterization of the whole grain rye flour and the water soluble and enzyme degraded arabinoxylans.

The first objective which includes evaluating the physical and chemical properties of whole grain rye flour with and without enzymes can be accomplished via the viscosity, water retention and the dough rheology of a model system (Aryee et al., 2006). The second objective includes evaluating the finished product attributes of cookies produced with whole grain rye flour with or without the incorporation of enzymes. The third objective involves screening various enzymes such as xylanase and arabinofuranosidase and enzyme levels.

The fourth objective includes a brief characterization the arabinoxylans that are water extractable and enzyme degradable.

The confirmation of this hypothesis will provide a greater understanding of whole grain rye flour and give product developers and researchers a greater insight on the appropriate actions to take in order to use whole grain rye flour more effectively. Finally, the increased use of this whole grain rye flour may help impart health benefits to the consumer.

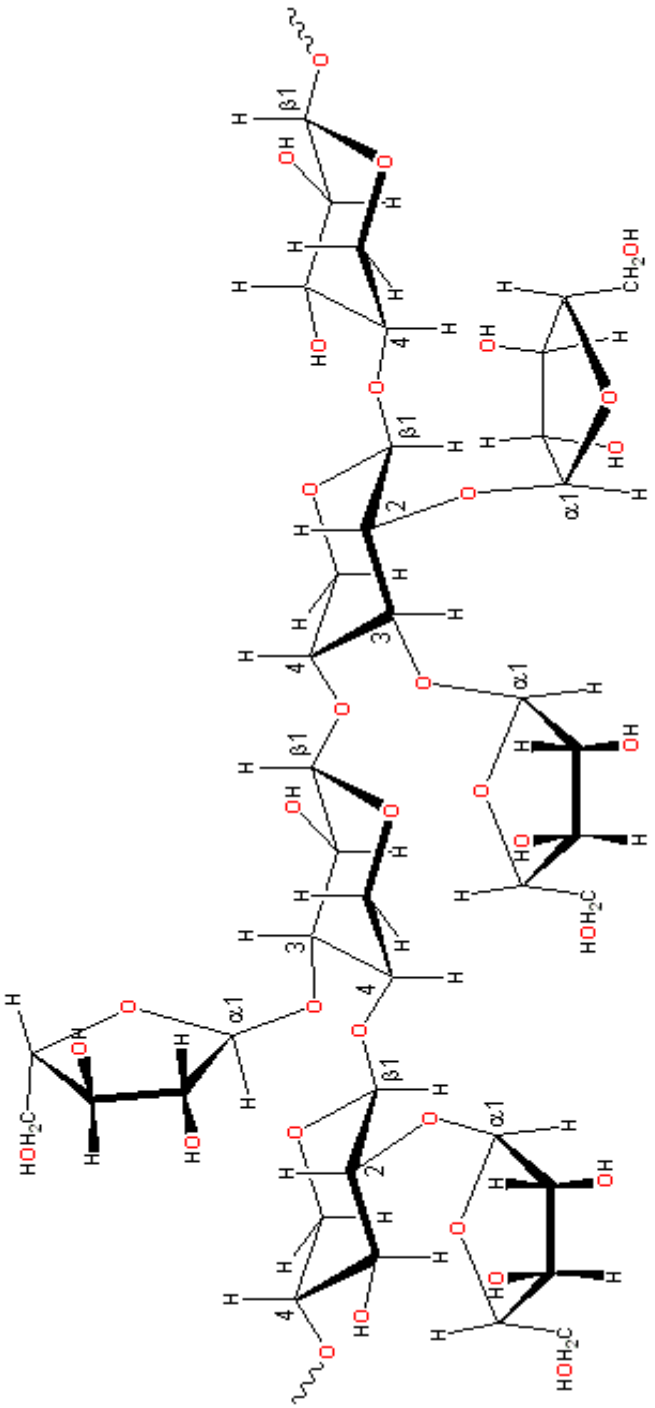

Figure 1. Structure of arabinoxylan

(www.lsbu.ac.uk)

II. LITERATURE REVIEW

A. Cereal Grains

1. Definition and Composition

Many researchers define cereal grains as members of the grass family mostly cultivated for their edible seeds, commonly referred to as the kernel portion of the grain. The most common cereal grains include wheat, rice, corn, barley, oat, rye, sorghum, triticale and millet. For the last several thousand years, humans have relied on grains as a main staple in their diet (Stoskopf, 1985a; Slavin, 2004). The strong appeal of grains is driven by the vast nutritional benefits they contribute to the diet. Many of these nutritional benefits are evident when they are consumed whole e.g. as whole grain flour verses a refined flour. The Whole Grain Council defines whole grains as cereal grains

> "or foods made from them contain all the essential parts and naturally-occurring nutrients of the entire grain seed. If the grain has been processed (e.g., cracked, crushed, rolled, extruded, and/or cooked), the food product should deliver approximately the same rich balance of nutrients that are found in the original grain seed." (www.wholegrainscouncil.org - b)

Figure 2 is a very general depiction of portions of a grain seed or kernel. The kernel contains the endosperm which is the starchy inner portion of the kernel. It typically comprises 80 – 85% of the kernel and most of the carbohydrates are stored in this portion. The endosperm contains a small portion of protein as well. The endosperm portion is typically refined/processed to produced refined flours such as white wheat

flour. Approximately 15% or less of the kernel consists of the germ which is also an interior portion of the kernel. The germ is rich in antioxidants such as vitamin E and B and other vitamins such as thiamin, niacin, riboflavin and pantothenic acid. The remainder of the kernel is composed of the bran at approximately 2.5%. This is the outer portion of the kernel and contains most of the fiber found in whole grain. A majority of the trace minerals such as calcium, magnesium, potassium, phosphorus, sodium and iron are present in the bran. The B vitamins which are present in the endosperm are present in this portion as well.

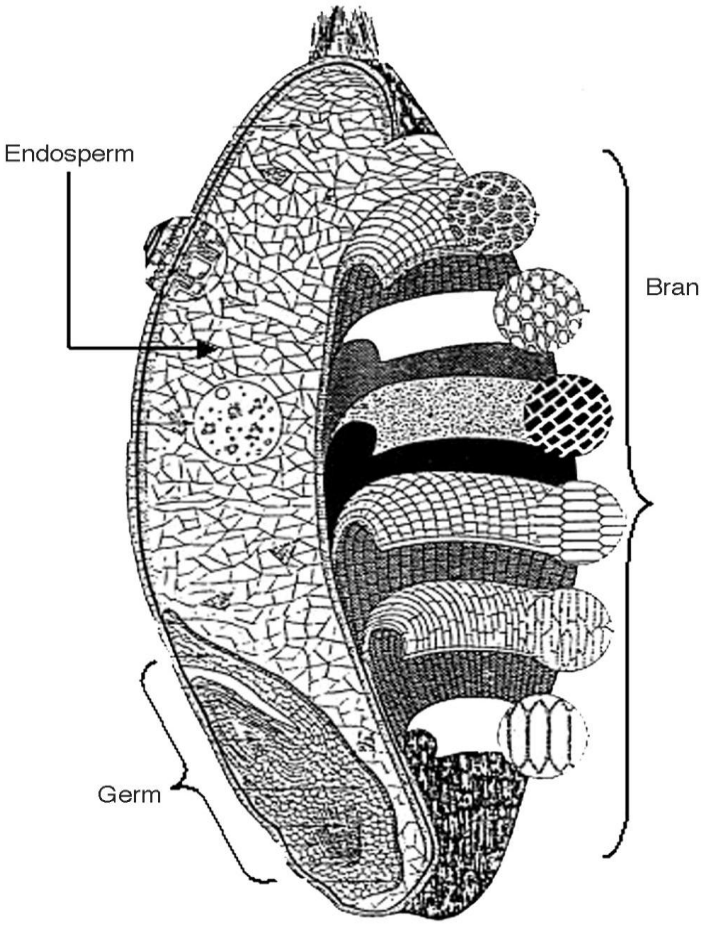

Figure 2. General diagram of a whole grain kernel

(Slavin, 2004)

2. Health Benefits

In recent years whole grains have been touted for their health benefits.

"The benefits of whole grains most documented by repeated studies include:

- stroke risk reduced 30-36%
- type 2 diabetes risk reduced 21-30%
- heart disease risk reduced 25-28%
- better weight maintenance"

Other benefits indicated by recent studies include: providing a source of dietary fiber, providing a prebiotic benefit, lowering the risk of colorectal cancer, reducing gum disease and tooth loss and reducing the risk of asthma (www.wholegrainscouncil.org - a; Grootaert et al., 2007; Slavin, 2004). Specifically, Slavin's (2004) work details the epidemiological studies which relate whole grain consumption to improved human health benefits such as reduced rates of severe forms of cancer and cardiovascular disease and the possible regulation of blood glucose. The Multi-Ethnic Study of Atherosclerosis (MESA) which involved researchers in the US and Norway, reported that "eating a "low risk food pattern" including more whole grains, fruits, green leafy vegetables, low-fat diary, and nuts/seeds, was associated with a 15% lower diabetes risk" (Nettleton et al., 2008). A study reported in the British Journal of Nutrition indicated that the consumption of whole grain cereal promoted the increase of bifidobacteria and lactobacillus, the beneficial bacteria in the gut. This is an indication that components in whole grain function as a prebiotic (Costabile et al., 2008).

B. Whole Grain Rye

1. Source and Viability

It is evident that there are many benefits to consuming whole grains and whole grain products. As previously mentioned, there are many types of whole grains with whole grain wheat being the most popular and most consumed in the United States. Whole grain rye and whole grain rye flour are used to a lesser extent in the United States but provide tremendous benefits.

Rye, *Secale cereale*, is a member of the wheat family. It is typically grown in the north-western portion of the Eastern Hemisphere (http://rye.vtt.fi), and previously was one of Europe's most commonly consumed grains. Rye is also grown in a small portion of the United States and Canada. One of the agricultural benefits of rye is its ability to withstand harsh and adverse climates and growing conditions not tolerated by many other cereal plants. It is the most cold tolerant cereal grain, able to withstand temperatures as low as 31ºF. Rye can thrive under a variety of soil conditions including a wide range of soil moistures, acidity and fertility (Stoskopf, 1985b). Although rye can withstand a variety of environmental conditions, it is still subject to many of the diseases common to cereals. Ergot sclerotia are forms of fungus which infect the rye spike under favorable conditions. Ergot can be very detrimental when present at toxic levels. The ergot can reduce feed conversion, reduce the lactation of sows and cause convulsions and loss of extremities. The ergot level in rye has been

regulated in order to prevent such deleterious occurrences. The maximum levels permitted are 0.05% for food and 0.1% for feed (Seibel and Weipert, 2001a).

2. Rye Usage

Typical uses of rye include rye bread, specifically sourdough and pumpernickel bread, buns, rolls and pastries. Rye may also be used in pasta and whisky (http://rye.vtt.fi). Rye is also used for animal feed as it is a good source of nutrients.

3. Composition

As previously mentioned, there are numerous benefits to whole grains. The unique composition of whole grain rye flour verses other cereal grains may position it as a healthier grain. Table 1 provides a quantitative assessment of some of the components of whole grain rye flour, whole grain wheat flour, white wheat flour and hulled oats.

Table 1. Nutritional properties of whole grain rye flour vs. other grains: a quantitative assessment

	Percent of dry matter			
Component	**Whole grain rye flour**	**Whole grain wheat flour**	**White wheat flour**	**Hulled oats**
Protein	10-15	12-14	13	13-16
Fat	2-3	3	1	6-7
Starch	55-65	67-70	84	54-64
Ash	2	2	0.5	2
Total dietary fiber	15-17	10-13	3	11-13
Soluble fiber	3-4	1-2	0.9-2.0	3-5

(Adapted from http://rye.vtt.fi)

The whole grain rye flour, whole grain wheat flour, white flour and hulled oats all have very similar protein contents. The fat level varies among the grains with whole grain rye flour falling towards the lower end of the spectrum. The starch level is similar to the other grains shown with the exception of white wheat flour which is much higher, most likely because white flour is mainly composed of the starchy endosperm of the grain. The total dietary fiber and soluble fiber values are of great interest. As indicated in Table 1, whole grain rye flour has a higher level of total dietary fiber, ranging from 15 – 17% on a dry solids basis verses whole grain wheat flour and hulled oats which have a total dietary fiber value ranging from 10 – 13% on a dry solids basis. As expected, the white wheat flour has a very low fiber value since the endosperm contains very little fiber. Along with the higher total dietary fiber value, whole grain rye flour has a high level of soluble fiber, similar to oats but greater than those of whole and white wheat flour.

Table 2 depicts the qualitative assessment of whole grain rye flour verses whole grain wheat flour. The relative amount of bran is similar in both whole grain wheat and rye flour. Lignin, a polymer linked to hemicellulose in the plant and cellulose levels are similar as well.

Table 2. Nutritional properties of whole grain rye flour vs. whole grain wheat flour: a qualitative assessment

Nutrient	Rye	Wheat
Gluten	++	++++
Bran	++++	++++
Lignin	++++	++++
Cellulose	++++	++++
β-Glucan	++++	++
Arabinoxylans	++++	++

(http://rye.vtt.fi)

In contrast, this source indicates that whole grain rye flour has much less gluten protein verses whole grain wheat flour. Gluten proteins are formed as a result of gliadin and glutenin storage proteins. These proteins form a polymeric network also known as a gluten network via intermolecular noncovalent interactions and disulfide bonds. A gluten network is very prevalent in wheat flour and greatly contributes to the physical and chemical properties of wheat products. The storage proteins in whole grain rye flour are significantly different than those in wheat flour with respect to the quantity and structure of the proteins (Verwimp et al., 2006). In rye products, the proteins have some ability to form aggregates but not to the extent of the gluten proteins in wheat flour (Fields et al., 1983 as reported by Verwimp et al., 2006). Due to the unique gluten network, the whole grain rye flour functions very differently in products than wheat flour.

Beta-glucans, a source of soluble fiber in whole grains, is present in whole grain rye flour at levels of approximately 2.3%, which is higher than the level present in whole grain wheat flour. Beta-glucan is typically know for its ability to reduce cholesterol, attenuate glycemic and insulin response and improves the body's immune system (Roneanelli et al., 2009).

Arabinoxylans, also a source of fiber are considerably higher in whole grain rye flour and are present at levels ranging from 6 – 12% (Bengtsson et al., 1992 as reported by Shewry and Bechtel, 2001). Since the beta-glucans and arabinoxylans are significantly higher in whole grain rye verses whole grain

wheat and are sources of fiber, they contribute to the healthy qualities of these whole grains. More specifically, arabinoxylans play an important part in the overall nutritional and functional properties of whole grain rye flour and will be discussed in more detail throughout this dissertation.

C. Arabinoxylan

1. Composition

Arabinoxylans are non-starch polysaccharides found in the cell walls of plants. They are generally classified as hemicelluloses or more specifically pentosans, a series of 5 carbon sugars. Their general structure is comprised of a β-(1,4) linked D-xylopyranosyl backbone with α-L-arabinofuranose units attached as side residues via α-(1,3) and/or α-(1,2) linkages (Figure 1). The ratio of the arabinose units to the xylose backbone can vary greatly. While the structure of arabinoxylans are not completely understood, it is known that the xylose moiety may be unsubstituted, monosubstituted at the O-2 or O-3 position or disubstituted at the O-2 and O-3 positions as shown in Figure 3 (Vinkx and Delcour, 1996). Vinkx and Delcour (1996) also reported that arabinoxylans can be chemically or physically associated with other components within the cereal grain. The arabinoxylan can be cross linked to diferrulic acid at the O-5 position, covalently linked to lignin and non-covalently linked to xylans or cellulose.

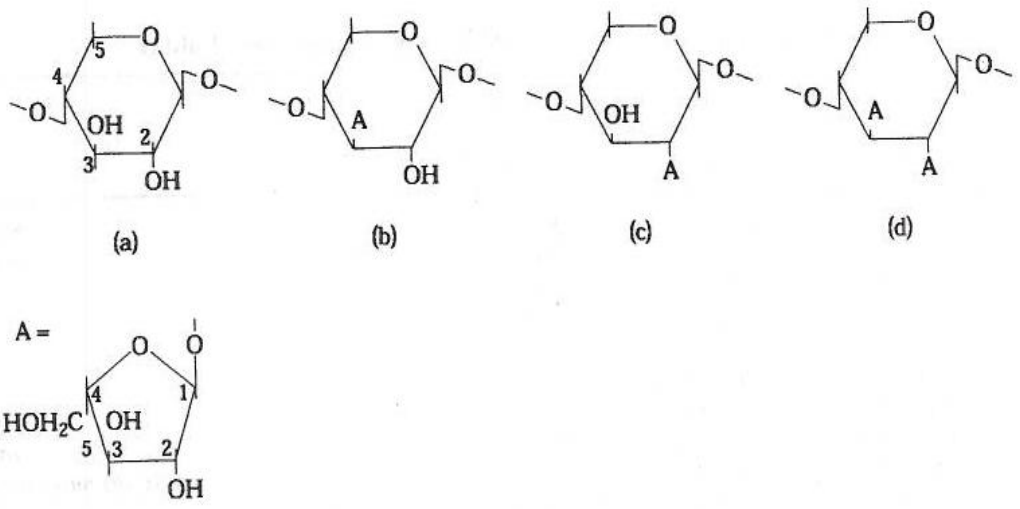

Figure 3. Arabinoxylans – substitution pattern of the arabinose side chains

(Vinkx and Delcour, 1996)

The potentially complex nature of the arabinoxylans greatly affects the solubility of the compounds. Arabinoxylans are typically segregated into two categories - water extractable arabinoxylans (WE – AX) and water unextractable arabinoxylans (WU – AX) or alkali extractable arabinoxylans. The water unextractable arabinoxylans are more abundant in the kernel verses the water extractable arabinoxylans (Fengler and Marquardt, 1988). It has been reported that the water unextractable arabinoxylans are retained in the cell walls by covalent and noncovalent interactions among arabinoxylans and between arabinoxylans and other cell wall constituents such as proteins, lignin, glucomannans, β-glucan and ferulic acid.

