

PHYSICOCHEMICAL PROPERTIES OF STARCH IN BRAN AND ENDOSPERM AND
RELATIONSHIP BETWEEN BRAN STARCH AND BRAN CHARACTERISTICS OF
SELECTED SOFT WHEATS GROWN IN MICHIGAN

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ABSTRACT

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Wheat bran is a major source of fiber as an ingredient in cereal-based products. However current utilization of bran represents only a small portion of total bran production as most is actually sold for animal feed at a reduced price. During wheat milling, bran is separated from the endosperm, though a clean separation is not possible and there is always some starch adherent to the bran. This bran starch still remains one of the main components of the bran fraction. Understanding the physicochemical properties of bran starch and its relationship with bran tissue is required to maximize bran utilization. Bran starch was hypothesized to have different properties than endosperm starch, and it was hypothesized that bran chemical composition and bran thickness are each related to bran particle size and bran starch quantity. The hypotheses were tested by following aims: (1) to develop methods of isolating bran starch and endosperm starch from wheat bran and endosperm flour, respectively, and identify a common method to isolate both types of starches to ensure comparability of results of further investigation, (2) to characterize bran starch properties compared with those of endosperm starch from the same wheat sample, (3) to determine bran particle thickness and biochemical composition of bran tissue and their relationships with bran particle size and bran starch quantity, and (4) to investigate the ultrastructure of milled bran particles and the starch morphology of isolated bran starch and endosperm starch.

Significantly different physicochemical properties of bran starch compared with endosperm starch were found. Bran starch was found to have higher percent B-type small granules, higher amylose content, higher crystallinity, broader gelatinization temperature range, higher enthalpy of gelatinization, lower retrogradation degree, and lower pasting peak and setback viscosities than the counterpart endosperm starch. A-type X-ray diffraction patterns were found for both bran starch and endosperm starch. Bran starch of variety Aubrey had highest crystallinity and gelatinization temperature, while bran starch of variety D8006 had highest percent B-type granules and lowest retrogradation degree. SEM images revealed that small sized starch granules were not lost during isolation steps. For both bran and endosperm starches, the A-type starch granules displayed a disk shape with diameters of 10-30 μm , and the B-type granules displayed a spherical shape with diameters of about 2 μm . A greater proportion of small granules was observed in bran starch than in endosperm starch. All granules seen in SEM images had smooth surfaces and intact structure, which may indicate that the isolation procedure did not damage the morphology of the starch granules.

Bran starch content was found to be negatively correlated with percent large bran particles. The neutral saccharide profile of the wheat bran was dominated by arabinose, xylose, and glucose, whereas mannose and galactose were present in small amounts. Bran thickness, bound ferulic acid (BFA) content and BFA to xylose ratio showed significant correlations with percent large bran particles and bran starch content. SEM images revealed that the outer layers of wheat bran were deformed after milling and the aleurone layer was no longer visible. Milled bran tissue was about twice as thick as intact outer layers of the wheat kernel. Observed relationship between bran characteristics and bran starch content explained why there was correlation between percent large bran particles and bran starch quantity.

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KEY TO ABBREVIATIONS

AX: arabinoxylans

Ara/Xyl: arabinose to xylose ratio

BFA: bound ferulic acid

CVD: cardiovascular disease

CWM: cell wall material

DP: degree of polymerization

DiFA: dehydrodiferulic acid

DSC: differential scanning calorimetry

FA: ferulic acid

GC-MS: gas chromatography-mass spectrometry

LBP: large bran particles

NE: nucellar epidermis (hyaline layer)

RS: resistant starch

SEM: scanning electron microscope

TFA: trifluoroacetic acid

CHAPTER 1 INTRODUCTION

Wheat is the major cereal grain in the world and wheat-based industries hold a multibillion dollar market. Evidence indicates that sufficient whole grain consumption has a protective effect against development of diet-related disorders, such as cardiovascular disease and type II diabetes, and also against cancers, such as digestive-tract cancer, hormone-related cancers and pancreatic cancer (Seal and Brownlee 2010). Cereal dietary fiber includes non-starch polysaccharides and resistant starch (ingested starch that cannot be digested in the small intestine of humans), which are the major health-beneficial carbohydrates in cereal whole grains and mainly found in the bran fraction (Collins et al 2010). Cereal DF provides benefits for colon health through its physicochemical properties, effects on bacterial community in the colon (colon microbiota), and colonic fermentation products.

During the dry milling process, wheat bran is removed from the endosperm in order to recover white flour. However, the endosperm does not completely separate from the bran tissue, and therefore bran starch still remains one of the main components of the milled bran fraction. The bran fraction comprises about 11% (w/w) of the total products in dry milling. However, only about 10% of commercial wheat bran is used as a source of insoluble dietary fiber for breakfast cereals and bakery products. The remaining 90% is sold as animal feed at very low price (Xie et al 2008). In order to add commercial value and benefit the food industry, bran utilization needs to be maximized and developed. Understanding the physicochemical properties of major components of the bran fraction is required to maximize its utilization.

It is well known that starch has many applications in foods and can also be processed into other products, for food and nonfood applications. Starch is unique among carbohydrates because it occurs naturally as discrete particles, called granules. There are two types of starch granules, each with its own distinct shape and size: A-type granules, which are disc-like or

lenticular in shape with diameters greater than 10 μ m; and B-type starch granules, which are roughly spherical or polygonal in shape with diameters smaller than 10 μ m (Whistler and Bemiller 1997). Normally, the number of A-type granules is fewer than those of B-type, and A-type granules represent the majority of the mass of the starch. A- and B-types of starch granules have different chemical compositions and functional properties, including differences in amylose and lipid contents, pasting properties, and baking qualities (Maningat and Seib 1997).

Bran starch comprises about 20% (w/w) of the milled wheat bran fraction (Liu and Ng 2012). Previous studies have shown that the physicochemical properties of bran starch are different from those of commercial wheat starch, with bran starch having higher resistant starch content, lower starting gelatinization temperature, and slower retrogradation than commercial wheat starch (Xie et al 2008). Furthermore, differences in bran starch quantity were found among different wheat varieties (Liu and Ng 2012). Xie et al (2008) characterized wheat bran starch compared to commercial starch, however there may have been some limitations in their sample sources and preparations: (1) commercial starch was used as a control that was prepared from a potentially different biological source (e.g., different wheat classes or varieties) than the isolated bran starch; (2) wheats used to produce commercial starch and isolated bran starch could have been from different locations and crop years and therefore subject to environmental effects; and (3) the isolation process of the commercial starch could have been different from the isolation process used for the bran starch. Different isolation processes have significant impact on starch properties.

The bran physical properties could affect the separation between bran and endosperm, and therefore the quantity of bran starch. It is known that the chemical composition of bran [such as arabinoxylans (AX), dehydrodiferulic acid (DiFA), etc.] plays an important role in

determining bran's physical properties, specifically extensibility. Bran thickness, the biochemical composition and the degree of AX cross-linking in the cell walls have been proposed to be the major factors controlling the physical properties of bran (Peyron 2002). The cross-linking of cell wall AX reaction (ferulic acid dimerization) is an oxidative mechanism, probably mediated by wheat bran endogenous peroxidases or phenol oxidases *in vivo* (Fry 2000 and Peyron et al 2001). A strong positive correlation between extensibility of the wheat grain outer layers and the proportion of larger size (> 2mm) coarse bran was reported by Greffeuille and coworkers (2006). However, the physicochemical properties of isolated bran starch compared with those of endosperm starch from the same wheat sample have not been determined and relationships among chemical composition of bran, bran particle size, and bran starch quantity and type are not known. Furthermore, the reasons why the characteristics of wheat bran starch are different from commercial wheat starch and why there are differences regarding bran starch quantity for bran samples from different wheat varieties are currently unclear.

The long term goals of this proposed study are to understand the benefits of bran starch for human health, to examine potential usage of bran starch as a functional ingredient for the food industry, and to increase bran utilization in cereal and food processing. The overall objective of this research work was to enhance understanding of the physicochemical properties of bran starch and its relationships with milled bran fractions. The central hypotheses for this dissertation were: bran starch has different properties than endosperm starch; bran chemical composition and bran thickness are each related to bran particle size and bran starch quantity.

The specific objectives of this research work were:

1. To develop methods of isolating bran starch and endosperm starch from wheat bran and endosperm flour, respectively, and identify a common method to isolate both types of starches to ensure comparability of results of further investigation
2. To characterize bran starch properties compared with those of endosperm starch from the same wheat sample
3. To determine the bran particle thickness and biochemical composition in bran tissue and their relationships with bran particle size and bran starch quantity.
4. To investigate the ultrastructure of milled bran particles of studied varieties and the starch morphology of isolated bran starch and endosperm starch.

Collectively, it is expected that results from the proposed studies will provide fundamental information on characteristics of bran starch and how these characteristics are related to the bran tissue, and results will also reveal any varietal and environmental effects on characteristics of wheat bran starch and wheat bran. Based on results of this research work, it is anticipated that bran starch can be potentially utilized as a naturally-derived functional ingredient which could benefit the food industry. In addition, this study will provide valuable comparative information for wheat breeders interested in developing new wheat varieties that provide desired bran starch quantity and type for different applications and bran with desired physical properties for milling. The following dissertation is divided into: (1) Literature review, (2) Isolation and characterization of wheat bran starch and endosperm starch of selected soft wheats grown in Michigan, (3) Relationship between bran characteristics and bran starch of selected soft wheat varieties grown in Michigan, (4) General conclusions, (5) Recommendation for future studies.

The chapters in this dissertation were written in the Cereal Chemistry journal paper format and thus some of the information presented in certain chapters is similar.

LITERATURE CITED

LITERATURE CITED

- Collins, H. M., Burton, R. A., Topping, D. L., Liao, M., Bacic, A., and Fincher, G. B. 2010. Variability in fine structures of noncellulosic cell wall polysaccharides from cereal grains: potential importance in human health and nutrition. *Cereal Chem.* 87:272-282.
- Fry, S. C., Willis, S. C., and Paterson, A. E. J. 2000. Intraprotoplasmic and wall-localised formation of arabinoxylan-bound diferulates and larger ferulate coupling-products in maize cell-suspension cultures. *Planta* 211:679-692.
- Greffeuille, V, Abecassis, J., Lapiere, C., and Lullien-Pellerine, V. 2006. Bran size distribution at milling and mechanical and biochemical characterization of common wheat grain outer layers: a relationship assessment. *Cereal Chem.* 83:641-646.
- Liu, Y., and Ng, P. K. W. 2012. Physicochemical properties of bran starches and endosperm starches of soft wheat grown in Michigan. AACC international annual meeting. Hollywood, FL.
- Maningat, C. C., and Seib, P. A. 1997. Update on wheat starch and its uses. Pages 261-284 in: *Proc. Int. Wheat Quality Conference*. J. L. Steele and O. K. Chung, eds. Grain Industry Alliance: Manhattan, KS.
- Peyron, S., Chaurand, M., Rouau, X., Abecassis, J., 2002. Relationship between bran mechanical properties and milling behaviour of durum wheat (*Triticum durum Desf.*). Influence of tissue thickness and cell wall structure. *J. Cereal Sci.* 36:377-386.
- Seal, C. J., and Brownlee, I. A. 2010. Whole grains and health, evidence from observational and intervention Studies. *Cereal Chem.* 87:167-174.
- Whistler, R. L., and Bemiller, J. N. 1997 Chapter 6-starch. Pages 117-152 in: *Carbohydrate Chemistry for Food Scientist*. AACC International: St. Paul, MN.
- Xie, X., Cui, S. W., Li, W., and Tsao, R. 2008. Isolation and characterization of wheat bran starch. *Food Research Intl.* 41:882-887.

CHAPTER 2 LITERATURE REVIEW

2.1 CEREAL DIETARY FIBER AND ITS HEALTH BENEFITS

Cereal dietary fiber includes non-starch cereal polysaccharides and resistant starch, which are the major functional carbohydrates in cereal whole grains and mainly found in the bran fraction. Cereal dietary fiber could impact colon health through modulation of the bacterial community (microbiota) in the colon (Zhang and Hamaker 2010). Non-starch cereal polysaccharides consist of different groups of carbohydrates, including cellulose, hemicelluloses, β -glucans, and a minor portion of pectin.

Resistant starch (RS) is the sum of dietary starch that cannot be digested in the upper gastrointestinal tract due to inaccessibility to physical hindrance (RS1), ungelatinized starch granules (RS2), amylose retrogradation (RS3), or chemical modification (RS4) (Sajilata et al 2006). The term “resistant starch” was first coined by Englyst et al (1982) to describe a small fraction of starch that was resistant to hydrolysis by α -amylase and pullulanase treatment *in vitro*. Subsequently resistant starch was defined as starch not hydrolyzed after 120 min of incubation with the two enzymes (Englyst et al 1992). But these definitions were just based on *in vitro* study. When starch reaches the large intestine, it can be fermented by the gut microflora to a certain extent. After reaching the large intestine, the resistant starch fractions are fermented by the colonic flora, and short-chain fatty acids are produced. The profiles of short-chain fatty acids derived from resistant starch are lower in acetate and higher in butyrate than those of conventional fibers (Sajilata et al 2006; Annison et al 2003).

Microbiota are the central link between dietary fiber and the health benefits related to dietary fiber fermentation. There are 10^{13-14} bacteria inside the adult colon representing over 1000 different species (Hooper 2004). The colon microbiota can digest the materials, including some types of DF, coming from the small intestine for growth and to generate short-chain fatty

acids that are important for human health (such as acetate, propionate, and butyrate) (Zhang and Hamaker 2010).

There is water-insoluble and water-soluble dietary fiber. Water-insoluble dietary fiber includes cellulose, hemicellulose, lignin, and water-insoluble arabinoxylans. Cellulose is the major structural component of plant cell walls, and has a high molecular weight. It is a linear homopolymer of D-glucopyranosyl residues linked together in beta-1,4-glycosidic linkages (Whistler and Bemiller 1997). Hemicellulose contains a variety of heteropolysaccharides in the backbone and side chains. Xylose, mannose, and galactose usually make up the backbone, joined with β -1,4-glycosidic linkages. Arabinose, galactose, and uronic acids are the most common side chains. Arabinoxylans consist of a β -1,4 linked xylose backbone with branches of α -L-arabinose residues attached at O-2 or O-3 positions; arabinose residues may also be linked to other groups such as glucuronic acid, ferulic acid cross-links, and acetyl groups. Water-insoluble dietary fiber can shorten transit time of feces in the colon due to water-holding or bulking properties and produce a laxative effect that is beneficial to alleviate constipation. Lignin with its large hydrophobic surface was found to be efficient in binding putative carcinogens and secondary bile acids into the fiber matrix (Bingham 1990).

