WHITE BREAD AND WHOLE WHEAT BREAD: COMPARISON OF END-PRODUCT QUALITY, STARCH CHARACTERISTICS AND NUTRITIONAL QUALITY

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Wheat, an important crop in North Dakota and the United States, is often used for bread. Health concerns related to chronic diseases have caused a shift towards consumption of whole wheat bread. This research investigated the differences between white and whole wheat bread related to the end-product and nutritional quality. Flours were milled from Glenn grown in Casselton in 2010, and Barlow, Glenn and Prosper grown in Casselton in 2012. White and whole wheat flours and breads were evaluated for chemical composition, baking quality by AACC method 10-09.01 and estimated glycemic index (eGI) by the Englyst assay. Whole wheat breads had significantly ($P<0.05$) lower loaf volumes than white breads. Whole wheat breads had significantly ($P<0.05$) higher mineral, protein and phenolic acid contents, as well as, significantly ($P<0.05$) lower eGI. Overall, several factors in the whole wheat bread composition can be found to affect the quality and starch hydrolysis.

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LIST OF ABBREVIATIONS

AEP……..Alkali extractable pentosans

AACCI……. American Association of Cereal Chemists - International

A/X……... Arabinose to xylose ratio

AX……..... Arabinoxylans

BU…… Braebender unit

CMWP…… Commercially milled white patent

CMWW…… Commercially milled whole wheat

CVD……. Cardiovascular disease

CRD……. Completely random design

Da…….Daltons

DM……Diabetes mellitus

DMSO……. Dimethyl sulfoxide

DWB…… Dry weight basis

eGI……... Estimated GI

GOPOD…….. Glucose oxidase peroxidase

GI……. GI

HRS……. Hard red spring

HPSEC……. High performance size exclusion chromatography

SDRI…… High starch digestion rate index

HI……..Hydrolysis index

LSD……..Least significant difference

MRP's……..Maillard reaction products

MTI…….. Mixing tolerance index

MB……Moisture basis

MALS……...Multi angle light scattering

NSP……. Non-starch polysaccharide

ND…… Not determined

RDS……. Rapidly digestible starch

RS……. Resistant starch

SEM……. Scanning electron microscopy

RS-I……..Type I resistant starch

RS-II…….Type II resistant starch

RS-III……Type III resistant starch

RS-IV……...Type IV resistant starch

WEP…….Water extractable pentosans

M_w…….Weight averaged molecular weight

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1. INTRODUCTION

Wheat, an important crop in North Dakota and the United States, is used to produce bread and many other products, such as, bagels and pizza crusts. However, health concerns related to chronic diseases such as diabetes mellitus (DM), cardiovascular disease (CVD), cancer and obesity have caused a shift away from consumption of white bread toward whole grain and whole wheat products. North Dakota ranks first in the production of hard red spring (HRS) wheat in the US, with approximately 6 to 7 million acres devoted to this crop each year (North Dakota Wheat Commission, 2012). Since bread is a staple product in many diets, as well as one of the main sources of dietary fiber (Johansson et al, 1984), it is important for bread products made in the US to be high quality and nutritious. The high protein content and superior gluten quality of HRS wheat make it ideal for use in some of the world's finest baked goods. HRS wheat often is used in a blend with other wheat to increase the gluten strength and performance qualities in a batch of flour. Adding HRS to lower protein or inferior quality wheat improves dough handling characteristics, mixing strength and water absorption. For example, the increased gluten strength of HRS wheat is beneficial when formulating whole wheat bread products.

Whole wheat bread is a standardized bread product in the US and must conform to the Code of Federal Regulations. Popularity of whole wheat breads may be due to their appeal as sources of good nutritional value or their perception by the consumer as healthful products. Whole wheat products have been gaining popularity as a result of awareness and trends in fitness, which has increased the demand and consumption of these types of products (Kapsak et al, 2011). During the past 20 years, more than a dozen governmental, non-profit health organizations, industrial and trade groups have encouraged the increase of whole-grain consumption (Slavin et al, 2001; Slavin, 2004). The consensus among US health organizations is that 14 grams of fiber in a 1,000 calorie a day diet (twice for 2000 cal./day) will provide health benefits (United States Department of Agriculture, 2005; Center for Disease Control, 2012). Incorporation of wheat bran into food matrices poses technical challenges for food manufacture's. Developing a whole wheat food product with added benefits does not simply mean incorporating the nutritional ingredient at the appropriate physiological level, but also supplying a product which meets consumers' requirements in terms of appearance, taste and texture (Siro et al, 2008).

Whole wheat bread offers many nutritional benefits over bread made from white flour. Whole wheat bread has increased dietary fiber, B vitamin, mineral content, and phytochemicals, such as phenolic compounds, phytates and avenathramides. While these components are beneficial for human health, another advantage of whole wheat bread is lower glycemic index (GI) (Slavin, 2004). Factors affecting the GI of food products include the susceptibility of starch to enzymatic degradation and gastric emptying. In turn, these factors are greatly affected by the botanical source and the food processing conditions (Holm and Björck, 1992). Previous research has found that the process of baking bread causes the formation of resistant starch (RS) in the form of retrograded starch, which is classified as type III resistant starch (RS-III) (Johansson et al, 1984; Holm and Björck, 1992). Along with the formation of RS-III, there may be other physicochemical causes of lower GI in whole wheat breads. These include the amylose to amylopectin ratio (Björck et al, 1994), presence of pentosans (arabinoxylan) (Choct and Annison, 1992), anti-nutrients (lectins, phytates and enzyme inhibitors) (Thompson and Yoon J.H., 1984), maillard reaction products (MRP's) (Slavin, 2004; Chung et al, 2011) and starch-protein interaction (Jenkins et al, 1987).

2. LITERATURE REVIEW

2.1. Whole Wheat Bread

2.1.1. History of whole wheat

The origin of wheat goes back to approximately 10,000 B.C., at which time the consumption of whole wheat bread began. A timeline, summarizing a brief history of wheat, flour and bread, can be seen in Figure 1. Stone Age man began the first wheat flour production by grinding wheat kernels between rocks. Around 5,500 B.C. the first millstones were developed for grinding wheat into flour. The Egyptians are credited as the first civilization to produce yeast-leavened breads. Milling technology advanced around 1180-1190 A.D. when windmills for milling grain were developed.

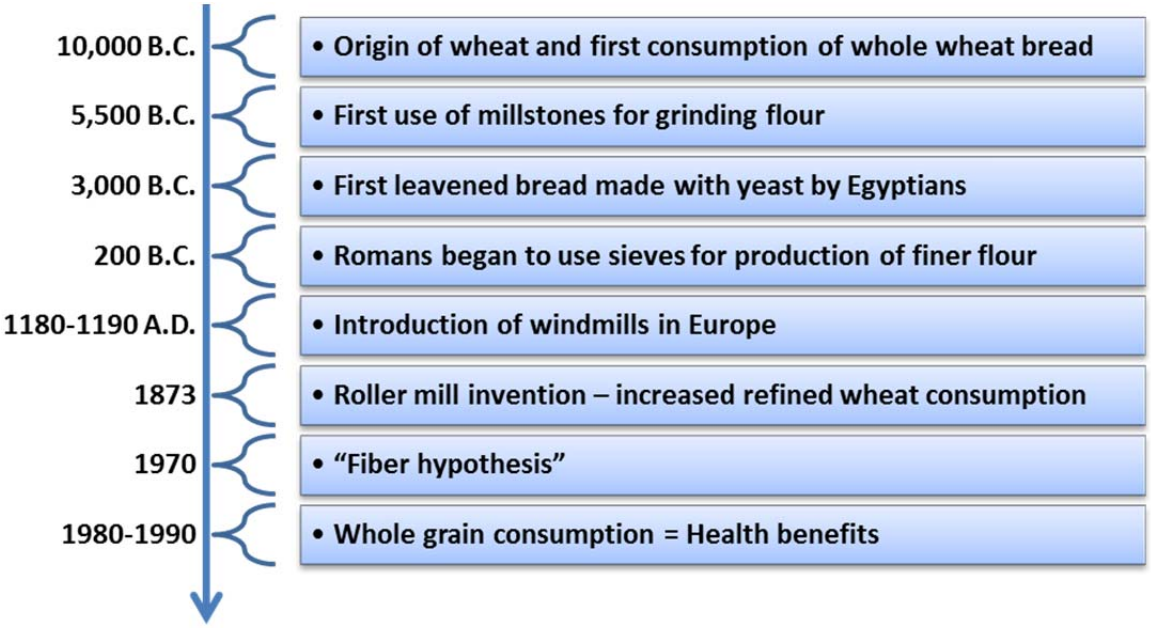

Figure 1. Timeline of Wheat and Bread Consumption
(Trowell, 1972; Anson, 2010; John Innes Centre and Institute of Food Research, 2013)

The modern roller mill was invented in 1873 and lead to the increase of refined flour consumption. Prior to the industrial revolution and invention of the roller mill, the use of white flour was too costly for most people (Anson, 2010; John Innes Centre and Institute of Food Research, 2013). Increased white flour and bread consumption continued in Western diets until the 1970's when the 'fiber hypothesis' was published by Trowell in 1972. The study recommended increased consumption of whole grains along with fruits and vegetables, because they are beneficial for health by providing fiber (Trowell, 1972; Slavin,

2004). Additional research conducted in the 1980's and 90's showed additional health benefits of whole wheat products, which lead to a gain in the popularity of whole wheat bread and other such products (Anson, 2010). The increased desires for whole wheat bread lead to an increase in the varieties which were available in stores. However, consumer acceptance of whole wheat bread can be lacking. The lower loaf volume, dense crumb, dark color and bitter flavors of whole wheat breads often prevent consumers from choosing whole wheat bread instead of white bread. This is especially true for consumers who are not so health conscious or care more about the appearance and taste of their food than the nutritional benefits.

2.1.2. Definition of whole wheat

To be considered whole wheat flour, the flour must comply with 21 CFR 137.200. This means that whole wheat flour must contain all portions of the wheat kernel in the correct biological ratios. For example, whole wheat flour must contain the same percentage of bran tissue that was contained in the wheat kernels prior to milling (U.S.Food and Drug Administration, 2012). Figure 2 depicts the anatomy of a wheat kernel and the components which must be included in whole wheat flour to comply with US Federal regulation.

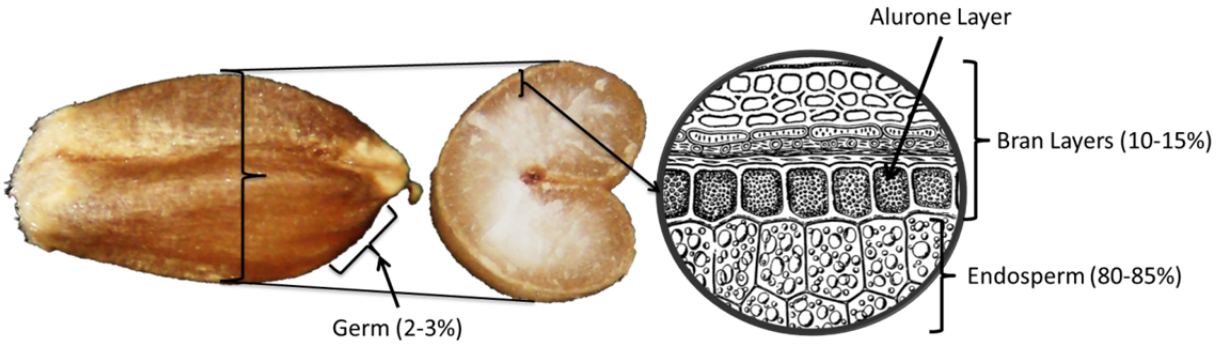

Figure 2. Anatomy of a Wheat Kernel Adapted from Bergen (1904) and Anson (2010)

The wheat kernel is made up of three main elements: the endosperm (80-85%), the bran (10-15%) and the germ (2-3%). The endosperm functions as storage for starch and protein in the seed. The bran layers form the outer coating of the wheat kernel. Within the bran layers, there is one highly specialized layer called the alurone layer. The alurone layer has significant functionality in the wheat seed as a repository for vitamins, minerals and phytochemicals. The germ portion of the wheat kernel contains

most of the wheat grain's lipids and is comprised of the embryonic axis and scutellum (Anson, 2010). All of these components must be included in or near their biological proportions to constitute whole wheat flour. The addition of the bran and germ components may cause reduction in shelf life and off flavors and colors in the whole wheat flour and resulting end-products. The phenolic compounds found in the bran causes the whole wheat flour and bread to have a dark color and can have bitter flavors. The lipids and enzymes in the bran and germ fractions may result in rancidity of the flour or bread (Doblado-Maldonado et al, 2012).

Consumers often consider whole wheat bread to be a healthful product and its popularity may be due to its appeal as a source of good nutritional value. Incorporation of wheat bran into food matrices poses technical challenges for food manufacturers. Whole wheat products have been gaining popularity as a result of awareness and trends in fitness which has increased the demand and consumption of these types of products (Kapsak et al, 2011).

2.2. Health Benefits of Whole Wheat Bread

2.2.1. Glycemic index

One reason for the encouragement of increased consumption of whole grain foods is the incidence of chronic diseases, such as diabetes mellitus (DM), cardiovascular disease (CVD), cancer and obesity. Specifically, DM is strongly associated with glucose and insulin responses. GI is one indicator that can be used to compare the glycemic response to foods (Slavin, 2004). The GI refers to the postprandial glycemic response of a test product compared to that of a reference food (glucose or white bread) (Björck et al, 1994; Augustin et al, 2002; Slavin, 2004). When *in vitro* assay methods are employed the term is referred to as estimated GI (eGI) and is measured based on the glucose released from the test food compared to the glucose released by the reference food (Ovando-Martínez et al, 2011a). Foods can be classified as high, medium or low GI foods. Foods with a GI above 70 are considered to be high GI foods. Medium GI foods have a GI between 56 and 69, and foods with a GI below 55 are low GI foods (Venn and Green, 2007; American Diabetes Association, 2013). Figure 3 shows a list of foods in each of the 3 GI categories.

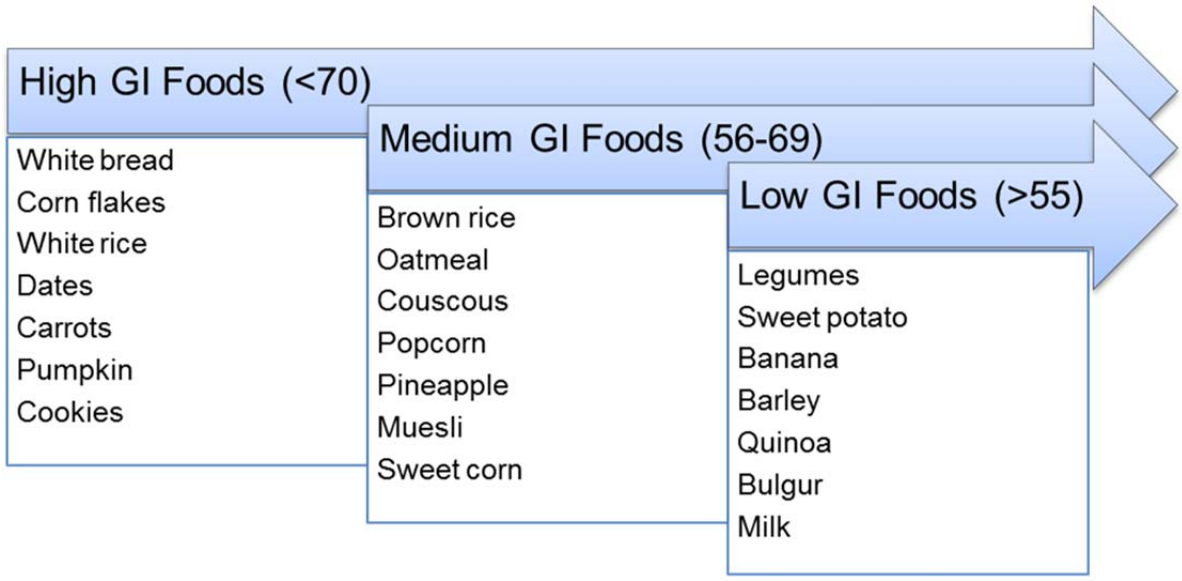

Figure 3. List of Foods in Each of the Three GI Categories
(Foster-Powell et al, 2002; Venn and Green, 2007; Atkinson et al, 2008; Jones, 2010; American Diabetes Association, 2013)

Although GI is often used as a guideline for food selections, there has been some debate about the effectiveness of GI. The main argument is that GI values and the methods for GI measurement tend to have high variation from one source to the next (Jones, 2011). For example, one source reports the GI for carrots as 92 (Jones, 2010) and another reports the GI for carrots as 39 (Atkinson et al, 2008). This is a large difference which puts carrots either in the high GI or the low GI category. The variability in the data reported for GI could be due to characteristics of the food itself. The same type (but not the exact same sample) of food analyzed by different labs may have different formulations, processing conditions or physical structures which result in differences in GI (Jones, 2011). Another important factor may be the method that is used for measurement of GI. The GI can be measured by *in vivo* assays or *in vitro* assays, of which there are many variations which could be used. When conducting *in vitro* assays different researchers have used different enzyme mixes for hydrolysis, different sample preparation methods and different buffers to simulate conditions of the gut. *In vivo* assays tend to be just as variable, and more complex, than the *in vitro* assays. The population of the human subjects and the overall diet consumed can have effects on the results of the *in vivo* measurements of GI (Wolever et al, 1991; Englyst et al, 1992; Goñi et al, 1997; Venn and Green, 2007; Butterworth et al, 2011; Butterworth et al, 2012)

Whole wheat bread offers many nutritional benefits over bread made from white flour. Whole wheat bread has increased dietary fiber, B vitamins, mineral content, and phytochemicals such as, phenolic compounds, phytates and avenathramides. While these components are beneficial for human health, another advantage of whole wheat bread is lower GI (Slavin, 2004). Factors affecting the GI of food products include the susceptibility of starch to enzymatic degradation and gastric emptying. In turn, these are greatly affected by the botanical source and the food processing conditions (Holm and Björck, 1992). Previous research has found that the process of baking bread causes the formation of resistant starch (RS) in the form of retrograded starch, which is classified as type III resistant starch (RS-III) (Johansson et al, 1984; Holm and Björck, 1992). Along with the formation of RS-III, there may be other physicochemical causes of lower GI in whole wheat breads. These include the amylose to amylopectin ratio (Björck et al, 1994), presence of non-starch polysaccharides (arabinoxylans) (Choct and Annison, 1992), anti-nutrients (lectins, phytates and enzyme inhibitors) (Thompson and Yoon J.H., 1984), MRP's (Chung et al, 2011) and starch-protein interaction (Jenkins et al, 1987).