The terminology water extractable and water unextractable arabinoxylans is typically mis-used by many researchers. Courtin and Delcour (2002) clearly discuss the use of the terms water extractable and water unextractable arabinoxylans. Alkali extractable arabinoxylans are discussed as well. The use of alkali during the extraction of arabinoxylans breaks the bridges between the arabinoxylan molecules. The arabinoxylans are freed from the cell wall and become water soluble, referred to as alkali soluble.

Alternately, enzymes may be used to influence the extraction of arabinoxylans. When endoxylanase, enzymes used to hydrolyze the arabinoxylan backbone, are applied to the water unextractable arabinoxylans, they may be referred to as enzyme extractable arabinoxylans. As expected, the

use of enzymes alter the molecular weight and functionality of the arabinoxylans. It is also known that enzymes affect water extractable arabinoxylans as well.

Water extractable arabinoxylans make up approximately 1.5% - 3.0% of the kernel. The water extractable arabinoxylans are loosely bound at the cell wall surface (Figuerooa-Espinoza et al., 2002; Hansen et al., 2003, Hansen et al., 2004; Frederix et al., 2004a). Their relative ease of extraction may also be caused by incomplete crosslinking with other grain components, slight structural differences or enzyme activity (Courtin and Delcour, 2002).

A schematic of the extraction routes of arabinoxylans is shown in Figure 4. This figure has been adapted from Courtin and Delcour's work (2002) referring to wheat flour but it is believed that the general description of the extraction process applies to whole grain rye flour as well.

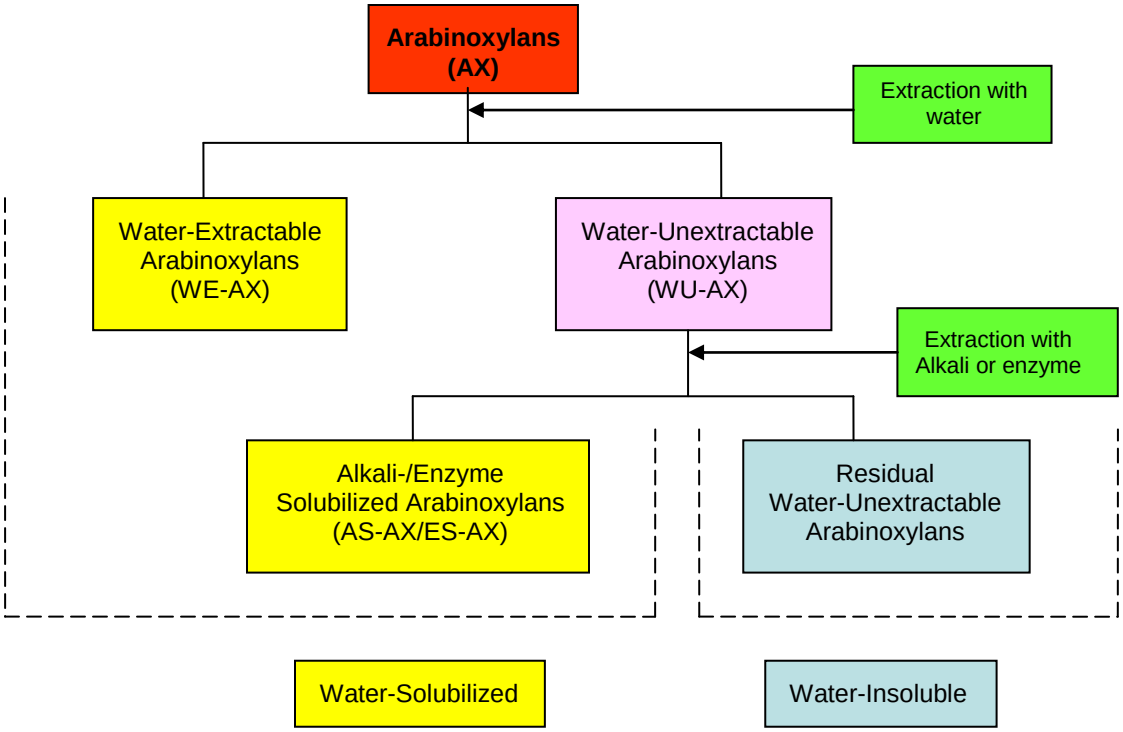

Figure 4. Classification of arabinoxylans in flour based on extractability (adapted from Courtin and Delcour, 2002)

2. Health Benefits

While much has been reported regarding the importance of whole grain on the impact of health, several studies have been conducted regarding the specific components of whole grains and their specific contribution to health. Health benefits have been attributed to arabinoxylans specifically. Due to their structure, it has been documented that arabinoxylans are not digested in the small intestine, a criteria which allows arabinoxylans to meet the definition of fiber. Arabinoxylans can be present at approximately 6 – 12% in whole grain rye (Bengtsson et al., 1992 as reported by Shewry and Bechtel, 2001). It has been reported that

> "whole grain rye contains high amounts of soluble arabinoxylans, which theoretically may have a similar function in lowering cholesterol levels as β-glucan in oats. A diet high in rye, especially rye fibre, has been shown to have positive effects in reducing serum total and LDL cholesterol in men with elevated serum cholesterol." (http://rye.vtt.fi)

According to Izydorczyk and Biliaderis (2007) a rat study was conducted which indicated that water soluble arabinoxylans effect lipid metabolism and mineral absorption. Grootaert et al., (2007) have shown that enzymes present in the colon can specifically hydrolyze arabinoxylans, resulting in arabinoxylan oligosaccharides. These oligosaccharides are said to have a prebiotic effect, meaning they promote the growth of beneficial bacteria in the gut. Soluble dietary fibers play a role in the reduction of blood cholesterol and postprandial blood glucose and insulin. Soluble arabinoxylans may posses these qualities as well (Vinkx and Delcour, 1996).

3. Physical and Chemical Properties

As indicated by several researchers, there are numerous health benefits associated with whole grain rye flour and arabinoxylans specifically. These health benefits should encourage product developers to develop products with more whole grain rye flour in order to provide consumers with healthy alternatives to white or refined flour. While this would be a very consumer conscience effort, there are many challenges with using whole grain flours in particular, whole grain rye flour. For whole grain rye flour, these challenges can be a result of the quantity and quality of the arabinoxylans. The arabinoxylan structure affects the physiochemical properties of the arabinoxylans. Arabinoxylans have the ability to bind water which may alter the dough rheology, processing and finished product attributes of many baked products. Multiple studies have shown the effect of whole grain rye flour on bread baking. Weipert (1997) has studied the effect of arabinoxylans and bread baking in particular, whole grain rye flours which contain a high quantity of water extractable arabinoxylans. The high water holding capacity of arabinoxylans delays starch gelatinization most likely by restricting the water available for starch gelatinization. The arabinoxylans also protect the starch from α-amylase enzyme degradation which results in increased bread volume, better crumb elasticity and increased shelf life.

More specifically, water extractable arabinoxylans have high viscosity forming potential in the dough's free water (Andrewartha et al., 1979 and Udy et

al., 1956 as reported by Frederix, 2004a; Courtin et al., 1999). In bread products, the water extractable arabinoxylans increase the bread volume and decrease the breads crumb firmness (Kuhn and Grosch, 1989). The water unextractable arabinoxylans have high water holding capacity (Andrewartha et al., 1979 and Udy et al, 1956 as reported by Frederix, 2004a). These water unextractable arabinoxylans have the opposite effect on bread by influencing the water distribution in the dough, decreasing bread volume and slightly increasing the crumb firmness (Kuhn and Grosch, 1989; Courtin et al., 1999).

4. Physical and Chemical Evaluations

There are numerous studies which show the arabinoxylans' influence on high moisture baked goods such as breads, rolls, etc. There are several physiochemical methods available to correlate a flour's attributes, specifically arabinoxylans to finished product properties.

a. Solvent Retention Capacity

One such physiochemical test is solvent retention capacity (SRC). Solvent retention capacity is an official American Association of Cereal Chemist (AACC) method (AACC 56-10 – Alkaline Water Retention Capacity and AACC 56 - 11 – Solvent Retention Capacity Profile) and was developed to evaluate the quality of soft wheat. The solvent retention capacity of a flour is defined as the weight of the solvent held after centrifugation and is expressed as a fraction of the original flour weight, on a 14% moisture basis. The four solvents typically

used in this process are sodium carbonate (5% w/w), lactic acid (5% w/w), sucrose (50% w/w) and water. In general, the sodium carbonate is an indication of the amount of damaged starch in the flour. Lactic acid relates to the glutenin characteristics. Sucrose relates to the pentosans and gliadins. Water, is influenced by all of the flour components (Slade and Levine, 1994 and Gains, 2000).

Typically, the information obtained from the solvent retention capacity helps predict how a flour will function in a baked good. A sugar cookie is typically used as a model bake system to evaluate flour and other functional ingredients. The work conducted by Pasha et al., (2009), showed how the use of the solvents on numerous varieties of spring wheat flour predicted the geometry of sugar cookies. The results indicated that the cookie spread ratio positively correlated to the water – solvent retention capacity. The lactic acid and sugar solvent retention capacity values positively correlated to the cookie thickness. In their study, there was not a positive correlation between the sodium carbonate solvent and the cookie geometry. While these four solvents provide a wealth of information regarding flour functionality, if only specific attributes are sought, not all solvents are required.

b. Farinograph

While the solvent retention capacity utilizes solvents to characterize the flour components and in turn predict the flour functionality in some baked good, specifically sugar snap cookies, other physical methods are used to predict the functionality of flour via its mixing properties. According to Bruemmer (1987) and American Association of Cereal Research (1994) (as reported by Seibel and Weipert, 2001b) while many lab trials utilize sensorial properties to determine the dough's functionality, the farinograph is a more scientific, practical and relatively quick tool. In general, the farinograph test determines the dough and gluten properties of a flour by measuring the dough's resistance against the mixing blades. It is the most commonly used flour quality test worldwide. More specifically, the farinograph test helps determine the quantity of water the dough required for proper functionality in processing and finished products performance and the effect of other ingredients added to the dough (www.wheatflourbook.org - a). Examples of typical farinographs are shown in Figures 5 and 6.

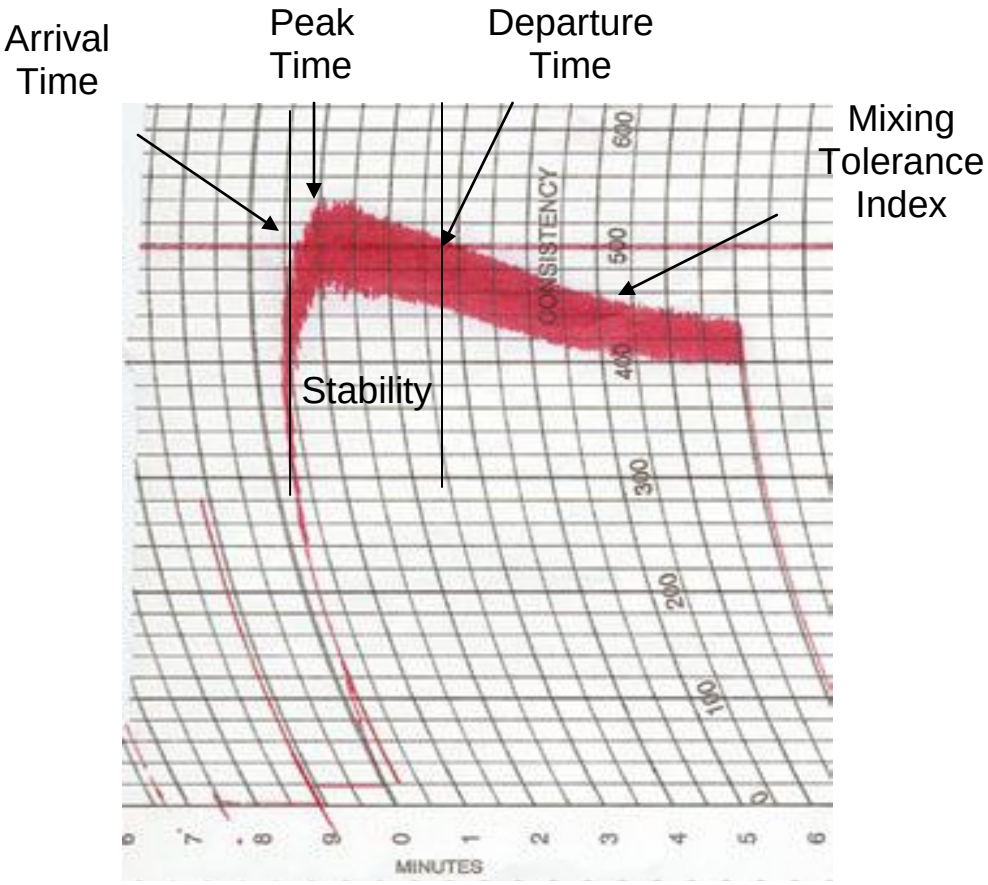

Figure 5 – Farinograph of weak gluten flour

(adapted from www.wheatflourbook.org - a)

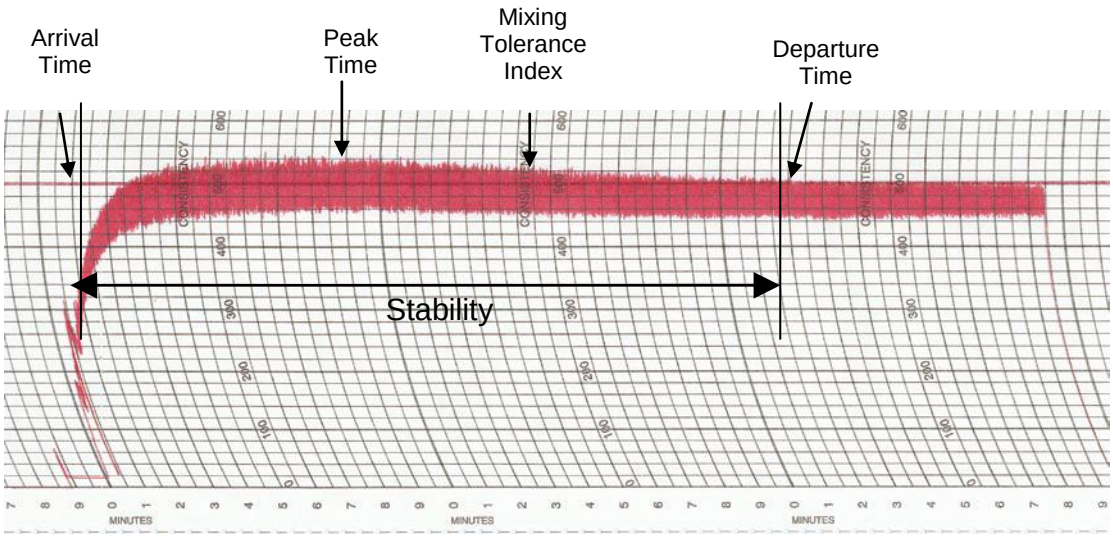

Figure 6 – Farinograph of strong gluten flour

(adapted from www.wheatflourbook.org - a)

The farinograph reports arrival time, peak time, departure time and mixing tolerance. During the test run, water is added to the flour. The absorption is defined as the amount of water required to center the peak of the curve on the 500 or 50% Brabender Unit line. This value is an indication of the amount of water required to optimally process the dough. The peak time is an indication of the dough development time which begins when the water is added to the flour and ends when the dough reaches maximum consistency, giving an indication of optimum mixing with standard mixing conditions. The arrival time is determined when the curve reaches the 500 or 50% BU line. It indicates the rate of flour hydration. The departure time is marked when the top of the curve leaves the 500 or 50% BU line. This is a result of the dough loosing its optimum structure. The mixing tolerance is the difference in the BU value at the top of the mixing curve at peak time and the BU value at the top of the mixing curve 5 minutes afterwards. This value is an indication of the dough softening during mixing.

The research has shown that flours with weak gluten networks (Figure 5) have low water absorption and shorter stability time verses a wheat flour with strong gluten networks (Figure 6) (www.wheatflourbook.org - a). Rye flours with large water soluble fractions have high water absorption and the pentosans e.g. arabinoxylans of the soluble portion have a greater effect on the dough viscosity verses the proteins. For wheat flour the proteins have a greater effect. It has been shown that the water absorption of rye flour and the dough viscosity are interrelated and influence the baking properties of the flour. For rye flour

specifically the dough viscosity is extremely important. For wheat flour, the dough viscosity is less important and it is more critical to balance/optimize the viscous and elastic properties. In rye baked goods, doughs with higher viscosity retain more water and yield a higher dough quantity but at the same time, the loaf volume is lower. This is possibly due to the release of the water during baking. Again, the quality and quantity of the pentosans, including the arabinoxylans have an effect on the dough characteristics and can be evident in the farinograph (Seibel and Weipert, 2001b)

c. Viscosity

The Rapid Visco Analyzer (RVA) is a commonly used tool to determine the rheological properties of ingredients, specifically starch containing ingredients. In general, the RVA allows the researchers to observe the viscosity profile but more specifically, the RVA shows the ingredient's peak viscosity, pasting temperature, peak time, breakdown, final viscosity and setback, all of which characterize the ingredient properties and provide an indication of its performance in finished products. Although the RVA is typically used to characterizes starch, I believe other viscosifying ingredients such as arabinoxylans can be detected using the RVA. It has been well documented that arabinoxylans contribute to dough viscosity and interact with starch. The effect of arabinoxylans can be emphasized or de-emphasized with the use of enzymes.

d. Color

The color of baked products can be critical and can lead to consumer acceptance of certain products. For fruits and vegetables, color is an indication of freshness or ripeness. For baked goods such as cookies, color is an indication of bake quality and can occur as a result of Maillard Browning or sugar carmalization.