Water-soluble dietary fiber, such as β -glucans and pectin, have high water dispersibility, facilitating their diffusion and promoting their degradation by microbes; this often results in high fermentability of water-soluble DF (Zhang and Hamaker 2010). Water-soluble β -glucans are the glucose polymers with β -(1, 3) and β -(1, 4) linkages. Pectins are considered the largest source of soluble fiber in plant food materials. The pectin backbone consists of α -1,4 linked galacturonic acid residues mixed with single α -1,2 linked rhamnose residues. The side chains of pectin consist of galactose, glucose, rhamnose, and arabinose (Bemiller 1986).

The benefits of cereal dietary fiber in reducing risk of cardiovascular disease (CVD) and cancer, and improving digestive health have been well documented (Seal and Browlee 2010). Moreau et al (2003) reported RS3 ingested by rats produced high levels of butyrate and restored the integrity of cecal-colonic mucosa. In a study by Qu et al (2005), wheat bran from different cultivars showed differences in protection against colon cancer that were related to lignin content through mediation of cytostatic and apoptotic mechanisms by lignan metabolites. Whole grains, containing bran, endosperm, and germ, are an important source of cereal dietary fiber for humans. Reports showed that CVD risk was dramatically reduced (about 30%) for the highest whole grain consumers compared to the lowest whole grain consumers (Seal and Browlee 2010). Strong protective effects of whole grain intake against colorectal and colon cancers, polyps, and gastric and other digestive-tract cancers were reported. Increasing whole grain consumption also showed strong associations with decreasing the risk of hormone-related cancers and pancreatic cancer (Schatzkin et al 2007, and Chatenoud et al 1998).

2.2 COMPOSITION AND PROPERTIES OF WHEAT BRAN TISSUES

2.2.1 Composition of wheat bran tissues

A wheat kernel is a multilayer system. Besides the embryo, from the center to the periphery of the grain, wheat kernel consists of endosperm, aleurone layer, the seed coats (composed of the hyaline layer and the testa), the outer pericarp (composed of the hypodermis and the epidermis), inner pericarp (composed of the tube cells and the cross cells) (Fig.2.1). The aleurone layer, the seed coats, and the pericarp are wheat outer layers, and called wheat bran. Wheat grains normally contain 14.5% (w/w) bran, 83% (w/w) endosperm, and 2.5% (w/w) germ (Hemery et al 2007; Antoine et al 2003; Martelli et al 2010).

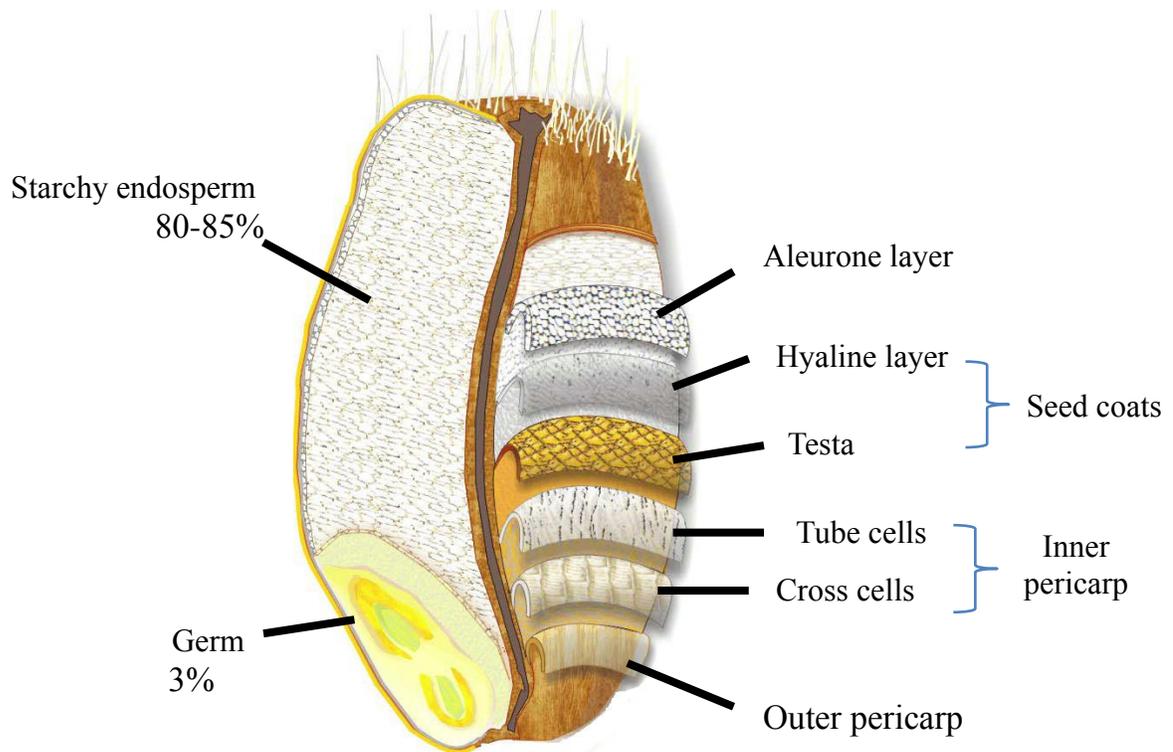


Figure 2.1. Histological composition of wheat grain. Source: Suget and Barron (2005).
For interpretation of the references to color in this and all other figure, the reader is referred to the electronic version of this dissertation.

The outermost layers of bran tissues are rich in insoluble fiber. They are composed of thick cell walls formed of cellulose, cuticle materials, and complex xylans with high arabinose to xylose ratios and substitution by dehydrodiferulic acid (DiFA), which act as cross-linkers between polymer chains. Pericarp and testa layers also contain high amounts of lignin, which is a phenolic polymer embedding the structural polysaccharides. The testa contains almost all of the grain alkylresorcinols (Landberg et al 2007). Kozubek and Tyman (1999) reported antioxidant properties and anticancer activity of alkylresorcinols. The aleurone layer is composed of living cells including bioactive compounds, surrounded by thick cell walls composed of relatively linear arabinoxylans with a low arabinose-to-xylose ratio (Saulnier et al 2007). The aleurone layer contains high levels of β -glucans which are the major soluble dietary fiber, as mentioned above, and certain amounts of ferulic acid, which is responsible for the antioxidant properties of the aleurone layer. Compared to the other peripheral layers, the aleurone layer comprises about 7% of the wheat grain dry mass. It has a high protein content with better balanced amino acids compared to the proteins of endosperm (Buri et al 2004; Rhodes and Stone 2002), and it also contains the major part of B vitamins, lignans (polyphenols that function as antioxidants, anticarcinogens, and estrogen modulators), and about half of the total mineral content of the wheat kernel (Buri et al 2004; Qu et al 2005).

2.2.2 Physical properties of wheat bran tissues

The physical properties of wheat bran tissues and the characteristics of the interfaces between these tissues may influence their behavior during milling and grinding. High milling efficiency with low bran contamination in flour requires low friability (high extensibility). Bran friability affects the extent of particle size reduction obtained in grinding. Bran particle size is especially related to tissue extensibility (Peyron et al 2002a). A strong positive correlation between extensibility of the wheat grain outer layers and the proportion of larger size (> 2mm) coarse bran was reported by Greffeuille and coworkers (2006). Since bran starch content is tightly related to the bran's fractionation from endosperm during the milling process, physical properties of wheat bran are expected to affect milling quality and therefore impact bran particle size and bran starch quantity and type.

In aleurone walls, where arabinoxylans (AX) account for 70% of the polysaccharide material, the degree of AX cross-linking controlled by DiFA in the aleurone layer significantly influences the strength and extensibility of the bran. Thus, the degree of AX cross-linking is one of the determinants of bran friability and must be taken into account in explaining bran physical properties (Peyron et al 2002a). The modulation of tissue mechanical properties by polymer cross-linking has also been suggested in other plant tissues such as rice and wheat seedlings (Tan et al 1992; Wakabayashi et al 2005a, b).

The ratio of endosperm and bran is an intrinsic grain characteristic that influences milling performance (Simmon and Meredith 1979; Evers and Withey 1989; Glenn and Johnston 1992). Bran thickness is genetically controlled; it may affect the mechanical resistance of bran and determines the ease of separation of the endosperm from bran. The plant tissue toughness and strength varies with the cell wall relative density, which is the volume fraction of cell wall

(Gibson and Ashby 1988). Intrinsic toughness must depend on wall composition, that is, the quantity of cellulose and lignin present. Toughness was found to correlate with thickness for outer layer tissues, as thickness was increased, toughness increased until reaching a plateau at about 1 mm in section thickness (Lucas et al 1995). The high toughness means the tissue has high strength and rigidity.

There are wide variations to milling process since milling is tailored, depending on wheat variety and food applications. It seems hard to relate bran chemical composition to milling behavior. However, the mechanical force applied to the tissues during grinding is much higher than the rupture strength of the bran layer and can be considered as a fixed stress force according to Peyron (2001). In this case, the impact of tissue strength may not have as much effect as extensibility on milling efficiency, thus bran particle size is more strongly related to tissue extensibility. Bran thickness, the biochemical composition and the degree of AX cross-linking in the cell walls have been proposed to be the major factors controlling the physical properties of bran (Peyron et al 2001).

2.2.3 Determination of non-cellulosic sugars in wheat bran

Plant cell wall tissue is composed of different proportions of the neutral monosaccharides, such as rhamnose, arabinose, xylose, mannose, galactose and glucose, as well as the acidic monosaccharides galacturonic acid, glucuronic acid (Melton and Smith 2001; Fig. 2.2.). Englyst et al (1994) reported several methods to determine non-starch polysaccharides. They found good agreement for a wide range of raw and processed food for three methods, which were gas chromatography (GC), high-performance liquid chromatography, and spectrophotometric measurements. The advantage of the GC method for measuring sugar alditol acetates is that the

procedure gives desired resolution without interference in a mixture of different sugars alditol acetates. GC also has the advantage of being able to determine the identity of the reducing sugar residues (Courtin et al 2000). The neutral monosaccharide composition of wheat bran can be determined by first hydrolyzing the polysaccharides to constituent monosaccharides with acid and then converting them to alditol acetates by reduction with sodium borohydride to the corresponding alditol, followed by acetylation of alditol acetates on each alditol. The product of sample preparation is volatile alditol acetates and can be identified and quantified by GC (Melton and Smith 2001). The tested monosaccharides need to be identified by comparing their retention times with the alditol acetates derivatized sugar standards such as D(+)-galactose, D(+)-glucose, D(+)-xylose, L(+)-arabinose, L(+)-rhamnose, D(+)-mannose (Ramirez-Truque et al 2011).

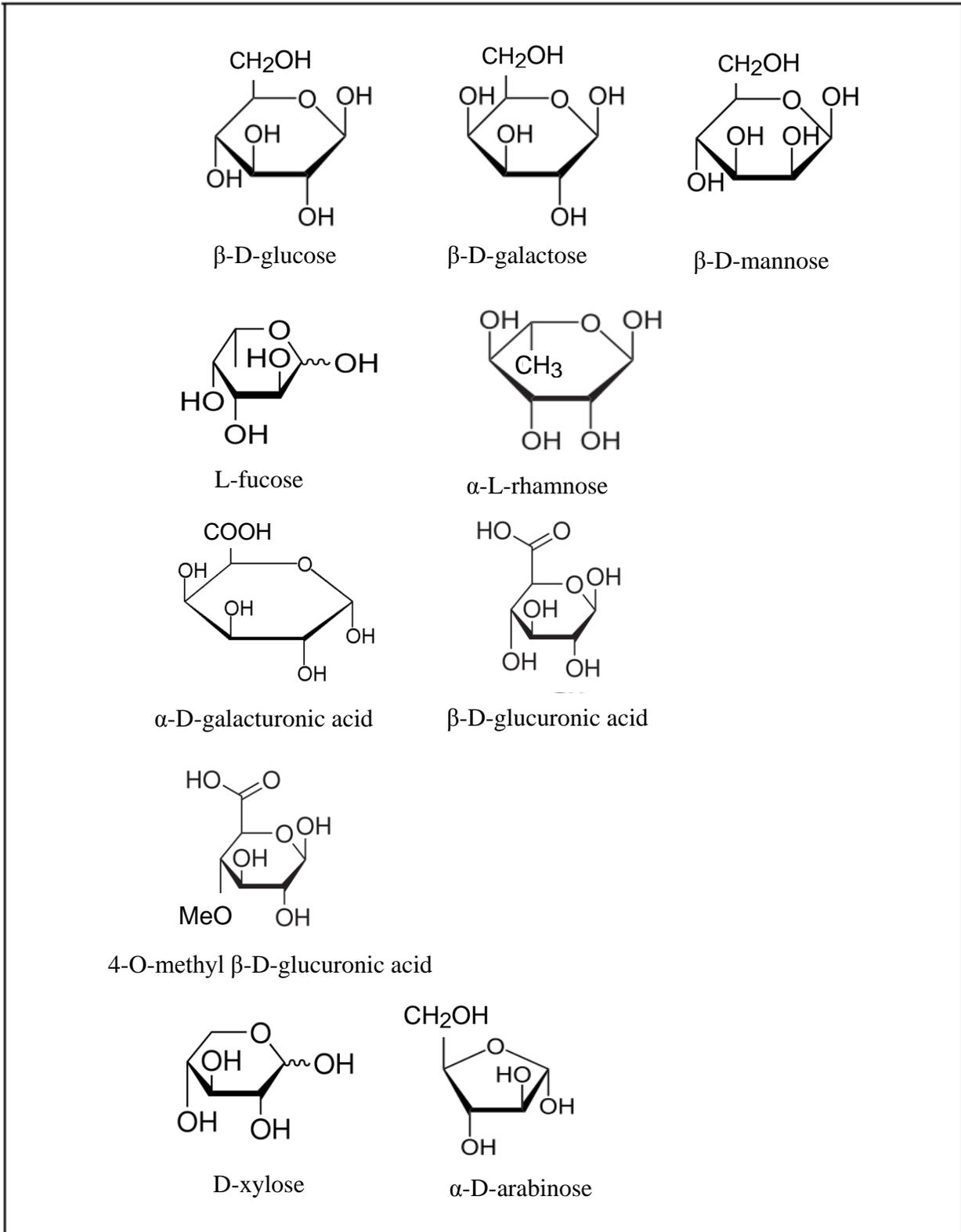


Figure 2.2. Structures of monosaccharides in plant cell walls. Source: Melton and Smith (2001).