2.2.2. Vitamins, minerals and antioxidant content

Not only does whole wheat bread have lower glycemic response, it also has considerably higher vitamin and mineral contents, as well as phenolic compounds and other bioactive compounds (Slavin, 2004; Anson, 2010). Whole wheat bread and flour are good sources of antioxidants. Processing and treatment methods have been studied to increase the availability of phenolic compounds in bread. Hemery et al (2010) determined that fractionation and particle size of bran will affect the accessibility of the phenolic compounds in whole wheat. There have been some reports that the antioxidant capacity of whole wheat has been underestimated (Slavin, 2004; Pérez-Jiménez and Saura-Calixto, 2005). Some research has reported that Maillard reaction intermediates may also contribute to increased antioxidant levels in baked bread products (Miller, 2001; Slavin, 2004). A substantial increase in the phenolic content of wheat flour and other cereal products after treatment with digestive enzymes was shown in a study conducted by Pérez-Jiménez and Saura-Calixto (2005). The total phenolics and antioxidant capacity of several cereals and cereal products were extracted and measured by traditional methods. The samples also were subjected to hydrolysis by digestive enzymes before determination of phenolic content and antioxidant capacity. The results of this study showed higher levels of phenolic compounds and higher

antioxidant activities in all samples when enzymatic extraction is conducted rather than aqueous-organic extraction. For most of the samples the phenolic compounds determined with enzymatic extraction were at least two times higher than when extracted with aqueous-organic solvents. There may be a much larger amount of phenolic compounds in cereals which reach the gut and become available after digestion than previously understood (Pérez-Jiménez and Saura-Calixto, 2005).

Phytic acid is another antioxidant compound found in wheat; however, it has been traditionally labeled an anti-nutrient due to its suppression of mineral absorption. Since phytic acid was considered an undesirable component in wheat, there have been some attempts to develop low phytic acid wheat lines (Guttieri et al, 2006), as well as attempts to increase bioavailability of minerals in whole wheat bread by the addition of phytases (Haros et al, 2001). Recently, there has also been some debate over whether or not the presence of phytic acid is completely deleterious. Phytic acid acts as a chelating agent that binds various metals and suppresses iron catalyzed redox reactions. However, phytic acid also acts as an antioxidant and anticarcinogen by suppression of oxidant damage in the human gut (Febles et al, 2002; Slavin, 2004); giving whole wheat breads a nutritional advantage over white bread.

Although, whole wheat bread does have considerable health benefits and a better nutritional profile overall. The importance of folic acid fortification in white flour and bread products which is required by U.S. law must also be considered. The US code of federal regulations requires white flour and bread to include 0.7mg/lb and 0.43mg/lb of folic acid, respectively (U.S.Food and Drug Administration, 2012). Folic acid is an important B vitamin which is involved in prevention of birth defects. It is currently recommended that adults consume 400 µg per day and pregnant women consume 600 µg per day of folic acid. The importance of folic acid, especially for pregnant women, resulted in requiring enriched products to also contain folic acid (Cohen, 2011). Enriched white bread typically has 37 µg folic acid per slice, while whole wheat bread contains only 14 µg per slice (Wheat Foods Council, 2011). The higher levels of folic acid in enriched white breads give them the advantage over whole wheat products. Whole grain foods are not required to be enriched and so their folic acid content is naturally occurring but at a lower level than enriched products. However, the increase in whole grain consumption there has been some suggestion to require fortification of whole grain products (Cohen, 2011).

2.3. Starch Characteristics of Whole Wheat Bread

Cereal products, such as bread, are a major source of starch, which is the only polysaccharide that can be digested by humans. Bread made from refined white flour contains starch, which is mostly rapidly digestible and causes high glucose and insulin responses. This is regrettable since, in Western diets, bread is the main source of starch and dietary fiber (Holm and Björck, 1992). Due to the high rate of starch digestibility and common consumption of white bread, it often is used as a reference standard when calculating the eGI of different food products (Englyst et al, 1992; Granfeldt et al, 1992; Ovando-Martínez et al, 2011b). However, whole wheat products have been gaining popularity as a result of awareness and trends in fitness. This has led to an increased demand and consumption of whole wheat/grain products (Kapsak et al, 2011). More than a dozen governmental, non-profit health, industrial and trade groups have encouraged the increase of whole-grain consumption, over the past 20 years (Slavin et al, 2001; Slavin, 2004). Not only do the increased dietary fiber, phytochemicals and nutrient/mineral content of whole wheat bread have health benefits, some of these components may result in decreased GI in whole wheat bread (Thompson and Yoon J.H., 1984; Choct and Annison, 1992).

One component affecting the GI in bread is the amount of resistant starch (RS), which is classified as dietary fiber, because it resists hydrolysis in the small intestine and is fermented by gut microbiota in the large intestine (Holm and Björck, 1992; Liljeberg et al, 1996). Resistant starch can be classified into four groups based on the mechanism of resistance. Type I resistant starch (RS-I) is physically inaccessible to enzymatic attack and is found in intact grains. Type II resistant starch (RS-II) consists of resistant starch granules that avoid hydrolysis due to their particular granular form. RS-II is commonly found as raw starch in bananas. Type III resistant starch (RS-III) is comprised of retrograded starch that is formed during the cooking and cooling of starch-based food products. RS-III makes up the majority of the RS found in bread. Type IV resistant starch (RS-IV) is classified as modified starch. These starches have been modified by chemical or physical means to result in their resistance to hydrolysis (Sajilata et al, 2006). Since RS-III makes up the bulk of the RS found in bread, it is important to specifically measure RS-III content and investigate its properties.

2.4. Factors Affecting Starch Hydrolysis in Whole Wheat Bread

Many factors can affect the starch hydrolysis and amount of RS (including RS-III) in wheat-based products. Several studies have determined the interaction between the components of whole wheat bread and GI. After starch, protein is the second largest component of the wheat grain. It can be expected that there would be intermolecular interactions between the starch and protein of wheat. In a study done by Jenkins et al (1987), the in vitro starch digestion and blood glucose levels were assessed for white bread, gluten-free bread and gluten-free bread plus gluten. The gluten-free bread was prepared using gluten-free wheat starch and the gluten-free bread plus gluten was prepared with wheat starch, which was free of gluten, and vital wheat gluten powder. The results of this study showed that the white wheat flour bread had significantly ($p<0.05$) lower total starch digestion products over 3 hours than both the gluten-free bread and the gluten-free bread plus gluten (Jenkins et al, 1987). Jenkins et al (1987), also found that the gluten-free bread and gluten-free bread plus gluten resulted in significantly ($p<0.05$) higher blood glucose levels in the study participants (Jenkins et al, 1987). These results obtained by Jenkins et al (1987) suggest that the interaction between the gluten proteins and starch in wheat bread provide a significant barrier to starch digestibility. However, since the gluten-free bread plus gluten showed similar results to the gluten free bread, it is important for the inherent starch-gluten interactions to remain intact.

Non-starch polysaccharides, specifically, arabinoxylans (AX) are another important constituent of whole wheat. AXs are polymers consisting of a β 1,4 linked xylose backbone which is substituted by arabinose. AXs are found in the cell wall structure of cereal grains and are most concentrated in the bran layer of wheat (Simsek et al, 2011). The determination of starch digestibility and non-starch polysaccharide (NSP) content of several wheat based products was determined in a study by Bravo et al (1998) (Bravo et al, 1998). Although this study determined that the products all had high levels of rapidly digestible starch (RDS) and high starch digestion rate index (SDRI), the whole meal bread was found to have significantly ($p \leq 0.05$) lower digestibility than the white bread. The whole meal bread also had higher NSP content than the white bread. This study did not determine any direct correlation between SDRI and NSP content of the samples. However, NSP in wheat may have some effect on gut viscosity and gastric emptying which may alter the starch digestion rate. Choct and Annison (1992) conducted a study to ascertain the inhibitory effect of wheat pentosans on nutrient digestion. This study was done by

adding water extractable pentosans (WEP) and alkali extractable pentosans (AEP) from wheat to the diets of broiler chickens. It was found that the addition of WEP and AEP reduced the apparent metabolizable energy and the digestibility coefficients of starch and protein (Choct and Annison, 1992). From these studies, it can be established that the presence of NSP, present in higher concentration in whole wheat, result in decreased starch digestibility.

There are other minor components that also affect the glycemic response of whole wheat bread. Examples of these are polyphenols and phytic acid. There are multiple presumed causes of reduced GI due to these components. The polyphenols and phytic acid may interact with amylases, proteins associated with the starch or with the starch itself; also phytic acid is known to bind calcium which catalyzes the amolytic reactions (Thompson and Yoon J.H., 1984). When polyphenols were added to wheat starch, it has been observed that starch digestibility was significantly ($p < 0.05$) reduced by tannic acid but not catechin. Phytic acid also significantly ($p < 0.05$) reduced the digestibility of wheat starch; the reduction in digestibility was even greater when tannic and phytic acids were added to starch (Thompson and Yoon J.H., 1984). Although, the levels of polyphenols and phytic acid were added at the concentration found in legumes, the increased phenolic content of whole wheat bread vs white bread may result in reduced GI in the whole wheat bread.

Other compounds, such as, those formed by the Maillard browning reaction, could also affect the glycemic response. Maillard browning involves the reaction of reducing sugars with free amino acids during heating at low moisture conditions. In bread this occurs in the crust as the bread bakes and the surface of the bread dries out (Chung et al, 2011). In a study done by Chung et al (2011), MRP's were prepared and extracted from rice that had been hydrolyzed with amylase by baking with glycine. The MRP's obtained from the rice were added to gelatinized rice starch before in vitro starch hydrolysis and blood glucose assays in mice. Chung et al (2011) found that the addition of the MRP's resulted in slower in vitro starch hydrolysis and lower blood glucose response in mice. It was also determined that the MRP's had an inhibitory effect on α-amylase. The α-amylase activity was reduced from 90% in gelatinized rice starch to approximately 60-70% in gelatinized rice starch with MRP's (Chung et al, 2011). Since

Maillard browning occurs in bread products, the reaction products of Maillard browning may be reducing glycemic response in bread.

Overall, bread is a major staple food in Western diets, and the increase in whole wheat bread consumption may lead to health benefits (Kapsak et al, 2011). These health benefits are due to the dietary fiber, phytochemicals, nutrient/mineral content and lower GI of whole wheat bread (Thompson and Yoon J.H., 1984; Choct and Annison, 1992). As seen in previous research, there are many possible causes of the reduced GI of whole wheat bread (Thompson and Yoon J.H., 1984; Jenkins et al, 1987; Bravo et al, 1998; Chung et al, 2011); however, these interactions need to be studied in more detail.

3. OBJECTIVES AND NEED STATEMENT

3.1. Research Objectives

Objective 1: To evaluate of differences in composition and end-product quality between white and whole wheat bread

Objective 2: To determine the difference in eGI and resistant starch content of white vs. whole wheat bread

Objective 3: To determine the relationships between eGI and the chemical composition of white and whole wheat bread

3.2. Need Statement

Currently there is a lack of consistent information found in literature about the end-product and nutritional quality comparisons between white and whole wheat breads. This study will investigate the effect of macromolecular interactions in whole wheat quality, specifically, the relationship between eGI and the changes in starch composition. It is important to investigate the variation in starch composition and characteristics between white and whole wheat breads, due to the increased popularity of whole grain breads and their health benefits relating to DM, CVD and obesity. By examining the changes in starch chemistry that occur in baking white and whole wheat flour breads, some conclusions may be drawn as to the basis for differences in eGI of these products. Also, it must be determined if the dilution of starch in whole wheat flour is the cause of lower GI in whole wheat bread.

4. MATERIALS AND METHODS

4.1. Experiment 1 Materials

4.1.1. Milling and sample preparation

A sample of Glenn (Mergoum et al, 2006) wheat, from Casselton, ND grown in 2010, was milled in two replicate batches of 2 Kg each. The growing season in 2010 at Casselton, ND had moderate temperatures and sufficient rainfall. Harvest was delayed due to rain and cloudy cool weather during maturation and harvest (Hareland, 2011). The wheat samples were tempered to 15.5% moisture for 16 hours and 0.5% water was added five minutes prior to milling. Milling was performed on a Buhler MLU-202 laboratory mill (AACC International, 1999c). The straight grade flour, bran and shorts fractions were collected off the mill. In this case straight grade flour is defined as the combination of the flour from the three break streams and the three reduction streams from the laboratory mill. The straight grade flour collected from the mill was blended on a cross flow blender and re-bolted over an 84 SS sieve to remove any foreign material from the milling process. The bran and shorts were blended together on a cross flow blender. The blended bran and short and duplicate (500 g) samples of the Glenn wheat kernels were ground using a hammer mill with a 0.8 mm screen (Perten Instruments Springfield, IL).

Starch was extracted from each replicate of the patent flour by washing the flour with NaCl solution on a Glutomatic system (Perten Instruments Springfield, IL). After washing, the NaCl solution containing the starch fraction was collected. The NaCl solution was removed by centrifugation (2500 g, 10 min). The starch was washed with 95% ethanol (300ml) 2 times, centrifuging (2500 g, 10 min) after each wash. The starch was then washed 4 times with deionized water (300 ml), centrifuging (2500 g, 10 min) after each wash. The top layer of precipitate, containing protein and damaged starch, was removed after the second, third and fourth washes with water. The final pure starch was lyophilized and ground for use in preparing flour blends.

The five flour formulations used for baking and additional analysis were prepared as follows in Table 1. Straight grade flour from the laboratory mill was used without modification for formula 1. Wheat ground on a hammer mill was used without modification for formula 2.

Table 1. Formulations of Flours Used in Experiment 1

		Base Flour %	Bran %	Starch %
Formula 1	**Straight Grade White Flour**	100.0	0.0	0.0
Formula 2	**Ground Whole Wheat**	100.0	0.0	0.0
Formula 3	**Blended Whole Wheat**	68.6	31.4	0.0
Formula 4	**Ground Whole Wheat + Starch**	98.7	0.0	1.3
Formula 5	**Blended Whole Wheat + Starch**	67.3	30.8	2.0

The blended whole wheat (formula 3) was prepared by blending the straight grade white flour (68.6%) with the ground bran and shorts (31.4%) at the ratios of material collected from the mill. For preparation of formulas 4 and 5, a portion of the whole wheat flour was replaced with the starch that was extracted from the straight grade flour. The ground wheat + starch (formula 4) was prepared by adding 1.3% starch to 98.7% ground whole wheat. The blended whole wheat + starch flour (formula 5) was prepared by blending 67.3% straight grade white flour with 30.8% bran and shorts and 2.0% starch.

4.2. Experiment 2 Materials

4.2.1. Flour preparation

Figure 3 illustrates the preparation of flours used in this experiment. A commercially milled white patent (CMWP) flour and commercially milled whole wheat (CMWW) flour were obtained from North Dakota State Mill (Grand Forks, ND, USA). It is important to note that the source of the wheat and milling procedure for the commercially milled flours are unknown. These flours were used as a check to reference the types of flours that would be similar to those available to commercial bakeries. However, in this case the CMWP flour was not enriched or bleached. White flour sold in the U.S. is enriched with thiamin, riboflavin, niacin, folic acid and iron according to 21CFR137.165 (U.S.Food and Drug Administration, 2012). The differences observed between the CMWP and CMWW flours, as well as, between the CMWP and varietal flours must be considered carefully with the realization that there are other unknown variables contributing to the differences. The HRS wheat varieties Glenn (Mergoum et al,

2006), Barlow (Mergoum et al, 2011) and Prosper (Mergoum et al, 2013) grown in 2012 at Casselton, ND were also used for this experiment. The environment conditions at Casselton, ND in 2012 were moist and slightly rainy at planting and then borderline dry all season. At harvest the conditions were drier and warmer than average (Wheat Quality Council and Ohm, 2013). The wheat was milled and tested in duplicate. First, the wheat samples were tempered to 15.5% moisture for 16 hours and, and then an additional 0.5% water was added five minutes prior to milling. The wheat was milled on a Buhler, type MLU-202 laboratory mill (AACC International, 1999c) and the white flour, bran and shorts fractions were all collected. The straight grade flour collected from the mill was blended on a cross flow blender and re-bolted over an 84 SS sieve to produce patent flour. After blending bran and shorts fractions in a cross flow blender, the particle size of the bran and shorts was reduced by grinding in a hammer mill with a 0.8mm screen (Perten Instruments Springfield, IL). Figure 4 diagrams the preparation of the flour components used in this study.