The color of a product, specifically baked goods, can be quantified using the L*, a* and b* scale. This scale provides a quantitative measurement of the black to white hue – L*, green to red hue – a* and the blue to yellow hue – b*. The MiniScan XE instrument is capable of measuring these values.

e. Baking Test

The assessment of the flour quality can be determined using such equipment as the RVA and farinograph. The quality of a flour can be determined in a finished baked good as well. To evaluate soft wheat flour, the American Association of Cereal Chemist 10-53 Baking Quality of Cookie Flour Method is used. The method uses a model sugar cookie to evaluate the cookies' geometry – length, width and height, moisture loss, crumb structure, etc. The changes in these attributes relate to the cookies' pentosans e.g. arabinoxylans, gluten structure and water absorption.

The model cookie system can be used to evaluate the function of other ingredients as well and their effect on the cookie. Delcour used a model cookie system to understand the effect of gluten, sugar and fat on the cookie. Uysal, et al., (2007) used a model cookie system, all be it different than the system used in this study to evaluate the effect of enzymes when fiber is added to the system. The use of enzymes on a model cookie system is of particular interest due to the infrequency of enzymes used in a cookie system. In cookies it is difficult to see the effect of enzymes due to high sugar and high fat in the system. Slade et al., (1994) have patented the use of an enzyme in baked good systems. In a further study conducted by Pareyt and Delcour (2008), they mention that the water extractable and water unextractable arabinoxylans are not beneficial for cookie baking because they may increase dough viscosity and reduce cookie spread.

D. Enzymes

While numerous researchers have reported utilizing enzymes such as proteinases to alter the structure of proteins in flour in order to optimize their functionality, the same can be said for the use of enzyme to alter the function of arabinoxylans. As previously mentioned, arabinoxylans are composed of a xylose backbone with various degrees of arabinose substitution. The structure can also be substituted with compounds such as ferulic acid. Various enzymes are responsible for the degradation of arabinoxylans at different bonding sites. As shown in Figure 7 endo -1,4 – β – xylanase (commonly referred to as xylanase) randomly cleaves the xylose backbone. β – xylanosidase releases the

xylose monomer from the non-reducing end. α – L – arabinofuranosidase detaches the arabinose side chain from the xylose backbone. Feruloyl esterase releases bound ferulic acid. Arabinoxylan arabinofuranohydrolase – D3 cleaves arabinose as well (Grootaert et al., 2007).

As indicated by Kuhn and Grosch (1989) endoxylanases can be used to breakdown water unextractable arabinoxylans and convert them to medium to high molecular weight soluble arabinoxylans. These newly created arabinoxylan structures improve rye flour bread making performance overall. More specifically, the newly formed water soluble high molecular weight arabinoxylans improve water distribution within the dough and increase the arabinoxylan interactions between the macromolecules within the dough as well (Courtin et al., 1999).

As previously mentioned xylanases can randomly cleave the xylose backbone. Some xylanases may have a preference for a water extractable or water unextractable arabinoxylan. The xylanase can solubilize water unextractable arabinoxylans or degrade water extractable arabinoxylans. Xylanase enzymes which solubilize water unextractable arabinoxylans increase batter viscosity and decrease gluten agglomeration. Xylanase enzymes which degrade water extractable arabinoxylans help increase dough viscosity and improve gluten agglomeration (Christopherson et al., 1997, Frederix et al., 2003, Frederix et al., 2004b).

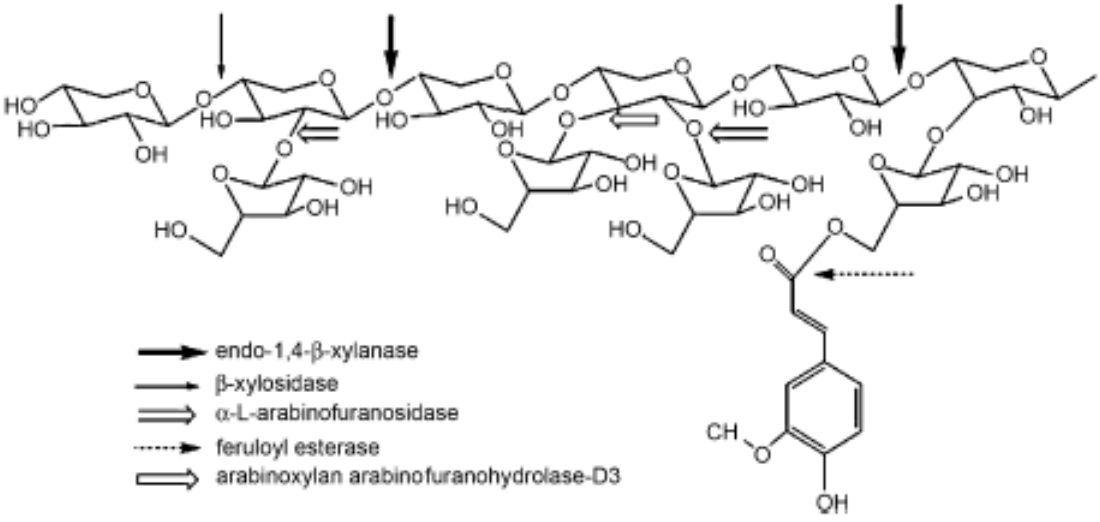

Figure 7. Chemical structure of arabinoxylan and the site of activity of the different arabinoxylan degrading enzymes

(Grootaert et al, 2007)

More specifically, Courtin and Delcour (2002) discuss the types of xylanases and give a general preference for these enzymes. Xylanases typically derived from *Aspergillus niger* degrade soluble arabinoxylans. Some can act on insoluble ones as well. Enzymes extracted from *Bacillus subtilis* typically degrade water unextractable/insoluble enzymes. The general preference of these enzymes also depend on their source of origin e.g. fungal or bacterial.

The use of enzymes to solubilize or degrade arabinoxylans impact the overall physical and chemical properties of rye flour and the impact on finished product. Other enzymes such as α – L – arabniofuranosidase also degrade the arabinoxylan by targeting a different portion of the arabinoxylan. This enzyme has an exospecifity and cleaves the arabinose moiety (Dornez et al., 2007). Sorensen et al., (2003) have studied various enzymes and their effect on the structure of water soluble wheat arabinoxylans. The researchers mention that substitution of the xylose backbone can inhibit the enzymatic degradation of the backbone by xylanases i.e. endo – 1,4 – β – xylanase. In order to obtain increased hydrolyses or degradation of the water soluble arabinoxylans, the side groups need to be cleaved by enzymes such as α – L – arabniofuranosidase.

E. Quantification

Douglas (1981) has developed a rapid method to determine the pentosan content in wheat flour. This method is based on previous work that utilizes a

gas-liquid chromatography methodology. Douglas' method utilizes the spectrophotometer and evaluates the color absorbance of the reaction. The method is based on extracting/hydrolyzing arabinoxylans with hydrochloric acid and the other reacting agents such as phloroglucinol help convert the pentosans to furfurals.

The method used xylose as a calibration curve and the percent xylose is determined. It is believed that other sugars such as arabinose can be estimated as well. Modifications of this method have been used many times by researchers such as Rouau and Surget (1994) to name a few, and have been reduced to semi-automation with reproducible results.

III. HYPOTHESIS

As indicated from the previous research, arabinoxylans are present in whole grain cereals. The arabinoxylans have purported health benefits such as reducing cholesterol and cardiovascular disease. The arabinoxylans also play a very important role in the physical and chemical functionality of the whole grain flour especially when incorporated into breads, a high moisture baked good. They influence dough viscosity, water absorption and gluten development. Because of their importance and the global popularity of whole wheat products many researchers have studied these compounds extensively in wheat flour products. Delcour and his colleagues have studied their effect on doughs and breads. Ordaz-Ortiz and Saulnier (2005) have looked at water extractable arabinoxylans. Izydorczyk and Billiarderis (1995) have characterized arabinoxylans. While there have been studies looking at the arabinoxylans in rye flour, there are less in relation to the work in wheat flour and the studies have mainly focused on the arabinoxylans in bread products. As a result of the amount of research on arabinoxylans in cereal grains and in mainly high moisture bread products, I hypothesize the following

The chemical structure of arabinoxylans in whole grain rye flour will affect the product attributes of low moisture baked goods such as cookies

IV. OBJECTIVES

In order to validate the hypothesis, the following objectives must be explored.

1. Evaluate the physical and chemical properties of whole grain rye flour with and without enzymes

Research has shown that the physical and chemical properties of certain flours can aid in the prediction of the flour's functionality in finished baked goods. Equipment such as the RVA and farinograph are useful in determining the properties of dough rheology i.e. viscosity and water holding capacity with and without enzymes. Testing such as solvent retention capacity can also define the physiochemical characteristics of flour.

2. Evaluate the finished product attributes of whole grain rye low moisture baked goods such as cookies with and without enzymes

The American Association of Cereal Chemist 10-53 Baking Quality of Cookie Flour Method has been utilized to determine the attributes of soft wheat flour in a model sugar cookie system. It has also been used to evaluate other functional ingredients in the cookie as well, such as fat type, sugar level, etc. As indicated from the literature, the functionality of wheat flour is very different from

rye flour. I believe this cookie system is adequate to evaluate the affect of whole grain rye flour on the sugar cookie attributes. The addition of arabinoxylan specific enzymes will alter the arabinoxylan structure and affect the dough and finished product characteristics and show a significant change in the cookies characteristics.

3. Evaluate the effect of various enzymes and enzyme levels on the physical and chemical properties of whole grain rye flour and rye cookies

It has been documented that various arabinoxylan specific enzymes target water extractable or water unextractable arabinoxylans. The resulting fragments can affect the arabinoxylans' solubility and their ability to interact with other macromolecules within flour. It has been documented that various enzymes attack the arabinoxylan structure at different bonding sites. It is believed that the type of enzyme and quantity of the enzyme will alter the arabinoxylan properties and those of the low moisture baked product.

4. Characterize the water soluble and enzyme degraded arabinoxylans

Delcour and numerous other researchers have studied the chemical composition of arabinoxylan. The effect of enzymes on the arabinoxylans has also been proven. However, in order to understand the impact of arabinoxylans on low moisture baked goods, it is beneficial to know the relative chemical

composition of the arabinoxylans. Extensive research has been conducted to characterize the arabinoxylans but a relatively simple arabinoxylan extraction followed by an arabinose and xylose sugar analysis will provide general information on the overall characteristics of the arabinoxylan compounds.

V. METHODOLOGY

A. Moisture Determination - Computrac

1. Materials

Computrac Max 2000 Moisture Analyzer and Max Waffle pans purchased from Arizona Instruments

2. Procedures

According to the program established, approximately 4.0 g of flour were added to the pan for analysis. The temperature program was set to start at approximately 40ºC and increase to achieve an end temperature of 140ºC. The heating cycle was completed when the weight change was less than 0.1%. The moisture content of the flour was determined and recorded as percent moisture.

B. Moisture Determination – Air Oven

Note: These procedures are a slight modification of the American Association of Cereal Chemist (AACC) 44-15A Moisture – Air Oven Method

1. Materials

Thelco, GCA Precision Scientific Model 26 Forced Air Oven

Aluminum sample pans with lid

Desiccator

Balance accurate to 0.0000g

2. Procedures

The pan and lid were weighted and the weight was recorded (wt of pan + lid). Approximately 2.5000g of flour were then added to the pan and the lid. The weight was recorded (wt of pan + lid + sample before heat). This procedure was repeated to result in three samples per flour tested. All samples were covered until placed in the air oven.

The oven was preheataed to 130ºC +/- 1ºC. The samples were uncovered and placed in the air oven. The samples were heated for 1 hr. Note: When the samples are placed in the oven, the oven temperature drops below target. The heating period begins once the target temperature is achieved. This is typically less then 5 min when 3 samples are added to the oven.

After the 1 hr heating, each sample was removed from the oven, covered with the lid and placed in the desiccator for approximately 30 min. The pan, lid and sample weight were recorded (wt of pan + lid + sample after heat)

3. Calculations

The moisture content of the samples was determined based on the calculation below

$$\frac{((\text{wt pan+lid+sample before heat}) - (\text{wt pan + lid})) - ((\text{wt pan+lid+sample after heat}) - (\text{wt pan + lid}))}{((\text{wt pan + lid + sample before heat}) - (\text{wt pan + lid}))} \times 100 = \% \text{ moisture}$$

C. Viscosity Determination

Note: These procedures are a slight modification of the AACC 76-21 General Pasting Method for Wheat or Rye Flour or Starch Using the Rapid Visco Analyzer.

1. Materials

The RVA Super 4, plain can 38 x 68 sample canister and double skirt paddle and Thermocline for Windows version 3.11 computer software from Newport Scientific

Julabo F10 Circulating water bath

Enzeco® Xylanase AN 60 (6,000 EDX/g +/- 5%), Enzeco® Arabinosidase 100 (10,000 ARF/g +/- 5%) and Enzeco® Xylanase SB 50 (5,000 NBXU/g +/- 5%) donated by Enzyme Development Corporation

Whole grain rye flour (rye meal fine pumpernickel: NIR moisture – 12.113%, ash NIR 14% MB – 1.6%, protein NIR 14% MB – 11.13%) donated by ConAgra.

Deionized water

Balance accurate to 0.0000g

Stir plate and stir bar

Erlenmeyer flask

Spatula

Weigh paper 3” x 3”

2. Procedures

a. Instrumentation Set Up

The RVA, circulating water bath and computer program were turned on at least 30 min prior to use. After instrumentation warm up and prior to sample preparation, the instrument was zeroed according to the procedures recommended by the manufacturer.

b. Sample Preparation

A flour sample of approximately 3.5 g was weighed onto weigh paper. The appropriate amount of enzyme was weighed onto weigh paper as well. Approximately 25.0 g of deionized water were weighed into a beaker. Note: The calculations to determine the exact amounts of flour and water to use are shown in the calculation section Methodology C.3.

A majority of the water was added to the sample canister. The flour and enzyme (when required) were then added. The remaining water was added to the canister. The sample paddle was used to agitate the suspension by moving it up and down within the suspension 30 times. The sample was then swirled 10

times and a spatula was used to break up a majority of the remaining rye flour lump. The paddle was used to scrape any residual flour from the spatula and to scrape down the side of the canister.

3. Calculations

a. Flour Weight

Theoretical solids of flour = 86%

Theoretical moisture of flour = 14%

Recommended flour weight based on a 14% moisture sample = 3.5 g

$$\frac{86 - 3.5}{100 - \text{actual flour moisture}} = \text{Flour weight used for testing}$$

b. Water Weight

Recommended flour weight based on a 14% moisture sample = 3.5 g

Recommended water weight based on a 14% moisture sample = 25 g

25 + (3.5 – calculated flour weight) = Water weight used for testing

4. Program

Segment	Time	Type	Value
1	0	Temp (ºC)	30
2	0	Speed (rpm)	960
3	30 sec	Speed (rpm)	160
4	2 min	Temp (ºC)	30
5	15 min	Temp (ºC)	95
6	30 min	Temp (ºC)	95
7	43 min	Temp (ºC)	30
8	53 min	Temp (ºC)	30
9	53 min, 10 sec	End	

D. Color

1. Materials

MiniScan XE with standardizing black and white plates

Petri dish

Osterizer Commercial Blender

2. Procedures

The MiniScan XE was calibrated using the black and white standardizing plates and the bottom portion of the petri dish. The instrument was adjusted to

standardization mode. The petri dish was placed on top of the black plate and the reading of the black plate with petri dish was taken. The procedure was repeated with the white plate and petri dish. The MiniScan was then changed to sample reading mode in preparation for the first sample reading.

Three of the four cookies per batch were ground and combined using the Osterizer Commercial Blender. Approximately 45 g of ground cookie were placed inside the petri dish and the lid was placed on the dish. The reading was taken and the L*, a* and b* values were recorded. The color values were repeated in triplicate per sample and the values were recorded.

E. pH

1. Materials

Corning 360i pH meter with pH 4 and pH 7 standardizing solutions

Osterizer Commercial Blender

Stir bar and stir plate

Beaker

Deionized water

2. Procedures

The pH meter was calibrated according to the procedures recommended by the manufacturer. A 10 g portion of dough and 90 mL of deionized water were

added to the blender and mixed for 1 min. The pH of the slurry was measured and recorded.

F. Farinograph

1. Materials

Do-Corder E330, Torque Console DCE 330 and mixing bowl type R.S. were purchased from Brabender

Enzeco® Xylanase AN 60 (6,000 EDX/g +/- 5%), Enzeco® Arabinosidase 100 (10,000 ARF/g +/- 5%) and Enzeco® Xylanase SB 50 (5,000 NBXU/g +/- 5%) donated by Enzyme Development Corporation

Whole grain rye flour (rye meal fine pumpernickel: NIR moisture – 12.113%, ash NIR 14% MB – 1.6%, protein NIR 14% MB – 11.13%) donated by ConAgra.