Different sample preparation procedures for determination of non-cellulosic sugars in wheat bran by GC analysis were investigated in previous studies. Wheat bran sample was pretreated by enzyme to remove starch and acid hydrolysis was performed before sugar measurement (Englyst et al 1994; Brillouet and Mercier 1981). A defatting procedure by soaking wheat bran in chloroform-methanol for 8h or acetone for 30 min has been reported (Brillouet and Mercier 1981; Englyst and Cummings 1984). Several procedures have been used to hydrolyze polysaccharides in cell walls. Hydrolyses with concentrated sulfuric acid (12 mol/L) for 60 min (Englyst et al 1994) and 1 M sulfuric acid for 2 to 3 hr at 100 °C (Selvendran and Ryden 1990) and 72% (w/w) sulfuric acid for 1 hour (Ramirez-Truque et al 2011) have been reported. One of the simplest procedures is the hydrolysis achieved by incubating samples in 2 M trifluoroacetic acid (TFA) at 121 °C for 1 hour (Albersheim et al 1967; Melton and Smith 2001). The advantages of the TFA procedure is that it is fast and the TFA can be removed by evaporation in nitrogen without residue. But it cannot hydrolyze cellulose, and is only suitable for non-cellulosic sugar analysis. Both dichloromethane (Melton and Smith 2001) and ethyl acetate were used as solvents to extract alditol acetates in previous studies (Englyst and Cummings 1984). Dichloromethane as an extraction solvent with (Melton and Smith 2001) or without (Courtin et al 2000) an evaporation step has been reported. Experiments comparing these two solvents of derivatization gave identical results for cereal, vegetable and legume products (Englyst and Cummings 1984). A more robust method using ethyl acetate rather than dichloromethane as a solvent for alditol acetates was recommended. Different approaches have been reported to obtain derivatization products of alditol acetates.

2.2.4 Phenolic compounds in wheat bran

In general, the term “phenol” chemically indicates a substance has an aromatic ring bearing a hydroxyl substituent and functional derivatives. Most phenolic compounds are of plant origin, although they are present in both plant and animal kingdoms. Phenylalanine and tyrosine are their precursors (Kozubek 1999). Phenolic compounds were studied for their well-known antioxidant feature at first. They function as free radical scavengers in many plants including cereals (Onyeneho and Hettiarachchy 1992; Deighton et al 2000; Zielinski and Kozłowska 2000). Because of valuable antioxidant functionality of phenolic compounds, whole grain products may be a valuable source of phenolic compound that can be responsible for health benefits.

Phenolic compounds have very diverse chemical structures, and occur in both free and bound form with other compound like saccharides and organic acids. Polyphenolic compounds can be divided in three groups by the structure of their basic carbon skeleton: derivatives of benzoic acid, e.g. gallic acid; derivatives of cinnamic acid, e.g., ferulic acid or caffeic acid; and derivatives of flavonoids that includes flavones and flavonols, flavanones, flavanols, anthocyanins, chalcones, and isoflavones and aurones (Klepacka and Fornal 2007). The flavonoids group is by far the largest group of phenolics. Ferulic acid occurs in the highest quantity in wheat grains (McKeehen 1999). The important groups of polyphenols that affect food properties include the anthocyanins, which are responsible for the red color of fruits and flowers; and phenolic acids, catechins, and proanthocyanidins. They are precursors of tanins and substrates for enzymatic browning for coloring products (Klepacka and Fornal 2007). Phenolic acids composition in wheat bran samples were analyzed by Klepacka and Fornal (2007) (Fig. 2.3). The results showed that ferulic acids accounts for about 95% of the cell wall-bound esterified phenolics. About 39% of ferulic acids was in dimer form and responsible for a high

degree of cell wall cross-linking. The highest contents of total phenolic acids were found in flours of winter wheat (1171 $\mu\text{g/g}$) with average total phenolics levels of 658 $\mu\text{g/g}$ across all of the wheat genotypes, based on a HEALTHGRAIN diversity screen study on phenolic acids in wheat varieties (Li et al 2008).

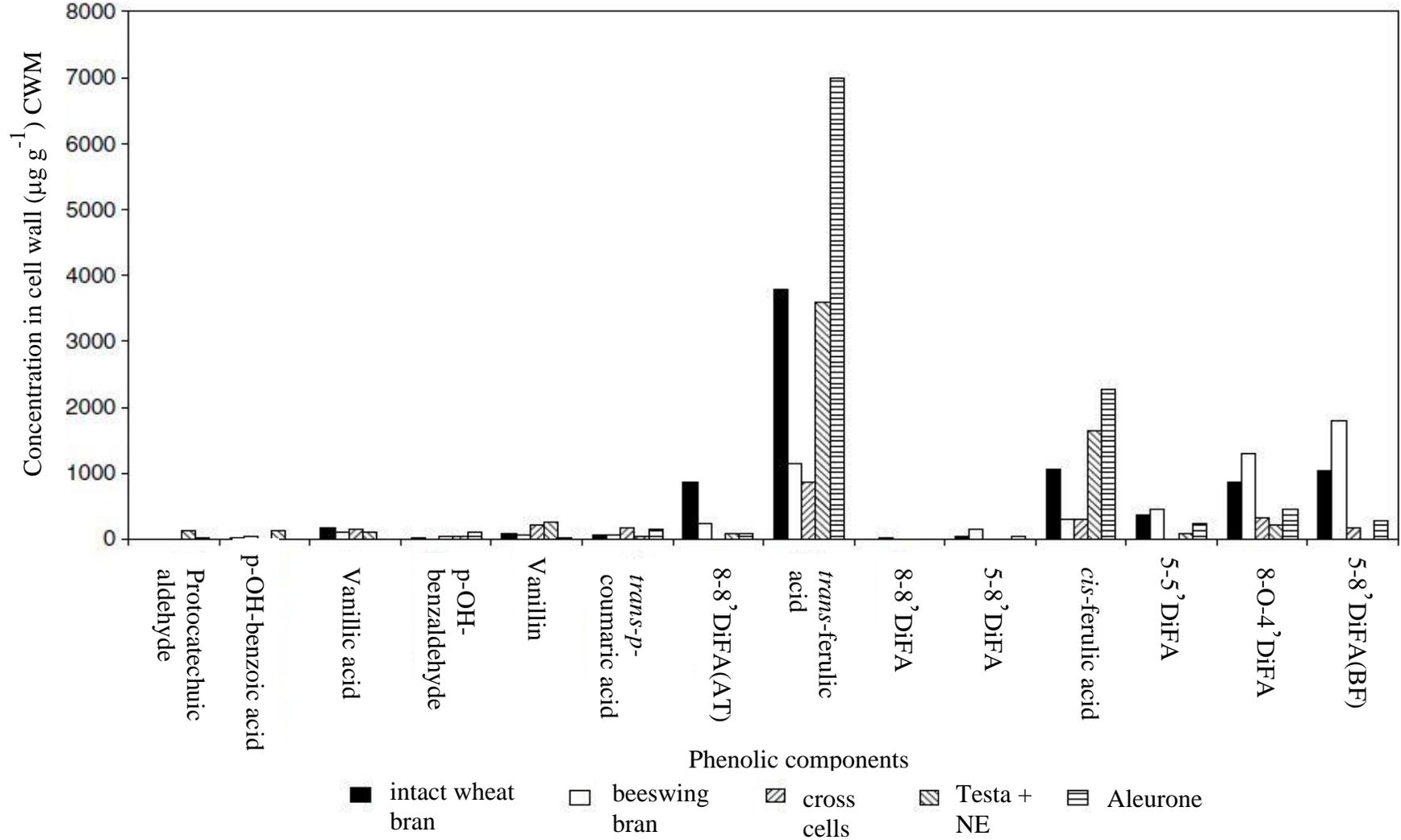


Figure 2.3. Phenolic compounds distribution in wheat bran cell walls. CWM: cell wall material; NE: nucellar epidermis (hyaline layer); DiFA: dehydrodiferulic acid; BF: benzofuran form; AT: aryltetralin form. Source: Klepacka and Fornal (2007).

The composition in phenolic acids in different layers wheat bran tissue was studied and was proved to be a useful as chemical marker for tissue separation and quantification to evaluate the milling efficiency, particularly the separation between bran and endosperm. The concentration of phenolic acids is more than 40 times higher in the aleurone layer than in the starchy endosperm, as shown in Table 2.1, for ferulic acid, which was quantified in durum wheat milling fractions (Lempereur et al 1997). High concentrations of ferulic acid esterified to cell wall arabinoxylans were found in the Aleurone layer (69%), germ and seedcoat (26.6%). Pericarp has the highest phenolic acid content among starchy endosperm, aleurone layer, and pericarp tissues (Peyron et al 2002c). In the bran of durum and soft wheat, ferulic acid can occur in bonds with polysaccharides and proteins (Saulinier et al 1999; Bartolome et al 2000). It can function as an esterified cross-linker with hemicellulose chains, mainly with arabinoxylans (Andreasen et al 2000; Hemery et al 2007). It may be bound with lignin via ether linkages as well (Andreasen et al 2000; Lozovaya et al 1999). Ferulic acid and its dimers play an important role in the formation and functional properties of dietary fiber (Bunzel et al 2000; Renger and Steihart 2000). The dominant dehydrodiferulic acid (DiFA) in wheat bran are 8-0-4', 5-8' - and 5-5' - DiFA (Bartolome et al 1997).

Table 2.1 Ferulic Acid (mg/g, dm) Contents of Different Histological Tissues and of Whole Grain of Durum Wheat Variety cv. Ardente

	Grain	Endosperm	Aleurone	Pericarp	Germ
FA (mg/g d.m.) ^x	0.87	0.17	8.82	3.71	0.08
Percent of whole grain	100	80-84	6-7	7.5-9.5	2.5-3.5
Concentration of FA (% of whole grain)	100	1.4	69	29	0.6

^x mean of duplicates. FA: Ferulic acid. Source: Lempereur et al (1997).

2.3 DRY MILLING PROCESS

The Buhler laboratory mill (MLU-202) is a type of roller mill which has six mill streams, three break and three reduction streams. Three breaks are arranged on one pair of rolls with corrugated surfaces. The cutting action created by corrugation can detach the bran tissue from the endosperm in the break system. Differential ratio (the difference in rpm of the rolls working as a pair) can be used to control the level of cutting created by corrugation. Three reduction stages are arranged on one pair of smooth rolls to minimize the cutting of bran particles and make more flour. Smooth rolls tend to flake the large chunks and middlings of endosperm. The objective of the break system is to open the wheat kernel and remove the endosperm and germ from the bran with least contamination and to obtain large middlings with minimum of flour. In the reduction system, large middlings are further reduced to flour while maintaining the desirable baking quality of flour by minimizing the amount of bran particles passing through the sifters into the flour (Posner and Hibbs 2005).

Increasing grain moisture by adding water followed by a rest period is called tempering. The amount of water added depends on the original moisture content of the wheat, the relative humidity and temperature in the milling room, and the desired final moisture content. The preconditioning of wheat is based on a trade-off between reducing endosperm crumbliness and decreasing outer layer friability (Mabille et al 2001). Water acts on wheat endosperm as a plasticizer and toughen the bran. The extensibility and plasticity of the bran increase as grain moisture increase. The outer layers with increased plasticity are believed to fuse and become more difficult to separate individually. The moisture distribution in wheat grain is not uniform during tempering (Song et al 1998). Water is highly accumulated in the germ and bran layers in

the early stages of tempering; the starch endosperm tissue hydrates unevenly as well upon tempering.

Environmental factors and many additional pretreatments have been studied to manipulate the milling performance of wheat. Gouveia (2002) reported that decreasing the temperature of the grain during grinding influenced the physical properties of bran tissue and produced smaller particle size of bran. Exposing durum wheat to UV radiation induced chemical modifications such as a decrease in DiFA in wheat bran, and thereby an increase in material stiffness and a decrease in extensibility of bran strips. As a result of that, the proportion of large bran particles from milling decreased (Peyron et al 2002b). A method of removing outer layers from cereal grain by preconditioning followed by a thermal shock using a cryogenic medium was reported (Van Bommel 2002). Biochemical pretreatment by including enzymes (Haros et al 2002; Moore et al 2006) and calcium chloride solution (Desvignes et al 2006) to a tempering solution were studied. Effects of ozone treatments on mechanical properties of the outer layers and milling behavior were reported by Desvignes et al (2007). The ozone treatment resulted in decreased extensibility of the aleurone layers and reduced total energy consumption for milling. The starch damage was minimized as well during milling.

2.4 WHEAT ARABINOXYLANS (AX)

2.4.1 General features

Wheat AX are the major polymer in the cell wall of grain. They are composed of a linear backbone of 1-4-linked β -D-xylopyranosyl units. The xylose accounts for over 50% of the constitutive sugars and a great diversity of side chains are present on the main chain on the O-2 or O-3 position. Single units of α -L-arabinofuranose and α -D-glucuronic acid are the most

frequent side-chains (Fig. 2.4). The arabinose to xylose ratio (Ara/Xyl) is often used to characterize the structure of AX. In addition, acetic acid, hydroxycinnamic acids, and ferulic and p-coumaric acids are found as esters. Ferulic and p-coumaric esters are linked to the O-5 of the arabinofuranosyl units (Saulnier et al 2007). The AX in endosperm of wheat (recovered in flour) are mostly composed of arabinose and xylose, and are found as water-extractable (WE-AX) and water-unextractable (WU-AX) fractions with very low amounts of ferulic acid [0.2-0.4% of WE-AX (w/w) and 0.6-0.9% of WU-AX] and DiFA present (10 to 15 times less than ferulic acid in WE-AX and 4 times less than ferulic acid in WU-AX). Aleurone AX are mostly heavily esterified WU-AX containing 3.2% (w/w) of ferulic acid and 0.45% DiFA. Since wheat bran consists of different tissues, the actual fine structure of bran AX is very diverse compared to AX in endosperm. Glucuronic acid and galactose are also present in bran AX. Arabinose residues associated with xylose and galactose are found as terminal side chains. Glucuronic acid and xylose residues are also found as terminal side-chains (Brillouet and Joseleau 1987). The Ara/Xyl ratio of WU-AX in bran is very high (close to 1), while the WE-AX in wheat bran are minimally substituted (Ara/Xyl = 0.45) (Maes and Delcour 2002). Bran AX are mostly WU-AX. Higher amounts of ferulic acid and DiFA are found in AX from wheat pericarp than those found in aleurone AX (Peyron et al 2002a). A molecular weight of 293 kDa has been reported for wheat bran AX (Saulnier et al 2007).