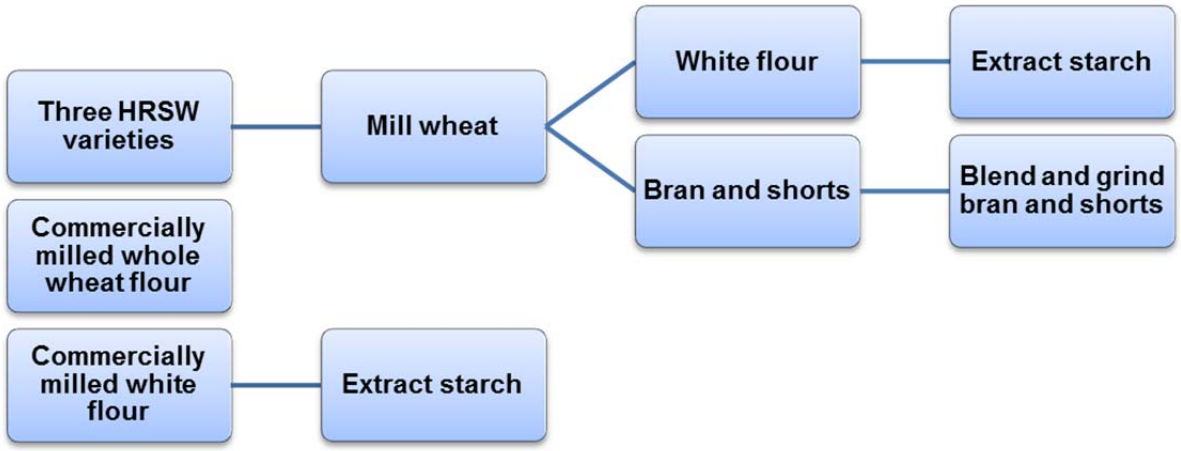

Figure 4. Preparation of Flour Formulation Components

The CMWP, CMWW flours, as well as the components collected from the mill (patent flour, bran and shorts) and the starch extracted from white flours were used to prepare the flour samples for additional analysis and preparation of bread formulations. The bread formulations are listed in Table 2. White flour and ground bran + shorts from the three wheat varieties were blended at the ratio of material obtained from milling to produce whole wheat flours (formulas 6-8). Starches extracted from each type of white flour were added to the whole wheat flour of the same type. For example, starch extracted from

Barlow white flour was added to Barlow whole wheat flour. Starch was added to the CMWW flour and the whole wheat flours from each of the three varieties (formulas 9-12) to increase their starch content to the same level as their corresponding white patent flour (formulas 1-4).

Table 2. Bread Formulations Prepared from Commercially Milled and Hard Red Spring Wheat Flours

White Breads	1. CMWP flour
	2. Barlow white flour
	3. Glenn white flour
	4. Prosper white flour
Whole Wheat Breads	5. CMWW flour
	6. Barlow whole wheat flour
	7. Glenn whole wheat flour
	8. Prosper whole wheat flour
Whole Wheat + Starch Breads*	9. CMWW flour + starch
	10. Barlow whole wheat flour + Starch
	11. Glenn whole wheat flour + starch
	12. Prosper whole wheat flour + starch

*Starch will be added to increase the starch content of formulas 9-12 to the level of their corresponding white bread formulation (1-4).

4.2.2. Starch extraction

Starch was extracted from the white flour using a Glutomatic gluten washing machine (Perten Instruments, Springfield IL). For starch extraction, 10 g samples of flour were placed into the washing cups and 4.8ml of 2% NaCl was added and spread on top of the flour. The flour was mixed and washed by the Glutomatic system with 2% NaCl solution (AACC International, 2000). After washing, the NaCl solution containing the starch fraction was collected. The NaCl solution was removed by centrifugation (2500 g, 10 min). The starch was washed with 95% ethanol (300 ml) 2 times, centrifuging (2500g, 10 min) after each wash. The starch was then washed 4 times with deionized water (300 ml), centrifuging (2500 g, 10 min) after each wash. The top layer of precipitate, containing protein and damaged starch, was removed after the second, third and fourth washes with water. The final pure starch was freeze dried and ground for use in preparing flour blends. The extracted starch will be added to the whole wheat flours in an effort to formulate whole wheat bread that will contain the same amount of starch as the white bread.

4.3. Methods

4.3.1. Flour composition and quality

Proximate analysis on all flour blends was done to determine the quality of flour used for these experiments. Determination of moisture, ash and protein content was done according to AACC approved methods 44-15.02, 08-01.01 and 46-30.01, respectively (AACC International, 1999a; AACC International, 1999d; AACC International, 1999e). Total starch and starch damage of the flour blends and bread samples were measured using AACC approved methods 76-13.01 and 76-30.02, respectively (AACC International, 1999f; AACC International, 1999g). The water absorption and dough strength of the flours were determined by the farinograph, according to the AACC method 54-21.02 (AACC International, 2011).

4.3.2. Arabinoxylan content

Arabinoxylans (AXs) are the main component of the dietary fiber portion of wheat flour. Arabinoxylan content of the flour and bread was measured according to the method of Blakeney et al 1983 (Blakeney et al, 1983). Samples (6-7mg) were hydrolyzed with triflouroacetic acid (250 µl 2 M) at 121°C for one hour. After hydrolysis, 75 µl *m*-inositol (20 mg/ml) was added to each tube as the internal standard. The samples were then dried at 55°C under nitrogen. Ammonium hydroxide (100 µl, 1 M) was added to each tube, before the addition of 0.5 ml sodium borohydride (20 mg/ml) in dimethyl sulfoxide (DMSO). The tubes were capped and heated at 40°C for 90 minutes. Then, six drops of glacial acetic acid was added to the tubes and used to rinse down the sides of the tubes. 1-methylimidazol (100 µl) and acetic anhydride (0.5 ml) were added to all tubes before incubation at room temperature for 10 minutes. Approximately 4 ml of water was added to each tube to stop the reaction. The samples were partitioned twice with 1 ml methylene chloride, which was transferred to a second tube. The methylene chloride fractions were pooled and dried at 45°C under nitrogen and the samples re-dissolved in 1ml acetone.

The derivitized sugars were quantified by an Agilent (Santa Clara, CA) 7890 gas chromatograph with flame ionization detector. A SP^{TM}-2380 column (30mx0.25mmx0.2µm, Supelco, Bellefonte, PA) was used for separation. The flow rate was kept constant at 0.8ml/min and the carrier gas was helium. The injector, oven and detector temperatures were set to 230, 100 and 250°C, respectively. The total

arabinoxylan content was calculated according to this formula: Total AX = (% arabinose + % xylose) × 0.88 (Henry, 1986).

4.3.3. Extractable polyphenols

Samples were extracted by shaking at room temperature with methanol:water acidified with HCl (50:50 v/v, pH 2, 50 mL/g sample, 60 min, room temperature; constant shaking) and acetone:water (70:30 v/v, 50 mL/g sample, 60 min, room temperature; constant shaking). After centrifugation (15 min, 25 °C, 3000 g), supernatants were combined and used to determine extractable polyphenols. Ferulic acid was used to prepare a standard curve. Extractable polyphenols were determined by the Folin-Ciocalteau procedure (Singleton et al, 1999). The results were expressed as ferulic acid equivalents.

4.3.4. Hydrolysable polyphenols

Hydrolysable polyphenols comprise hydrolysable tannins, phenolic acids, and hydroxycinnamic acids that are released from the food matrix by strong acidic hydrolysis. These compounds were extracted by a methanol/H_2SO_4 90:10 (v/v) hydrolysis at 85 °C for 20 h from the residues of methanol/acetone/water extraction that was done for determination of soluble polyphenols (Hartzfeld et al, 2002), after centrifugation (15 min, 25 °C, 3000 g) supernatants were combined and used to determine the hydrolysable polyphenols by the Folin Ciocalteu method with a ferulic acid standard curve (Singleton et al, 1999). The results were expressed as ferulic acid equivalents.

4.3.5. Phytic acid content

Phytic acid content of bread was determined according to the method of Haug and Lantzsh (1983) with modifications by Guttieri et al (2006). Phytic acid was extracted with 0.2M hydrochloric acid overnight. The extract was diluted and the sample extracts and standard solutions were boiled before the addition of ferric ammonium chloride. After cooling on ice, the samples were added to microplates along with 2, 2-bipyridine-thioglcolic acid and the absorbance as read at 530nm (Guttieri et al, 2006). The phytic acid content was determined by plotting the absorbance of the standard curve against concentration.

4.3.6. Baking and bread evaluation

Bread formulations were baked according to AACC approved method 10-09.01, with some modifications (AACC International, 1999b). Fungal α-amylase was used instead of malt powder and instant dry yeast was used instead of compressed yeast to improve the constancy of these ingredients used in the baking formula. Ammonium phosphate (5 ppm) was added to improve yeast function. The bread was prepared using a 2 hour fermentation schedule, rather than 3 hour fermentation to avoid over fermentation (Gonzalez-Gracia et al, 2012). The dough was punched once during fermentation. After baking, the bread was evaluated for loaf volume by rapeseed displacement (AACC International, 2001). The bread was lyophilized and ground to a fine and homogenous powder in a food processor before additional analysis.

4.3.7. Starch hydrolysis

The Englyst in vitro assay was conducted to determine the starch hydrolysis curves (Englyst et al, 1992). The bread samples were incubated at 37°C with an enzyme mix (amyloglucosidase, invertase and pancreatin) for 180 minutes. Aliquots of the digest were taken every 20 minutes to determine the amount of glucose released by reaction with glucose oxidase/peroxidase (GOPOD). A sample of commercial white bread (purchased from a local grocery store and air dried at room temperature) was analyzed as a reference. The hydrolysis index (HI) was obtained by dividing the area under the hydrolysis curve of the sample by the area obtained for commercial white bread (hydrolysis curve 0 min to 180 min). The estimated GI (eGI) of the samples was calculated using the equation described by Ovando-Martinez et al (2011b): eGI=8.198+0.862*HI.

The resistant starch content of the bread samples was measured using an assay kit according to AACC approved method 32-40.01 (AACC International, 2002). Samples were incubated with pancreatic α-amylase and amyloglucosidase for 16 hours. After centrifugation of the sample slurry, the pellet (containing the resistant starch) was washed. The resistant starch was dissolved in potassium hydroxide. The dissolved resistant starch was then hydrolyzed with amyloglucosidase and the amount determined by the reaction of glucose with GOPOD.

Due to the high level of retrogradation of starch occurring in baked bread, the amount of type-three (retrogradated starch) resistant starch (RS-III) was determined. The RS-III was measured using a modified enzymatic-gravimetric determination of dietary fiber. After incubation at 100°C with thermostable α-amylase for 35 minutes, protease was added to the samples for further incubation at 60°C for 35 minutes. Then, the samples were treated with AMG to hydrolyze the dextrans and physically inaccessible starch. The samples were centrifuged and the remaining pellets washed with distilled water, 96% ethanol and acetone. The pellets were dissolved in potassium hydroxide and hydrolyzed with AMG. The glucose was measured with GOPOD reagent (Saura-Calixto et al, 1993).

4.3.8. Starch characterization

Once the starch digestibility of the bread formulations was determined, additional tests were done to determine possible mechanisms affecting the starch digestibility of the whole wheat bread vs. white bread. Changes in physicochemical properties, such as molecular mass, were investigated in starch from the baked breads. For determination of starch molecular mass and apparent amylose content, the starch was extracted from bread and flour blends using the method of Simsek et al 2012 (Simsek et al, 2012). The extracted starch was dissolved in potassium hydroxide:urea solution and heated for 90 minutes at 100°C. The samples were then neutralized using hydrochloric acid and filtered before analysis by high performance size exclusion chromatography (HPSEC) with multi angle light scattering (MALS). The *dn/dc* value for calculation of the starch molecular mass was 0.146 (You et al, 1999; You and Lim, 2000; Simsek et al, 2012). The Debye model with a fit degree of one was used for calculation of the molar mass. The results were fitted to a first order polynomial model.

Portions of the wheat such as proteins or fiber may be coating the starch granules in the baked bread, which would block the access to hydrolytic enzymes. The bread was also evaluated using scanning electron microscopy to determine starch granule morphology and if there may be a physical barrier to the starch digestion. Thin slices of bread were dried at room temperature and mounted on aluminum mounts using colloidal silver or carbon adhesive tabs and coated with gold using a Balzers SCD 030 sputter coater (BAL-TEC RMC, Tucson, AZ). Images were obtained using a JEOL JSM-6300

Scanning Electron Microscope (JEOL USA, Peabody, MA) while using an accelerating voltage of 10 kV (Ovando-Martínez et al, 2011b).

4.3.9. Statistical analysis

The results of analysis were analyzed using SAS 9.3 statistical analysis software package. Analysis of variance (ANOVA) was conducted using completely random design (CRD). The mean separation was conducted by least significant difference (LSD) with $\alpha=0.05$. The ANOVA tables are presented in the appendix.

5. EXPERIMENT 1

5.1. Dough and Bread Quality

The farinograph often is used to evaluate the water absorption capacity and dough strength of wheat flours. Having the correct water absorption for flour is critical for production of the best quality bread and flours with higher water absorptions often are desired by bakers. Water absorption and dough strength measured by farinograph are reported in Table 3. The white flour had the lowest water absorption (64.7%) of all the flour samples. The water absorption in the ground wheat and blended wheat had absorptions of 67.5 and 71.9%, respectively. The bran component in the whole wheat flours absorbs water at a higher rate, due to the presence of arabinoxylans and other non-starch polysaccharides (D'Appolonia and Kunerth, 1984). The addition of starch also increased the water absorption in the ground wheat + starch and blended wheat + starch samples.

Table 3. Water Absorption and Dough Strength of White and Whole Wheat Flours

	Absorption 14% MB	**Peak Time Min.**	**Stability Min.**	**MTI BU**
White	64.7	3.0	12.5	30.0
Ground Whole Wheat	67.5	8.5	12.5	20.0
Blended Whole Wheat	71.9	7.0	16.5	30.0
Ground Whole Wheat + Starch	70.0	9.0	13.5	20.0
Blended Whole Wheat + Starch	72.3	7.5	16.0	25.0

Analysis was not replicated
MB = Moisture basis, MTI = Mixing tolerance index, BU = Braebender unit

The dough strength which is indicated by the peak time, stability and mixing tolerance index (MTI), is another important characteristic of wheat flour. The peak time will suggest to the baker how much energy it will take to mix dough to optimum consistency. Conversely, the stability and MTI reveals how tolerant the dough is to over-mixing. The whole wheat samples all had higher peak times than the white flour sample (3.0 minutes) (Table 2). The peak times of the whole wheat samples ranged from 7.0 to 9.0 minutes. The increase in peak time is a result of the competition for water between the protein and bran. The arabinoxylans, which are concentrated in the bran portion of the wheat, have been determined to increase dough development time (D'Appolonia and Kunerth, 1984). The gluten in the dough takes longer to develop and reach the peak consistency because of this competition for water. There was no difference in stability between the white flour and ground wheat flour (12.5 minutes). The blended wheat

had 16.5 minute stability and was the longest of the samples. The MTI of the samples ranged from 20 to 30 BU, and the whole ground wheat and whole ground wheat + starch flours had the lowest MTI (20). The blended wheat and blended wheat + starch seemed to have better dough quality than the ground wheat samples

After assessment of the dough quality, the flours were baked to determine the end product quality. The bake absorptions, mix times and loaf volumes of breads made from white and whole wheat flours are given in Table 4. The bake absorption followed a similar trend as the absorption determined using the farinograph. The white bread had a bake absorption of 70.6%, which was significantly ($P<0.05$) lower than the whole wheat breads. The blended wheat and blended wheat + starch samples had the highest bake absorptions, 76.2 and 76.7, respectively. The addition of the starch did not significantly ($P<0.05$) affect the bake absorption of the ground wheat + starch or blended wheat + starch breads. The dough mixing is conducted at room temperature (approximately 25°C) (AACC International, 1999b), so the starch will not swell and hold water (Shibanuma et al, 1996).

The mix time during the baking procedure is a critical value for bakers. Bakers want a mixing time which is not too short, as to be easily exceeded or too long which takes valuable time and energy. All of the whole wheat flours had significantly ($P<0.05$) lower mix times (3.5-3.9 minutes) than the mix time of the white flour (4.6 minutes). The pin mixer and additional ingredients, used for baking, will result in slightly different mixing characteristics than the farinograph presents. In this case, the additional ingredients will change the rate of water uptake and gluten matrix formation in the dough. Also, the pin mixer pulls the dough around the pins in thin sheets, while the farinograph mixes the dough in a kneading fashion between two sigmoidal blades. The action of the pin mixer may cause the bran particles to tear at the gluten more severely than the farinograph mixer, resulting in the lower mix times of the whole wheat dough. In general, mixing of whole meal dough must be more carefully monitored to avoid over or under mixing, because of the disruption of the gluten matrix by bran particles (Lai et al, 1989).

The loaf volume (Table 4) of the bread was measured after the bread was baked and cooled. Similar to the results of other research (Lai et al, 1989; Gonzalez-Gracia et al, 2012), the loaf volumes of the whole wheat breads were significantly ($P<0.05$) lower than the loaf volume of the white bread.