Chart recorder with graph paper

Burette with stop cock and Erlenmeyer flask

Circulating water bath

Balance accurate to 0.00g

2. Procedures

The farinograph was calibrated according to the procedures recommended by the manufacturer. Approximately 200 g of flour and the appropriate amount of enzyme (when required) were added to the mixing bowl. The mixer was started and the water was added within 30 sec into the top slot of the mixer via the burette. Once the determined peak height was achieved, the mixer was allowed

to continue for an additional 12 min according to the recommended farinograph procedures. All samples with added enzymes were mixed the same amount of time as the control. The mixing bowl and blades were cleaned between each sample run.

a. Settings

1) Rpm = 65
2) Condenser temperature setting = 30ºC
3) Chart Speed = 1 mm/min x 10
4) Damp = 1
5) Suppression = 0%
6) Range = 10

G. Cookie Baking Test

Note: These procedures are a slight modification of the AACC 10-53 Baking Quality of Cookie Flour Method. For this research the procedures were modified to produce 4 cookies per batch.

1. Materials

Nonfat dry milk, salt, sodium bicarbonate, fine granular sugar, ammonium bicarbonate and 42 DE high fructose corn syrup were purchased from the appropriate chemical or ingredient companies.

Enzeco® Xylanase AN 60 (6,000 EDX/g +/- 5%), Enzeco® Arabinosidase 100 (10,000 ARF/g +/- 5%) and Enzeco® Xylanase SB 50 (5,000 NBXU/g +/- 5%) donated by Enzyme Development Corporation

Whole grain rye flour (rye meal fine pumpernickel: NIR moisture – 12.113%, ash NIR 14% MB – 1.6%, protein NIR 14% MB – 11.13%) donated by ConAgra

C-100 Hobart Mixer, 3 quart mixing bowl and mixing paddle purchased from Hobart

Computrac Max 2000 Moisture Analyzer

TA-XT2 Analyzer, ½" ball probe and 3 pt bend apparatus purchased from Texture Technologies

Dough rheology test pan

Digital Thermometer with thermocouple

National Test Baking Oven

Aluminum Cookie sheet with the following approximate dimensions: 25.4 cm wide X 33.0 cm long with 2 gauge bars placed length wise with the following approximate dimensions: 7 mm high and the length of the baking sheet

Cookie cutter with 60 mm inner diameter

Rolling pin with sleeve

Spatula

Brown absorbent paper

Stop watch accurate to 0.00 minutes

Balance accurate to 0.00 g

2. Formulation

Stage 1		Stage 2		Stage 3	
Ingredient	**Weight (g)**	**Ingredient**	**Weight (g)**	**Ingredient**	**Weight (g)**
Nonfat Dry Milk Powder	2.3	Ammonium Bicarbonate	1.1	Flour	225.00*
Salt	2.8	42 DE High Fructose Corn Syrup	3.4		
Sodium Bicarbonate	2.3	Enzyme	TBD		
Fine Granular Sugar	94.50	Tap Water	49.5*		
Fat	90.00				

*Weight varies according to flour moisture.

3. Flour moisture determination

Determine the flour moisture according to the procedures in section Methodology A. The target flour moisture is 13%. If the flour deviates from this number the moisture is adjusted using the calculations

((87)/(100 – actual flour moisture)) + 225 g = Flour weight

49.5 g + 225 g – Flour weight used = Water weight

4. Mixing procedures

a. Stage 1

Fat (temperature of fat should be approximately 21ºC and recorded) was added to the mixing bowl. The nonfat dry milk powder, salt, sodium bicarbonate and fine granular sugar were dry blended and add to the mixing bowl. The ingredients were mixed at low speed for 3 min, scraping the bowl and paddle after each 30 sec. The temperature of the stage 1 mixture was recorded.

b. Stage 2

A small amount of water (temperature of water should be approximately 21ºC and recorded) was added to the ammonium bicarbonate, high fructose corn syrup and enzyme. The ammonium bicarbonate solution was added to the mixing bowl. The enzyme suspension was then added to the mixing bowl. The high fructose corn syrup solution was added to the enzyme container to “wash” the container and ensure all residue was removed and added into the mixing bowl. The remaining amount of water was used to wash any residual enzyme suspension into the mixing bowl. The ingredients were mixed at low speed for 1 min, scraping the bowl and paddle after 30 seconds. The mixing speed was then adjusted to medium for 2 min and the bowl and paddle were scraped after each 30 sec.

c. Stage 3

Flour was added to the mixing bowl and folded in 3 times. The ingredients were mixed for 2 min at low speed, scraping the bowl and paddle after each 30 sec. The dough temperature was recorded.

5. Dough Blanks

The slightly greased cookie sheet was weighed and the weight was recorded (baking sheet cold). Dough blanks, 4 – 60 g, were equally spaced on the cookie baking sheet. They were gently pressed by placing the rolling pin with the sleeve across the gauge bars. The dough blanks were then rolled one time in the direction away from you. The appropriate cookie dimensions in each dough blank were cut using the cookie cutter. The dough scraps were carefully removed without damaging the shape of the cookie. The baking sheet with the cookie dough blanks was weighed and the weight was recorded (dough blank + baking sheet cold)

6. Baking

The oven was pre-heated to oven to 400ºF. The baking sheet with cookies was placed in the oven and bake for 10 – 13.5 minutes. Note: The baking time is determined with pre-baking test which results in a 13.58% moisture loss. At the end of the baking cycle, the cookies were immediately removed from the oven and the cookies along with the cookie sheet were weighed. The weight

was recorded (wt. of cookies + baking sheet hot). The cookies were then removed from the baking sheet and placed on the absorbent paper, evenly spaced. Excess crumbs were removed from the baking sheet and the weight of the cookie sheet hot was recorded (baking sheet hot). Samples were packaged approximately 30 min after removal from the oven.

7. Moisture loss

The moisture loss of the cookies was determined using the calculation below

$$\% \text{ moisture loss} = \frac{((\text{dough blank} + \text{baking sheet cold}) - (\text{baking sheet cold})) - (\text{cookies} + \text{baking sheet hot}) - (\text{baking sheet hot}))}{(\text{dough blank} + \text{baking sheet cold}) - (\text{baking sheet cold})} \times 100$$

8. Cookie Geometry

The geometry measurements are taken at least 30 minutes after baking.

a. Stack Height

All 4 cookies were stacked on top of each other and the height was measured in cm from the bottom of the first cookie to the top of the fourth cookie. The cookies were shuffled and the measurement was repeated.

b. Length

The rolling pin sleeve lines on each cookie should be perpendicular to the meter stick. All four cookies should be aligned next to each other. The length was measured in cm starting from the far end of the first cookie to the far end of the fourth cookie.

c. Width

The rolling pin sleeve lines on each cookie should be parallel to the meter stick. All four cookies should be aligned next to each other. The width was measured in cm starting from the far end of the first cookie to the far end of the fourth cookie.

9. Dough Rheology

The dough rheology measurements were taken from the dough remaining in the mixing bowl. Measurements should be taken while cookies are baking.

a. Sample Preparation

A 150 g sample of dough was gently formed into a ball. The ball was placed into the dough rheology test pan. The surface of the dough ball was flattened by gently placing the rolling pin with sleeve against the top edges of the test pan.

B. Analysis

1) The TA-XT2 with the ½ inch ball probe was calibrated according to the procedures required by the instrument

2) The analyzer was run using the following program

a. Pre-test speed = 2 mm/s
b. Test speed = 2 mm/s
c. Post-test speed = 10 mm/s
d. Distance = 15 mm

3) The measurements should be conducted in triplicate and evenly spaced on the surface of the dough. The force values (+ and –) should be recorded in grams.

10. Cookie Texture

The cookie texture measurements were on three of the four cookies baked per batch. The cookie was placed on the support with the rolling pin sleeve lines parallel to the rig support. The TA-XT2 with a modified 90 mm 3 pt bend blade was calibrated according to the procedures required by the instrument. The test conditions for the analysis are proprietary and will not be discussed. The force value (+) is recorded in grams.

D. Arabinose and Xylose Quantification

1. Materials

The D-(+)-xylose, D-(-)-arabinose, hydrochloric acid, acetic acid, phloroglucinol, ethanol, and glucose were purchased from Sigma-Aldrich

100 mL and 2mL volumetric flask

30 mL heating vials and heating block

Stir plate and stir bar

3” x 3” weigh paper

10 mL disposable pipets

20 uL – 5000 uL autosample pipet

Polypropylene conical bottom 50 mL centrifuge tubes with screw cap lids from VWR

HP 8453 spectrophotometer with HP 845x UV-Vis System Computer Software

Thermolyne 37600 Mixer

Spectrophotometer vials

Enzeco® Xylanase AN 60 (6,000 EDX/g +/- 5%), Enzeco® Arabinosidase 100 (10,000 ARF/g +/- 5%) and Enzeco® Xylanase SB 50 (5,000 NBXU/g +/- 5%) donated by Enzyme Development Corporation

Whole grain rye flour (rye meal fine pumpernickel: NIR moisture – 12.113%, ash NIR 14% MB – 1.6%, protein NIR 14% MB – 11.13%) donated by ConAgra

Controlled Environmental Incubator Shaker from New Brunswick Scientific

Deionized water

2. Procedures

a. Standard Preparation

1) Xylose Standard

Xylose, 0.1000 g +/- 0.0005 g was weighed onto weigh paper and then added to a 100 mL volumetric flask. Deionized water was added to make the 100 mL solution. A stir bar was added to the flask and the mixture was stirred on the stir plate in order to dissolve the xylose. Aliquots of 0.1 mL, 0.5 mL, 1.0 mL, 1.5 mL and 2.0 mL were added to a 2.0 mL volumetric flask. Deionized water was added to make up the 2 mL solutions. All of the flask were shaken with the vortex. The solutions were labeled solution 1, 2, 3, 4 and 5.

2) Arabinose Standard

Arabinose, 0.1000 g +/- 0.0005 g was weighed onto weigh paper and then added to a 100 mL volumetric flask. Deionized water was added to make the 100 mL solution. A stir bar was added to the flask and the mixture was stirred on the stir plate in order to dissolve the arabinose. Aliquots of 0.1 mL, 0.5 mL, 1.0 mL, 1.5 mL and 2.0 mL were added to a 2.0 mL volumetric flask. Deionized water was added to make up the 2 mL solutions. All of the flask were shaken with the vortex. The solutions were labeled solution 6, 7, 8, 9 and 10.

b. Extraction Solution Preparation

1) Phloroglucinol solution – 20% w/v

Phloroglucinol, 1.000 g +/- 0.005 g was weighed on weigh paper and added to a 5 mL volumetric flask. Ethanol was added to make a 5 mL solution. A stir bar was added to the solution in order to disperse the phloroglucinol.

2) Glucose solution – 1.75%

Glucose, 0.0175 g +/- 0.005 g was weighed on weigh paper and added to a 1 mL volumetric flask. Deionized water was added to make a 1 mL solution. The solution was shaken in order to disperse the glucose.

3) Extracting solution

Acetic acid - 110 mL, hydrochloric acid - 2 mL, phloroglucinol solution - 5 mL and glucose solution - 1 mL were added to a beaker and stirred until use.

c. Standard Calibration Curve

Each xylose standard, solutions 1 - 5 was transferred into 30 mL sample vials. The vials were placed in a heating block and boiled for 30 min. The samples were then cooled by placing them in a cool water bath. Each sample was then analyzed for it's absorbance at 552 nm and 510 nm (see Methodology section D.j.). A calibration curve (concentration vs. absorbance) was then generated for xylose solutions at each absorbance.

The same procedure was utilized to generate a calibration curve for the arabinose solution 6 - 10.

d. Sample Preparation

Note: Section D.d – h are based on the AACC 56 – 11 Solvent Retention Capacity Profile Method.

1) Weighing sample

It is best to number all centrifuge tubes and lids intended for use e.g. centrifuge tube = 1, centrifuge lid = 1, etc. The centrifuge tubes with lids were weighed accurately and the weight was recorded (wt. tube + lid). On weigh paper, 5 g of flour to +/- 0.03 g were weighed. The appropriate amount of enzyme (when required) was weighed. The flour was added to the centrifuge tube and the centrifuge tube, lid and flour were weight was recorded (wt. tube + lid + flour).

2) Weighing solvent

Deionized water, 25 g +/- 0.05 g, was weighed into a tared centrifuge tube. It was covered with the lid until use.

e. Solvation

The deionized water was added to the centrifuge tubes and the tubes were shaken by hand. They were then securely placed in the environmental shaker and shaken at 250 rpm for 1 hr. During this period, the samples were

shaken by hand at 5 min, 10 min, 30 min, 45 min and 60 min in order to ensure the sample did not settle in the tube.

f. Centrifugation of the flour/solvent tubes

After 60 min of solvation, each centrifuge tube sample was placed in the centrifuge. The centrifuge was set for 1000 x g for 15 min.

g. Decanting the centrifuge tubes

After the tubes were removed from the centrifuge, the lids were removed and the supernatant was transferred to a clean centrifuge tube. The samples were then inverted into the new tubes and the remaining solvent was allowed to drain for 10 minutes.

h. Determining the solvent retention capacity of the flour

1) The corresponding lids were placed on the centrifuge tubes. The tube, lid and pellet were weighed and the weight was recorded (wt. pellet + lid + tube).

2) The solvent retention capacity of each sample was determined using the equation below.

$$\%\ SRC = \frac{(\text{wt. pellet + lid + tube})}{(\text{wt. tube + lid + flour}) - (\text{wt. tube + lid})} \times \frac{86}{(100 - \%\ \text{flour moisture})} - 1 \times 100$$

i. Sample Heating

25 uL of sample (control or sample with enzyme), 2 mL of deionized water and 10 mL of extracting solution were added to the 30 mL reaction vial. Each vial was loosely sealed with the screw cap and placed in the heating block. The samples were heated for 30 min. After heating, the screw caps were firmly secured and the samples were placed in a slightly cool water bath and tap water was allowed to flow pass the samples for approximately 5 min. The samples were then removed from the bath and set aside for analysis.

j. Analysis

The spectrophotometer was calibrated with a deionized water blank according to the procedures specified by the manufacturer. Approximately 2 mL of the standard solutions were placed in the spectrophotometer tube. Absorbance readings were taken at 510 nm and 552 nm. The flour samples were measured in the same manor. Note: It is important to take measurements quickly as the solution will begin to change color while sitting.

VI. RESULTS

A. Flour Moisture

The flour moisture is critical for running such experiments as the baking test and viscosity measurements using the RVA. In both of these methods, the analysis is based on specific solid and moisture levels in order to compare control vs. test samples. For the baking test and viscosity test the flour moistures are assumed to be 13% and 14% respectively. In both methods if the target moisture is not achieved, water is added to or removed from the required water level in the dough.

The flour moisture was determined using the Computrac Moisture Analyzer according to the procedures mentioned in methodology section A. The flour moisture was determined prior to each baking or viscosity experiment if more than a week passed since the last measurement. The constant monitoring of the flour moisture was necessary since changes in flour moisture can occur over time due to moisture pick up during storage. The flour moisture values determined by the Computrac are shown in Table 3.

Table 3. Moisture content of whole grain rye flour – Computrac

Moisture Test	Moisture (%)	Average Moisture (%)
Test 1	11.661	11.383
	11.277	
	11.210	
Test 2	11.563	11.403
	11.489	
	11.157	
Test 3	11.463	11.362
	11.370	
	11.253	

The AOAC 45 – 15A Flour Moisture method is a validated method to determine the moisture of grains. This method was used to determine the moisture of the whole grain rye flour as well. The value was compared to the moisture value of whole grain rye flour as determined by the Computrac method. The air oven method was compared to Computrac Test 1 only since both experiments were conducted during the same time period. The comparison values are shown in Table 4. Since the values are similar, further moisture analysis was conducted using the Computrac due to the rapidness of the test. Computrac testing was approximately 5 min and the air oven testing was approximately 1 hr.

Table 4. Moisture content of whole grain rye flour – Computrac and air oven

Moisture Test	Moisture (%)	Average Moisture (%)
Test 1	11.661	11.383
Computrac	11.277	
	11.210	
Test 1	11.528	11.486
Air Oven	11.392	
	11.537	

B. Whole Grain Rye Flour Sugar Cookie

When evaluating flour functionality, it is beneficial to evaluate the ingredient in finished products such as baked goods. The American Association of Cereal Chemist have approved a baking test to evaluate flour in a sugar cookie model system. The AACC Baking Quality of Cookie Flour Method determines how the flour attributes effect cookie geometry – length, width and height. It also gives an indication of the moisture loss, crumb structure, color, etc. While this method was specifically designed to evaluate soft white wheat flour varieties, it is believed other flours and/or other functional ingredients can be evaluated using this model system as well.

Sugar cookies with whole grain rye flour were produced according to the Cookie Method discussed in the methodology section G. As hypothesized for this research topic, enzymes will alter the properties of whole grain rye flour by reducing the arabinoxylan molecular weight, making some more water soluble, and some more easily extractable. These alterations can in turn alter the properties of low moisture baked goods such as cookies.

1. Design

In order to understand the effect of rye flour and specific enzymes on the sugar cookie, three types of enzymes were added to the cookie and evaluated against a control whole grain rye flour cookie without enzymes. The *Bacillus subtilis* xylanase enzyme was expected to aid in solubilizing the arabinoxylans

previously deemed water unextractable. The *Aspergillus niger* xylanase enzyme was expected to modify water extractable arabinoxylans. Arabinofuranosidase was expected to cleave the arabinose side chain from the xylose backbone. Each enzyme was evaluated at a low and high level and each enzyme sample was produced in triplicate in order to evaluate statistical differences between samples. The control cookie sample was produced four times.