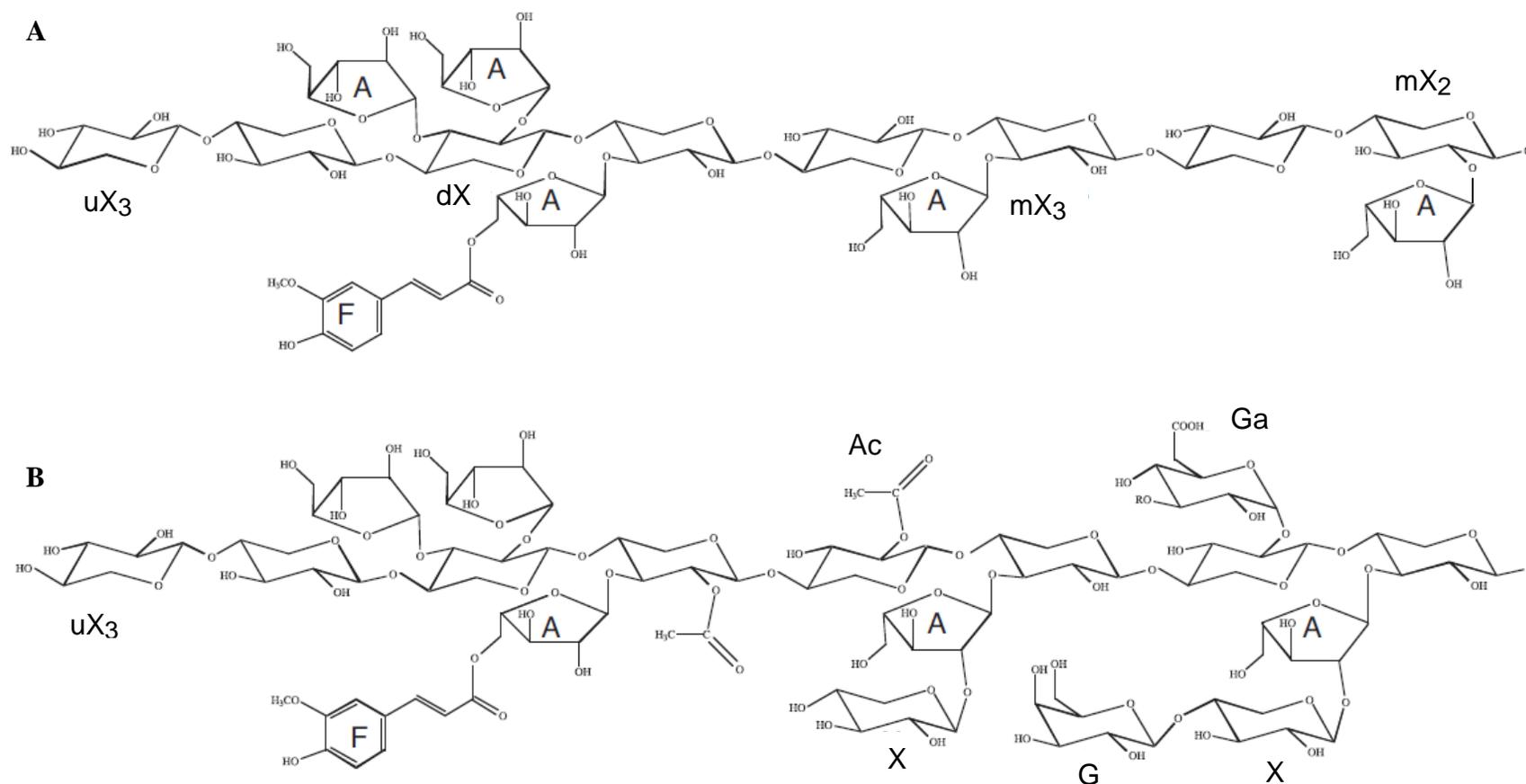


Figure 2.4. Main structural features of AX from endosperm (A) and outer tissues (B) of cereal grains. A: arabinose; X: xylose; G: galactose; Ga: glucuronic acid; F: ferulic acid; uX: unsubstituted xylose; dX: di-substituted xylose; mX₃: O-3 mono-substituted xylose; mX₂: O-2 mono-substituted xylose (rare in wheat endosperm AX). Hydroxyl functional groups (-OH), carboxyl functional group (-COOH), methoxy functional groups (-OCH₃), and acetyl group (-COCH₃) are denoted in AX structure. Reprinted from Saulnier et al (2007).

2.4.2 Interaction between AX through diferulic bridges

Ferulic acid contributes to wall assembly, promotes tissue cohesion, and restricts cell expansion, and thereby influences the physical properties of tissues. The cross-linking reaction of cell wall AX (ferulic acid dimerization) is an oxidative mechanism, probably mediated by wheat bran endogenous peroxidases or phenol oxidases *in vivo* (Fry 2000 and Peyron et al 2001). Feruloylation of AX is an essential aspect of cell-wall development and contributes to tissue properties. The outer tissues of the kernel and the aleurone layer are very rich in ferulic acid and dehydrodimers (Antoine et al 2003).

2.5 STARCH AND ITS MAJOR PROPERTIES

Starch is the second most abundant carbohydrate in nature next to cellulose. Starch is synthesized in a white and semi-crystalline granular form in special organelles and plastids. Native starch is synthesized in chloroplasts or amyloplasts of plant organs including seeds, stems, tubers, roots, and fruits as long-time energy storage over long periods (Robyt 1998). Biosynthesis of starch granules in cereal is initiated at the hilum, which is the organic center of the granule, and the granule grown by apposition, from the hilum towards the periphery (Yoshida et al 1958). Therefore the core of the starch granule corresponds to the small granule synthesized at the early stage of the starch granule development. The granules occur in all shapes and sizes (spheres, ellipsoids, polygon, platelets and irregular tubules) depending upon the botanical source (Perez and Bertoft 2010; Jane et al 1994; Gallant and Bouchet 1986). Starches are used in their native form as well as modified form. Both can have enormous numbers of food uses, including adhesion, binding, clouding, dusting, film-formation, foam strengthening, antistaling, gelling, glazing, moisture retention, stabilizing, texturizing, and thickening applications

(Whistler and Bemiller 1997). Starch is unique among carbohydrates because it occurs naturally as discrete particles, called granules. Starches vary in their inherent characteristics, such as molecular structure, molecular organization of starch granules, gelatinization and pasting properties, starch retrogradation, and starch damage (Jane 2004).

2.5.1 Molecular structure

Starch is composed of a mixture of two polymers: amylose and amylopectin. Amylose is essentially a linear polymer of α -(1 \rightarrow 4) linked D-glucose units with a few α -(1 \rightarrow 6) branches (0.3-0.5%), either very long or short, separated by large distances (Takeda et al 1989; Takeda et al 1986; Takeda et al 1993). The average molecular weights of high-amylose maize starches range from 1.5×10^5 to 10.4×10^5 Da (Li et al 2008). Amylopectin is a very large branched polymer with many α -(1 \rightarrow 6) linked glucose side chains attached to the main α -(1 \rightarrow 4) polymer; its MW is about 7.0×10^7 to 5.7×10^9 Da (French 1984; Yoo and Jane 2002). Normal starch consists of 15-30% (w/w) amylose, depending on the botanical sources, degree of maturity, growing conditions and the determination method (Chung et al 2009; Hasjim et al 2009; Jane et al 1999; Li et al 2007; Lu et al 1996; Onoet al 1998; Reddy and Seib 1999; Srichuwong et al 2005; Wu et al 2007; Yoo et al 2009). The amylose content of waxy starches is low, about 1%-8% (Yoo and Jane 2002; Perez and Bertoft 2010). High amylose starch contains more than 50% and up to 80% (w/w) amylose (Campbel et al 2007; Li et al 2008; Regina et al 2006; Shi et al 1998).

In order to distinguish and describe the unit chain composition of amylopectin, the chains are grouped into certain categories (Fig 2.5). The structure of amylopectin was depicted as a cluster model by Hizukuri (1985), with a number of branch chains (A, B, and C chains) making up the clusters. Short A chains possess degrees of polymerization (DP) from 6-12. The A chains

have reducing ends attached to B or C chains but do not carry any other chains. The B chains have reducing ends attached to B or C chains and also carry A or other B chains. The C chains possess a reducing end and carry A or B chains. Hizukuri (1986) found that the chains of amylopectin possessed a characteristic periodicity in length, and subdivided B chains into B₁, B₂ and B₃ chains. B₁ chains range from DP 13-24, B₂ chains range from 25-36, and B₃ and longer chains possess DP greater than 36. A and B₁ chains dominate the distribution of branch chains of amylopectin molecules. Amylose molecules are linear molecules and tend to form double helical inclusion complexes with lipids (French and Murphy 1977; Gidley 1989; Zobel 1988).

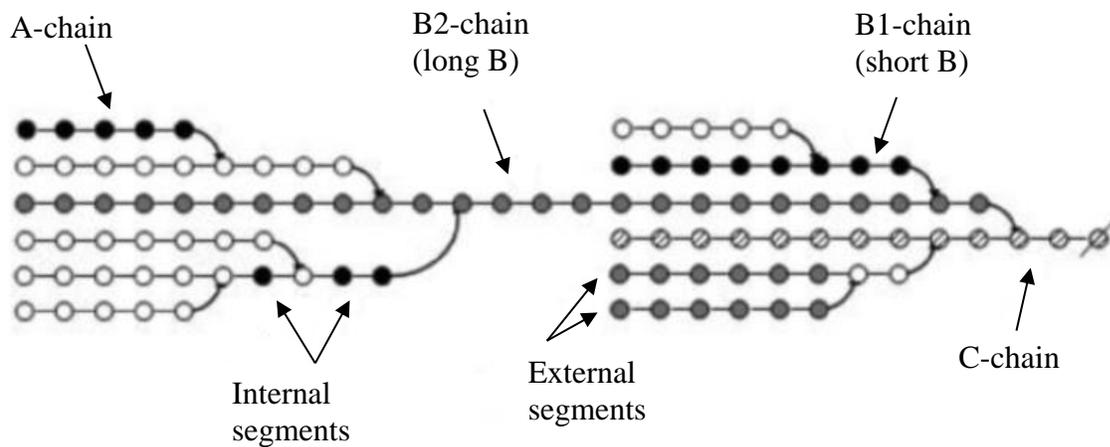


Figure 2.5. Basic labeling of chains in amylopectin. Circles indicates glucosyl residues, horizontal lines (1-4) and bent arrows (1-6) linkages. The reducing-end residue is on the right.
 Source: Perez and Bertoft (2010).

Starch molecules are synthesized in chloroplast or amyloplast by a series of enzymes. Four major enzymes include ADP-glucose pyrophosphorylase, granule-bound starch synthase (GBSS), soluble starch synthases, branching enzymes (BE) and debranching enzymes (DBE). During starch synthesis, each starch biosynthetic enzyme exist in several isoforms (James et al 2003; Morell and Myers 2005; Myers et al 2000; Smith 2001; Li 2007), even in the same tissue. The express patterns of different enzyme isoforms are different during seed development. Li et al (2007) found that the amylose contents of the endosperm and pericarp starches have different amylose content (Table 2.2). Amylose content of small granules in early development was much lower than in mature granules. As mentioned earlier, starch granules are synthesized via apposition, from hilum towards the periphery. The core of the starch granule corresponds to the small granule synthesized during the early stage of starch granule development and has been found to be lower in amylose content. However, amylose content of starch in pericarp was found to have little increase during starch granule development. The different amylose contents of the endosperm and pericarp starches were due to different isoforms of granule-bound starch synthases present in the endosperm and the pericarp tissues (Li et al 2007).

Table 2.2 Amylose Contents^x of Endosperm and Pericarp Starches of Maize at Different Developmental Stages

Days after pollination	Endosperm (% w/w)	Pericarp (% w/w)
0	nd ^y	19.6 ± 0.8
6	nd	19.7 ± 1.9
8	nd	19.0 ± 1.3
10	nd	14.7 ± 0.9
12	9.2 ± 0.8	14.4 ± 1.4
14	11.1 ± 0.6	16.2 ± 3.5
20	21.4 ± 0.9	18.3 ± 1.2
30	24.2 ± 0.8	19.3 ± 2.3
45 (mature and dried)	24.4 ± 0.7	nd

^x Values given are means ± standard deviation obtained from two replicates.

^y Not determined. Source: Li et al (2007)

2.5.2 General features of starch granules

Amylopectin and amylose are organized in semi-crystalline structures of double helices, which are comprised of crystalline and non-crystalline lamellae. Starch granules are made up of alternating amorphous and semi-crystalline shells which are between 100 and 400 nm thick (Gallant et al 1997). These structures are called growth rings. The radial organization of the amylopectin within this structure causes optical polarization (Perez and Bertoft 2010), therefore most native starch granules exhibit a Maltese cross when observed under polarized light (Jane et al 1994). The birefringence indicates a radial orientation of the principle axis of the crystallites. The branch chains of amylopectin molecules form double helices and contribute to starch crystallinity, while amylose molecules are in an amorphous form (Jane et al 1992). The amylose content is greater at the periphery than at the core of the starch granule, and amylopectin molecules at the center of the granule have greater proportions of the long B-chains than those at the periphery of the granule (Jane and Shen 1993; Pan and Jane 2000).