Table 4. Baking Quality of White and Whole Wheat Flours

	Bake Absorption % As Is	Mix Time Min.	Loaf Volume cc
White	70.6	4.6	1045
Ground Whole Wheat	71.8	3.5	695
Blended Whole Wheat	76.2	3.5	710
Ground Whole Wheat + Starch	71.0	3.9	710
Blended Whole Wheat + Starch	76.7	3.5	730
LSD (P<0.05)	0.8	0.3	56

LSD = Least significant difference

The white bread had a loaf volume of 1045cc, while the whole wheat breads had loaf volumes around 700cc. The blended wheat + starch bread had the highest loaf volume (730cc), among the whole wheat breads. There has been extensive research investigating the effect that bran and other components of whole wheat have on the loaf volume of whole wheat bread. Many of these studies show contradictory results as to the mechanism by which the loaf volume of whole wheat bread is decreased (Lai et al, 1989; Gan et al, 1992; De Kock et al, 1999; Zhang and Moore, 1999; Seyer and Gelinas, 2009; Noort et al, 2010; Gonzalez-Gracia et al, 2012). The cause of reduction in loaf volume is likely a complex combination of many factors, which are greatly affected by the bran source and extraction procedure (i.e. milling) (Lai et al, 1989). The loaf volume of the bread is not the only parameter which is affected by the inclusion of bran in the bread. The chemical composition and nutritional quality of the bread also could be affected (Slavin, 2004; Anson, 2010).

5.2. Proximate Analysis

The ash, protein and starch contents were measured in the flour and bread samples (Table 5). The ash content of the white flour is significantly (P<0.05) lower than all of the whole wheat flour samples. This is to be expected since the bran and germ portions of the wheat kernel contain most of the mineral content, which also can be seen in the higher ash content of the bran fraction. The ash content of the starch was not measured since it would be too low for an accurate measurement and would not comprise a significant portion of the starch. All of the bread samples had significantly (P<0.05) higher ash contents than their respective flours. Ash levels increased in the bread samples because of the addition of the baking ingredients, such as, yeast, sugar and salt. The ash content of the white bread is significantly

($P<0.05$) lower than the whole wheat breads. One of the health benefits of whole wheat bread consumption is the increased mineral content (Slavin, 2004), indicated by the high ash levels.

The whole wheat flours had significantly higher ($P<0.05$) protein than the white flour. The ground wheat and blended wheat flours had protein contents of 15.53 and 15.60 % (DWB), respectively. The ground wheat + starch and blended wheat + starch samples had significantly ($P<0.05$) lower protein than the whole wheat flours without starch. All of the bread samples had significantly ($P<0.05$) lower protein content than their corresponding flours, which is likely due to a dilution effect of the other ingredients used in baking.

The starch content was measured to calculate the amount of starch which was added to the whole wheat + starch samples. In the preliminary samples the white flour contained approximately 16% more starch than the ground wheat or the blended wheat samples. An additional 1.2g/100g starch was added to the ground wheat and an additional 1.9 g/100g starch was added to the blended wheat. Theoretically, this should have resulted in whole wheat flours having similar starch contents as the white flour. After measuring the starch content of the whole wheat + starch samples, the actual values were significantly ($P<0.05$) higher than the whole wheat samples by approximately 3%. However, the starch content of the whole wheat + starch samples was still significantly ($P<0.05$) lower than the white flour (Table 5). There may be several reasons why the theoretical and measured starch contents of the whole wheat + starch samples are not the same. Inadequate blending of the sample may be a partial cause since it may be difficult to evenly distribute the small amount of starch in the whole wheat samples.

There were also significant ($P<0.05$) differences in starch content between the flours and breads. All of the bread samples, except for the ground wheat bread, had lower starch content than their corresponding flour. The reduction in starch content in the bread samples is also a result of a dilution effect caused by the addition of the other ingredients used in baking. Overall, the bran component of the whole wheat flours and breads (Lai et al, 1989) resulted in significant ($P<0.05$) differences in the composition of the samples.

Table 5. Proximate Analysis of White and Whole Wheat Flour and Bread

		Ash % DWB	Protein % DWB	Starch % DWB
Flour	**White**	0.68	15.00	75.52
	Ground Whole Wheat	2.10	15.53	58.41
	Blended Whole Wheat	2.17	15.60	60.48
	Ground Whole Wheat + Starch	2.08	15.03	61.41
	Blended Whole Wheat + Starch	2.10	15.23	63.45
	Starch	ND	0.40	93.87
	Bran	5.18	17.86	28.49
Bread	**White**	1.55	14.35	70.67
	Ground Whole Wheat	2.77	15.21	58.48
	Blended Whole Wheat	2.79	15.47	55.59
	Ground Whole Wheat + Starch	2.75	15.20	59.34
	Blended Whole Wheat + Starch	2.73	15.08	58.73
	LSD ($p<0.05$)	0.04	0.12	1.15

DWB = Dry weight basis, ND = Not determined, LSD = Least significant difference

5.3. Bread Crumb Structure

Scanning electron microscopy images were taken to investigate possible changes to the bread crumb structure and gluten matrix (Figure 5). Several differences can be seen between the crumb structure of the white bread sample and the whole wheat bread samples. The crumb surfaces are relatively smooth in the white bread, whereas in the whole wheat samples the surfaces are rough. A coating, which has been observed in other research (Rojas et al, 2000), can be seen covering the starch granules. This coating is most likely composed of the gluten protein matrix and starch molecules which have leached from the starch granules. The starch granules which are visible in the white bread are relatively smooth and intact. However, these granules seem to be more highly visible and less thickly coated than the granules in the whole wheat breads. Wheat kernels also have small hairs located on their surface, known as brush hairs. Since these hairs are attached to the bran layer they will be found in the whole wheat bread and can cause additional disruption of the gluten matrix. A brush hair from a wheat kernel can be seen in the image of the ground wheat bread in Figure 5. The structures in these images show that the starch granules in whole wheat breads may have more physical barriers to hydrolysis by digestive enzymes.

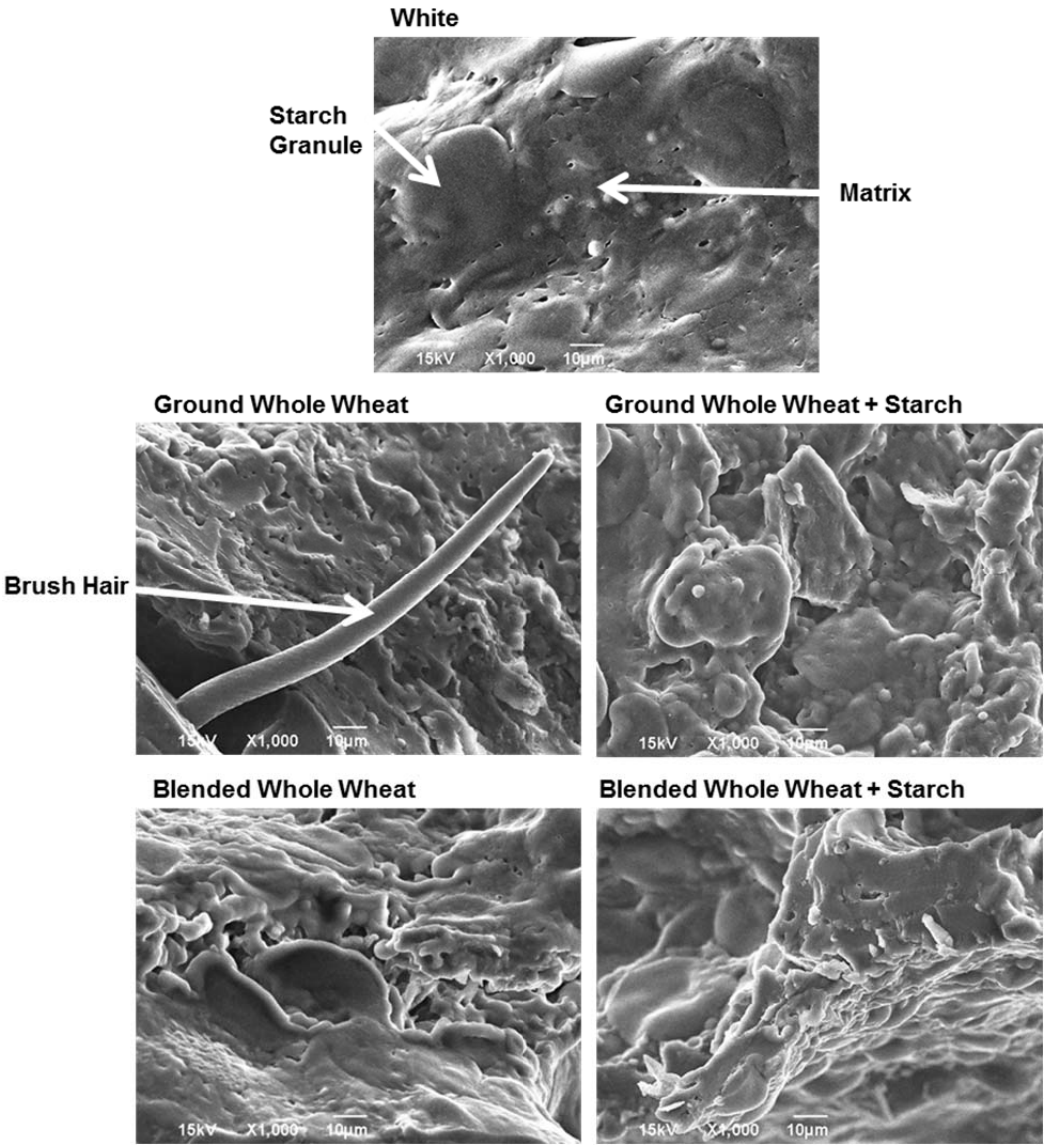

Figure 5. Scanning Electron Microscopy Images of White and Whole Wheat Bread Crumb
*Images taken at X1,000 magnification

5.4. Estimated GI

The end-use quality of whole wheat bread is considered lower than that of white bread due to lower loaf volume and dense crumb structure. Yet, whole wheat bread has gained popularity among consumers due to health benefits of whole grain consumption (Slavin et al, 2001; Slavin, 2004). The lower GI is one benefit to whole wheat bread consumption. Although, whole wheat bread is still considered a high GI food (Venn and Green, 2007; American Diabetes Association, 2013), its GI is

considerably lower than white bread. The hydrolysis index (HI) and estimated GI (eGI) of white and whole wheat breads are shown in Table 6.

Table 6. Hydrolysis Index and Estimated GI of White and Whole Wheat Breads

	HI	eGI
White	111.7	104.5
Ground Whole Wheat	82.8	79.6
Blended Whole Wheat	84.0	80.6
Ground Whole Wheat + Starch	87.5	83.6
Blended Whole Wheat + Starch	80.6	77.7
LSD (p<0.05)	9.6	8.3

HI = Hydrolysis index, eGI = Estimated GI, LSD = Least significant difference

The HI and eGI of the white bread were 111.7 and 104.5, respectively. All of the whole wheat bread samples had significantly ($P<0.05$) lower HI and eGI than the white bread. There were no significant ($P<0.05$) differences between HI and eGI with respect to the milling method. The blended whole wheat + starch bread had the lowest HI (80.6) and eGI (77.7) of all the samples. The HI and eGI of the whole wheat breads were not significantly ($P<0.05$) different from each other. This means that even when increasing the starch content of the whole wheat bread to the same level as the white bread, the eGI is still significantly ($P<0.05$) lower than the white bread. From this evidence we may be able to disprove the lower starch content as the reason for the lower eGI in whole wheat bread. Since we have some evidence that the starch content is not the factor causing the reduction of eGI, more investigation is needed. As seen in Figure 4, the structure of the crumb and physical barriers in the bread could be contributing to the decrease in eGI. There could also be some other chemical components present in the whole wheat bread that are not present in white bread which are affecting the starch hydrolysis. These components need additional investigation. The levels of phenolic compounds, phytic acid and resistant starch and changes in starch molecular weight should be measured to determine the relationship between them and the eGI.

5.5. Conclusions of Experiment 1

Overall, there are significant differences between white and whole wheat breads with respect to both end-product quality and nutritional value. The inclusion of bran in the whole wheat flours increased the farinograph water absorption and mixing stability. However, the increased mixing stability seen in the

farinograph analysis of whole wheat flours is deceptive. The whole wheat flour acts differently in a more intensive mixing process used in baking and the whole wheat dough may breakdown more easily in different types of mixers. The milling method used to produce the whole wheat flours had an effect on the farinograph absorption and mixing stability. The bake absorption was also significantly ($P<0.05$) higher in blended whole wheat. However, the milling method did not have any significant ($P<0.05$) effect on loaf volume. Because of the increase in water absorption, blended whole wheat should be used for additional studies.

Even though the whole wheat breads had lower loaf volume than the white bread the whole wheat breads do have some advantages in their nutritional quality. The whole wheat breads have increased mineral content, seen in the higher ash values (Table 5). The whole wheat breads also have significantly ($P<0.05$) higher protein content than the white bread. Another nutritional benefit of the whole wheat breads is their lower eGIs. A thicker coating can be seen in the microstructure of the bread (Figure 4). This coating may act as a barrier to digestive enzymes, which would slow the rate of digestion and result in lower eGI than the white bread. However, this is likely not the only explanation so, more investigation is needed to ascertain which components of the whole wheat flours may affect the eGI. It will also be useful to determine if there is any difference in eGI between several wheat varieties.

6. EXPERIMENT 2

6.1. Quality and Composition of White and Whole Wheat Flours and Breads

6.1.1. Flour extraction of wheat varieties

In the process of milling wheat into flour, the amount of white flour obtained after milling is reported as milling extraction. Table 7 gives the flour extraction as percent wheat basis and the percent flour and bran and shorts as product basis. The flour extraction calculated on a percent wheat basis is used by millers to determine the total amount of flour that is extracted from the total weight of wheat milled. There were no significant (P<0.05) differences in flour extraction or the percentages of flour and bran and shorts obtained between any of the three wheat varieties milled in this study.

Table 7. Flour Yield and Milling Extraction

	Flour % Product Basis	**Bran + Shorts % Product Basis**	**Flour Extraction % Wheat Basis**
Barlow	76.14	23.86	72.72
Glenn	73.05	26.95	69.57
Prosper	74.02	25.98	71.01
LSD (P<0.05)	4.08	4.08	3.47

LSD = Least significant difference

Although there were no significant (P<0.05) differences in the flour yield, it was important to determine the appropriate ratio of flour to bran and shorts for blending the whole wheat flours. To be considered a whole wheat flour, the flour must comply with 21CFR137.200 and contain all portions of the wheat kernel in the correct biological ratios (U.S.Food and Drug Administration, 2012). For this experiment it was important to have high quality flour for all samples and that the milling method was the same for the white and whole wheat flours. The sample set in this experiment also includes commercially milled white and whole wheat flours. The commercially milled samples will be used as a reference to the type of flours available to commercial bakeries.

6.1.2. Dough quality of white and whole wheat flours

After milling and blending of the whole wheat flours, the water absorption and dough strength was determined by Farinograph. The results of the Farinograph analysis can be seen in Table 8. The results show that there were significant (P<0.05) differences in absorption and peak time between the white and

whole wheat flours. There also were some significant (P<0.05) differences in Farinograph parameters between the wheat varieties.

Table 8. Dough Quality of White and Whole Wheat Flours Measured by Farinograph

	Absorption 14% MB	Peak Min	Stability Min	MTI BU
CMWP	61.4	7.9	11.7	28.5
Barlow White	66.5	8.8	11.2	20.5
Glenn White	63.9	9.9	22.7	15.0
Prosper White	63.2	7.2	9.7	27.5
CMWW	65.6	5.7	10.2	19.5
Barlow Whole Wheat	73.9	6.4	8.7	24.0
Glenn Whole Wheat	71.7	7.7	10.2	20.5
Prosper Whole Wheat	68.9	5.5	6.3	33.0
CMWW + Starch	66.2	5.8	11.1	20.5
Barlow Whole Wheat + Starch	74.0	7.2	10.4	18.5
Glenn Whole Wheat + Starch	71.7	6.9	11.5	15.5
Prosper Whole Wheat + Starch	69.2	5.9	6.5	36.5
LSD (P<0.05)	**0.3**	**1.3**	**2**	**5.8**

CMWP=Commercially milled white patent, CMWW= Commercially milled whole wheat, MB = Moisture basis, BU = Braebender unit, LSD = Least significant difference

Of the white flours, Barlow had significantly (P<0.05) higher absorption (66.5%) than the Glenn or Prosper white flours. Barlow also produced the whole wheat flour and whole wheat + starch flours with the highest absorptions, 73.9 and 74.0 %, respectively. Overall, the whole wheat flours and whole wheat + starch flours all had significantly (P<0.05) higher absorptions than their corresponding white flours. Higher water absorption in whole wheat flours was also seen in previous research by Gonzalez-Gracia et al (2012), who found that whole wheat flours had approximately 8% higher water absorption than white flour. High water absorption is desired by bakers, so that they can have more water and less flour in a loaf of bread.

In this experiment, the peak times of the whole wheat and whole wheat + starch flours were all significantly (P<0.05) lower than the white flours of the same variety. This is most likely due to the interference of the bran particles in the whole wheat flours. The peak times of the whole wheat flours were not significantly (P<0.05) different than those of the whole wheat + starch flours. Since starch does not absorb a significant amount of water at 30°C, it is unlikely that the addition of starch would have any

effect on the farinograph parameters. The white flour from the variety Glenn had the highest peak time (9.9 min.) of all the samples. The Prosper whole wheat had a peak time of 5.5, which was the lowest of all the samples.