Table 5 shows the enzymes used during this study and the codes used to represent these enzymes. The level of enzymes is also indicated in the table. These levels were established based on preliminary work (not discussed in this dissertation) which showed a significant difference when 1.5000 g of enzyme were added to the cookie. This level was then doubled to represent a high level in the cookie system. It is important to note that the enzymes were used based on a gram level added and not based on the enzyme activity level.

Table 5. Whole grain rye flour sugar cookie – sample codes

Sample Description	Enzyme	Enzyme Level (g)
C1	Control - no enzyme	N/A
C2	Control - no enzyme	N/A
C3	Control - no enzyme	N/A
C4	Control - no enzyme	N/A
BS1	*Bacillus subtilis*	1.5000
BS2	*Bacillus subtilis*	1.5000
BS3	*Bacillus subtilis*	1.5000
BS4	*Bacillus subtilis*	3.0000
BS5	*Bacillus subtilis*	3.0000
BS6	*Bacillus subtilis*	3.0000
AN1	*Aspergillus niger*	1.5000
AN2	*Aspergillus niger*	1.5000
AN3	*Aspergillus niger*	1.5000
AN4	*Aspergillus niger*	3.0000
AN5	*Aspergillus niger*	3.0000
AN6	*Aspergillus niger*	3.0000
AF1	Arabinofuranosidase	1.5000
AF2	Arabinofuranosidase	1.5000
AF3	Arabinofuranosidase	1.5000
AF4	Arabinofuranosidase	3.0000
AF5	Arabinofuranosidase	3.0000
AF6	Arabinofuranosidase	3.0000

In order to better understand the effects of the flour and enzymes on the cookie, each variable was baked in triplicate, on different days and in a random order. Typically each day a variable is produced, a control is produced. This aids in eliminating possible day effects of baking. Table 6 shows the random design used for the baking portion of the study. If a sample encountered an error during mixing or baking, this sample was removed from that day's production and produced on the back up day. As a result of some samples being removed and produced on the back up day, day to day effects were not considered. The variable used are highlighted in Table 6.

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The statistical analysis for all samples was conducted using a one-way analysis of variance (ANOVA) and pairwise comparison of differences evaluated by Tukey. For analysis, a P value < 0.05 was considered significant. Also note, differences within a sample set were not considered. Only differences between samples were analyzed. For example, dough texture for the control was conducted three times per sample. These numbers were averaged and noted as sample C1. The same procedure was conducted for C2, C3 and C4. When results are reported, they are noted as average of C1 – C4. Based on the procedures, samples C1 - C4 were composed of 12 individual sample points.

Table 6 – Whole grain rye flour sugar cookie – baking design

Random Order	Day 1	Random Order	Day 2	Random Order	Day 3
0.4360	AF - H	0.7710	AN - L	0.9470	AN - L
0.0760	C	0.2800	BS - L	0.6650	AN - H
0.9260	BS - H	0.1230	C	0.6990	AF - L
0.5980	AN - H	0.3350	AN - L	0.2260	C
		0.3530	AN - H	0.2390	AF - H
		0.2210	BS - H	0.7290	BA - H
		0.9290	AN - H	0.7620	BS - L

Random Order	Day 4	Random Order	Day 5
0.4010	AN - L	N/A	C
0.5470	AF - L	N/A	AN - L
0.3300	C	N/A	BS - H
0.9810	BS - L	N/A	AN - H

C = Control
BS - L = BS samples 1, 2, 3
BS - H = BS samples 4, 5, 6
AN - L = AN samples 1, 2, 3
AN - H = AN samples 4, 5, 6
AF - L = AF samples 1, 2, 3
AF - H = AF samples 4, 5, 6

C. Baking

As indicated in the official Flour Quality Method, the temperature of the ingredients is critical and should be kept constant for all test bakes. If the temperature of an ingredient such as fat is too high, it may result in a high dough temperature and a softer final dough texture. This type of dough may produce cookies with larger geometry and not show the effect of the ingredients inherent attributes at constant temperature. Table 7 shows the temperature of the critical ingredients and the temperature of the mixture at various stages. For the fat, the temperature varied approximately 1ºC. For the water, stage 1 and dough temperature, a range of approximately 2ºC was noted.

According to the baking method, it is important to adjust the flour and water level based on the flour moisture. Based on the flour moistures given in Table 3, the flour and moisture values required for baking were calculated using the formulation provided in methodology section G. The total added water and flour are shown in Table 8.

Table 7. Whole grain rye flour sugar cookie – ingredient and mixing temperatures

Sample Description	Fat Temperature (ºC)	Water Temperature (ºC)	Stage 1 Temperature (ºC)	Dough Temperature (ºC)
C1	20.0	22.2	21.9	22.4
C2	19.6	22.0	21.9	22.1
C3	20.5	22.3	22.4	22.5
C4	20.0	21.8	21.7	21.9
BS1	19.8	22.5	22.2	22.8
BS2	20.3	22.8	22.4	22.7
BS3	20.3	22.0	21.8	21.9
BS4	19.6	22.4	21.9	22.4
BS5	19.5	22.3	22.1	22.5
BS6	19.9	21.3	22.0	21.9
AN1	20.0	22.8	22.4	23.1
AN2	20.3	22.7	22.0	22.2
AN3	19.7	21.8	21.7	22.0
AN4	19.6	22.2	21.7	22.5
AN5	19.8	22.1	22.0	22.3
AN6	20.3	21.7	22.1	21.9
AF1	19.8	22.8	22.5	22.5
AF2	19.8	22.2	22.0	22.5
AF3	20.3	22.3	*	22.0
AF4	19.8	22.4	22.3	22.7
AF5	19.9	23.0	22.4	23.0
AF6	19.8	22.1	21.8	22.3

*Value not recorded

Table 8. Whole grain rye flour sugar cookie – ingredient levels

Sample Description	Water Weight (g)	Flour Weight (g)	Enzyme Weight (g)
C1	53.61	220.89	N/A
C2	53.61	220.89	N/A
C3	53.56	220.94	N/A
C4	53.56	220.94	N/A
BS1	53.61	220.89	1.5001
BS2	53.61	220.89	1.5001
BS3	53.56	220.94	1.5001
BS4	53.61	220.89	3.0005
BS5	53.61	220.89	3.0000
BS6	53.56	220.94	3.0001
AN1	53.61	220.89	1.5000
AN2	53.61	220.89	1.5001
AN3	53.56	220.94	1.5001
AN4	53.61	220.89	3.0005
AN5	53.61	220.89	3.0008
AN6	53.56	220.94	3.0001
AF1	53.61	220.89	1.5000
AF2	53.61	220.89	1.5000
AF3	53.56	220.94	1.5002
AF4	53.61	220.89	3.0003
AF5	53.61	220.89	3.0004
AF6	53.61	220.89	3.0009

D. Moisture Loss

In order to achieve a cookie product with the appropriate moisture value, a baking moisture loss of approximately 13.58% was targeted. To obtain this value, the control product was baked at 400ºF for a series of bake times. For whole grain rye flour bake times of 11.5 min, 12 min, 12.5 min, 13 min and 13.5 min were evaluated and a bake time of 13.5 min was chosen to be most appropriate. All prototypes were baked using this condition and moisture loss was compared to the control sample – whole grain rye flour without enzymes.

The moisture loss of the cookies is shown in Table 9. The ANOVA statistical analysis for cookie moisture generated a P value of 0.008, indicating significant differences between some of the samples. The ANOVA analysis is shown in Table 10. The Tukey pairwise comparison provided more insight to the differences between samples. The control sample had a similar moisture loss as the BS, AN and AF series of cookies. This indicated that the enzymes did not influence the moisture loss and the same bake time was appropriate to bake the control and enzyme sample to approximately the same moisture loss using the same baking conditions. There was not a significant difference between the low and high enzyme values within a sample set e.g. no statistical difference between BS 1 – 3 and BS 4- 6. The BS 1 – 3 series was significantly different and had a significantly higher moisture loss than the AN 1 – 3 and AN 4 – 6 series, indicating that a longer bake time would be required if the moisture loss of

the AN series is intended to be the same as the BS 1 – 3 series. The BS 4 – 6 was significantly different and had a significantly higher moisture loss than the AN 4 - 6 also indicating a longer bake time would be required for the AN 4-6 set in order to achieve a comparable moisture loss to the BS 4 - 6 set.

Table 9. Whole grain rye flour sugar cookie – moisture loss during baking

Sample Description	Cookie Moisture Loss (%)	Avg Cookie Moisture Loss (%)
C1	13.68	13.69
C2	14.04	
C3	13.40	
C4	13.63	
BS1	14.20	14.09
BS2	14.24	
BS3	13.82	
BS4	14.15	13.94
BS5	13.91	
BS6	13.77	
AN1	13.80	13.36
AN2	13.11	
AN3	13.16	
AN4	13.22	13.24
AN5	13.26	
AN6	13.24	
AF1	13.64	13.62
AF2	13.87	
AF3	13.34	
AF4	13.87	13.74
AF5	13.53	
AF6	13.82	

Table 10. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie moisture

Source	DF	SS	MS	F	P
Sample Number	6	1.6186	0.2698	4.51	0.008
Error	15	0.8971	0.0598		
Total	21	2.5157			

S = 0.2446 R-Sq = 64.34% R-Sq(adj) = 50.07%

Level	N	Mean	StDev	Individual 95% CIs For Mean Based on Pooled StDev
C	4	13.688	0.265	(-----*------)
BS1-3	3	14.087	0.232	(------*-------)
BS4-6	3	13.943	0.192	(-------*------)
AN1-3	3	13.357	0.385	(-------*------)
AN4-5	3	13.240	0.020	(-------*-------)
AF1-3	3	13.617	0.266	(------*-------)
AF4-6	3	13.740	0.184	(------*-------)
				13.20 13.60 14.00 14.40

Pooled StDev = 0.245

D. Geometry

The geometry of the cookie is very important as it visually impacts consumer perception of the product and also effects the ability of manufactures to package the product. The geometry of the rye cookies includes the length, width and height of the cookie. As shown in Figure 8, the width is measured parallel to the impressions, the length is measured perpendicular to the impressions and the height is the thickness of the cookie. When the length, width and height are recorded, it is a summation of the four cookies baked per batch.

The width, length and height of each series is shown in Table 11. The ANOVA statistical analysis shows P values of 0.000, 0.000 and 0.012 for the width, length and height respectively. Although the differences in the width, length and height values seem small, all of these P values were less than 0.05 and indicate there were significant differences between some samples. The ANOVA analysis is shown in Tables 12, 13 and 14.

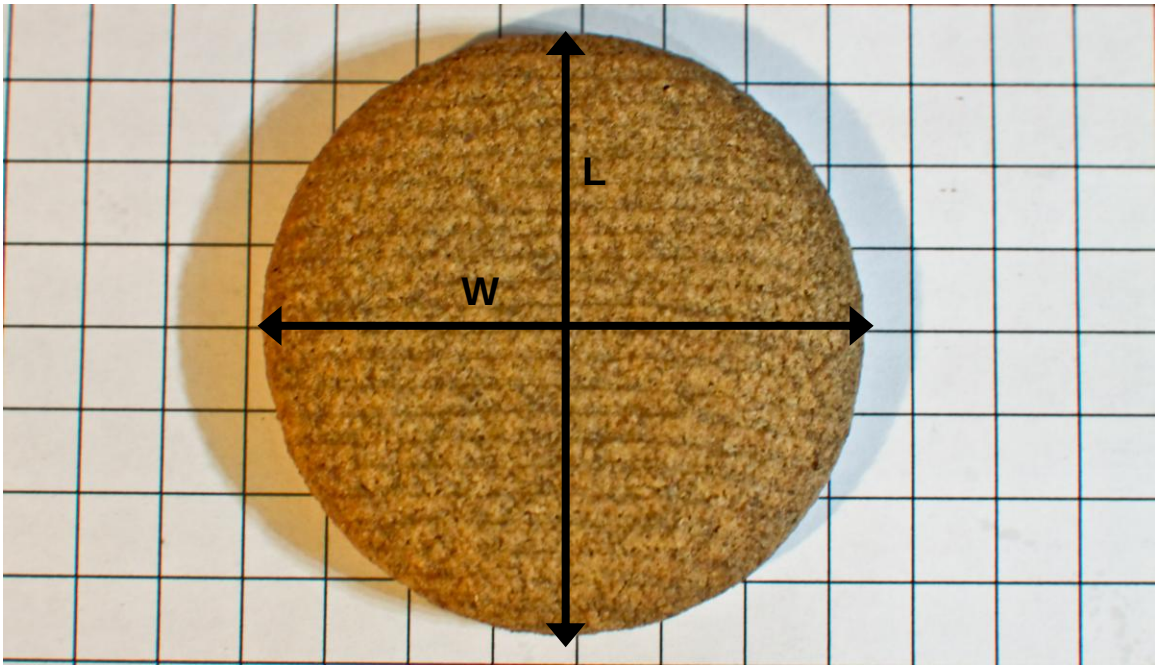


Figure 8 – Whole grain rye flour sugar cookie – geometry orientation

For the cookie width, there was no significant difference between the low and high levels of enzymes within each series e.g. the width of series BS 1 – 3 is not significantly different than the width of series BS 4 – 6. Figure 9 depicts these similarities. However, there were differences between the control sample and cookies produced with some of the enzymes or between various types of enzymes. The width of the control was significantly smaller than samples BS1 – 3 and BS 4 – 6 with an average value of 28.5 cm for the control and 29.5 cm and 29.3 cm for the BS 1 -3 and BS 4 – 6 respectively. This is depicted in Figure 10. The BS 1 – 3 and 4 – 6 series was wider than the AN low and high level enzymes series 1 – 3 and 4 – 6. The BS 1 – 3 series was also wider than AF 1 – 3.

Table 11. Whole grain rye flour sugar cookie - geometry

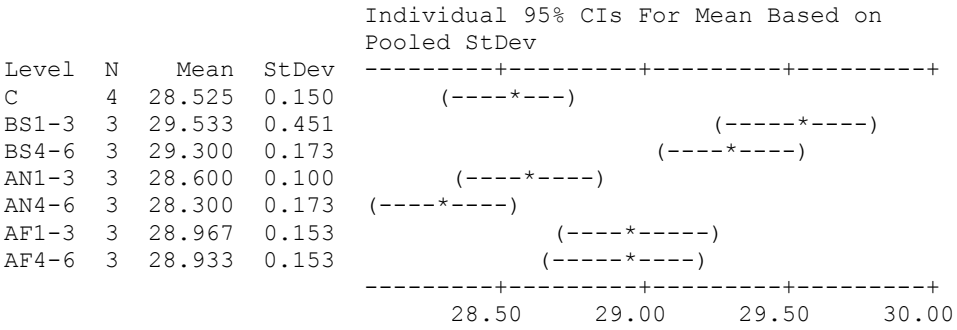
Sample Description	Width (cm)	Avg Width (cm)	Length (cm)	Avg Length (cm)	Height (cm)	Avg Height (cm)
C1	28.4	28.5	29.0	29.0	4.4	4.5
C2	28.7		29.3		4.4	
C3	28.4		28.8		4.5	
C4	28.6		28.9		4.5	
BS1	30.0	29.5	30.0	30.0	4.1	4.2
BS2	29.5		30.1		4.2	
BS3	29.1		29.9		4.3	
BS4	29.4	29.3	30.0	29.9	4.2	4.2
BS5	29.4		30.0		4.1	
BS6	29.1		29.8		4.3	
AN1	28.7	28.6	29.2	29.0	4.2	4.3
AN2	28.5		28.8		4.4	
AN3	28.6		29.0		4.4	
AN4	28.1	28.3	28.8	28.8	4.4	4.4
AN5	28.4		28.9		4.4	
AN6	28.4		28.7		4.5	
AF1	28.8	29.0	29.1	29.4	4.4	4.3
AF2	29.0		29.5		4.3	
AF3	29.1		29.6		4.3	
AF4	28.9	28.9	29.3	29.4	4.4	4.3
AF5	28.8		29.1		4.4	
AF6	29.1		29.7		4.2	

Table 12. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie width

Source	DF	SS	MS	F	P
Sample Number	6	3.5834	0.5972	12.66	0.000
Error	15	0.7075	0.0472		
Total	21	4.2909			

S = 0.2172 R-Sq = 83.51% R-Sq(adj) = 76.92%

Level	N	Mean	StDev
C	4	28.525	0.150
BS1-3	3	29.533	0.451
BS4-6	3	29.300	0.173
AN1-3	3	28.600	0.100
AN4-6	3	28.300	0.173
AF1-3	3	28.967	0.153
AF4-6	3	28.933	0.153



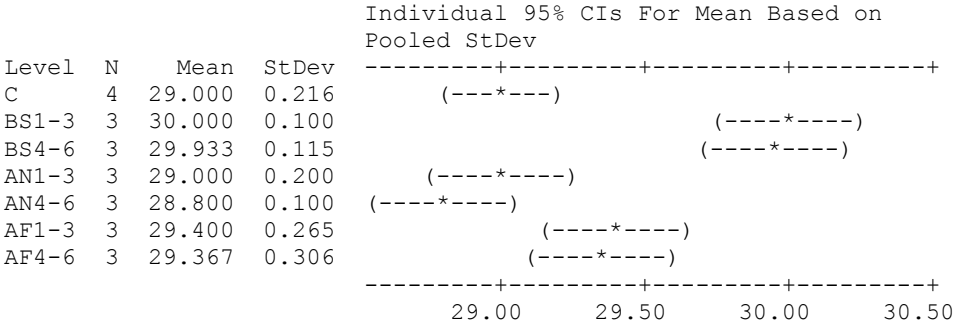
Pooled StDev = 0.217

Table 13. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie length

Source	DF	SS	MS	F	P
Sample Number	6	4.0598	0.6766	16.55	0.000
Error	15	0.6133	0.0409		
Total	21	4.6732			