All native starches have one of three X-ray diffraction patterns for the packing of the double helices in the granules: A-, B-, and C-types (Jane 2004). The crystalline amylose-lipid complex displays a V-type X-ray diffraction pattern with 2θ peaks at 8° , 13° , and 20° (Zobel 1988). Starches with amylopectins of relatively short average branch chain lengths, such as maize, rice, wheat, tapioca, and sweet potato, display the A-type X-ray diffraction pattern with peaks at $2\theta = 15^\circ$, 17° , 18° and 23° (Jane et al 1997). Other starches that have amylopectins with long branch chains, such as potato, canna, and high amylose maize, display the B-type X-ray pattern with peaks at $2\theta = 5.5^\circ$, 15° , 17° , 22° , and 24° . Starches with amylopectins of branch chain length in between the above two groups display the C-type X-ray pattern which is a mixture of the A- and B-type crystalline structures (Jane et al 1997). The branch chain-length of

amylopectin has been confirmed to be an important factor in determining the crystalline diffraction pattern (Hizukuri et al 1983). In general, starch granules are confirmed to have a semi-crystalline character based on X-ray diffraction experiments. About 70% of the mass of starch granules is regarded as amorphous and about 30% as crystalline. The amorphous regions mainly consist of amylose but also a considerable part of the amylopectin molecules. Amylopectin is mainly responsible for the crystallinity of starch granules (Sajilata et al 2006). Water content, temperature and the presence of other solutes and solvents can affect crystallinity.

There are two types of starch granules, each with its own distinct shape and size: A-type granules, which are disc-like or lenticular in shape with diameters greater than 10 μm ; and B-type starch granules, which are roughly spherical or polygonal in shape with diameters smaller than 10 μm (Whistler and Bemiller 1997). Normally, the numbers of A-type granules are fewer than those of B-type, and A-type granules represent the majority of the mass of the starch.

A- and B-types of starch granules have different chemical compositions and functional properties, including differences in amylose and lipid contents, pasting properties, and baking qualities (Maningat and Seib 1997). Published reports differ on types of granules, amylose content, and starch crystallinity in A-type granule starch (starch with a high A-type granule concentration) and B-type granule starch. Some researchers (Raeker et al 1998, Chiotelli and Lemeste 2002) indicated that A-type granule starch is more crystalline (higher in amylose content) than B-type granule starch. Others reported there was no statistical difference in amylose contents of A- type versus B-types starch granules (Dengate et al 1984). Xie and coworkers (2008) found that wheat bran starch containing a higher content of B-type granules exhibited higher crystallinity and amylose content compared with commercial wheat starch.

2.5.3 Gelatinization and pasting

Native starch is not soluble in cold water, but can imbibe water during heating. Gelatinization is a process of disruption of molecular order within starch granules when they are heated in the presence of water. There is evidence for the loss of native structure during this process, such as irreversible granule swelling, solubilization of starch granules, loss of birefringence, and loss of crystallinity (Jane 2004). Amylose leaches out of the starch granules during gelatinization. When shear force is applied during gelatinization, starch granules can be disrupted and a paste is formed. Factors such as the composition of amylose and amylopectin in the starch, pH of the medium, type of mixing during heating, and other ingredients in the mixture will result in different types of pasting profiles for starches. Starch gelatinization is an energy-absorbing process; temperature and enthalpy of gelatinization can be determined by differential scanning calorimetry (DSC) (Whistler and Bemiller 1997), from which, gelatinization onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c) can be obtained. The gelatinization properties are affected by the starch structures and components present in the starch granules, which are determined by botanical origin of the starch (Jane 2004). Starch consisting of a larger proportion of the short branch-chain (DP 6-9) amylopectin molecules has a lower gelatinization temperature. This is attributed to defects in the crystalline structure of the granule. That occurs since it is difficult for very short branch-chain amylopectin molecules to form double helix structures (Jane et al 1997).

2.5.4 Retrogradation

Cooking or processing of a starch with water normally causes starch gelatinization. The behavior of gelatinized starches upon cooling and storage, is generally termed retrogradation.

Retrogradation is a process in which solubilized polymers and insoluble fragments in gelatinized starch, such as amylose, will reassociate during cooling. Precipitation, gelation, and changes in consistency and opacity of gelatinized starch will occur. Crystallites begin to form, and this is accompanied by gradual increases in rigidity of the pastes or gels and phase separation between polymer and water or solvent, this separation process is termed syneresis (Karim et al 2000). Retrogradation is important to food scientists since it greatly affects quality, acceptability, texture, and shelf-life of starch-based foods (Biliaderis 1991). For example, retrogradation is highly related to many food quality deteriorations such as bread staling, loss of viscosity of soups, and texture changes of sauces (Whistler and Bemiller 1997).

The rate of retrogradation depends on several variables, including the structures of amylose and amylopectin, molecular ratio of amylose to amylopectin within the starch, starch concentration in the solution, cooling temperature and time, and other ingredients present, such as surfactants and salts. Although amylose is mainly responsible for starch retrogradation, amylopectin with long branch-chain length also has a high percent retrogradation. Amylopectin molecules with branched structures can be equally effective for crystalline structure formation, depending on the structure of the branch chains (Jane et al 1999). Retrogradation can be considered as a time and temperature dependent polymer recrystallization process (Biliaderis 1990; Slade and Levine 1991). Short-term development of retrogradation is driven by amylose crystallization, whereas long-term reordering of amylopectin during retrogradation is a much slower process involving recrystallization of the outer branches of amylopectin (Miles et al 1985; Ring et al 1987). A previous study found that after 15 days storage at 5 °C, native waxy starch retrogradation enthalpy change (ΔH) reached a near-constant final value (Aytunga Arik Kidar et al 2011).

Methods to study starch retrogradation can be classified into two types: macroscopic techniques and molecular techniques. Macroscopic techniques monitor changes in physical properties as a result of retrogradation such as mechanical or textural changes in the paste. Molecular techniques are methods that study changes in starch polymer conformation or water mobility in starch gels at the molecular level. Differential scanning calorimetry (DSC), light scattering, turbidometry, and measurement of syneresis are approaches to study the macroscopic manifestations of retrogradation. While X-ray diffractometry, nuclear magnetic resonance spectroscopy, and fourier transform infra-red spectroscopy are among the molecular techniques utilized (Karim et al 2000). DSC has proven to be one of the most useful methods for providing basic information on starch retrogradation. In the case of retrograded starch, the value of enthalpy change (ΔH) provides a quantitative measure of the energy transformation that occurs during the melting of recrystallized amylopectin as well as precise measurements of the transition temperatures (i.e., onset, T_O ; peak, T_P ; and conclusion, T_C) of this endothermic process. The percentage retrogradation can be calculated by the ratio of the gelatinization enthalpy of the starch after storage to that of the original starch (Xie et al 2008).

2.5.5 Starch damage

The dry milling of wheat causes physical damage to a proportion of the starch granules of flour. Damaged starch can rapidly hydrate and swell, even in cold water, and thus has increased water absorption. Greater amounts of water need to be added to a dough to maintain adequate handling properties. During dough fermentation, damaged starch is more susceptible to enzymatic hydrolysis than native starch, thus more carbohydrate in the form of maltose will be produced. This provides an additional supply of substrate for yeast in the later stages of dough

processing and, as a result, more gas will be produced and a higher bread loaf volume may be obtained (Evers and Stevens 1985). A modest level of starch damage is therefore beneficial for breadmaking. However, too much damaged starch results in a sticky dough that is difficult to handle and has reduced water holding capacity and gas retention (Gibson et al 1992). Starch damage causes great changes to a starch granular structure and amylopectin molecules, so the rheological and functional properties of gelatinized starch paste made from damaged starch are different than those of the counterpart paste made from undamaged starch. Previous studies have shown that extensive starch damage can decrease the viscosity of pastes (Han et al 2002).

2.6 BRAN STARCH CHARACTERIZATION

Xie and coworkers (2008) isolated and characterized wheat bran starch and compared the findings with those from commercial wheat starch. The number of B-type granules (small granules) in bran starch was found to be 45% (w/w), which was greater than the 15% (w/w) found in the commercial wheat starch sample. The differences in the proportions of A- and B-type granules in bran starch contribute to the difference in swelling power, since B-type granules have a larger total surface area per unit volume of granules than that of A-type granule, and therefore have higher swelling power. Wheat bran starch exhibited higher crystallinity (16.4%) than commercial wheat starch (14.7%). A positive correlation was observed between crystallinity and percent amylose content in starch. Wheat bran starch contained more amylose than commercial starch. Compared with commercial wheat starch, bran starch also had lower starting gelatinization temperatures, pasting peaks, and final viscosities (Xie et al 2008).

In Xie et al's (2008) study, wheat bran starch was found to have slower retrogradation (retrogradation rate of 17.8%) during storage at 4 °C for 2 weeks compared to that of commercial

wheat starch (retrogradation rate of 40.7%). The rate of digestibility of wheat bran starch by α -amylase was slightly slower than the digestibility rate of commercial starch; wheat bran starch contained higher resistant starch content than commercial starch. The grinding steps during isolation caused little or no starch damage, since the starch damage level in bran starch was very low ($1.03 \pm 0.15\%$). While the above research is relevant, it has several key limitations. First of all, the above authors used a commercial starch as a control that could have come from a different biological source (i.e., different wheat classes or varieties) than the bran starch. Secondly, many milling factors could have affected the results obtained from characterization of the bran and commercial starch, such as different types of mills and milling procedures for preparation of the two types of starches. Wheats used to produce commercial starch and isolated bran starch could have been from different locations and crop years and therefore subject to environmental effects. The isolation of the commercial starch could have been different from the isolation process used for bran starch. Different isolation processes have significant impact on starch properties. Accordingly, more detailed research studies were planned in this dissertation to address these limitations.

LITERATURE CITED

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- Albersheim, P., Nevins, D. J., English, P. D., and Karr, A. 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. *Carbohydr. Res.* 5:340-345.
- Andreasen, M. F., Christensen, L. P., Meyer A. S., and Hansen, A. 2000. Content of phenolic acids and ferulic acid dehydrodimers in 17 rye (*Secale cereale*L.) varieties. *J. Agric. Food Chem.* 48:2837-2842.
- Annison G, Illman R. J., Topping D. L. 2003. Acetylated, propionylated or butyrylated starches raise large bowel short-chain fatty acids preferentially when fed to rats. *J Nutr.* 133:3523-3528.
- Antoine, C., Peyron, S., Mabilie, F. R., Lapierre, C., Bouchet, B., Abecassis, J. and Rouau, X. 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat Bran. *J. Agric. Food Chem.*:2026-2033.
- Aytunga Arik Kibar, E., Gonenc, I., Us, F. 2011. Modeling of retrogradation of waxy and normal corn starches. *Intl. J. Food Properties* 14:954-967.
- Bartolome B., Faulds C. B., Kroon P. A., Waldron K., Gilbert H. J., Hazlewood, G. and Williamson, G. 1997. An *Aspergillus niger* esterase (ferulic acid esterase III) and a recombinant *Pseudomonas fluorescens* subsp. *cellulosa* esterase (XylD) release a 5-5' ferulic dehydrodimer dehydrodiferulic acid) from barley and wheat cell walls. *Appl Environ Microbiol* 63:208-212.
- Biliaderis, C. G. 1990. Thermal analysis of food carbohydrates. Pages 168-220 In: *Thermal Analysis of Foods*. Harwalkar, V.R. and Ma, C.Y., eds. Elsevier Applied Science Publishers: New York.
- Biliaderis, C. G. 1991. The structure and interactions of starch with food constituents. *Canadian J. of Physiology and Pharmacology* 69:60-78.
- Bingham, S. A. 1990. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proc. Nutr. Soc.* 49:153-171.
- Brillouet, J. M., and Mercier, C. 1981. Fractionation of wheat bran carbohydrates. *J. Sci. Food Agric.* 32:243-251.
- Bunzel, M., Ralph, J., Marita, J., and Steinhart, H. 2000. Identification of 4-0-5'- coupled diferulic acid from insoluble cereal fiber. *J. Agric. Food Chem.*48:3166-3169.

- Buri, R. C., von Reding, W., Gavin, M. H., 2004. Description and characterization of wheat aleurone. *Cereal Foods World* 49: 274-282.
- Chatenoud, L., Tavani, A., La Vecchia, C., Jacobs, D. R. Jr., Negri, E., Levi, F., and Franceschi, S. 1998. Whole grain food intake and cancer risk. *Int. J. Cancer* 77:24-28.
- Courtin, C. M., Van den Broeck, H., and Delcour, J. A. 2000. Determination of reducing end sugar residues in oligo- and polysaccharides by gas-liquid chromatography. *J. Chromatogr. A* 866:97-104.
- Chiotelli, E., and LeMeste, M. 2002. Effect of small and large wheat starch granules on thermo-mechanical behavior of starch. *Cereal Chem.* 79:286-293.
- Chung, H. J., Liu, Q., and Hoover, R. 2009. Impact of annealing and heat-moisture treatment on rapidly digestible, slowly digestible and resistant starch levels in native and gelatinized corn, pea and lentil starches. *Carbohydr. Polymers.*75:436-447.
- Deighton, N., Brennan, R., Finn, C., and Davies, H. V. 2000. Antioxidant properties of domesticated and wild *Rubus* species. *J. Sci. Food Agric.* 80:1307-1313.
- Dengate, H., and Meredith, P. 1984. Variation in size distribution of starch granules from wheat grain. *J. Cereal Sci.* 2:83-90.
- Desvignes, C., Olive, C., Lapierre, C., Rouau, X., Pollet, B., Lullien-Pellerin, V., 2006. Effects of calcium chloride treatments on wheat grain peroxidase activity and outer layer mechanical properties. *J. Sci. Food Agric.* 86:1596-1603.
- Desvignes, C., Chaurand, M., Dubois, M., Sadoudi, A., Abecassis, J., Lullien-Pellerin, V., 2008. Changes in common wheat grain milling behavior and tissue mechanical properties following ozone treatment. *J. Cereal Sci.* 47:245-251.
- Englyst, H. N., and Cummings, J. H. 1984. Simplified method for the measurement of total non-starch polysaccharides by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 109:937-942.
- Englyst H. N., Kingman S. M., Cummings J. H. 1992. Classification and measurement of nutritionally important starch fractions. *Eur J. Clin. Nutr.* 46:S33-S50.
- Englyst H. N., Wiggins H. S., Cummings J. H. 1982. Determination of the non-starch polysaccharides in plant foods by gas-liquid chromatography of constituent sugars as alditol acetates. *Analyst* 107:307-318.
- Englyst, H. N., Quigley, M. E., and Hudson, G. J. 1994. Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119:1497-1509.