Mixing stability and tolerance to over-mixing are important parameters of bread flours. The stability of the flour samples ranged from 6.3 min (Prosper whole wheat flour) to 22.7 min. (Glenn white flour). The stabilities of Glenn and Prosper white flours were significantly ($P<0.05$) higher than their corresponding whole wheat and whole wheat + starch flours. There were no significant ($P<0.05$) differences between the white, whole wheat and whole wheat + starch flours for the Barlow flours. The differences, or lack thereof, in the commercially milled samples might be because the flour in the CMWP and CMWW are likely not from the same source. The quality of the protein or the composition of the other components in the Barlow flours may allow for the retention of similar stabilities between the white and whole wheat flours of that variety. MTI is an indication of resistance to over-mixing. In these samples there were significant ($P<0.05$) differences in MTI.

6.1.3. End-Product quality of white and whole wheat breads

Test baking of flour is generally done to assess the end-product quality. The bake absorption, mix time and loaf volume were measured during the baking process and are presented in Table 9. The Farinograph can give the baker a general idea about the water absorption of flour, but the absorption determined by the Farinograph can be different than when preparing bread. The type of mixer and additional ingredients used in baking will have an effect on how much water the flour can optimally absorb. Also, the desired consistency of the dough for certain products could be different than the consistency formed in the Farinograph, which would also result in differences in absorptions. Although the bake absorptions of the flours were not the same as the Farinograph absorptions, they do show a similar trend. The bake absorptions of the whole wheat and whole wheat + starch flours were all significantly ($P<0.05$) higher than their corresponding white flours. The high water absorption of the whole wheat and whole wheat + starch flours occurs because of the high water holding capacity of the bran.

Table 9. Quality of Bread Prepared from White and Whole Wheat Flours

	Bake Absorption %	Mix Time Min.	Loaf Volume cc
CMWP	62.7	3.0	1072.5
Barlow White	69.0	2.9	1060.0
Glenn White	67.7	4.1	1122.5
Prosper White	65.1	2.9	902.5
CMWW	71.6	3.0	717.5
Barlow Whole Wheat	75.0	3.1	762.5
Glenn Whole Wheat	72.7	4.0	747.5
Prosper Whole Wheat	71.3	3.0	685.0
CMWW + Starch	67.6	3.1	707.5
Barlow Whole Wheat + Starch	74.8	3.3	752.5
Glenn Whole Wheat + Starch	73.5	3.5	730.0
Prosper Whole Wheat + Starch	70.9	3.3	725.0
LSD (P<0.05)	1.2	0.3	35.8

CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat, LSD = Least significant difference

The mix time for baking was evaluated for the white, whole wheat and whole wheat + starch samples (Table 9). The mix times ranged from 2.9 to 4.1 min., and there were some small significant ($P<0.05$) differences among samples. The bread made from Glenn flours had significantly ($P<0.05$) longer mix times than most of the other samples. The white flour samples from Barlow and Prosper had the lowest mix times (2.9 min.).

The largest differences in end-product quality of the flours can be seen in the loaf volumes of the samples. Similar to the preliminary experiments and the results obtained by other researchers (Lai et al, 1989; Gan et al, 1992; De Kock et al, 1999; Zhang and Moore, 1999; Seyer and Gelinas, 2009; Noort et al, 2010; Gonzalez-Gracia et al, 2012), the whole wheat and whole wheat + starch breads had significantly ($P<0.05$) lower loaf volumes than the white breads. The loaf volume of the Prosper white bread (902.5 cc) was significantly lower than the other white bread samples. This result is reflected in the weaker dough strength shorter mix time determined by the Farinograph (Table 8).

Overall, the whole wheat and whole wheat + starch breads are on average 300cc lower in volume than the white bread samples. The bread from the variety Barlow had the highest loaf volume (762.5 cc) of the whole wheat and whole wheat + starch breads. Glenn resulted in the highest loaf volume (1122.5

cc) among the white bread samples. The addition of starch to the whole wheat formulas only resulted in a significant increase in loaf volume for the Prosper whole wheat breads. The Prosper whole wheat + starch bread (725.0 cc) had significantly ($P<0.05$) higher loaf volume than the Prosper whole wheat bread (685 cc). The composition of the Prosper whole wheat + starch flour may allow for hydrolysis of the additional starch and increased gas production leading to higher loaf volume. The lower loaf volumes of the whole wheat breads are caused by a complex combination of many factors, which are greatly affected by the bran source and extraction procedure (i.e. milling) (Lai et al, 1989). The loaf volume of the bread is not the only parameter which is affected by the inclusion of bran in the bread. The chemical composition and nutritional quality of the bread could also be affected (Anson, 2010).

6.1.4. Microstructure of bread crumb

The crumb structure of bread is another important factor in evaluating end-product quality of bread. The crumb structure is often subjectively evaluated by visual inspection by the baker and given a score. Another technique involves photographing a slice of bread under carefully controlled conditions and evaluating the crumb structure attributes with a special software package. In this study SEM was used to take images of the bread microstructure. This method was used because the microstructure of the bread crumb is of more interest since these microstructures may have some influence on the digestibility of the starch in the bread. The SEM images showing the bread microstructure are shown in Figure 5.

The most obvious difference between the microstructure of the white breads and the whole wheat breads is the visibility of the starch granules. The starch granules in the white bread can be seen far more clearly than in the whole wheat and whole wheat + starch breads. The starch granules in the white bread samples have become deformed and pitted by gelatinization and the action of amolytic enzymes. The degradation of the starch granules will allow for more rapid hydrolysis by human digestive enzymes when the bread is eaten.

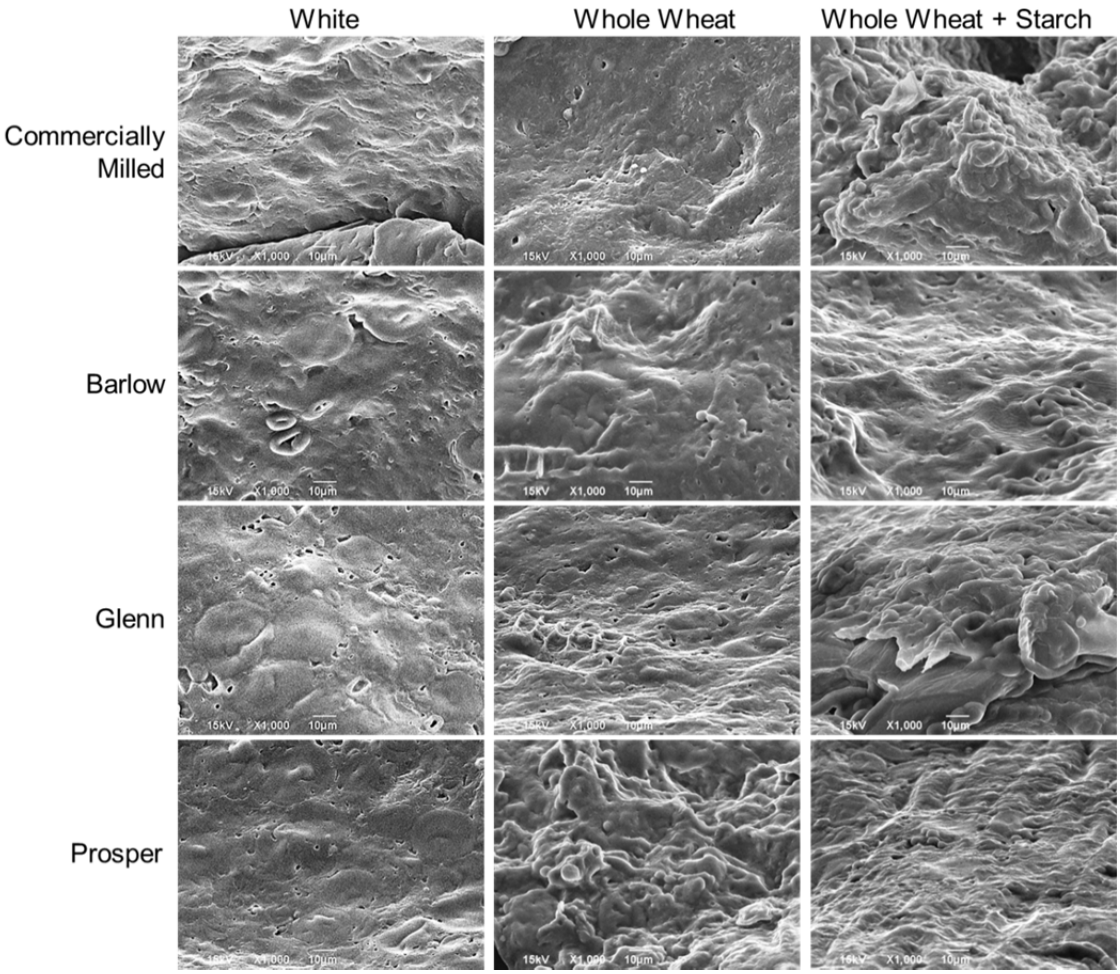

Figure 6. Scanning Electron Microscopic Images of White and Whole Wheat Bread Crumb
*Images taken at X1000 magnification

The starch granules in all the bread samples will have a matrix of protein and leached starch coating them (Rojas et al, 2000). This matrix can be seen in all of the bread samples in this study (Figure 6). The whole wheat and whole wheat + starch samples seem to have a thicker matrix coating the starch granules. The thick matrix in these samples blocks the starch granules from view in the SEM images of the bread. The matrix in the whole wheat samples may be thicker because of arabinoxylans that have leached from the bran during the mixing and fermentation processes of baking. A thicker matrix will act more strongly as a physical barrier to digestive enzymes. There will also be a chemical barrier to starch digestion if arabinoxylans are forming a portion of the matrix covering the starch granules, since arabinoxylans are not hydrolyzed by human digestive enzymes. Studies done by Bravo et al,(1998) and Choct and Annison (1992) determined that wheat products containing higher levels of arabinoxylans had

slower starch digestibility. Based on previous research and the SEM images in this study, it is possible that the arabinoxylans are forming a barrier to prevent starch hydrolysis. The microstructure of the bread and possible components making up these structures lead us to investigate the chemical composition of the bread in more detail to determine any link between the chemical composition of the bread and its end-use quality and starch digestibility.

6.1.5. Composition of white and whole wheat flours and breads

The composition of flour can have a considerable effect on its end-use and nutritional quality. One minor but important component of wheat flour is the ash content. In white flour the ash content should be low since low ash content reflects better milling efficiency and high ash levels can have a negative effect on bread quality. Whole wheat flours will have higher ash content since the majority of the minerals in wheat are concentrated in the bran and germ fraction. The higher mineral content of the whole wheat flour and bread gives these products a nutritional advantage (Slavin, 2004).

The ash content of white and whole wheat flours and breads is given in Table 10. No significant ($P<0.05$) differences were observed in the ash content of the white flour samples. The ash contents of the whole wheat and whole wheat + starch samples did have significant ($P<0.05$) differences between each other. The whole wheat and whole wheat + starch samples also had significantly ($P<0.05$) higher ash content than the white flours, which is expected due to the high mineral content of the bran. The Barlow whole wheat had the highest ash content (1.97%, DWB). Addition of other ingredients during baking resulted in higher ash content in the bread samples. The ash content of the bread samples are significantly higher than the flours they were made from, but follow a similar trend. The white bread samples all have significantly ($P<0.05$) lower ash contents than the whole wheat and whole wheat + starch breads.

Protein is a major component of hard spring wheat and it provides for both nutritional and end-use quality of the flour. As is typical, the whole wheat flours and bread have higher protein content than their white flour and bread counterparts. The bran layer contains high levels of amino acids which increase the overall protein content of the whole wheat flour and bread (Slavin, 2004).

Table 10. Composition of White and Whole Wheat Flours and Breads

		Ash % DWB	Protein % DWB	Starch % DWB	Starch Damage % As Is
Flour	**CMWP**	0.77	15.12	74.54	7.15
	Barlow White	0.76	17.61	70.76	7.58
	Glenn White	0.72	16.74	71.80	7.39
	Prosper White	0.72	14.19	74.91	8.66
	CMWW	1.94	16.41	60.92	3.38
	Barlow Whole Wheat	1.97	18.43	58.66	6.73
	Glenn Whole Wheat	1.95	17.55	59.18	7.09
	Prosper Whole Wheat	1.81	15.03	62.39	8.01
	CMWW + Starch	1.92	16.14	63.89	3.35
	Barlow Whole Wheat + Starch	1.95	18.21	61.13	6.24
	Glenn Whole Wheat + Starch	1.92	17.35	63.72	7.14
	Prosper Whole Wheat + Starch	1.82	14.77	66.16	7.27
Bread	**CMWP**	1.56	14.93	69.15	20.80
	Barlow White	1.52	17.15	64.80	19.14
	Glenn White	1.49	16.34	70.00	19.22
	Prosper White	1.50	14.05	69.91	18.08
	CMWW	2.52	16.16	56.24	13.24
	Barlow Whole Wheat	2.57	18.02	52.87	13.64
	Glenn Whole Wheat	2.52	17.40	55.99	16.54
	Prosper Whole Wheat	2.52	14.85	58.07	14.60
	CMWW + Starch	2.51	16.12	58.53	12.57
	Barlow Whole Wheat + Starch	2.58	17.76	55.46	13.15
	Glenn Whole Wheat + Starch	2.48	17.00	56.97	14.32
	Prosper Whole Wheat + Starch	2.46	14.73	58.35	12.75
	LSD (P<0.05)	0.05	0.17	1.32	0.77

CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat, DWB = Dry weight basis, LSD = Least significant difference

The whole wheat + starch flours and breads was slightly, and in some cases, significantly ($P<0.05$) lower than the whole wheat flours and breads. The added starch acts to dilute the protein portion of these samples. The variety of wheat also resulted in significant ($P<0.05$) differences in protein content among all flour and bread types. Barlow had the highest protein contents for white, whole wheat and whole wheat + starch flours and breads. The protein content of Barlow white, whole wheat and whole wheat + starch flours were 17.61, 18.43 and 18.21%, respectively. The protein content of Barlow white, whole wheat and whole wheat + starch breads were 17.15, 18.02 and 17.76%, respectively. Although, high protein flours are generally desired for bread making; the quality of the gluten forming proteins in the

flour will have a substantial effect on the end-product quality. However, protein is an essential part of the human diet and it is beneficial to the nutritional quality of the bread to have higher protein content.

Another component of wheat that affects flour functionality, end-product and nutritional quality is starch. The major portion of both white and whole wheat flours is starch, although in whole wheat flours, the starch is lower because of dilution by the bran and germ. As expected, the total starch contents of the whole wheat flours and breads are significantly ($P<0.05$) lower than their white flour and bread counterparts. The starch content of the white and whole wheat flours in this study are within the range that is typically found in wheat (Shewry, 2009).

The whole wheat + starch flours have been supplemented with additional starch, which was extracted from the white flours. This is to increase the starch content of the whole wheat flour to a similar level as the white flour. Approximately, 1.1% starch was added to whole wheat flours. This was done to determine if the dilution of starch in whole wheat bread was the factor causing the lower eGI in whole wheat bread. The whole wheat + starch fours were predicted to have similar starch content as the white flours. However, as seen in Table 10 the whole wheat + starch samples had significantly ($P<0.05$) lower starch content than the white flours. The whole wheat + starch flours did have significantly ($P<0.05$) higher starch contents than the whole wheat flours of the same variety. The difference in theoretical and measured starch contents of the whole wheat + starch samples may have several causes. The blending of the sample may have been inadequate since it may be difficult to evenly distribute the small amount of starch in the whole wheat samples.

During milling and processing of wheat, a portion of the starch can become damaged by mechanical action or gelatinization. The amount of damaged starch in a sample is measured by treating the sample with α-amylase for a short period of time and measuring the amount of hydrolyzed starch. The amount of starch damage in wheat flour is affected by the wheat type and quality and the method of milling (Prabhasankar and Rao, 2001). Starch damage will affect water absorption of flour and end-product quality. Flours with high starch damage will have higher water absorption, but the dough may over ferment more rapidly or the bread crumb may be sticky. Flours with starch damage that is too low may require the addition of extra amylase in the baking formula (Boyaci et al, 2004).

Barlow whole wheat had significantly ($P<0.05$) lower starch damage than the Barlow white flour. The reduction in starch damage in the whole wheat flours from Barlow, Glenn and Prosper could be due to two reasons. There could be some component of the whole wheat which is inhibiting the starch hydrolysis during the assay procedure or the starch which is removed from the flour along with the bran could be less damaged than the starch in the flour portion.

The starch damage increased significantly ($P<0.05$) in all samples after baking. This is caused by the starch hydrolysis and gelatinization that occurs in the baking process. The white breads all had significantly ($P<0.05$) higher starch damage than their whole wheat and whole wheat + starch counterparts. Barlow and Glenn breads had the largest increase in starch damage after baking. The large increase in starch damage after baking of the white bread samples may indicate that the starch in the white breads is more susceptible to hydrolysis than the starch in the whole wheat and whole wheat + starch breads. These main components, of wheat flour, greatly affect the end-product and nutritional quality. However, there are other components that may be minor but can also have strong effects on the quality.

Arabinoxylans (AX) are considered a minor component of wheat, but they have important effects on the quality of wheat flour. AX content and structure can affect the dough consistency and water absorption of flour (Goesaert et al, 2005; Dornez et al, 2008). They may also act to stabilize gas cells during fermentation and baking (Goesaert et al, 2005). The majority of the dietary fiber in wheat is comprised of AX (Anson, 2010). The structure of arabinoxylans is a β-1, 4 linked xylose backbone which is substituted with arabinose at the C(O)-2 and/or C(O)-3 positions (Goesaert et al, 2005). The arabinoxylan content and A/X ratio of the white and whole wheat flours and breads are shown in Table 11.