S = 0.2022 R-Sq = 86.88% R-Sq(adj) = 81.63%

Level	N	Mean	StDev
C	4	29.000	0.216
BS1-3	3	30.000	0.100
BS4-6	3	29.933	0.115
AN1-3	3	29.000	0.200
AN4-6	3	28.800	0.100
AF1-3	3	29.400	0.265
AF4-6	3	29.367	0.306



Pooled StDev = 0.202

Table 14. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie height

```
Source          DF       SS       MS     F      P
Sample Number    6  0.19106  0.03184  4.09  0.012
Error           15  0.11667  0.00778
Total           21  0.30773

S = 0.08819   R-Sq = 62.09%   R-Sq(adj) = 46.92%

                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean   StDev  ---------+---------+---------+---------+
C      4  4.4500  0.0577                       (-------*-------)
BS1-3  3  4.2000  0.1000  (--------*--------)
BS4-6  3  4.2000  0.1000  (--------*--------)
AN1-3  3  4.3333  0.1155            (--------*--------)
AN4-6  3  4.4333  0.0577                     (--------*--------)
AF1-3  3  4.3333  0.0577            (--------*--------)
AF4-6  3  4.3333  0.1155            (--------*--------)
                          ---------+---------+---------+---------+
                                 4.20      4.32      4.44      4.56

Pooled StDev = 0.0882
```

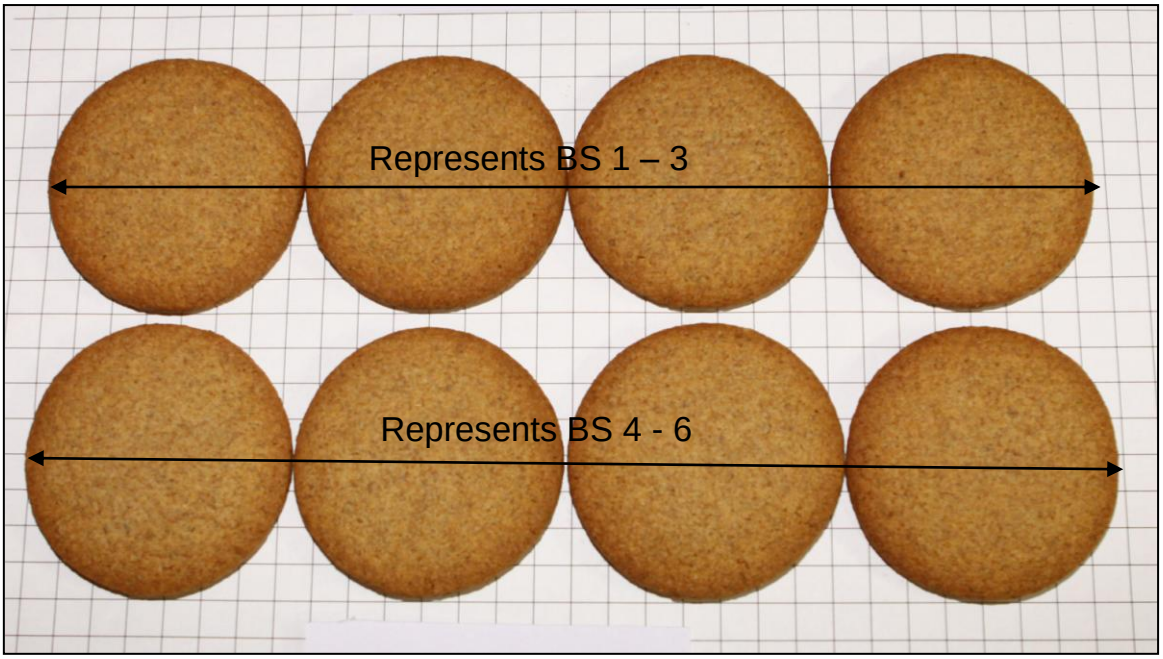


Figure 9. Whole grain rye flour sugar cookie – representative of BS 1 – 3 series vs. representative of BS 4 – 6 series

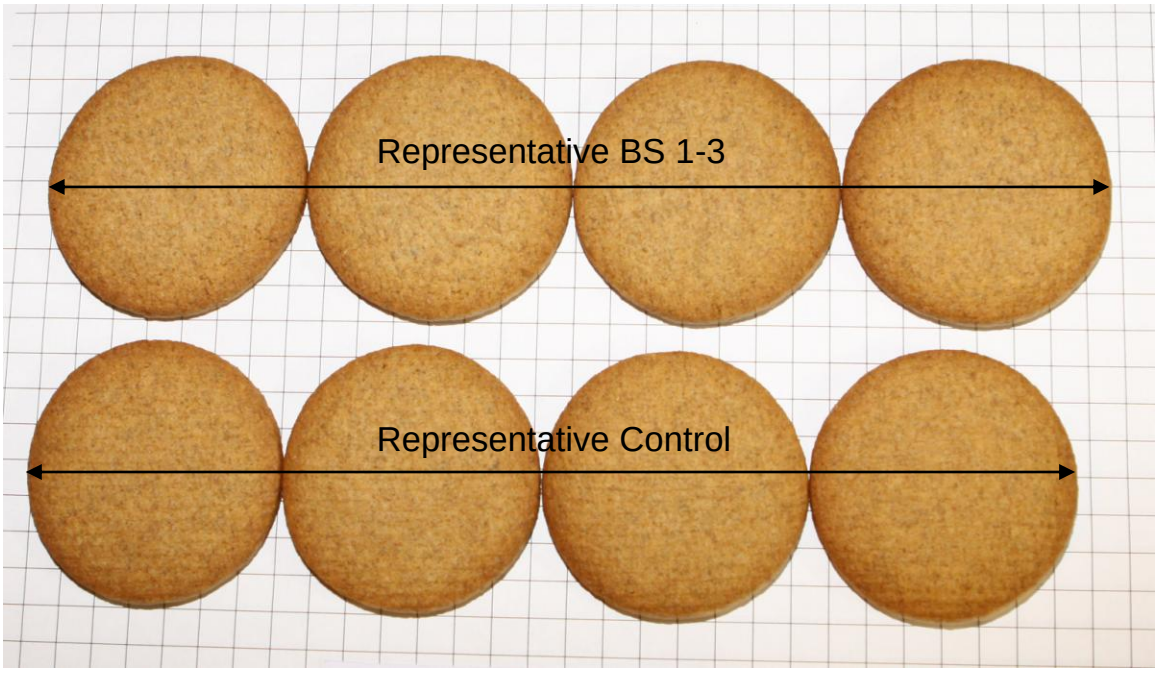


Figure 10. Whole grain rye flour sugar cookie – control vs. representative BS 1-3 series

There were also differences in the cookies' length. Similar to the width of the samples, the control was similar in length to all samples except the BS 1 – 3 and 4 – 6 series which were much longer than the control. The BS 1 - 3 series was also longer than the AN and AF series. The BS 4 -6 series alone was longer than all samples with the AF 1 – 3 being the exception.

The thickness of the cookies also referred to as the cookie height shows differences as well. In this instance, the control was significantly thicker than the BS low and high enzyme series but not significantly thicker than the other enzyme series. There were no other significant differences between high and low enzyme levels within a series or between cookies produced with different enzymes.

Overall, the *Bacillus subtilis* enzyme at either a high or low level produces cookies with significantly different width, length and height vs. the control sample. The enzyme also produced differences between other enzyme types as well. It is believed that the *Bacillus subtilis* creates more water soluble arabinoxylans out of water unextractable arabinoxylans. The excess, lower molecular weight enzymes now in the soluble phase allows for more spread of the cookie.

E. Dough Texture

The TA-XT2 was used to measure the firmness and stickiness of the dough. For each sample, three measurements were taken and averaged. These

averages were then combined to represent one sample e.g. C1. An example of a dough texture graph is shown in Figure 11. The positive force value represents the firmness and the negative force value represents stickiness. For all samples, the ANOVA analysis was used to determine statistical differences between the samples and the standard deviation. The firmness and stickiness values of the samples along with the standard deviation are shown in Table 15. Other researchers indicate a standard deviation of +/- 10% can be typical for cookie dough samples. For some samples, the standard deviation may be high due to difficultly in forming a uniform dough mass in which to accurately measure the rheological properties. The P values for the doughs were 0.679 and 0.933 for the firmness and stickiness respectively. Since these values were well above a 0.05, there was not a significant difference between the firmness or stickiness of the samples. Table 16 and 17 show the ANOVA analysis.

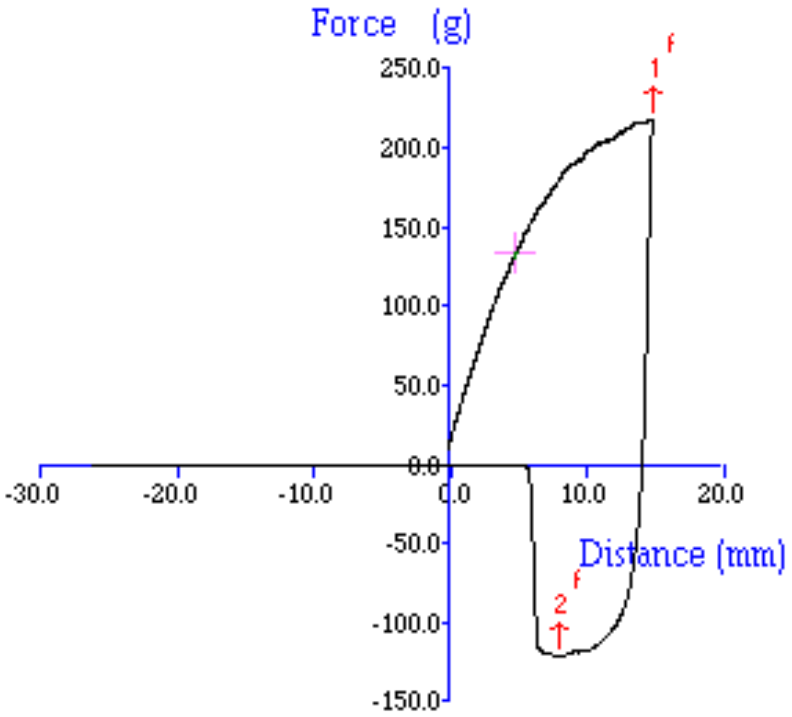


Figure 11. Whole grain rye flour sugar cookie – dough texture

Table 15. Whole grain rye flour – dough texture

Sample Description	Firmness (g)	Avg Firmness (g)	Std Dev	Stickiness (g)	Avg Stickiness (g)	Std Dev
C1	204.00	220.60	22.11	-105.00	-122.60	14.24
C2	201.90			-119.00		
C3	228.20			-127.70		
C4	248.30			-138.70		
BS1	184.10	219.60	47.61	-96.40	-119.37	31.91
BS2	201.00			-105.90		
BS3	273.70			-155.80		
BS4	202.20	228.00	44.69	-109.60	-125.37	29.86
BS5	202.20			-106.70		
BS6	279.60			-159.80		
AN1	188.80	225.50	31.79	-100.40	-122.17	18.86
AN2	244.60			-132.40		
AN3	243.10			-133.70		
AN4	253.80	255.13	15.04	-124.50	-135.73	15.80
AN5	240.80			-128.90		
AN6	270.80			-153.80		
AF1	273.30	245.67	24.73	-133.90	-128.20	6.71
AF2	238.10			-120.80		
AF3	225.60			-129.90		
AF4	217.00	221.07	3.54	-115.10	-117.80	4.5
AF5	223.50			-115.30		
AF6	222.70			-123.00		

Table 16. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie dough firmness

```
Source          DF     SS   MS     F      P
Sample Number    6   3647  608  0.66  0.679
Error           15  13714  914
Total           21  17361

S = 30.24   R-Sq = 21.01%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on
                          Pooled StDev
Level  N    Mean  StDev  ---------+---------+---------+---------+
C      4  220.25  22.10    (---------*----------)
BS1-3  3  219.60  47.61  (-----------*-----------)
BS4-6  3  228.00  44.69     (-----------*-----------)
AN1-3  3  225.50  31.79    (-----------*-----------)
AN4-6  3  255.13  15.04              (-----------*-----------)
AF1-3  3  245.67  24.73          (------------*-----------)
AF4-6  3  221.07   3.54  (------------*-----------)
                          ---------+---------+---------+---------+
                                 210       240       270       300

Pooled StDev = 30.24
```

Table 17. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie dough stickiness

```
Source          DF    SS   MS     F      P
Sample Number    6   666  111  0.29  0.933
Error           15  5768  385
Total           21  6434

S = 19.61   R-Sq = 10.35%   R-Sq(adj) = 0.00%


                          Individual 95% CIs For Mean Based on Pooled
                          StDev
Level  N     Mean  StDev     +---------+---------+---------+---------
C      4  -122.60  14.24             (----------*---------)
BS1-3  3  -119.37  31.91             (-----------*-----------)
BS4-6  3  -125.37  29.86          (-----------*-----------)
AN1-3  3  -122.17  18.86            (-----------*-----------)
AN4-6  3  -135.73  15.80     (-----------*-----------)
AF1-3  3  -128.20   6.71         (-----------*-----------)
AF4-6  3  -117.80   4.50              (-----------*-----------)
                          +---------+---------+---------+---------
                        -160      -140      -120      -100

Pooled StDev = 19.61
```

F. Cookie Color

As previously mentioned, the color of a product may strongly effect the consumer perception and acceptance of the product. This holds true for baked goods such as cookies. The color may be a result of the baking time, moisture loss or chemical reactions occurring during the baking process. The whole grain rye flour sugar cookie samples with and without enzymes were ground and the L*, a* and b* values were measured and statistical differences were determined. The values are shown in Table 18. According to the ANOVA analysis the P values were 0.004, 0.002 and 0.100 for the L*, a* and b* respectively. The P values for both the L* and a* indicate that there were significant differences among the samples. There was not a significant difference in the b* values. The ANOVA analysis for the L* and a* values are shown in Tables 19 and 20 respectively. The ANOVA analysis of the b* value is not shown since a significant difference was not detected.

The L* value represents the black to white hues of the cookie. Within an enzyme set, there was no difference between the L* value e.g. the BS low enzyme series did not have a significantly different L* value vs. the BS high enzyme series. The control was similar to all samples except the BS series. The BS series contained significantly more black hues. The BS high enzyme level was only different than the high level of AN.

The a* value represents the green to red hues of the cookie. Similar to the L* value, there was no difference in a* values within a low-high set. The control sample is significantly different than the BS series which has a more red hue. The BS4-6 also had a more red hue vs. the AN1-3 and 4-6 series. As previously mentioned, there was no significant difference between the b*, blue to yellow hues, in the samples.

Although the samples were ground, the uniformity of the ground sample may be a factor in the color differences. Prior to grinding, the cookie color top and bottom were measured. The data is not shown but the results indicate that the top color of all the cookies were the same. Some differences were detected for the L* and b* bottoms. Some of these differences may translate into the ground sample color differences.

Table 18. Whole grain rye flour sugar cookie – ground cookie color

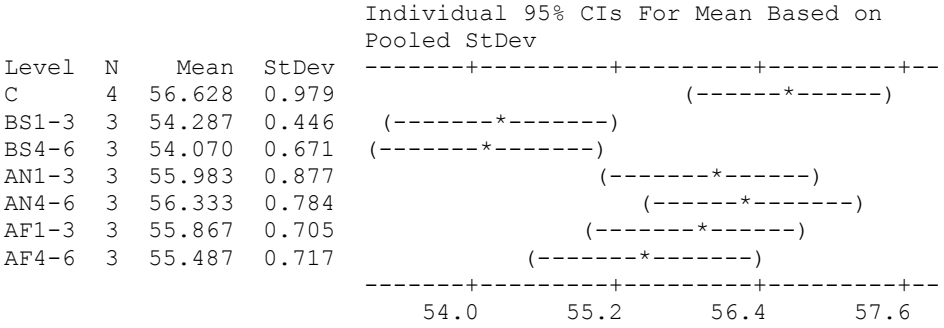
Sample Description	L*	Avg L*	a*	Avg a*	b*	Avg b*
C1	56.77	56.63	11.61	11.50	31.42	31.46
C2	55.71		11.66		31.82	
C3	57.94		11.19		30.94	
C4	56.09		11.53		31.66	
BS1	54.80	54.29	12.34	12.43	32.35	32.32
BS2	53.99		12.49		32.37	
BS3	54.07		12.45		32.23	
BS4	53.48	54.07	12.92	12.54	31.74	32.02
BS5	53.93		12.63		32.34	
BS6	54.80		12.07		31.99	
AN1	56.31	55.98	11.73	11.72	31.54	30.71
AN2	54.99		12.11		31.30	
AN3	56.65		11.33		29.28	
AN4	57.04	56.33	11.68	11.70	31.26	31.69
AN5	55.49		11.88		31.98	
AN6	56.47		11.55		31.83	
AF1	56.67	55.87	11.67	11.93	31.77	31.90
AF2	55.35		12.13		31.96	
AF3	55.58		11.99		31.97	
AF4	56.17	55.49	11.60	11.97	30.71	31.62
AF5	55.55		12.10		31.83	
AF6	54.74		12.21		32.31	

Table 19. Whole grain rye flour sugar cookie – ANOVA Analysis of ground cookie color L* value

Source	DF	SS	MS	F	P
Sample Number	6	18.709	3.118	5.22	0.004
Error	15	8.963	0.598		
Total	21	27.672			

S = 0.7730 R-Sq = 67.61% R-Sq(adj) = 54.66%

Level	N	Mean	StDev
C	4	56.628	0.979
BS1-3	3	54.287	0.446
BS4-6	3	54.070	0.671
AN1-3	3	55.983	0.877
AN4-6	3	56.333	0.784
AF1-3	3	55.867	0.705
AF4-6	3	55.487	0.717

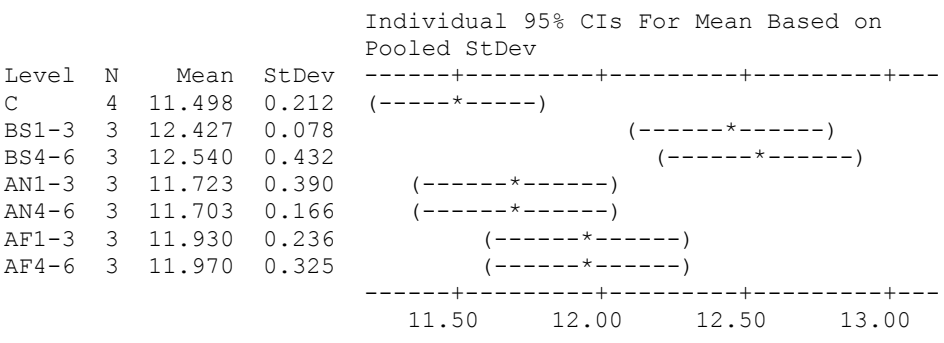


Pooled StDev = 0.773

Table 20. Whole grain rye flour sugar cookie – ANOVA Analysis of ground cookie color a* Value

Source	DF	SS	MS	F	P
Sample Number	6	2.8840	0.4807	6.00	0.002
Error	15	1.2023	0.0802		
Total	21	4.0863			

S = 0.2831 R-Sq = 70.58% R-Sq(adj) = 58.81%



Pooled StDev = 0.283

G. Cookie Texture

The cookie texture also effects the consumer perception. The cookie texture either hard or soft should match the consumer expectation. Similar to the color of the cookie, the texture can also be influenced by the moisture loss, baking conditions and ingredient reactions. The cookie texture was measured using the TA-XT2 with a 3-pt bend apparatus. The data is shown in Table 21. Similar to dough texture, the cookie texture had a large standard deviation. These differences have also been seen in researchers' previous experiments. The ANOVA analysis showed a P value of 0.007 indicating significant differences between some samples. The ANOVA analysis is in Table 22. The control was similar to all samples. The BS low – high enzyme series was significantly less hard than the AN high series.