- Evers, A. D., and Stevens, D. J. 1985. Starch damage. *Adv. Cereal Sci. Technol.* 7:321-349.
- Evers, A. D., and Withey, R. P. 1989. Use of image analysis to predict milling extraction rate of wheats. *Food Microstructure* 8:191-199.
- French, A. D., and Murphy, V. G. 1977. Computer modelling in the study of starch. *Cereal Foods World* 22:61-70.
- French, D. 1984. Organization of starch granules. Pages 183-247 In: *Starch: Chemistry and Technology*. R. L. Whistler, J. N. BeMiller, and E.F. Paschall, eds. Academic Press: New York.
- Gallant, D. J., and Bouchet, B. B. 1986. Ultrastructure of maize starch granules. A review. *J. Food Microstruct.*5:141-155.
- Gallant, D. J., Bouchet, B., Baldwin, P. M. 1997. Microscopy of starch: Evidence of a new level of granule organization. *Carbohydr. Polymer* 32:177-191.
- Gidley, M. J. 1989. Molecular mechanism underlying amylose aggregation and gelation. *Macromolecules* 22:351-358.
- Gibson, L. J., and Ashby, M. F. 1988. *Cellular Solids*. Oxford Pergamon Press: New York.
- Gibson, T. S., Al Qalla, H., and McCleary, B. V. 1992. An improved enzymatic method for the measurement of starch damage in wheat flour. *J. Cereal Sci.* 15:15-27.
- Glenn, G. M., and Johnson, R. K. 1992. Moisture dependant changes in the mechanical properties of isolated wheat bran. *J. Cereal Sci.* 15:223-236.
- Grefeuille, V., Abecassis, J., Lapierre, C., and Lullien-Pellerin, V. 2006. Bran size distribution at milling and mechanical and biochemical characterization of common wheat grain outer layers: a relationship assessment. *Cereal Chem.* 83:641-646.
- Han, X. Z., Campanella, O. H., Mix, N. C., and Hamaker, B. R. 2002. Consequence of starch damage on rheological properties of maize starch pastes. *Cereal Chem.* 79:897-901.
- Haros, M., Rosell, C. M., and Benedito, C. 2002. Improvement of flour quality through carbohydrases treatment during wheat tempering. *J. Agric. Food Chem.* 50:4126-4130.
- Hasjim, J., Srichuwong, S., Scott, M. P., and Jane, J. 2009. Kernel composition, starch structure, and enzyme digestibility of opaque-2 maize and quality protein maize. *J. Agric. Food Chem.* 57:2049-2055.
- Hemery, Y., Rouau, X., Lullien-Pellerin, V., Barron, C., and Abecassis, J. 2007. Dry processes to develop wheat fractions and products with enhanced nutritional quality. *J. Cereal Sci.* 46:327-347

- Hizukuri, S., Kaneko, T., and Takeda, Y. 1983. Measurement of the chain-length of amylopectin and its relevance to the origin of crystalline polymorphism of starch granules. *Biochimica et Biophysica Acta* 760:188-191.
- Hizukuri, S. 1986. Polymodal distribution of the chain lengths of amylopectins, and its significance. *Carbohydr. Research*.147:342-347.
- Hooper, L. V. 2004. Bacterial contributions to mammalian gut development. *Trends Microbiol.* 12:129-134.
- James, M. G., Denyer, K., and Myers, A. M. 2003. Starch synthesis in the cereal endosperm. *Current Opinion in Plant Biology* 6: 215-222
- Jane, J. 2004. Starch: structure and properties. Pages 81-101 in: *Chemical and Functional Properties of Food Saccharides*. P. Tomasik, eds. CRC Press: New York..
- Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., and Kasemsuwan, T. 1999. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* 76:629-637.
- Jane, J., and Chen, J. F. 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. *Cereal Chem.* 69:60-65.
- Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., and Kasemsuwan, T. 1999. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* 76:629-637.
- Jane, J., Kasemsuwan, T., Leas, S., Zobel, H., Robyt, J. F. 1994. Anthology of starch granule morphology by scanning electron microscopy. *Starch/Starke* 46:121-129.
- Jane, J., and Shen, J. J. 1993. Internal structure of the potato starch granule revealed by chemical gelatinization. *Carbohydr. Res.* 247:279-290.
- Jane, J., Wong, K., and McPherson, E. 1997. Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrans. *Carbohydr. Res.* 300: 219-227.
- Karim, A. A., Norziah, M. H., and SeoW, C. C. 2000. Methods for the study of starch retrogradation. *Food Chem.* 71:9-36.
- Klepacka, J., and Fornal, L. 2007. Ferulic acid and its position among the phenolic compounds of wheat. *Critical Reviews in Food Sci. and Nutrition* 46:639-647.
- Kozubek, A., and Tyman, J. H. P. 1999. Resorcinolic lipids, the natural nonisoprenoid phenolic amphiphiles and their biological activity. *Chemical Reviews* 99:1-26.

- Landberg, R., Kamal-Eldin, A., Salmenkallio-Marttila, M., Rousset, M., Aman, P. 2008. Localization of alkylresorcinols in barley, wheat and rye kernels. *J. Cereal Sci.* 48:401-406.
- Lempereur, I., Rouau, X., and Abecassis, J. 1997. Genetic and agronomic variation in arabinoxlan and ferulic acid contents of durum wheat grain and its milling fractions. *J. Cereal Sci.* 25:103-110.
- Li, L., Shewry, P. R., and Ward, J. L. 2008. Phenolic acids in wheat varieties in the Healthgrain diversity screen. *J. Agric. Food Chem.* 56:9732-9739.
- Li, L., Blanco, M., and Jane, J. 2007. Physicochemical properties of endosperm and pericarp starches during maize development. *Carbohydr. Polymers* 67:630-639.
- Li, L., Jiang, H., Campbell, M., Blanco, M., and Jane, J. 2008. Characterization of maize amylose-extender (ae) mutant starches. Part I: Relationship between resistant starch contents and molecular structures. *Carbohydr. Polymers* 74:396-404.
- Lim, S. T., Kasemsuwan, T., and Jane, J. 1994. Characterization of phosphorus in starches using ³¹P-NMR spectroscopy. *Cereal Chem.* 7:488-493.
- Lozovaya, V. V., Gorshkova, T. A., Yablokova, E. V., Rumyantseva, N. I., Valieva, A., Ulanov, A., and Widholm, J. M. 1999. Cold alkali can extract phenolic acids that are ether linked to cell wall components in dicotyledonous plants (buckwheat, soybean and flax). *Phytochemistry*, 50:395-400.
- Lucas, P. W., Darwell, B. W., Lee, P. K. D., Yuen, T. D. B., and Choong, M. F. 1995. The toughness of plant cell walls. *Philosophical Transactions-Royal Society of London. Biological Sciences* 328:363-372.
- Lu, T. J., Jane, J., Keeling, P. L., and Singletary, G. W. 1996. Maize starch fine structures affected by ear developmental temperature. *Carbohydr. Res.* 282:157-170.
- Mabille, F., Gril, J., Abecassis, J., 2001. Mechanical properties of wheat seed coats. *Cereal Chemistry* 78:231-235.
- Maningat, C. C., and Seib, P. A. 1997. Update on wheat starch and its uses. Pages 261-284 in: *Proc. Int. Wheat Quality Conference*. J. L. Steele and O. K. Chung, eds. Grain Industry Alliance: Manhattan, KS.
- Martelli, M. R., Barron, C., Mabille, F., Rouau, X., Sadoudi, A. 2010. Adherence within biological multilayered systems: Development and application of a peel test on wheat grain peripheral tissues. *J. Cereal Sci.* 52:83-89.
- McKeehen, J. D., Busch, R. H., and Fulcher, R. G. 1999. Evaluation of wheat (*Triticum aestivum* L.) phenolic acids during grain development and their contribution to *Fusarium* resistance. *J. Agric. Food Chem.* 47:1476-1482.

- Melton, L. D., and Smith, B. G. 2001. Determination of neutral sugar by gas chromatography of their alditol acetates. Pages E3.2.1-E3.2.13 in: Current Protocols in Food Analytical Chemistry. John Wiley and Sons, Inc.: Hoboken, NJ.
- Miles, M. J., Morris, V. J., and Ring, S. G. 1985. Gelation of amylose. Carbohydrate Research 135:247-269.
- Moore, J., Cheng, Z., Su, L., Yu, L., 2006. Effects of solid-state enzymatic treatments on the antioxidant properties of wheat bran. J. Agric. Food Chem 54:9032-9045.
- Moreau, N. M., Marin, L. J., Toquet, C. S., Laboisse, C. L., Nguyen, P. G., Siliart, B. S., Dumon, H. J., and Champ, M. M. J. 2003. Restoration of the integrity of rat caeco-colonic mucosa by resistant starch, but not by fructo-oligosaccharides, in dextran sulfate sodium-induced experimental colitis. Br. J. Nutr. 90:75-85.
- Morell, M. K., and Myers, A. M. 2005. Towards the rational design of cereal starches. Current Opinion in Plant Biology 8:204-210.
- Myers, A. M., Morell, M. K., James, M. G., and Ball, S. G. 2000. Recent progress toward understanding biosynthesis of the amylopectin crystal. Plant Physiology 122:989-997.
- Ono, K., Seib, P. A., and Takahashi, S. 1998. Properties of hydroxypropylated wheat starches. Nippon Kasei Gakkaishi 49:985-992.
- Onyeneho, S. N., and Hettiarachchy, N. S. 1992. Antioxidant activity of durum wheat bran. J. Agric. Food Chem. 40:1496-1500.
- Pan, D. D., and Jane, J. 2000. Internal structure of normal maize starch granules revealed by chemical surface gelatinization. Biomacromolecules 1:126-132.
- Perez, S., and Bertoft, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. Starch/Starke 62:389-420.
- Peyron, S., Abecassis, J., Autran, J. C., Rouau, X., 2001. Enzymatic oxidative treatments of wheat bran layers: effects on ferulic acid composition and mechanical properties. J. Agric. Food Chem. 49:4694-4699.
- Peyron, S., Chaurand, M., Rouau, X., Abecassis, J., 2002a. Relationship between bran mechanical properties and milling behaviour of durum wheat (*Triticum durum Desf.*). Influence of tissue thickness and cell wall structure. J. Cereal Sci. 36:377-386.
- Peyron, S., Abecassis, J., Autran, J. C., Rouau, X., 2002b. Influence of UV exposure on phenolic acid content, mechanical properties of bran, and milling behavior of durum wheat (*Triticum durum desf.*). Cereal Chem. 79:726-731.

- Peyron, S., Surget, A., Mabillet, F., Autran, J. C., Rouau, X., Abecassis, J. 2002c. Evaluation of tissue dissociation of durum wheat grain (*Triticum durum* Desf.) generated by the milling process. *J. Cereal Sci.* 36:199-208.
- Posner, E. S., and Hibbs, A. N. 2005. Chapter 5. Grinding. Pages 200-219 in: *Wheat Flour Milling*. 2nd edition. E. S. Posner and A. N. Hibbs, eds. Am. Assoc. Cereal Chem., Inc: St. Paul, MN, USA.
- Qu, H., Madl, R. L., Takemoto, D. J., Baybutt, R. C., and Wang, W. 2005. Lignans are involved in the antitumor activity of wheat bran in colon cancer SW480 cells. *J. Nutr.* 135:598-602.
- Raeker, M. O., Gaines, C. S., Finney, P. L., and Donelson, T. 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. *Cereal Chem.* 75:721-728.
- Ramirez-Truque, C., Esquivel, P., and Carle, R. 2011. Neutral sugar profile of cell wall polysaccharides of pitaya (*Hylocereus* sp.) fruits. *Carbohydr. Polymers* 83:1134-1138.
- Reddy, I., and Seib, P. A. 1999. Paste properties of modified starches from partial waxy wheats. *Cereal Chem.* 76: 341-349.
- Renger, A., and Steinhart, H. 2000. Ferulic acid dehydrodimers as structural elements in cereal dietary fibre. *Eur. Food Res. Technol.* 211:422-428.
- Rhodes, D. I., Stone, B. A. 2002. Proteins in walls of wheat aleurone cells. *J. Cereal Sci.* 36:83-101.
- Ring, S. R., Colonna, P., I'Anson, K. J., Kalichevsky, M. T., Miles, M. J., Morris, V. J., and Orford, P. D. 1987. The gelation and crystallization of amylopectin. *Carbohydr. Res.* 162:277-293.
- Robyt, J. F. 1998. Chapter 1. Beginnings. Pages 1-18 in: *Essentials of Carbohydrate Chemistry*, Springer-Verlag, New York.
- Sajilata, M. G., Singhal, R. S., and Kulkarni, P. R. 2006. Resistant starch—A review. *Compr. Rev. Food Sci. Food Safety* 5:1-17.
- Saulnier, L., Crépeau, M. J., Lahaye, M., Thibault, J. F., Garcia- Conesa, M. T., Kroon, P. A., and Williamson, G. 1999. Isolation and structural determination of two 5,5'- diferuloyl oligosaccharides indicate that maize heteroxylans are covalently cross- linked by oxidatively coupled ferulates. *Carbohydr. Res.* 320:82-92.
- Saulnier, L., Sado, P. E., Branlard, G., Charmet, G., and Guillon, F. 2007. Wheat arabinoxylans: Exploiting variation in amount and composition to develop enhanced varieties. *J. Cereal Sci.* 46:261-281.