The AX content of the whole wheat and whole wheat + starch flours and breads were significantly ($P<0.05$) higher than the AX content of the white flours and breads. The higher AX content of the whole wheat products is expected since the majority of the AX in wheat is found in the bran. The AX content of the whole wheat products ranged from about 8-11%, while the AX content in the white flour and bread was about 2.5-3%.

Table 11. Arabinoxylan Content and A/X Ratio of White and Whole Wheat Flours and Breads

		Arabinoxylan % DWB	A/X Ratio
Flour	**CMWP**	2.84	0.95
	Barlow White	2.78	0.89
	Glenn White	2.39	0.90
	Prosper White	2.51	0.84
	CMWW	11.04	0.89
	Barlow Whole Wheat	9.54	0.92
	Glenn Whole Wheat	9.53	0.94
	Prosper Whole Wheat	7.94	0.94
	CMWW + Starch	10.39	0.89
	Barlow Whole Wheat + Starch	11.02	0.92
	Glenn Whole Wheat + Starch	10.11	0.90
	Prosper Whole Wheat + Starch	8.00	0.93
Bread	**CMWP**	2.96	0.83
	Barlow White	2.80	0.80
	Glenn White	2.46	0.81
	Prosper White	2.33	0.90
	CMWW	10.27	0.87
	Barlow Whole Wheat	11.20	0.85
	Glenn Whole Wheat	10.14	0.92
	Prosper Whole Wheat	8.40	0.93
	CMWW + Starch	7.97	0.94
	Barlow Whole Wheat + Starch	8.63	0.93
	Glenn Whole Wheat + Starch	9.29	0.87
	Prosper Whole Wheat + Starch	8.31	0.86
	LSD (P<0.05)	0.48	0.06

A/X Ratio= Arabinose to xylose ratio, DWB= Dry weight basis, LSD = Least significant difference

Glenn white flour had 2.39% AX, which was lower than the other white flours, but not significantly different (P<0.05). The AX content of white wheat flour has been reported to be between 1.5-2.5% (Goesaert et al, 2005; Simsek et al, 2011) Prosper had the lowest AX contents in the whole wheat (7.94%) and whole wheat + starch (8%) flours, which were significantly (P<0.05) lower than the other whole wheat flours. The AX content of the white breads were not significantly (P<0.05) different than the white flours. However, there were significant (P<0.05) differences between some of the whole wheat breads and the whole wheat flours. As a staple product and one of the main sources of dietary fiber in many diets (Johansson et al, 1984), it is important for bread products to be high quality and nutritious.

The significant ($P<0.05$) increase in AX (dietary fiber) improves the nutritional quality of the whole wheat and whole wheat + starch breads. AX have also been found to slow starch digestion, improving glycemic response of whole wheat products (Choct and Annison, 1992; Bravo et al, 1998). When looking at AX functionality in bread, the A/X ratio is another factor to consider.

The rate of substitution with arabinose is referred to as the A/X ratio and plays an important role in the AX functionality (Goesaert et al, 2005). Table 11 gives the A/X ratios of the white and whole wheat flours and breads. There were some small significant ($P<0.05$) difference between wheat varieties and between the white and whole wheat products. However, there is no clear trend to these differences. Among the flour samples, Prosper white had the lowest A/X ratio (0.84) and Barlow white bread (0.80) had the lowest A/X ratio of the bread samples. The AX content and A/X ratio may also effect the phenolic content of the wheat, since ferulic acid is often found bound to the arabinose residues of the AX (Goesaert et al, 2005).

Whole grains, such as wheat, are a good source of antioxidants, though the antioxidant capacity of whole wheat has typically been under estimated (Slavin, 2004; Pérez-Jiménez and Saura-Calixto, 2005). Phytic acid is one anti-oxidant compound found in wheat. Phytic acid acts as a chelating agent which binds various metals and suppresses iron catalyzed redox reactions (Slavin, 2004). The phytic acid content of the white and whole wheat flour and bread samples is reported in Table 12. The white flours had significantly ($P<0.05$) lower phytic acid content than their whole wheat counterparts. There was a 1.5 to 2 mg/g reduction as a result of the removal of the bran during milling. Prosper white flour had significantly ($P<0.05$) lower phytic acid content than Barlow and Glenn white flours. There were no significant ($P<0.05$) differences in the phytic acid content of the Barlow, Glenn or Prosper whole wheat and whole wheat + starch flours. There is a wide range of phytic acid contents reported for wheat in literature (Lolas et al, 1976; Haros et al, 2001; Febles et al, 2002; Guttieri et al, 2006). This is due to the differences in genotypes and growing environments of the wheat tested. There may be additional variation due to the methods of phytic acid determination. The levels of phytic acid in this research are similar to those reported by Lolas et al (1976).

The phytic acid content of all the bread samples was significantly ($P<0.05$) lower than the original flour samples they were produced from. The most drastic reduction in phytic acid content occurred in the white bread samples. The white breads had nearly 4 mg/g less phytic acid than the white flours. While the whole wheat and whole wheat + starch breads had significantly ($P<0.05$) lower phytic acid than the whole wheat and whole wheat + starch flours, the decreases were less than 1 mg/g. The higher phytic acid content of the whole wheat breads may reduce-end product quality by binding Ca^{2+}, which would inhibit amylase activity during baking (Thompson and Yoon J.H., 1984; Haros et al, 2001). However, phytic acid acts as an antioxidant and anticarcinogen by suppression of oxidant damage due to the high amount of oxygen radicles produced by the bacteria in the human gut (Febles et al, 2002; Slavin, 2004). This makes the whole wheat breads more desirable from a nutrition stand point.

Table 12 also presents the levels of extractable and hydrolysable phenolic compounds in the flour and bread samples. Extractable phenolic compounds are those that can be extracted by shaking with acidified methanol:water and acetone:water at room temperature. There was no significant ($P<0.05$) difference in extractable phenolic compounds in Barlow, Glenn or Prosper flours. All of the flour samples contained approximately 4 mg/g extractable phenolic compounds. However, there were significant ($P<0.05$) differences between the hydrolysable phenolic compounds in Barlow, Glenn and Prosper white and whole wheat flours. The hydrolysable phenolic compounds are extracted from the residue after removal of extractable phenolic compounds by hydrolysis with strong acid at 85°C. The hydrolysable phenolic content of the white flours ranged from 6.30 to 7.33 mg/g. The whole wheat and whole wheat + starch flours had about twice as much hydrolysable phenolic compounds as their white flours. Glenn whole wheat + starch had the highest amount of hydrolysable phenolic compounds (12.69). The Prosper whole wheat flour had significantly ($P<0.05$) lower hydrolysable phenolic compounds than the whole wheat flours from Barlow and Glenn. Two of the most common phenolic compounds in wheat, ferulic and cinnamic acids, are found bound to the AX in wheat, making them insoluble until hydrolysis by gut microflora (Slavin, 2004). The levels of extractable and hydrolysable phenolic compounds are similar to those found in literature (Pérez-Jiménez and Saura-Calixto, 2005; Beta et al, 2005).

Table 12. Phytic Acid and Phenolic Compound Content of White and Whole Wheat Flours and Breads

			Phenolic Compounds	
		Phytic Acid* mg/g	Extractable* mg/g	Hydrolyzable* mg/g
Flours	**CMWP**	4.11	3.82	6.78
	Barlow White	4.13	4.35	6.69
	Glenn White	4.20	4.28	7.33
	Prosper White	3.89	3.83	6.30
	CMWW	5.55	4.26	12.33
	Barlow Whole Wheat	5.83	4.18	12.11
	Glenn Whole Wheat	5.76	4.13	12.55
	Prosper Whole Wheat	5.83	3.86	11.44
	CMWW + Starch	5.57	4.24	12.61
	Barlow Whole Wheat + Starch	5.81	4.38	12.29
	Glenn Whole Wheat + Starch	5.75	4.04	12.69
	Prosper Whole Wheat + Starch	5.84	4.01	11.89
Bread	**CMWP**	0.13	1.45	10.16
	Barlow White	0.24	1.37	10.23
	Glenn White	0.22	1.52	9.65
	Prosper White	0.21	1.47	9.44
	CMWW	5.35	2.23	13.75
	Barlow Whole Wheat	5.22	1.69	14.21
	Glenn Whole Wheat	5.34	2.12	14.25
	Prosper Whole Wheat	5.02	2.49	13.04
	CMWW + Starch	5.27	2.16	14.05
	Barlow Whole Wheat + Starch	5.30	1.97	14.03
	Glenn Whole Wheat + Starch	5.48	1.98	14.60
	Prosper Whole Wheat + Starch	5.07	2.45	12.82
	LSD (P<0.05)	0.10	0.30	0.54

CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat, DWB = Dry weight basis, LSD = Least significant difference

The extractable phenolic compound content was significantly (P<0.05) reduced after baking bread for all samples. The largest reductions in extractable phenolic compounds were found in Barlow and Glenn white breads. The whole wheat and whole wheat + starch breads had significantly (P<0.05) higher extractable phenolic content than their white bread counter parts. The higher level of extractable phenolic compounds in the whole wheat breads may be due to hydrolysis of these compounds from the AX during the fermentation process of baking. Conversely, the hydrolysable phenolic compound content was significantly (P<0.05) higher in the bread samples than the flours they were prepared from. In the case of the hydrolysable phenolic compounds the white bread samples had larger increases than the

whole wheat and whole wheat + starch breads. A study done by Pérez-Jiménez and Saura-Calixto (2005) showed a substantial increase in the phenolic content of wheat flour and other cereal products after treatment with digestive enzymes. The enzyme hydrolysis that occurs during baking could be increasing the detectable levels of the hydrolysable phenolic compounds in the bread. The level of phenolic compounds found in bread is not only important for their antioxidant activity but also for their effect in slowing starch digestibility (Thompson and Yoon J.H., 1984).

6.2 Starch Characteristics of White and Whole Wheat Flour and Bread

6.2.1. Amylose content of white and whole wheat flour and bread

Since starch is a major component of wheat flour and bread it is important to evaluate how the starch properties are affecting end-product and nutritional quality. It has been determined that there are significant ($P<0.05$) differences between samples in regards to the total starch content (Table 10). To fully investigate the effects of starch on the bread quality and eGI, the components and properties of the starch must be determined. Starch is composed of two large glucose polymers, amylose and amylopectin. The first is a linear chain of α-1, 4 linked glucose units with a very minor occurrence of α-1, 6 linked branch points. The second is a highly branched molecule made up of α-1, 4 linked glucose chains linked by α-1, 6 linkages. The proportion of amylose in most native cereal starches is approximately 25% (Eliasson, 2004; Simsek et al, 2012). The proportion of amylose and other starch properties, such as, molecular mass can have an influence on the end-product and nutritional quality.

The amylopectin and amylose contents of the starches extracted from the flour and bread samples are presented in Table 13. The amylopectin and amylose contents of the white, whole wheat and whole wheat + starch flour samples are very similar to each other. There are some significant ($P<0.05$) differences in the amylopectin and amylose content of the samples. Barlow white flour has significantly higher amylose than the Barlow whole wheat and whole wheat + starch flours, as well as, the Glenn and Prosper white and whole wheat flours. All of the flours have amylose contents which are in the normal range for native wheat starch. Native cereal starches typically have around 25% amylose content (Eliasson, 2004; Simsek et al, 2012).

Table 13. Amylose Content White and Whole Wheat Flour and Bread Samples

		Amylopectin %	Amylose %
Flours	**CMWP**	74.60	25.40
	Barlow White	73.86	26.14
	Glenn White	74.83	25.17
	Prosper White	74.75	25.25
	CMWW	75.04	24.96
	Barlow Whole Wheat	76.19	23.81
	Glenn Whole Wheat	74.95	25.05
	Prosper Whole Wheat	74.96	25.04
	CMWW + Starch	75.73	24.27
	Barlow Whole Wheat + Starch	74.98	25.02
	Glenn Whole Wheat + Starch	74.39	25.61
	Prosper Whole Wheat + Starch	75.24	24.76
Bread	**CMWP**	80.66	19.34
	Barlow White	80.09	19.91
	Glenn White	79.90	20.10
	Prosper White	79.00	21.00
	CMWW	74.61	25.39
	Barlow Whole Wheat	76.16	23.84
	Glenn Whole Wheat	78.16	21.84
	Prosper Whole Wheat	76.37	23.63
	CMWW + Starch	77.15	22.85
	Barlow Whole Wheat + Starch	75.85	24.15
	Glenn Whole Wheat + Starch	77.78	22.22
	Prosper Whole Wheat + Starch	76.58	23.42
	LSD (P<0.05)	0.76	0.76

CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat, LSD = Least significant difference

After baking, the amylose content significantly ($P<0.05$) decreased for all samples except for the commercially milled and Barlow whole wheat breads. Since the amylose leaches from the starch granule first during starch gelatinization (Eliasson, 2004), the amylose will be more readily available for hydrolysis than the amylopectin. The hydrolysis of amylose during baking causes the bread samples to have lower percentage of amylose content. The largest reduction in amylose content was in the white bread samples. The Barlow, Glenn and Prosper white bread samples have significantly ($P<0.05$) lower amylose content than the corresponding whole wheat and whole wheat + starch samples bread samples (Table 13). The reduction of amylose in the white bread samples may indicate that the starch is more readily hydrolyzed and may have more rapid digestion in the human gut than the starch in the whole wheat breads.

6.2.2. Molecular mass of starch in white and whole wheat flour and bread

A reference chromatogram illustrating the integration of the refractive index signal peaks is given in Figure 7. The HPSEC profiles of the starch extracted from the flour and bread samples are shown in Figure 8. The amylopectin fraction is the first peak of the chromatogram, and it can be seen as a single peak, a split peak or a peak having shoulders. The amylose peak shows up in the chromatogram at a retention time between 30 and 40 minutes. The amylose peak is very flat and broad and due to the stacking of the chromatograms, is difficult to distinguish in some cases.

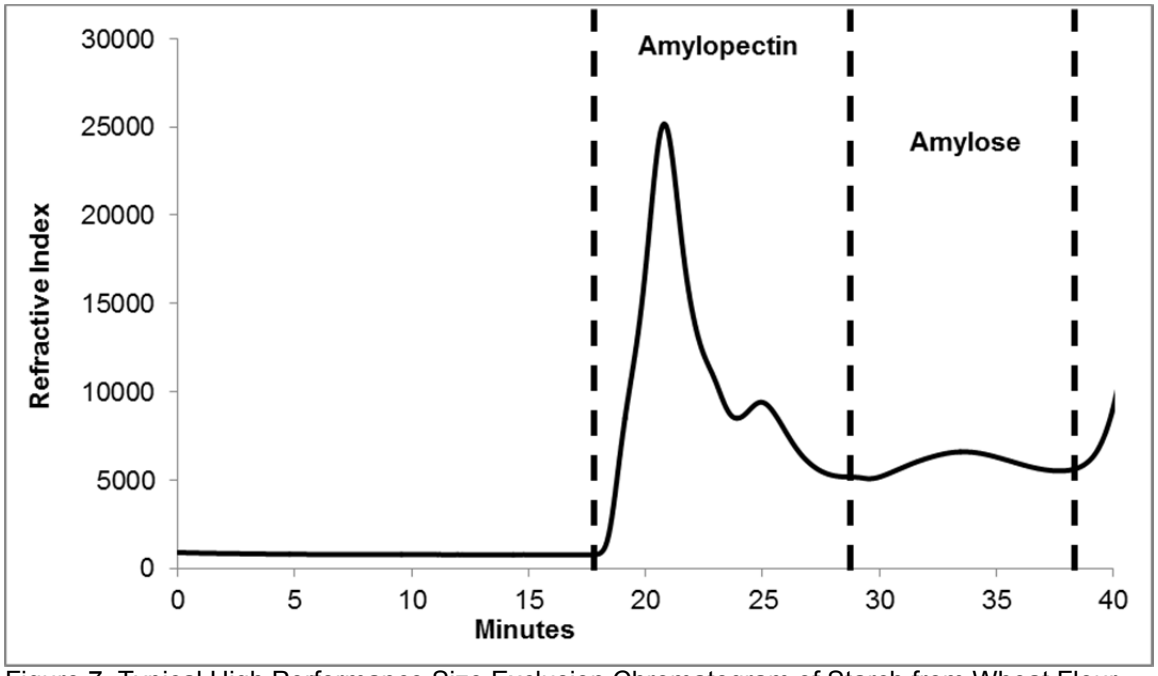

Figure 7. Typical High Performance Size Exclusion Chromatogram of Starch from Wheat Flour

The hydrolysis of the starch due to baking can be seen as shift in retention time between the chromatograms of the flour starch and bread starch. Peaks with later retention times will have smaller molecular mass since the small molecules are retained longer on size exclusion chromatography column packing material. Some of the bread starch chromatograms also show a splitting of the amylopectin peak which is not present in the flour starch from the same source. This shows the degradation and increasing polydispersity of the amylopectin molecules in the bread starch. The molecular mass of the amylose and amylopectin in the flour and bread samples is given in more detail in Figures 10 and 11.