H. Organoleptic Evaluation

No formal organoleptic evaluation was conducted for the whole grain rye flour cookie samples. Informal tasting of the control indicated acceptable texture with earthy and brown notes.

Table 21. Whole grain rye flour sugar cookie – cookie texture

Sample Description	Hardness (g)	Avg Hardness (g)	Std Dev
C1	3334.00	3044.23	344.9
C2	2678.70		
C3	3343.20		
C4	2821.00		
BS1	2211.50	2547.30	291.7
BS2	2737.60		
BS3	2692.80		
BS4	2393.10	2873.60	457.3
BS5	2924.10		
BS6	3303.60		
AN1	2918.30	3347.50	385.9
AN2	3458.50		
AN3	3665.70		
AN4	3823.40	3827.70	13.9
AN5	3816.50		
AN6	3843.20		
AF1	3103.57	3080.35	275.2
AF2	2794.27		
AF3	3343.20		
AF4	3277.27	3068.86	248.2
AF5	3135.03		
AF6	2794.27		

Table 22. Whole grain rye flour sugar cookie – ANOVA Analysis of cookie texture

```
Source          DF       SS      MS     F      P
Sample Number    6  2857067  476178  4.70  0.007
Error           15  1518123  101208
Total           21  4375190

S = 318.1   R-Sq = 65.30%   R-Sq(adj) = 51.42%

                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level  N    Mean  StDev  ----+---------+---------+---------+-----
C      4  3044.2  344.9           (-----*----)
BS1-3  3  2547.3  291.7  (-----*------)
BS4-6  3  2873.6  457.3       (------*-----)
AN1-3  3  3347.5  385.9               (------*-----)
AN4-6  3  3827.7   13.9                       (------*-----)
AF1-3  3  3080.3  275.2           (-----*------)
AF4-6  3  3068.9  248.2           (-----*------)
                           ----+---------+---------+---------+-----
                            2400      3000      3600      4200

Pooled StDev = 318.1
```

I. Farinograph

The farinograph is a relatively quick and simple way to evaluate the rheological properties of dough. It evaluates the dough by measuring the dough's resistance against the blades during mixing. The resulting graph shows the dough's development time, the maximum consistency of the dough, rate of hydration and dough breakdown. The arrival time is defined by curve reaching 50% or 500 BU and is an indication of hydration. The peak time is defined as the dough development time when the dough reaches maximum consistency. The departure time is defined as the time when the curve leaves this line as a result of the dough breakdown.

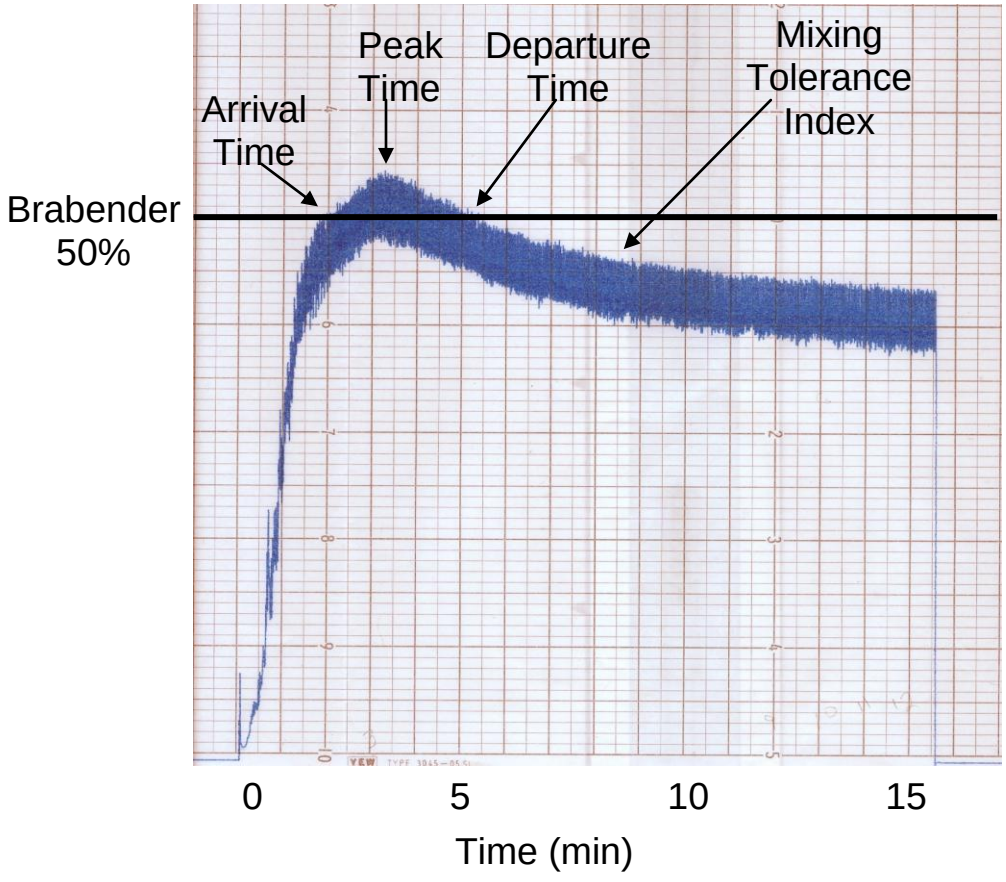


Figure 12 Whole grain rye flour - farinograph.

The farinograph in Figure 12 is a result of 200 g of rye flour and 107 mL of water. For the mixing bowl capacity, typically a 300 g dry basis sample is used but with the addition of water and the mixing required, the overall volume of dough appeared to exceed the capacity of the bowl. After several mixing trials, 107 mL of water was determined to be optimal. According to the farinograph of the whole grain rye flour, it showed the characteristics of a weak dough. As described by Figure 5 reference of wheat flour. The arrival time for the whole grain rye flour was at 2 minutes. The peak time was at 3.5 min, indicating a quick water absorption. The dough had minimal development and then broke down after 5 min. The weak dough characteristics of whole grain rye flour is consistent with previous research which has proven that there is less gluten development and it plays a less critical role in the structure of the flour.

Typically water is added to flour in order to optimally develop the dough. For the following experiment, reaching the critical BU value was not the objective. The purpose of the experiments was to add the enzymes at low and high levels proportional to a 200 g sample of rye flour with the same amount of water that produced the optimum mixing curve show in Figure 12 – whole grain rye flour without enzyme. When the enzyme is added to the flour, it alters the arabinoxylans which along with gluten provide some structure. As a result a dough weaker than the sample without enzyme was shown. Also, research shows that arabinoxylans, specifically the water extractable arabinoxylans

influenced by xylanase, can interfere with gluten development, creating even less of a structural network and providing a weaker dough.

The enzyme levels were based on the amount of enzyme to effectively alter the characteristics of the sugar cookie. An average flour weight of 220.9 g was used during the cookie baking. The effective low and high level of enzymes were determined to be 1.5000 g and 3.0000 g respectively. A proportional enzyme level for 200 g is 1.3581 g and 2.7162 g for a low and high value respectively.

Figure 13 show whole grain rye flour with the addition of *Bacillus subtilis* at a low enzyme level. The *Bacillus subtilis* enzyme is intended to affect the water unextractable enzymes, making them more water soluble. Although there is very little gluten in the rye flour, this enzyme may weaken the limited gluten and arabinoxylan network resulting in a poorly developed dough with this quantity of water. Although only one water level, 107 g/200 g flour was used during this experiment, one advantage of using this enzyme was the requirement of less water to produce a farinograph curve similar to the control sample without enzyme. It should also be noted that the farinograph with the higher level of *Bacillus subtilis* enzyme (figure not shown) produced the same curve as the low level, indicating it was not advantageous to double the amount of enzyme in order to see an effect in the flour/water system.

The farinograph of whole grain rye flour with the addition of *Aspergillus niger* is shown in Figure 14. The low enzyme level, approximately 1.3581 g was used and 107 g of water/200 g of flour. The *Aspergillus niger* is intended to reduce the molecular weight of the water extractable arabinoxylans. This reduced weight contributes to the weakened structure of the flour. The weaken structure is represented by the inability of the flour to achieve the 50% Brabender value with the same amount of water as the control. In order to achieve a curve similar to the control, less water was required. The arabinofuranosidase sample required less water vs. the control as well, results not shown.

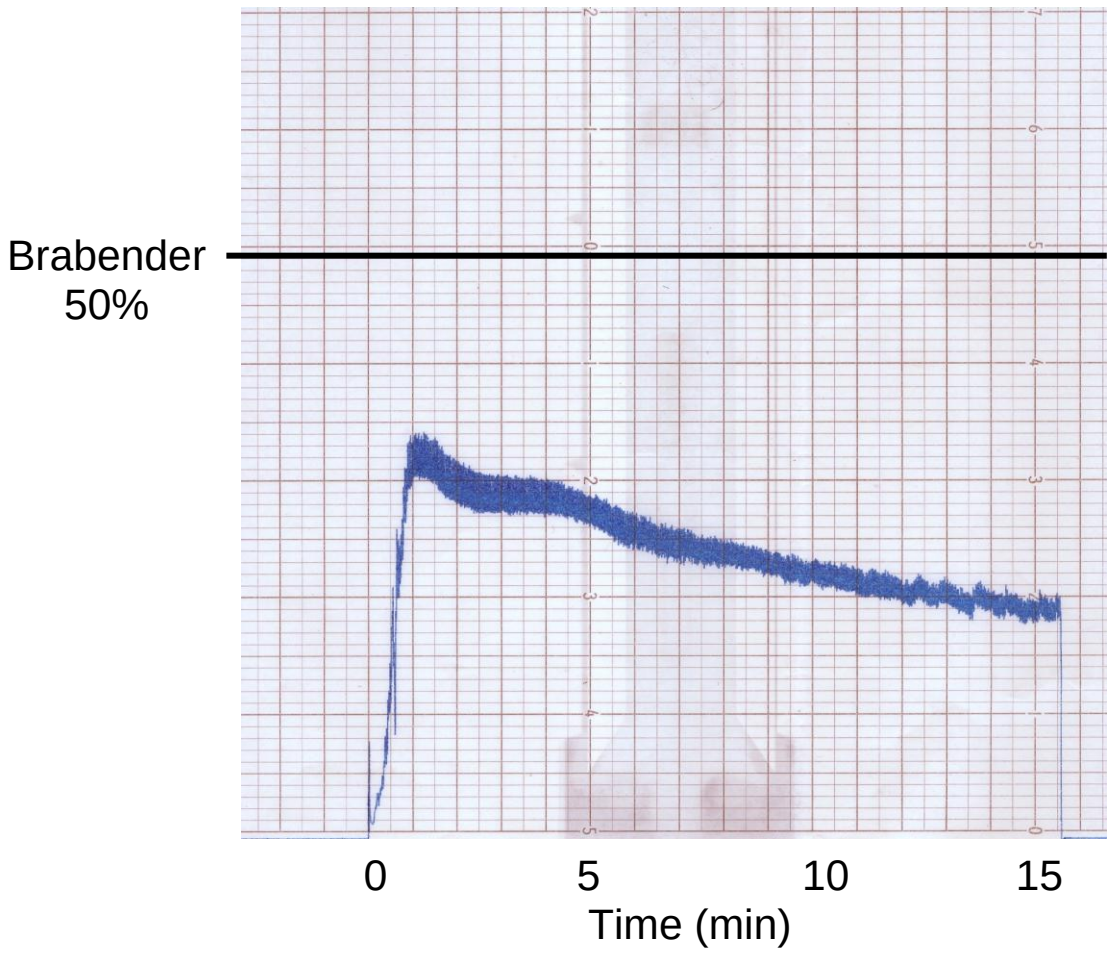


Figure 13. Whole grain rye flour with *Bacillus subtilis* low level enzyme – farinograph

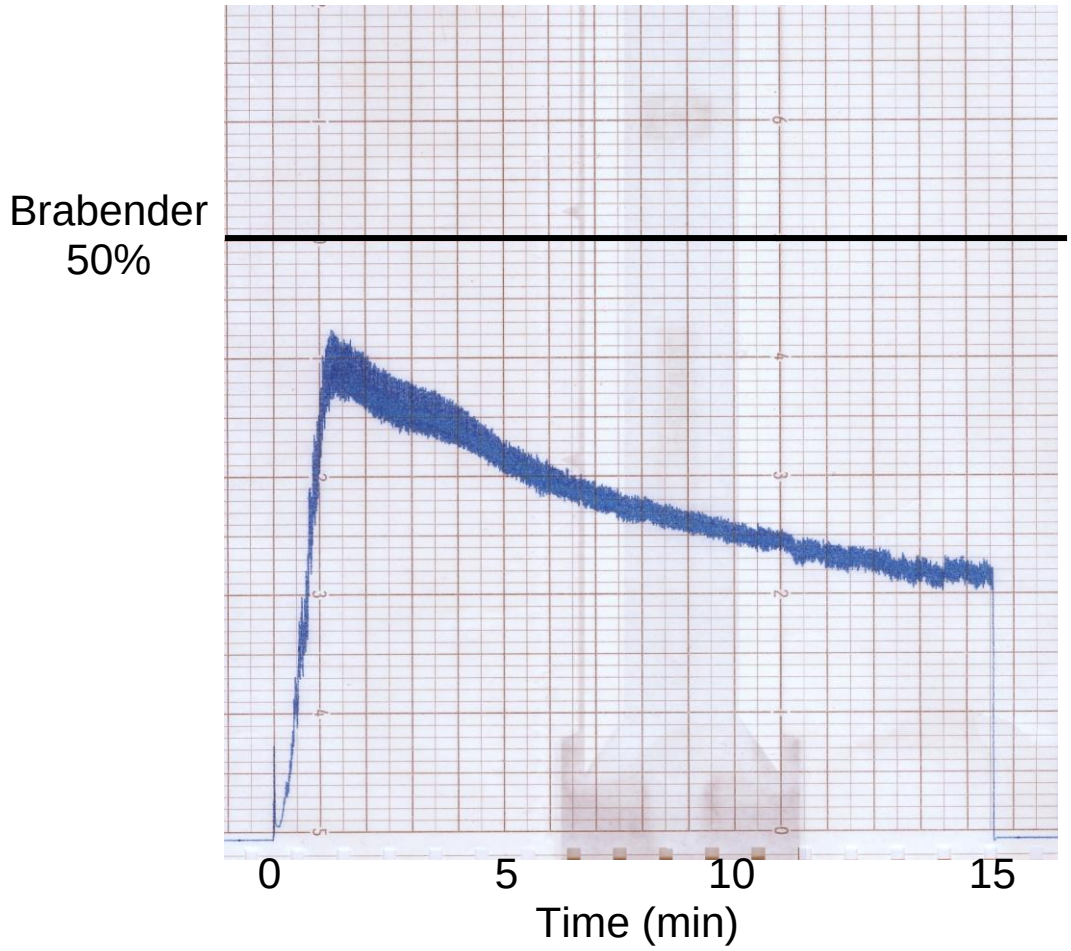


Figure 14. Whole grain rye flour with *Aspergillus niger* low level enzyme – farinograph

J. Viscosity

The RVA can be used to determine the viscosity of an ingredient. Typically the starch pasting profile is generated but it is believed that since arabinoxylans can have a major contribution to the viscosity of flour, differences in the viscosity profile with and without enzymes can be seen. The samples were prepared by adding whole grain rye flour at 3.4 g, deionized water at 25.1 g and the appropriate amount of enzyme when needed according to the procedures discussed in the methodology section C. Statistical differences between samples was not determined since each sample was run one time under these conditions. For all samples, the peak viscosity and final viscosity were determined according to the temperature and time profile selected. For many starch pasting curves, the initial temperature is 50ºC with an increase to 95ºC followed by a cool down to 50ºC. The heating and cooling rate varies depending upon the starch type and the profile information desired. This experiment was run with a starting temperature of 30ºC, increased to 95ºC and then cooled back to 30ºC. The lower temperature profile was chosen in order to have a starting temperature closer to the cookie dough temperature and to allow the enzymes time to properly function before inactivation at high temperatures. The initial mix time of 30 sec was also use to allow for proper interaction between the arabinoxylans and the enzymes. Typical initial mix times are 10 sec.