- Schatzkin, A., Mouw, T., Park, Y., Subar, A. F., Kipnis, V., Hollenbeck, A., Leitzmann, M. F., and Thompson, F. E. 2007. Dietary fiber and whole-grain consumption in relation to colorectal cancer in the NIHAARP Diet and Health Study. *Am. J. Clin. Nutr.* 85:1353-1360.
- Seal, C. J. and Brownlee, I. A. 2010. Whole grains and health, evidence from observational and intervention Studies. *Cereal Chem.* 87:167-174.
- Selvendran, R. R., and Ryden, P. 1990. Isolation and analysis of plant cell walls. Pages 549-579 In: *Methods In Plant Biochemistry*, Vol. 2, Carbohydrates. P.M. Dey and J.B. Harbourne, eds. Academic Press, London.
- Shi, Y. C., Capitani, T., Trzasko, P., and Jeffcoat, R. 1998. Molecular structure of a low amylopectin starch and other high-amylose maize starches. *J. Cereal Sci.* 27:289-299.
- Simmons, L., and Meredith, P. 1979. Width, weight, endosperm and bran of the wheat grain as determinants of flour milling yield in normal and shivelled wheats. *New Zealand J. Sci.* 22:1-10.
- Slade, L., and Levine, H. 1991. Beyond water activity: Recent advances based on an alternative approach to the assessment of food quality and safety. *Crit. Rev. Food Sci. Nutr.* 30:115-360.
- Smith, A. M. 2001. The Biosynthesis of Starch Granules. *Biomacromolecules* 2: 335-341.
- Song, H. P., Delwiche, S. R., and Line, M. J., 1998. Moisture distribution in a mature soft wheat grain by three-dimensional magnetic resonance imaging. *J. Cereal Sci.* 27: 191-197.
- Takeda, C., Takeda, Y., and Hizukuri, S. 1989. Structure of amylozyme amylose. *Cereal Chem.* 66:22-25.
- Tan, K. S., Hoson, T., Masuda, Y., Kamisaka, S., 1992. Effect of ferulic and P-coumaric acids on *Oryza* coleoptile growth and the mechanical properties of cell walls. *J. Plant Physiology* 140:460-465.
- Takeda, Y., Hizukuri, S., and Juliano, B. O. 1986. Purification and structure of amylose from rice starch. *Carbohydr. Res.* 148:299-308.
- Takeda, Y., Tomooka, S., and Hizukuri, S. 1993. Structures of branched and linear molecules of rice amylose. *Carbohydr. Res.* 246:267-272.
- Wakabayashi, K., Soga, K., Kamisaka, S., Hoson, T., 2005a. Changes in levels of cell wall constituents in wheat seedlings grown under continuous hypergravity conditions. *Advances in Space Research* 36:1292-1297.
- Wakabayashi, K., Soga, K., Kamisaka, S., Hoson, T., 2005b. Increase in the level of arabinoxylan-hydroxycinnamate network in cell walls of wheat coleoptiles grown under continuous hypergravity conditions. *Physiologia Plantarum* 125:127-134.

- Whistler, R. L. and Bemiller, J. N. 1997. Chapter 6-starch. Pages 117-152 in: Carbohydrate Chemistry for Food Scientist. AACC International: St. Paul, MN.
- Xie, X., Cui, S. W., Li, W., and Tsao, R. 2008. Isolation and characterization of wheat bran starch. *Food Research Intl.* 41:882-887.
- Yoshida, M., Fujii, M., Nikuni, Z., and Maruo, B. 1958. The appositive growth of starch granules in beans as revealed by autoradiography. *Bulletin of Agricultural and Chemical Society* 21:127.
- Zhang, G., Hamaker, B. R. 2010. Cereal carbohydrates and colon health. *Cereal Chem.* 87:331-341.
- Zielinski, H., and Kozłowska, H. 2000. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. *J. Agric. Food Chem.* 48:2008-2016.
- Zobel, H. F. 1988. Molecules to granules. A comprehensive starch review. *Starch/Starke* 40:44-50.

**CHAPTER 3 ISOLATION AND CHARACTERIZATION OF WHEAT BRAN STARCH
AND ENDOSPERM STARCH OF SELECTED SOFT WHEATS GROWN IN
MICHIGAN AND COMPARISON OF THEIR PHYSICOCHEMICAL PROPERTIES**

3.1 ABSTRACT

During wheat milling, bran is separated from the endosperm, although a clean separation is not possible and there is always some starch adherent to the bran. Understanding the physicochemical properties of bran starch and its relationship with bran tissue is required to maximize bran utilization. The objective of this study was to characterize bran starch properties compared with those of its counterpart endosperm starch from the same wheat sample. Three varieties (Aubrey, Caledonia, and D8006) with relatively high crop yield and different levels of milling softness equivalence were chosen for this study. Bran starch and its counterpart endosperm starch were isolated by an alkaline extraction method, and their morphology, crystalline structures and properties were analyzed. Chain length distribution of debranched amylopectins by HPAEC-PAD showed that bran starch had more short chains (A and B1 chains) than endosperm starch; SEM images revealed morphology of starch granules present in both bran starch and endosperm starch after starch isolation and a greater proportion of small granules in bran starch. Bran starch was found to have higher percent B-type granules, higher amylose content, higher crystallinity, broader gelatinization temperature range, higher enthalpy of gelatinization, lower retrogradation degree, and lower pasting peak and setback viscosities than the counterpart endosperm starch. A-type X-ray diffraction patterns were found for both bran starch and endosperm starch. Bran starch of variety Aubrey had highest crystallinity (21.75%) and gelatinization temperature (62.9 °C). While bran starch of variety D 8006 had highest percent B-type granules and lowest retrogradation degree (21.7%). Results of this study provide a foundation for a better utilization of bran starch during whole grain food processing.

3.2 INTRODUCTION

Wheat flour is the major product from a dry milling process and has been fully utilized by the grain industry. However, most commercial wheat bran is sold as animal feed at a very low price (Xie et al 2008). In order to add commercial value and benefits for the food industry, bran utilization needs to be maximized and developed. Previous research has confirmed that wheat bran contains a number of high value components, such as phenolic compounds, starches, water-soluble and water-insoluble dietary fibers, and proteins (Peng et al 1999). Cereal dietary fibers (non-starch polysaccharides and resistant starch) are the major health-beneficial carbohydrates in cereal whole grains, and mainly found in their bran fractions. Sufficient consumption of dietary fibers has a protective effect against development of diet-related disorders, such as cardiovascular disease and type II diabetes, and also against cancers, such as digestive-tract cancer, hormone-related cancers and pancreatic cancer (Seal and Brownlee 2010).

In the milled bran fraction of wheat, starch still remains one of the main components (Liu and Ng 2012). Wheat bran starch is the starch adherent to bran tissue after the dry milling process. Bran starch comprises about 20% (w/w) of the milled wheat bran fraction (Liu and Ng 2012). Bran starch was reported to be higher in resistant starch content (starch or a fraction of starch that is not digested by amylolytic enzymes in the digestive tract), and have lower starting gelatinization temperature and slower retrogradation than commercial wheat starch (Xie et al 2008). Thus, bran starch can potentially be used as a functional ingredient together with bran for food product development. Starch is unique among carbohydrates because it occurs naturally as discrete granules and is composed of a mixture of two polymers, amylose and amylopectin. There are two types of starch granules, each with its own distinct shape and size: A-type starch granules, which are disk-like or lenticular in shape with diameters greater than 10 μm ; and B-

type starch granules, which are roughly spherical or polygonal in shape with diameters smaller than 10 μm (Whistler and Bemiller 1997).

The structure of amylopectin was depicted as a cluster model by Hizukuri (1985), with a number of branch chains making up the clusters. Short A chains possess degrees of polymerization (DP) from 6-12, B₁ chains range from DP 13-24, B₂ chains range from 25-36, and B₃ and longer chains possess DP greater than 36. A and B₁ chains dominate the distribution of amylopectin branch chains. Amylopectin branch chain-length has been confirmed to relate with starch crystalline structure (Hizukuri 1985; Gidley and Bulpin 1987), gelatinization, retrogradation (Miles et al 1985; Gudmundsson and Eliasson 1990; Jan et al 1992), and pasting properties of starch (Wang et al 1993; Jane and Chen 1992). The branch short chains of amylopectin molecules form double helices structure and therefore contribute to starch crystallinity. Understanding the physicochemical properties of bran starch is needed to maximize bran utilization. However, the physicochemical properties of isolated bran starch compared with those of endosperm starch from the same wheat sample have not been reported, and thus the reason for different functional properties is not clear.

Wheat starch has been produced by a wet-milling process from flour or whole kernels, but limited publications have reported isolation of starch from wheat bran. Developing a common method of isolating both wheat bran starch and its counterpart endosperm starch is necessary to ensure compatibility of results of further investigation since many processing conditions can change the properties of starch. For example, starch isolated with a strong-alkaline method showed a significantly higher gelatinization temperature than starch isolated from the same sample by a mild alkaline and enzymatic method; the pasting viscosities also were significantly different (Jane et al 1999).

Xie et al (2008) characterized wheat bran starch compared to commercial starch, however there may have been some limitations in their sample sources and preparations, as follows: (1) commercial starch was used as a control that was prepared from a potentially different biological source (e.g., different wheat classes or varieties) than the isolated bran starch; (2) wheats used to produce commercial bran and isolated bran starch could have been from different locations and crop years and therefore subject to environmental effects; and (3) the isolation of the commercial starch could have been different from the isolation process used for bran starch and, as discussed above, different isolation processes have significant impact on starch properties. Accordingly, the aims of this study were (1) to develop a starch isolation procedure feasible for both bran starch from milled bran fractions and endosperm starch from milled flour fractions; and (2) to characterize and compare bran starch properties and the counterpart endosperm starch properties from the same wheat sample.

3.3 MATERIALS AND METHODS

3.3.1 Materials and milling

Wheat variety Caledonia harvested at Huron in 2010 was used for isolation procedure development. Wheat varieties Aubrey, Caledonia, and D8006 harvested at Lenawee in 2011 were used for starch characterization. All three wheat varieties are soft white wheat grown in Michigan. Bran fractions and flour fractions from all three varieties were obtained with a Buhler laboratory mill (MLU-202) (Buhler, INC, Uzwil, Switzerland) according to AACCI Method 26-31. Wheat grain samples were all tempered at 14.5% grain moisture and preconditioned for 18 hr before milling.

3.3.2 Isolation method development

An alkaline extraction isolation method was developed (Fig. 3.1) based on Xie et al (2008) and Verwimp et al (2004) for obtaining bran starch from the milled wheat bran fraction.

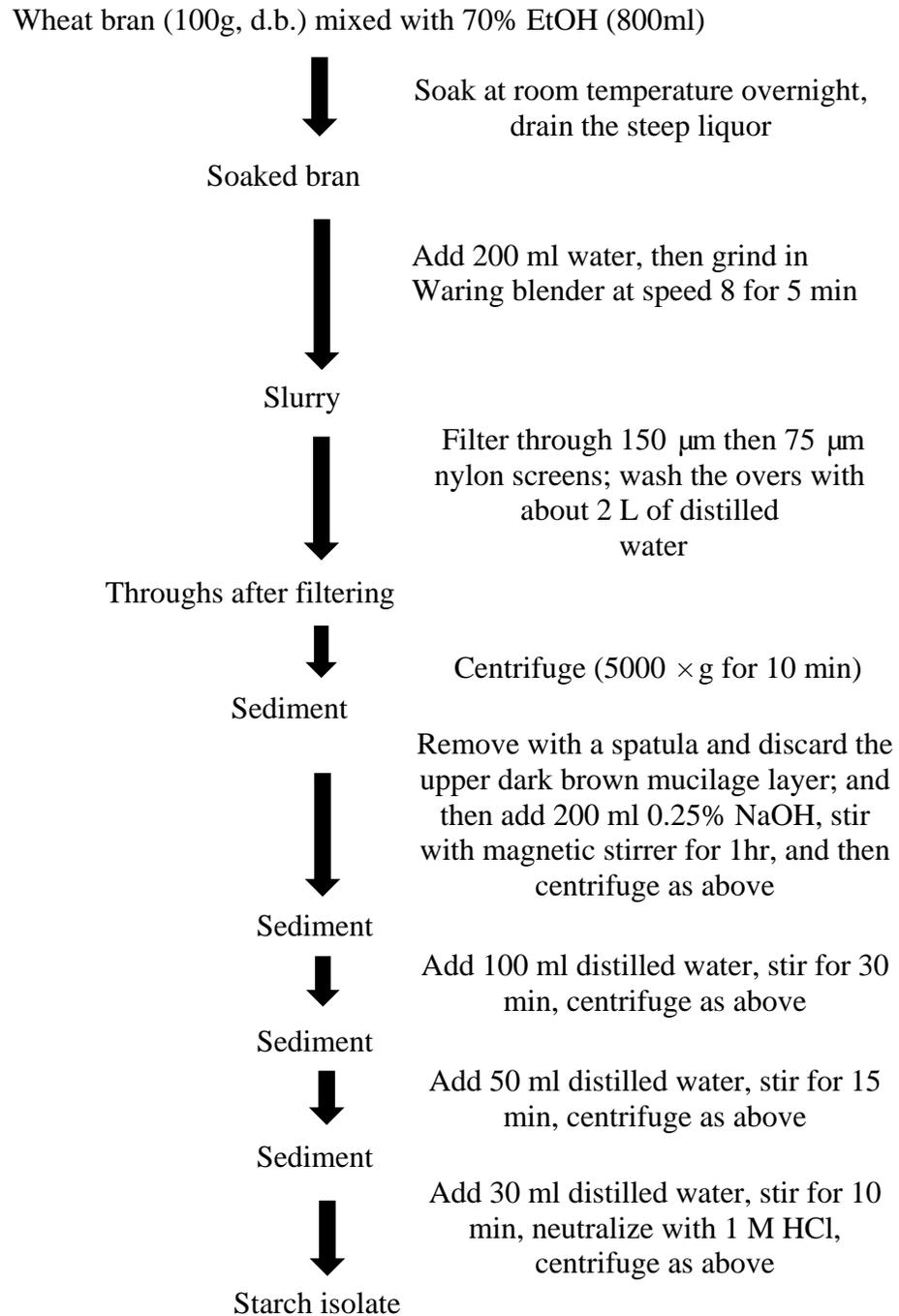


Figure 3.1. Flow chart of a wet-milling process for isolation of wheat bran starch by alkaline extraction [developed based on Xie et al (2008) and Verwimp et al (2004)].

Endosperm starch was obtained from the milled wheat flour fraction based on a method published by Verwimp et al (2004) with some modifications. The modifications were as follows: centrifugation steps were at $5000\times g$ (10 min, 20 °C); wheat flour (100 g) was suspended in 700 ml 0.25% (w/v) NaOH solution with stirring for 60 min; the slurry was centrifuged, then the sediment was washed with 350 ml of water by stirring for 30 min; after centrifugation, the sediment was washed with 160 ml water for 15 min followed by centrifugation; the sediment was suspended in 80 ml water and stirred for 10 min and neutralized by 1.0 M HCl solution, followed by centrifugation; the white starch was suspended in 200 ml water and passed through a 75 μm nylon screen; about 300 ml water was used to wash the overs; the throughs were centrifuged and sediment was the endosperm starch isolate.

All starch isolates were freeze-dried and gently ground using a mortar and pestle, sieved through a 425 μm stainless steel screen (40US), and stored at 4°C until further analyses. Starch isolation yields were recorded.