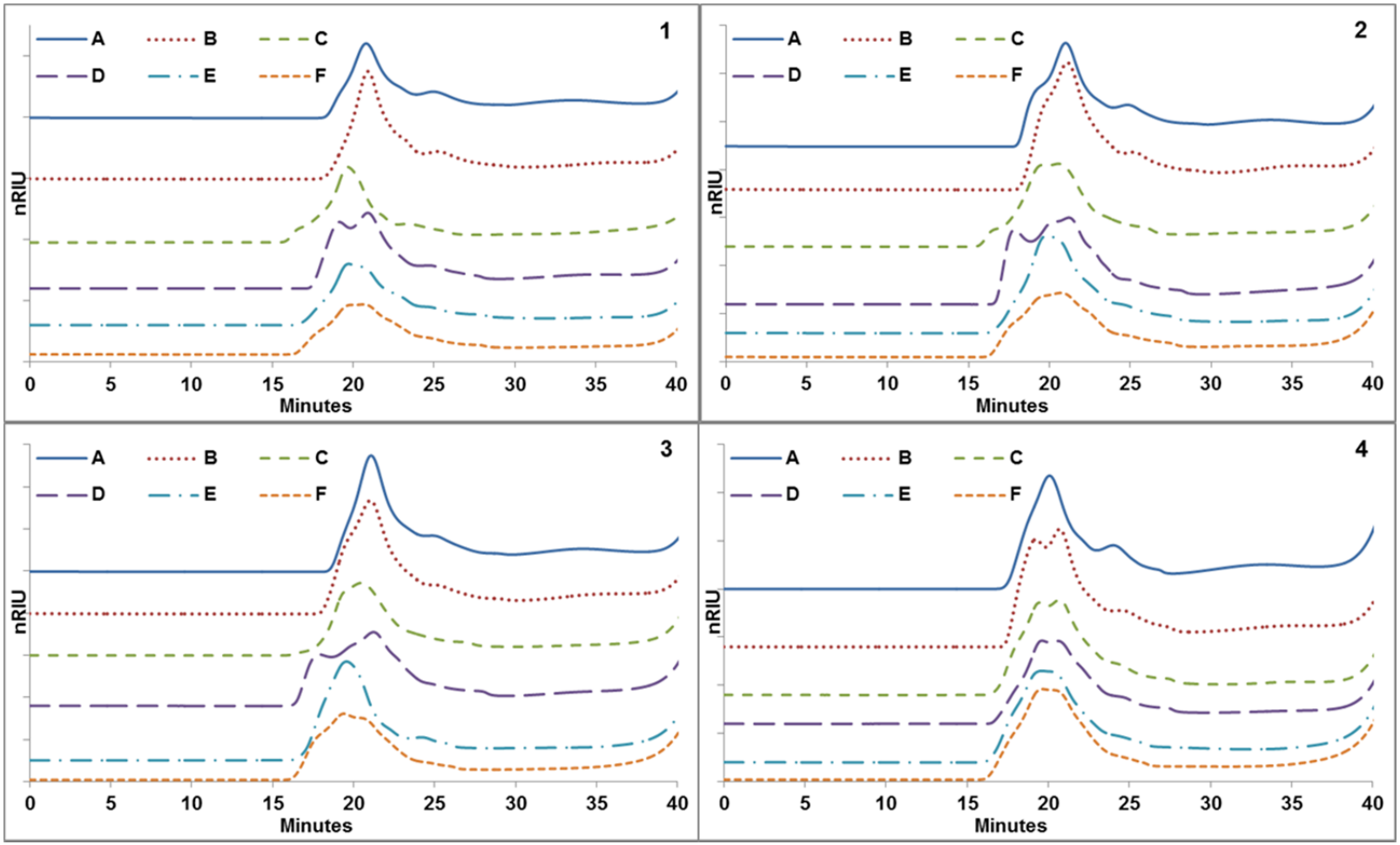

Figure 8. Chromatograms of Starch from White and Whole Wheat Flours and Breads
1 = Commercially milled, 2 = Barlow, 3 = Glenn, 4 = Prosper, A = White Flour, B = White Bread, C = Whole Wheat Flour, D = Whole Wheat Bread, E = Whole Wheat + Starch Flour, F = Whole Wheat + Starch Bread

Molecular mass is another important characteristic of starch which could impact end-product and nutritional quality. Starch molecular mass can vary based on genetic and environmental differences in a sample (Simsek et al, 2012). The variation in starch molecular mass can alter wheat starch swelling and pasting characteristics (Shibanuma et al, 1996; Sasaki and Matsuki, 1998), which may influence end-product quality. The molar mass of the starch samples was determined by MALS. The signal of a typical MALS chromatogram, along with a RI signal, is shown in Figure 9.

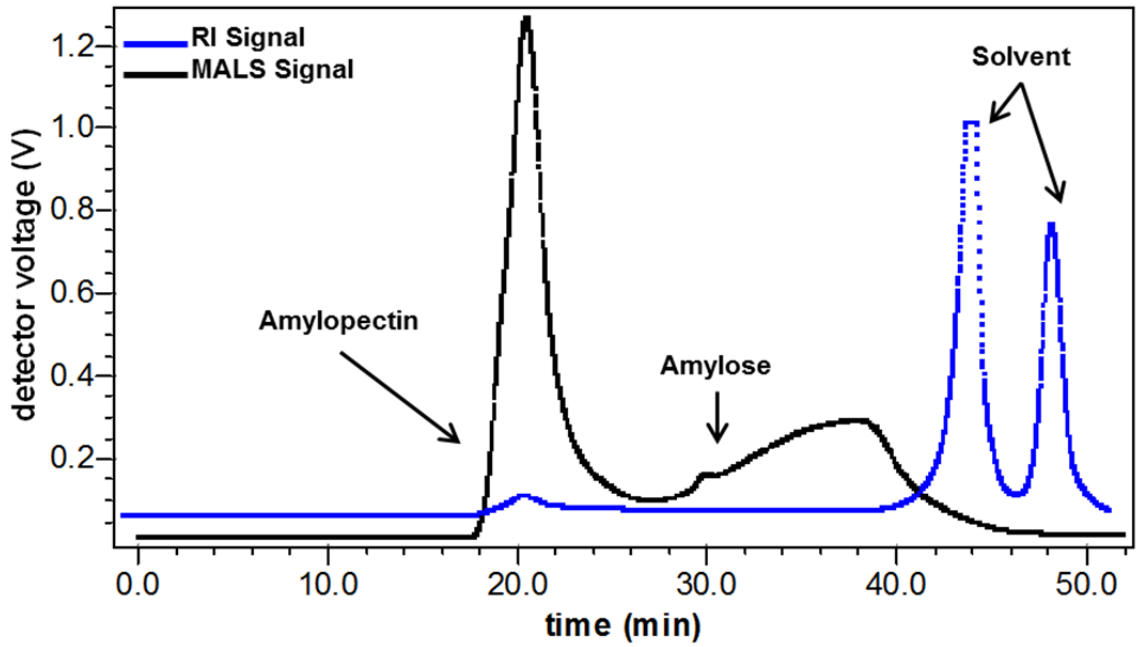

Figure 9. Reference Chromatogram of Refractive Index and Multi Angle Laser Light Scattering Signals
RI= Refractive index, MALS= Multi angle laser light scattering

The weight averaged molecular mass of amylopectin in the flour and bread samples are shown in Figure 10. There were no significant ($P<0.05$) differences between the molecular mass of amylopectin from flours of the same wheat source, except for Prosper white and whole wheat + starch flours. However, the difference in molecular mass of amylopectin from the Prosper flours was not that large. The molecular mass of the commercially milled white flour amylopectin is significantly ($P<0.05$) higher than the commercially milled whole wheat flour, but these flours did not come from the same source so cannot be directly compared. The amylopectin molecular mass was significantly ($P<0.05$) different between varieties. The amylopectin from the Barlow flours, at approximately $1.7x10^7$ daltons (Da), was the largest among all the flour samples. The amylopectin from the Prosper flours had the smallest molecular mass

(aprox. $1.3x10^7$Da). Amylopectin is one of the largest known biopolymers, and as such the large molecular mass of the amylopectin in this study is within the acceptable range for amylopectin (Eliasson, 2004; Gidley et al, 2010).

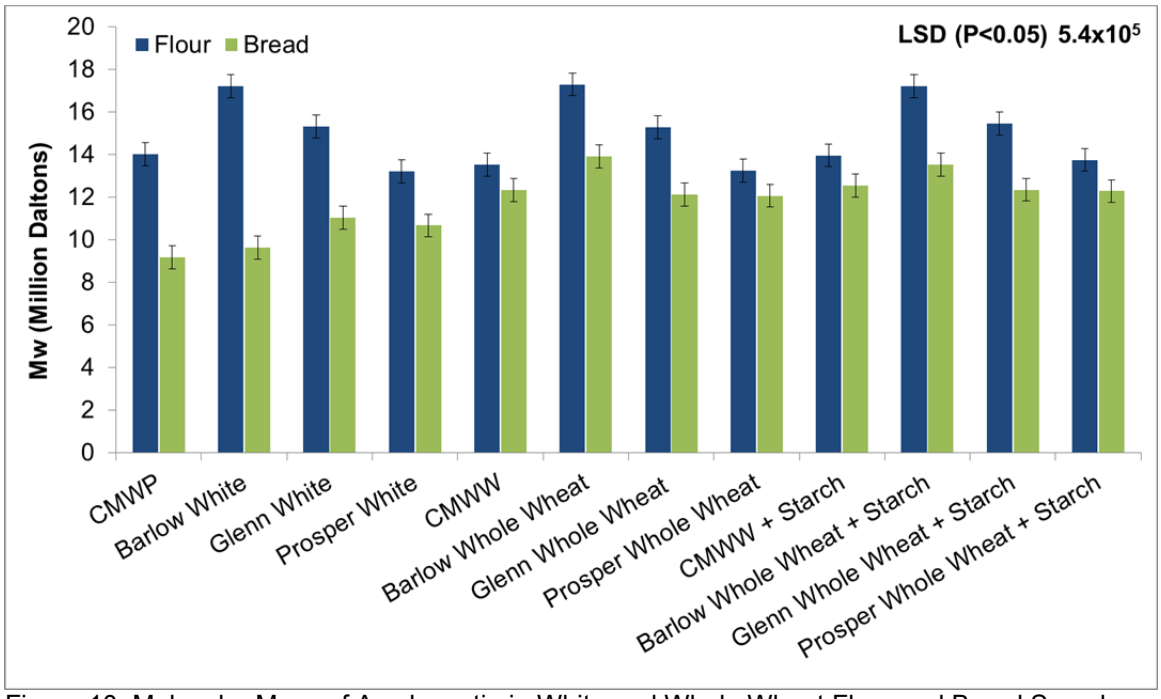

Figure 10. Molecular Mass of Amylopectin in White and Whole Wheat Flour and Bread Samples *M_w = Weight averaged molecular mass, Error bars represent ± LSD (P<0.05), CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat

There were many more significant (P<0.05) differences between the molecular mass of the amylopectin in the bread samples. The amylopectin from the white bread samples was significantly (P<0.05) lower than the amylopectin in the whole wheat and whole wheat + starch breads from the same source. Also, the amylopectin in the Barlow white bread had significantly (P<0.05) lower molecular mass than the Glenn and Prosper breads, but Barlow whole wheat and whole wheat + starch breads had significantly (P<0.05) higher molecular mass than the Glenn and Prosper breads. The molecular mass of the amylopectin in the Barlow white bread was $9.63x10^6$ Da and the amylopectin from Barlow whole wheat bread had a molecular mass of $1.39x10^7$ Da. The reduction in molecular mass of the amylopectin in the bread samples is related to the level of starch hydrolysis that occurred during fermentation and baking of the bread. These results indicate that the amylopectin in white bread is more easily hydrolyzed than the amylopectin in whole wheat breads. There must be some component present in the whole wheat flour which is lacking in the white flour which prevents the starch hydrolysis. This may be affecting the

end-product quality by lowering the amount of starch hydrolyzed for yeast metabolism and gas production. However, it may have some benefit to the nutritional quality of the bread. The prevention of starch hydrolysis in the whole wheat breads may lead to slower starch digestion in the human digestive tract.

Amylose has a smaller molecular mass than amylopectin, but it has significant functionality in wheat flour. The amylose molecule provides for a significant amount of the structure and texture of bread. Bread made from wheat flour which has low or no amylose will have a very poor texture and may collapse (Hung et al, 2007). The molecular mass of amylose in wheat flour may also affect the end-product and nutritional quality. The weight averaged molecular mass of amylose in white and whole wheat flour and bread samples are shown in Figure 11. As seen in the molecular mass of amylopectin, there were significant ($P<0.05$) differences in the molecular mass of amylopectin. However, these differences were not as large as the differences in amylopectin molecular mass. The molecular mass of the amylose determined in this study was relatively high but within the range reported in literature (Eliasson, 2004; Gidley et al, 2010).The differences in amylose molecular mass between flours from the same source were not significant ($P<0.05$). The molecular mass of the amylopectin from the Prosper flours was about $6.5x10^6$ Da. The amylose in the Glenn flours had the highest molecular mass, approximately $7.5x10^6$ Da, of the flours.

The molecular mass of amylose in the bread samples were all significantly ($P<0.05$) lower than the amylose from the flours from which they were prepared. As was the case with amylopectin, the white breads also had significantly ($P<0.05$) lower molecular mass amylopectin than the whole wheat and whole wheat + starch breads from the same source. However, there was less change in molecular mass of amylose in all samples between the flours and the breads than was seen with the molecular mass of the amylopectin. The reason for this is the manner and rate that each of these molecules undergoes hydrolysis. If the amylopectin has few molecules of glucose hydrolyzed from the ends of the branches it will retain most of its molecular mass. However, if the amylopectin becomes debranched the molecular mass will decrease rapidly. The branching of amylopectin can inhibit complete hydrolysis of the molecule. Amylose has minimal branching and may be more completely hydrolyzed. Only the amylose which has

relatively less degradation will be precipitated during starch extraction, and so this may explain why there seems to be less degradation of the amylose than amylopectin in the bread.

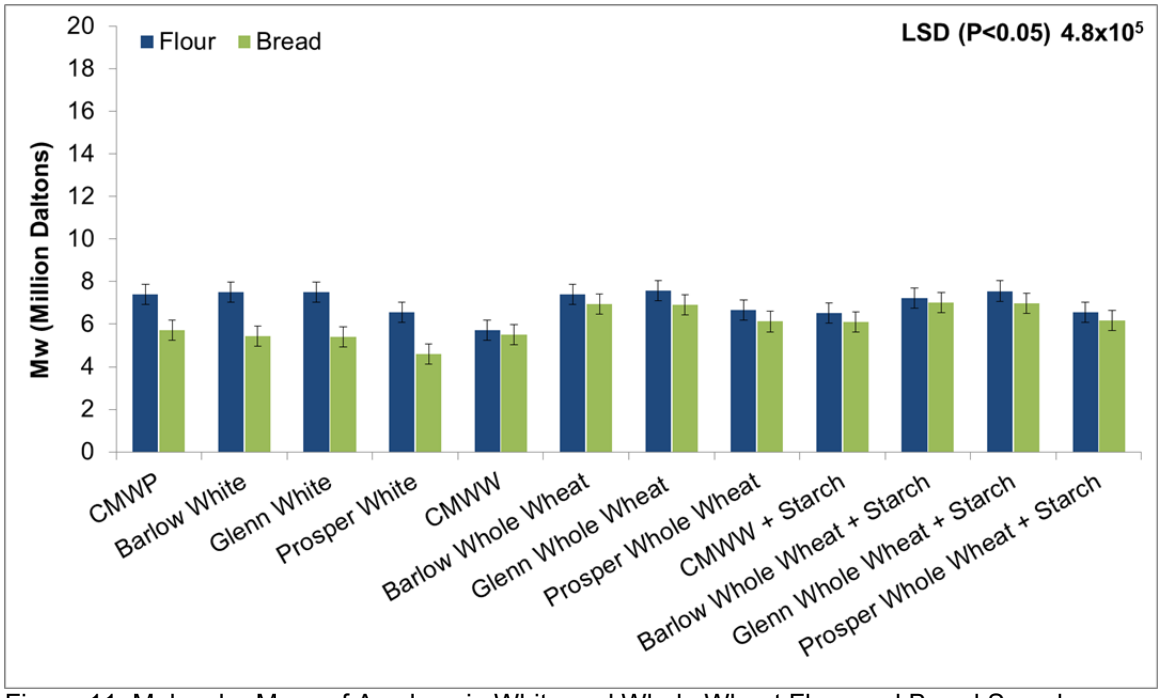

Figure 11. Molecular Mass of Amylose in White and Whole Wheat Flour and Bread Samples
* M_w = Weight averaged molecular mass, Error bars represent ± LSD (P<0.05), CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat

6.2.3. Starch digestibility of white and whole wheat bread

Resistant starch (RS) is defined as starch which resists complete digestion in the small intestine. RS is able, at least in part, to be fermented in the colon and is classified as dietary fiber. There are several types of RS, which are classified based on their mechanism of resistance. (Sajilata et al, 2006). In this study we focused on the total RS content and type III resistant starch (RS-III). RSIII is classified as starch that composed of retrograded starch, most of which is retrograded amylose (Sajilata et al, 2006). Table 14 shows the RS and RS-III content of the bread samples. Overall, the RS content of the breads was quite low. This is expected since bread generally has a high glycemic response. Typical levels of RS in bread range from 0.5 to 2 percent. The RS content of the breads in this study ranged from 0.49 to 1.41%. The RS content of Barlow white and Prosper white breads was significantly (P<0.05) higher than the RS of the whole wheat and whole wheat + starch breads made from the same source. Barlow white bread had the highest RS content (1.41%) and Glenn whole wheat + starch bread had the lowest RS

content of the bread samples. Glenn whole wheat and whole wheat + starch breads had significantly ($P<0.05$) lower RS than the whole wheat and whole wheat + starch breads from Barlow and Prosper.