The whole grain rye flour sample's viscosity profile is shown in Figure 15. The peak viscosity is 784 cp and the final viscosity is 1640 cp. The low level of

Bacillus subtilis enzyme, graph shown in Figure 16, had a peak viscosity of 485 cp and a final viscosity of 929 cp. This was much lower than the control sample, indicating the enzyme decreased the arabinoxylan network and its possible interactions with starch and gluten. This weak structure was also evident when observing the farinograph profile and the increased geometry during cookie baking. The low level of enzyme for the *Aspergillus niger* and Arabinofuranosidase showed peak viscosities of 564 cp and 599 cp respectively and final viscosities of 1186 cp and 1118 cp respectively. For both of these enzyme samples, their peak and final viscosities were much less than that of the control but higher than the *Bacillus subtilis* sample. This indicated that the enzymes did not have a significant impact on the arabinoxylan structure and its interaction with other flour components to the extend that the *Bacillus subtilis* did.. This was also seen with their farinograph profiles. A summary of the viscosity data is shown in Table 23.

Table 23. Whole grain rye flour - viscosity

Sample Description	Peak Viscosity (cp)	Final Viscosity (cp)
C - No Enzyme	784	1640
BS - Low Enzyme Level	485	929
BS - High Enzyme Level	449	833
AN - Low Enzyme Level	564	1186
AN - High Enzyme Level	554	1143
AF - Low Enzyme Level	599	1118
AF - High Enzyme Level	539	1046

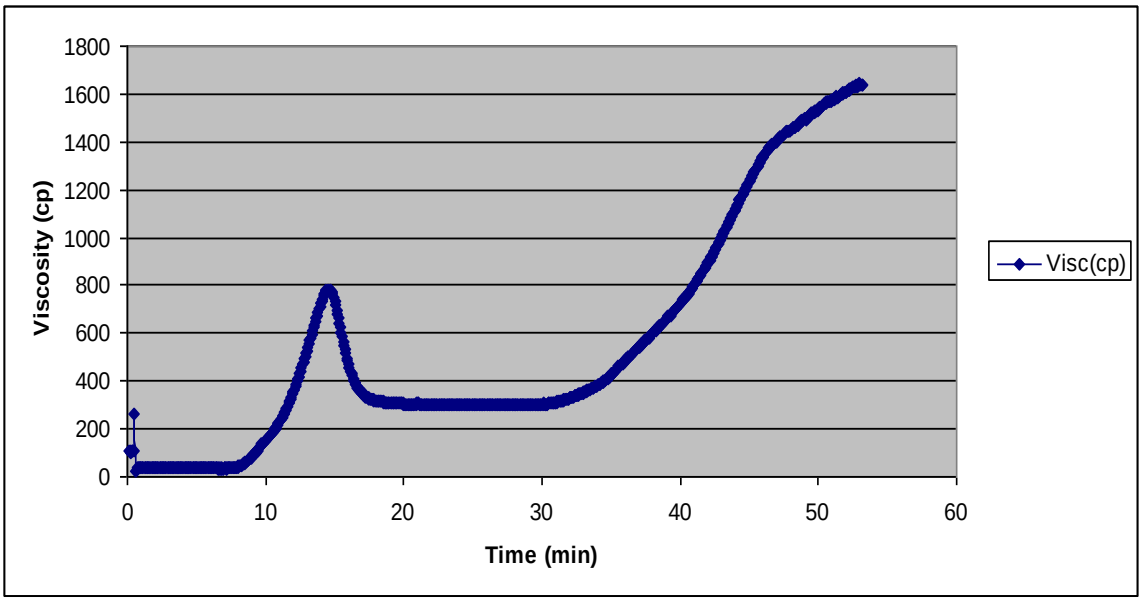


Figure 15. Whole grain rye flour – viscosity profile

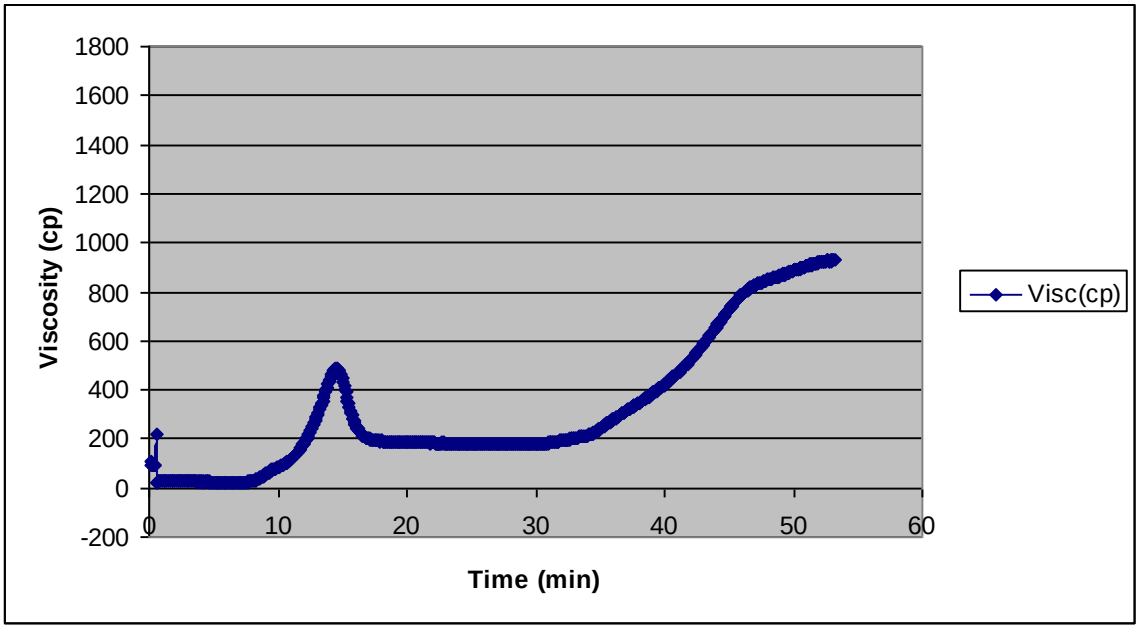


Figure 16. Whole grain rye flour with *Bacillus subtilis* low level enzyme – viscosity profile

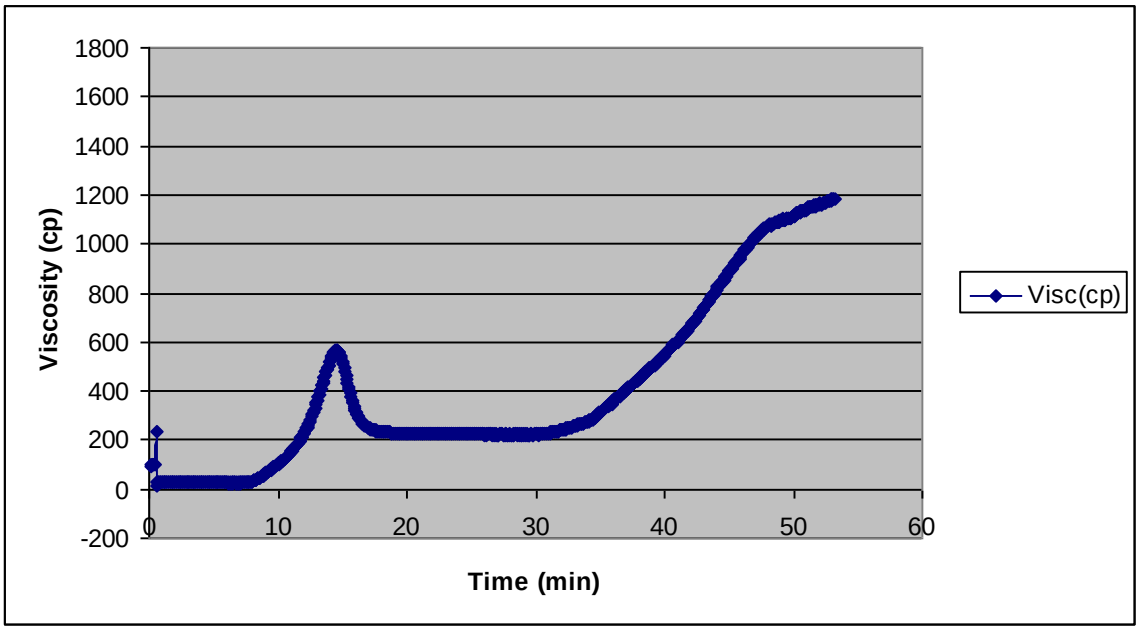


Figure 17. Whole grain rye flour with *Aspergillus niger* low level enzyme – viscosity profile

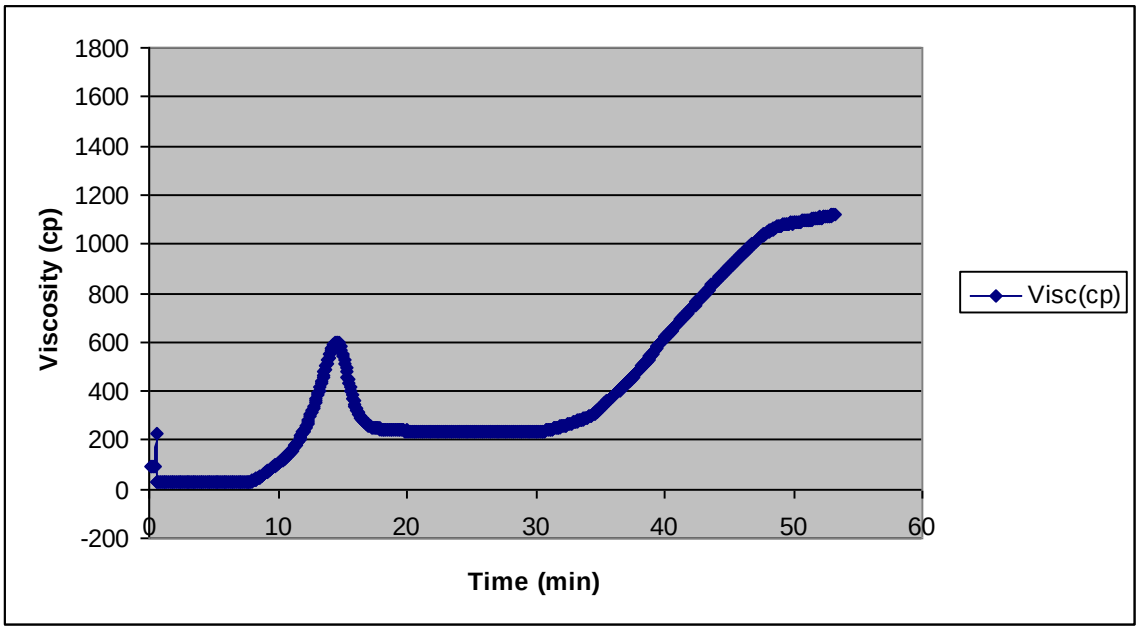


Figure 18. Whole grain rye flour with Arabinofuranosidase low level enzyme – viscosity profile

K. pH

The pH values for the dough were taken in order to understand the environment the enzymes would be exposed to before baking. There was not a significant difference between the pHs. All values were approximately 8.0. The P value was 0.084. The data is shown in Table 24.

Table 24. Whole grain rye flour sugar cookie – dough pH

Sample Description	Dough pH	Avg Dough pH
C1	8.09	8.04
C2	7.97	
C3	7.99	
C4	8.12	
BS1	8.00	7.96
BS2	7.93	
BS3	7.96	
BS4	7.94	7.94
BS5	7.95	
BS6	7.94	
AN1	8.14	7.99
AN2	7.91	
AN3	7.93	
AN4	7.81	7.90
AN5	7.95	
AN6	7.93	
AF1	8.00	8.10
AF2	8.22	
AF3	8.08	
AF4	7.95	7.96
AF5	7.94	
AF6	8.00	

L. Quantification of Arabinose and Xylose

As previously mentioned, Douglas (1981) developed a colorimetric method for the determination of pentosans, based on the presence of xylose in wheat flour. The modifications made to the method during this research indicated that it showed potential to not only provide info on the quantity of xylose but arabinose as well.

According to the Douglas method, 5 mg of flour are used as the starting material. For this experiment, the water extractable/water soluble arabinoxylans were analyzed but first a very general solvent retention capacity utilizing water was determined.

The SRC determines the amount of solvent retained by the flour after centrifugation and expressed as a fraction of the original flour weight, on a 14% moisture basis. Four solvents are typically used to obtain a more complete profile but for this experiment only water was used which represents the overall characteristics of the flour components. Typically solvation is allowed to occur for 20 min but in this experiment 1 hr was used with the addition of continuous shaking in order to ensure complete mixing of the enzyme with the flour and water. Table 25 shows the SRC of the whole grain rye flour sample without enzymes and with high levels of enzymes (low level enzyme samples were not evaluated). As indicated, when enzyme was not added, the flour SRC was approximately 98%. When the *Bacillus subtilis* enzyme was added, it was

believed that the enzyme solubalized the water unextractable arabinoxylans, which are more abundant than the water extractable arabinoxylans and less water was held by the flour. The *Aspergillus niger* and Arabinofuranosidase enzymes/flour also held less water but not to the same extent as the *Bacillus subtilis*/flour.

Table 25. Whole grain rye flour – solvent retention capacity for water

Sample	% SRC
Control	98.92
BS - High	4.17
AN - High	10.70
AF - High	10.70

The preliminary data indicated that absorbance differences can be determined between a control rye flour and a rye flour sample with particular added enzymes. The absorbance values were determined at 552 nm and 510 nm. As indicated in Table 26 the absorbance values at both wavelengths showed more absorbance for the BS enzyme system. This increase in the arabinoxylans as altered by the *Bacillus subtilis* enzyme may be a cause for the increased geometry in the finished product samples. Quantification of the xylose and arabinose are required in a follow up study. In order to achieve this, further method modifications are required i.e. optimization of the calibration curve, further dilution of the flour sample and modification of the extracting/hydrolyzing solution. Note: The calibration curve procedures are provided but the results are not shown as more work is required to optimize the dilution levels and extracting reagent in order to achieve reproducible results. Again, the results shown for the flour/enzyme systems are preliminary and may be repeated at a later time after the procedures are optimized.

Table 26. Absorbance of rye flour and enzyme samples at 552 nm and 510 nm

Sample Description	Abs @ 552 nm	Abs @ 510 nm
Control	0.5136	0.6861
BS	0.6272	0.7101
AN	0.6083	0.5646
AF	0.5219	0.4241

VII. CONCLUSION

Whole grain rye flour provides important health benefits to the consumer. It is a source of vitamins, minerals, phytonutrients and most importantly fiber. The fiber component of the whole grain rye flour can be strongly attributed to the arabinoxylans present. Not only do the arabinoxylans provide a source of fiber but they also greatly affect the functionality of low moisture baked goods. They alter dough viscosity and increase cookie geometry, just to name a few. The arabinoxylan functionality can be controlled by the use of enzymes. Enzymes, such as xylanases can decrease the molecular weight of the arabinoxylans, generate more water soluble arabinoxylans and change their interaction with other components within the system. The three enzymes evaluated during this study – *Aspergillus niger, Bacillus subtilis* and Arabinofuranosidase are all enzymes commonly used in baked goods. This research shows that the use of the *Bacillus subtilis* - BS 1-6 series changes the geometry of the cookie and generates more water soluble arabinoxylans in solution, decreases the dough viscosity, requires less water when mixing a dough and generates more xylose and arabinose in the soluble phase. Some of these changes are evident when *Aspergillus niger* and arabinofuranosidase are used as well but to a lesser extent.

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CURRICULUM VITAE

Michelle Denine Beaver

Dates	**Education**
May 1992	BA – Chemistry Cook Collage, Rutgers University
January 1995	MA – Food Science Rutgers University
January 2010	PhD – Food Science Rutgers University
	Occupation
January 1995	Associate Scientist II Kraft Foods
June 1998	Scientist Kraft Foods
June 2000	Senior Scientist Kraft Foods
December 2003	Associate Principal Scientist Kraft Foods
	Publications
February 1993	Izzo, Henry V.; **Lincoln, Michelle D**.; Ho, Chi-Tang. Effect of Temperature, Feed Moisture, and pH on Protein Deamidation in an Extruded Wheat Flour. Journal of Agricultural and Food Chemistry 1993, 41, 199-202.
January 1995	Volatile Compounds Generated from the Thermal Degradation of Thiamine: Identification of Novel Compounds – **Michelle Denine Beaver** MS Thesis
January 2010	The Effect of Whole Grain Rye Flour Arabinoxylans on the Physical and Chemical Characteristics of a Low Moisture Baked Good **– Michelle Denine Beaver** PhD Dissertation