By determination of RS-III, it can be seen that the RS content of the bread samples is comprised of mostly (if not completely) RS-III. The RS-III content of the bread samples followed the same trend as the RS content. Barlow white bread had significantly ($P<0.05$) higher RS-III (1.57%) than the other bread samples. The Glenn white, whole wheat and whole wheat + starch breads had the lowest levels of RS-III, 0.70, 0.56 and 0.59%, respectively. The RS-III, which is retrograded starch, was formed during the storage of the bread prior to lyophilization as part of the staling process. There seems to be more RS in most of the bread samples when determining the RS-III than in the determination of RS. However, this is likely due to the differences in enzymes and treatments used by the two separate assay procedures. Also, RS is not the only factor of importance when evaluating the nutritional value of starch.

Table 14. Starch Hydrolysis Properties of White and Whole Wheat Breads

	Resistant Starch	**Type III Resistant Starch**	**HI**	**eGI**
	% DWB	**% DWB**	**DWB**	**DWB**
CMWP	1.20	1.47	101.86	96.00
Barlow White	1.41	1.57	102.66	96.69
Glenn White	0.58	0.70	106.26	99.79
Prosper White	1.29	1.42	109.76	102.81
CMWW	0.79	0.90	85.98	82.31
Barlow Whole Wheat	1.17	1.18	82.80	79.57
Glenn Whole Wheat	0.52	0.56	86.23	82.53
Prosper Whole Wheat	1.13	1.18	83.70	80.34
CMWW + Starch	0.85	0.91	86.06	82.38
Barlow Whole Wheat + Starch	1.15	1.22	83.60	80.26
Glenn Whole Wheat + Starch	0.49	0.59	88.12	84.15
Prosper Whole Wheat + Starch	1.13	1.15	86.04	82.36
LSD (P<0.05)	0.08	0.08	8.77	7.56

HI = Hydrolysis index, eGI = Estimated GI, DWB= Dry weight basis, CMWP= Commercially milled white patent, CMWW= Commercially milled whole wheat, LSD = Least significant difference

GI and starch digestibility are important nutritional factors in high-starch foods such as bread. The GI refers to the postprandial glycemic response of a test product compared to that of a reference food (glucose or white bread) (Björck et al, 1994; Augustin et al, 2002; Slavin, 2004). When *in vitro* assay methods are employed the term is referred to as estimated glycemic index (eGI) (Ovando-Martínez et al,

2011a). Foods can be classified as high, medium or low GI foods. Wheat breads are typically high GI foods, but whole wheat breads often have lower GI than white flour bread (McKevith, 2004; Anson, 2010). The starch hydrolysis properties of the white and whole wheat bread samples are shown in Table 14.

The hydrolysis index (HI) is determined by the rate of starch hydrolysis in the target food compared to the rate of starch hydrolysis in a references food (Goñi et al, 1997). Typically white bread is used as a reference and white bread purchased at a local grocery store was used as a reference in this study. The HI of the breads in this study ranged from 109.76 to 82.80. The white bread samples all had HI above 100, meaning that the starch in these samples had a higher rate of starch hydrolysis than the reference food. It is well known that starch in white bread is easily hydrolyzed and may give a spike in glucose response (Slavin, 2004). The HI of the whole wheat and whole wheat breads were significantly ($P<0.05$) lower than their white bread counterparts. Starch was added to whole wheat flours to counteract the effect of the difference in starch content between white and whole wheat bread. The results of this study show that there were no significant ($P<0.05$) differences between the HI of the whole wheat and whole wheat + starch breads from the same source. Because of this we can assume that the lower starch content of whole wheat bread was not the factor which results in lower HI. When conducting *in vitro* starch hydrolysis assays, the HI can be used to calculate the eGI.

The eGI of the bread samples in this study followed a similar trend as the HI (Table 14). The white bread samples had significantly ($P<0.05$) higher eGI than the whole wheat and whole wheat + starch breads. The eGI ranged from 96.00 to 102.81 in the white breads and from 79.57 to 84.15 in the whole wheat and whole wheat + starch breads. The difference in eGI of the whole wheat and whole wheat + starch breads was not significant ($P<0.05$). As stated previously, starch was added to white breads to eliminate the effect of starch content on starch hydrolysis in the bread samples. Bravo et al (1998), determined a starch digestion rate index of about 90 in a whole wheat bread sample. This is slightly higher than the HI and eGI of the whole wheat breads in this study. The results of this study show that although the whole wheat and whole wheat + starch breads are still in the high GI category, their eGI is still significantly lower than in the white breads. This might be beneficial to consumers who want to continue consuming bread products but desire a more health conscious option.

Since it was determined that the lower starch content in whole wheat bread does not decrease the eGI, there must be some other component of the whole wheat bread resulting in the lower eGI. From the results of the chemical analysis of the white and whole wheat flours and breads the components that may be responsible for lowering the eGI in whole wheat bread may be determined. When examining the bread crumb structure by SEM (Figure 6) a thicker matrix can be seen coating the starch granules in the whole wheat bread. This matrix will act more strongly as a physical barrier to digestive enzymes and could reduce the eGI. The matrix covering the starch granules is composed of the gluten matrix and possibly arabinoxylan. Both of these components have been shown to result in a reduction in starch hydrolysis. Studies done by Bravo et al,(1998) and Choct and Annison (1992) determined that wheat products containing higher levels of arabinoxylans had slower starch digestibility. Jenkins et al (1987), determined that in bread which had the native protein-starch interaction remaining intact the glycemic response and *in vitro* starch digestion was lower than in bread which was made from starch and protein which had been extracted from each other. Based on their previous research and the SEM images in this study, it is possible that the arabinoxylans and the gluten protein matrix are forming a barrier to prevent starch hydrolysis.

Phytic acid and phenolic compounds are other relatively minor components of wheat flour and bread, but they may influence the starch hydrolysis and eGI of the whole wheat bread samples. In the case of phytic acid there was an inverse relationship with the eGI of the bread samples. The whole wheat bread samples, which had significantly ($P<0.05$) higher phytic acid levels (Table 12), had significantly ($P<0.05$) eGI than the white breads. The phenolic compounds measured in the bread samples (Table 12) had the same inverse trend as the phytic acid. Thompson and Yoon (1984) found that starch hydrolysis was inhibited by the presence of phytic acid and some phenolic compounds. It is reasonable to presume that the higher levels of phytic acid and phenolic compounds in the whole wheat samples are affecting the eGI of the whole wheat breads.

The differences in eGI between the white and whole wheat breads can also be examined by comparing the properties of the starch in these samples. The amount of amylose in the bread starches was measured and the white breads had significantly ($P<0.05$) less amylose than the whole wheat breads

(Table 13). The reduction of amylose in the white bread samples may indicate that the starch is more readily hydrolyzed and may have more rapid digestion in the human gut than the starch in the whole wheat breads. Changes in molecular mass of both amylopectin and amylose in the bread samples also allude to differences in starch hydrolysis between the white and whole wheat breads. The molecular mass of the amylopectin and amylose were significantly ($P<0.05$) lower in the white bread samples than in the whole wheat bread samples (Figures 10 and 11). The bread samples with higher eGI also tended to have lower molecular mass starch. The prevention of starch hydrolysis in the whole wheat breads may lead to slower starch digestion in the human digestive tract.

6.3. Conclusions

When examining the starch hydrolysis properties and eGI of these bread samples along with the results of the physicochemical analysis of the flour and bread samples, several interesting conclusions can be made. These conclusions are related to the end-product quality, as well as the nutritional quality of the white and whole wheat bread samples analyzed in this research.

In reference to Objective 1 of this research, there were significant differences in the composition and end product quality between white and whole wheat bread. Primarily, the end-product quality of the whole wheat breads is reduced by the action of the bran and some other chemical components, which have deleterious effects on the gluten matrix and gas cell formation Overall, the presence of the bran in the whole wheat flours increased the water absorptions of the whole wheat flours. Whole wheat flours produced weaker dough and had less stability during mixing, probably due to interference by bran and other components in the whole wheat flour which are not in the white flour. The largest differences in end-product quality of the flours can be seen in the loaf volumes of the samples. The whole wheat breads had relatively small loaf volumes compared to the white breads. Based on this study, there are many components of the whole wheat that alter the end-product quality in a complex manner and the results will vary depending on wheat type and source, and the methods used for milling and baking.

This study was also able to determined differences in the eGI of white and whole wheat breads (Objective 2). The results of this research show that the lower starch content in whole wheat bread does not decrease the eGI, so there must be some other component of the whole wheat bread resulting in the

lower eGI. Since the dilution of starch in the whole wheat bread was determined not to be the factor causing lower eGI in whole what breads, The relationships between eGI and the composition of the bread samples was investigated to meet the third objective of this study.

Several connections between the eGI and the composition of the bread samples were found. The white bread had larger increases in starch damage after baking than the whole wheat breads and may indicate that the starch in the white breads is more susceptible to hydrolysis and gelatinization. The nutritional quality of the whole wheat and whole wheat + starch breads is better because of higher AX (dietary fiber) content. The other component of the dietary fiber content of the breads is RS. The majority of the RS in the bread samples is a result of retrogradation of the starch during storage of the bread and is classified as RS-III. The eGI of the bread samples was not related to the level of RS. However, the presence of the resistant starch is a significant portion of the dietary fiber content in all the bread samples.

The phytic acid in the whole wheat bread may be partially responsible for the lower loaf volumes of the whole wheat breads. The levels of phytic acid and phenolic compounds were found to have a notable connection to the eGI of the bread samples. The most drastic reduction in phytic acid and hydrolysable phenolic compound content occurred in the white bread samples. The results of this study showed an inverse relationship between the eGI and the levels of phytic acid and phenolic compounds in the breads.

There were significant ($P<0.05$) reductions in the molecular mass of amylopectin and amylose after baking. In this instance, samples (white breads) with the higher eGI had starches with the lowest molecular mass. The lower amount of starch hydrolysis may be affecting the end-product quality by reducing the glucose available for yeast metabolism and gas production. However, prevention of starch hydrolysis in the whole wheat breads may lead to slower starch digestion in the human digestive tract.

On the whole, the end-product quality of whole wheat bread may be somewhat lower than white bread; however, the nutritional quality of whole wheat bread may outweigh these faults in end-product appearance. The results of this study show that although the whole wheat breads are still in the high GI

category, their eGI is still significantly ($P<0.05$) lower than in white bread. Also, there are several components of the whole wheat contributing to the reduction of the eGI in whole wheat bread. This might be beneficial to consumers who want to continue consuming bread products but desire a more health conscious option.

7. STUDY LIMITATIONS

This study was undertaken to compare the end-product quality, starch characteristics and nutritional quality of white and whole wheat bread. However, due to the nature of this study and the experimental approach there were several limitations. One limiting factor in this study is the small size of the sample set. There are only three wheat varieties and one growing location that were investigated. The limited number of samples may limit the scope of assumptions and generalizations about the results relating to all white or whole wheat breads. Some comparisons and inferences can be made based on the results of the analysis presented in this research. However, a more detailed analysis with a more direct approach is necessary to draw more definite conclusion from the data. Another limitation of this study is that the eGI is measured *in vitro*. Ideally, an *in vivo* study would be done to determine the actual GI values.

8. FUTURE RESEARCH DIRECTIONS

The results of this study present some interesting research questions and additional opportunities for further investigation, which are listed below,

1. Determination of amino acid profile, dietary fiber content and the specific phenolic compounds in the flour and bread samples would give a more complete picture of the nutritional quality of these samples. Also, *in vivo* digestibility studies would give a more accurate representation of the GI of the bread samples.
2. Analysis of a larger set of wheat varieties which could include red and white wheat varieties. This would determine if there is a genetic effect to the nutritional quality and eGI of white and whole wheat breads. While including samples from different growing locations and seasons would allow for investigation of environmental effect on the nutritional quality and eGI of white and whole wheat breads.
3. Reconstitution studies would also shed a greater light on the effects of the wheat components on the eGI of white and whole wheat breads. For example, the phenolic compounds or AX could be extracted and added into white and whole wheat flours to determine their effects on the eGI.

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APPENDIX

Table A.1. ANOVA of Flour Yield for Barlow, Glenn and Prosper Wheat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.96796117	4.98398059	3.03	0.1903
Error	3	4.92927743	1.64309248		
Corrected Total	5	14.89723860			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.2. ANOVA of Bran + Shorts Yield for Barlow, Glenn and Prosper Wheat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.96796117	4.98398059	3.03	0.1903
Error	3	4.92927743	1.64309248		
Corrected Total	5	14.89723860			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.3. ANOVA of Flour Extraction for Barlow, Glenn and Prosper Wheat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	2	9.98973213	4.99486606	4.19	0.1353
Error	3	3.57391782	1.19130594		
Corrected Total	5	13.56364995			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.4. ANOVA of Farinograph Absorption for Flour Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	392.6571922	35.6961084	1515.44	<.0001
Error	12	0.2826589	0.0235549		
Corrected Total	23	392.9398511			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.5. ANOVA of Farinograph Peak Time of Flour Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	39.85000000	3.62272727	9.51	0.0002
Error	12	4.57000000	0.38083333		
Corrected Total	23	44.42000000			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.6. ANOVA of Farinograph Stability of Flour Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	376.9045833	34.2640530	38.52	<.0001
Error	12	10.6750000	0.8895833		
Corrected Total	23	387.5795833			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.7. ANOVA of Farinograph Mixing Tolerance Index of Flour Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1008.458333	91.678030	13.02	<.0001
Error	12	84.500000	7.041667		
Corrected Total	23	1092.958333			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.8. ANOVA of Bake Absorption for Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	323.4512500	29.4046591	94.73	<.0001
Error	12	3.7250000	0.3104167		
Corrected Total	23	327.1762500			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.9. ANOVA of Baking Mix Time for Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3.76458333	0.34223485	23.47	<.0001
Error	12	0.17500000	0.01458333		
Corrected Total	23	3.93958333			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.10. ANOVA of Loaf Volume of Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	579095.8333	52645.0758	194.38	<.0001
Error	12	3250.0000	270.8333		
Corrected Total	23	582345.8333			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.11. ANOVA of Ash Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	17.98662243	0.78202706	1290.36	<.0001
Error	24	0.01454528	0.00060605		
Corrected Total	47	18.00116771			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.12. ANOVA of Protein Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	83.26318866	3.62013864	524.85	<.0001
Error	24	0.16553886	0.00689745		
Corrected Total	47	83.42872751			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.13. ANOVA of Total Starch Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	1919.513132	83.457093	204.83	<.0001
Error	24	9.778554	0.407440		
Corrected Total	47	1929.291686			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.14. ANOVA of Starch Damage of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	1223.517312	53.196405	381.16	<.0001
Error	24	3.349585	0.139566		
Corrected Total	47	1226.866896			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.15. ANOVA of Arabinoxylan Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	542.0422022	23.5670523	434.59	<.0001
Error	24	1.3014653	0.0542277		
Corrected Total	47	543.3436675			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.16. ANOVA of Arabinose/Xylose Ratio in Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	0.08755251	0.00380663	4.32	0.0003
Error	24	0.02113083	0.00088045		
Corrected Total	47	0.10868334			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.17. ANOVA of Phytic Acid Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	183.2155254	7.9658924	3576.26	<.0001
Error	24	0.0534585	0.0022274		
Corrected Total	47	183.2689839			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.18. ANOVA of Extractable Phenolic Acid Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	62.89568169	2.73459486	124.82	<.0001
Error	24	0.52581433	0.02190893		
Corrected Total	47	63.42149602			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.19. ANOVA of Hydrolysable Phenolic Acid Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	305.9647004	13.3028131	190.86	<.0001
Error	24	1.6727824	0.0696993		
Corrected Total	47	307.6374828			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.20. ANOVA of Amylopectin Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	177.3186732	7.7095075	57.13	<.0001
Error	24	3.2387053	0.1349461		
Corrected Total	47	180.5573785			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.21. ANOVA of Amylose Content of Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	177.3186732	7.7095075	57.13	<.0001
Error	24	3.2387053	0.1349461		
Corrected Total	47	180.5573785			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.22. ANOVA of Amylopectin Molecular mass in Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	2.1998493E14	9.5645622E12	139.69	<.0001
Error	24	1.64325E12	68468750000		
Corrected Total	47	2.2162818E14			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.23. ANOVA of Amylose Molecular mass in Flour and Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	23	3.1766225E13	1.3811402E12	25.90	<.0001
Error	24	1.2799E12	53329166667		
Corrected Total	47	3.3046125E13			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.24. ANOVA of Resistant Starch Content of Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2.20366228	0.20033293	144.69	<.0001
Error	12	0.01661474	0.00138456		
Corrected Total	23	2.22027703			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.25. ANOVA of Type III Resistant Starch Content of Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2.53907403	0.23082491	159.72	<.0001
Error	12	0.01734177	0.00144515		
Corrected Total	23	2.55641580			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.26. ANOVA of Hydrolysis Index of Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2218.204546	201.654959	12.45	<.0001
Error	12	194.303750	16.191979		
Corrected Total	23	2412.508296			

DF = Degrees of freedom, Pr>F = Probability > F

Table A.27. ANOVA of Estimated GI of Bread Samples

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1648.223579	149.838507	12.45	<.0001
Error	12	144.376236	12.031353		
Corrected Total	23	1792.599814			

DF = Degrees of freedom, Pr>F = Probability > F