3.3.3 Starch analyses

Total starch content of milled bran fraction and flour fraction were determined in triplicate according to the procedure in the Total Starch Assay Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland). Milled bran fraction was ground into powder using a Cyclone Sample Mill (UDY Corp., Fort Collins, Co) equipped with 0.5 mm screen. Ground bran (100 mg) was mixed with 0.2 ml of 80% (v/v) ethanol in a glass tube. Three ml of diluted alpha amylase [alpha amylase (3000 U/ml of Ceralpha reagent): sodium acetate buffer (pH 5, 100 mM) = 1:30 (v/v)] was added to each tube, followed by incubation in a boiling water bath for 6 min with vortexing at 2, 4, and 6 min. The tubes were placed in another water bath at 50 °C and 0.1

ml of amyloglucosidase (3300 U/ml of soluble starch) was immediately added. After incubation for 30 min, the content of each tube was transferred to 100 ml volumetric flasks to adjust volume to 100 ml with distilled water. The contents of the volumetric flasks were then transferred to 150 ml beakers and 3 ml aliquot of slurry was transferred to a centrifuge tube and centrifuged at $3200 \times g$ for 10 min at 25 °C. To 0.1 ml aliquot of supernatant, 3 ml of glucose oxidase-peroxidase-aminoantipyrine (GOPOD) reagent was added. Samples were incubated at 50 °C for 20 min. A spectrophotometer (Spectronic 5, Spectronic Instruments Inc., Rochester, NY, USA) was used to measure the absorbance for each sample at 510 nm against the reagent blank (0.1 ml of deionized water and 3 ml of GOPOD reagent). Total starch (%) on a dry weight basis was calculated. Milled flour (100 mg) was analyzed as above for total starch content.

Amylose content, damaged starch content, and resistant starch content were determined in triplicate according to Megazyme kit methods (Megazyme International Ireland Ltd. Co., Wicklow, Ireland, 2006) for each sample of endosperm starch and bran starch with the following modifications: for resistant starch determination, centrifugation at $12900 \times g$ was used to recover resistant starch after adding 99% ethanol to terminate hydrolysis by enzymes (amylase and amyloglucosidase) as well as after adding 50% ethanol in washing steps; for amylose content determination, centrifugation at $5440 \times g$ was used to precipitate starch in ethanol and recovering the precipitated starch.

3.3.4 Proximate analyses

Total lipid content in starch was determined based on the method of Morrison et al (1980). Each sample (500-750 mg) was accurately weighed into screw-cap tubes and 2 ml methanol was added. Each tube was flushed thoroughly with nitrogen and 1.8 ml concentrated

HCl was added. The tubes were vortex mixed, heated in a 100 °C heating block for 1 h (with vortexing every 5 min), and cooled to room temperature. After hydrolysis, chloroform (2 ml) was added, the tubes were vortexed, then centrifuged (1000×g, 15 min). About 2 ml chloroform was withdrawn from the bottom layer in the tube and the solvent was removed under nitrogen. Hexane (2 ml) was added into the dried tube to solubilize lipid, and then the solution was transferred to a clean pre-weighed tube (dried at 100 °C for 1 hr). The solvent was removed again under nitrogen. The tube with lipid was re-weighed and total lipid content was calculated. Protein content of starch isolate was determined by Leco nitrogen analyzer (LECO Corp., St. Joseph, MI, USA) (AACCI Method 46-30).

3.3.5 Particle size distribution

Granule size distribution of a starch was determined on a laser-diffraction particle analyzer (Malvern, Hydro 2000 SM). The instrument is based on the principle of laser-light-scattering and capable of measuring sizes down to 0.01 μm. The starch suspension was transferred into the dispersion tank containing isopropyl alcohol and the starch granule particle size was measured. Each starch sample (25-30 mg) was slurried with 3-5ml of distilled water in a glass vial, and was vortexed for 10 s and sonicated for 30 s. The size distribution was expressed in terms of the volumes of equivalent spheres.

3.3.6 Pasting properties

Pasting properties of bran starch and endosperm starch at a concentration of 8% (w/w, dry starch basis) were determined using a Rapid Visco Analyzer RVA-4 (Newport Scientific Pty.

Ltd, Warriewood, NSW, Australia) (AACCI Method 76-21). Each sample was analyzed in at least duplicate.

3.3.7 Crystalline structure of starches

X-ray diffraction patterns of the starch samples were obtained using a diffractometer (XDS 2000, Scintag Inc, CA) with copper K-alpha emission radiation. The X-ray source was operated at 35 mA and 35 kV. Two theta scans with an angular range from 5 ° to 43 ° at a scan speed of 0.02 deg/min in continuous mode were performed. Starch samples were equilibrated in a 98% humidity chamber for 24 h at 25 °C. The crystallinity (%) was calculated by equation: $\text{Crystallinity (\%)} = 100 \times A_c / (A_c + A_a)$, where A_c is the crystalline area on the X-ray diffractogram and A_a is the amorphous area.

3.3.8 Thermal properties

Thermal properties of starches were determined using a differential scanning calorimeter (DSC) (Q100 Differential Scanning Calorimeter; TA Instruments, New Castle, DE) for evaluating starch gelatinization and retrogradation. Starches were weighed into aluminum hermetic pans and distilled water was added to make suspensions containing 70% moisture. Pans were sealed and equilibrated for 2 h at room temperature before testing. The scanning temperature range and heating rate were 20-120 °C and 5 °C/min, respectively. An empty pan of the same size was used as a reference. The enthalpy of gelatinization (ΔH), onset temperature (T_o), peak temperature (T_p) and the conclusion temperature (T_c) were measured from the endotherm in DSC thermograms using a software (Universal Analysis, TA Instruments). Each sample was analyzed in triplicate. For retrogradation, DSC-gelatinized samples in the pans were

stored at 4 °C for 14 days and subsequently rescanned with the same heating rate and temperature range.

3.3.9 Amylopectin bran chain-length distribution

Starch polymer was debranched using isoamylase and incubation at 40 °C for 24 hr. The amylopectin branch chain-length distributions were analyzed by a high performance anion exchange chromatography system (HPAEC, Dionex Co., Sunnyvale, CA). A Carbopac PA200 (3×250 mm) column and a Carbopac guard column (3×50 mm) were employed. The eluents A and B were 100 mM sodium hydroxide, and 100 mM sodium hydroxide in 500 mM sodium acetate solution, respectively. The gradient of eluent B was 0% at 0 min, 30% at 2 min, 40% at 5 min, 55% at 20 min, 60% at 25 min, and 80% at 45 min. The eluent gradient was operated at a 0.4 ml/min flow rate. The results were obtained from at triplicates of HPAEC for each amylopectin sample.

3.3.10 Morphology of starch granules

The scanning electron micrographs of wheat bran starch compared with endosperm starch were taken with a Hitachi tabletop microscope TM 3000 (Hitachi Corp., Tokyo, Japan). Starch samples were affixed onto the center of conducting double-sided tape which was then attached to the specimen stub. Specimen stub was placed on the specimen height gauge to adjust its height. Hitachi TM 3000 application software was used for observation and data recording. Visual observation was conducted with ×1500 magnifications. The scale bar indicates 50 μm is noted in each image.

3.3.11 Statistical analysis

A completely randomized design was used for all experiments. The data were analyzed using two-way analysis of variance (ANOVA) in Statistical Analysis System (SAS, Inc, 2006). When differences among results for starch type (bran starch or endosperm starch) and variety (Aubrey, Caledonia, and D8006) were found to be statistically significant ($\alpha = 0.05$), comparisons were conducted using Fisher's Least Significant Difference (LSD) procedure.

3.4 RESULTS AND DISCUSSION

3.4.1 Starch isolation and starch purity

An isolation procedure using NaCl and toluene for wheat bran starch (Xie et al 2008) was initially attempted for current study. Bran starch can be isolated by this procedure with 86.77% starch recovery and 0.17% protein content, however, starch recovery for endosperm starch isolated by this procedure was extremely low (Table 3.1).

Table 3.1 Starch Yield, Starch Recovery and Comparison of Purity of Wheat Bran Starch and Endosperm Starch Isolated using Two Different Methods^{x,y} from Soft Wheat Variety Caledonia Grown at Huron in 2010^z

Sample	Total Starch (%)	Starch Yield (%)	Starch Recovery (%)	Protein Content (%)	Starch Damage (%)
Br Starch ^x	15.96 ± 0.12	13.85 ± 0.54	86.77 ± 2.73	0.17 ± 0.01	1.03 ± 0.15
Br Starch ^y	15.88 ± 0.25	13.49 ± 0.03	84.97 ± 0.17	0.19 ± 0.08	1.68 ± 0.17
En Starch ^x	80.22 ± 2.53	6.38 ± 1.08	7.95 ± 1.35	0.68 ± 0.43	—
En Starch ^y	77.08 ± 4.44	70.64 ± 1.11	91.75 ± 3.84	0.16 ± 0.05	1.51 ± 0.20

^x Starch isolated by 0.5 M NaCl and toluene according to Xie et al (2008).

^y Starch isolated by alkaline extraction described in section 3.3.2.

^z Values are means ± standard deviation. Br: bran; En: endosperm.

An isolation procedure by alkaline extraction was then developed (Fig. 3.1) based on Verwimp et al (2004) and Xie et al (2008) with modifications. This alkaline extraction procedure proved to be suitable for both bran starch and endosperm starch isolation (Table 3.1). The modifications were made based previous studies showing that alcohol helps to solubilize alcohol-soluble protein in bran and is removed by centrifugation through the supernatant (Karlsson et al 1983), and that alkaline solution disperses protein matrix and leaves the starch free of protein (Mistry et al 1992).

The starch yield varied between bran starch and endosperm starch isolation using the two different procedures (Table 3.1). Starch recovery was the highest (91.75%) for endosperm starch isolated by alkaline procedure. Protein contents of isolated starch samples were less than 0.2% for both extraction procedures. Starch purity was acceptable for either of the tested procedures. In addition, the levels of starch damage were less than 1.68%, revealing the grinding steps did not cause further starch damage. According to the results obtained, bran starch isolated by 0.5 M NaCl and toluene had lower protein residue than that from the alkaline extraction method, and starch recovery was a little higher than what was obtained by the alkaline method (Table 3.1). However, starch recovery for endosperm starch isolated by 0.5 M NaCl and toluene was extremely low (7.95%), thus no starch analyses were conducted. The toluene to water ratio (1:4, Xie et al 2008) probably was not adequate to separate substantial protein from the starch in endosperm flour. A higher toluene to aqueous solvent ratio might be needed to dissolve endosperm protein (Lopez-Ahumada et al 2010). In order to compare bran starch with endosperm starch isolated under the same conditions, alkaline extraction was then chosen for all starch isolations in the remainder of this study for consistent recovery and testified feasibility.

In the samples isolated for starch characterization and comparison studies, a higher recovery of starch was obtained from endosperm than from bran (Table 3.2). Protein contents for all isolated samples were less than 0.36%. Starch damage levels were less than 2.5%, which means the grinding step during isolation did not cause further starch damage. The differences in percent starch damage and protein content of Caledonia samples between isolations for starch characterization (Table 3.2) and isolation procedure development (Table 3.1) may be attributed to different growing environments (crop year 2010 vs 2011). Total lipid contents were generally less than 0.45% (w/w) (Table 3.3) and were not significantly different from each other, thus it is unlikely they had a significant impact on the studied starch properties.

Table 3.2 Yield, Starch Recovery and Comparison of Purity of Bran Starch and Endosperm Starch Isolated from Three Wheat Varieties for Starch Characterization^x

Sample ^y	Total Starch (%)	Starch Yield (%)	Starch Recovery (%)	Protein Content (%)	Starch Damage (%)	Total Lipid Content (%)
Br-Aubrey	21.64 ± 0.67	19.47	89.97	0.36 ± 1.85	1.67 ± 0.04	0.37 ± 0.02
En-Aubrey	76.30 ± 1.87	70.06	91.82	0.29 ± 1.90	2.48 ± 0.03	0.42 ± 0.01
Br-Caledonia	20.39 ± 0.45	17.93	87.94	0.27 ± 0.99	1.71 ± 0.02	0.35 ± 0.02
En-Caledonia	77.62 ± 0.30	73.42	94.58	0.36 ± 1.07	2.34 ± 0.08	0.39 ± 0.03
Br-D8006	20.51 ± 0.27	19.16	93.42	0.31 ± 1.29	1.86 ± 0.07	0.45 ± 0.02
En-D8006	77.44 ± 0.56	72.62	93.78	0.34 ± 0.68	2.48 ± 0.03	0.41 ± 0.03

^x Values are means ± standard deviation.

^y Br: bran starch; En: endosperm starch.

Table 3.3 Physicochemical Properties of Bran Starch and Endosperm Starch^x
Isolated from Three Wheat Varieties

Sample	Small Granule (%)	Amylose Content (%)	Total Lipid Content (%)	Crystallinity (%)
Br-Aubrey	34.59b	24.63b	0.37a	21.75b
En-Aubrey	30.90a	23.67a	0.42a	18.71a
Br-Caledonia	32.02b	25.87b	0.35a	21.39b
En-Caledonia	29.17a	23.03a	0.39a	19.99a
Br-D8006	37.88b	25.16b	0.45a	21.06b
En-D8006	26.20a	23.02a	0.41a	18.69a

^x Values marked with the same letters are not significantly different from each other ($\alpha = 0.05$). Comparisons were conducted between Br and En starches within the same variety. Br: bran starch; En: endosperm starch.

3.4.2 Granule size distribution

It is widely acknowledged that wheat contains two types of starch granules at maturity: large A-type granules ($>10\ \mu\text{m}$) small B-type granules ($\leq 10\ \mu\text{m}$) (Ever et al 1974; Eliasson and Karlsson 1983; Karlsson et al 1983; Dengate and Meredith 1984; and Soulaka and Morrison 1985). Different granule types are developed in the endosperm during different periods of grain development. The A-type granules appear four days after anthesis and then continuously increase in size throughout the grain-filling period, whereas the B-type granules started being synthesized at 12-14 days after anthesis and remain relatively small in size (Karlsson et al 1983 and Parker 1985). Bechtel et al (1990) reported the existence of another distinct class of small granules (C-type) that was synthesized 21 days after flowering. A-, B-, and C-type granules are more a function of when their syntheses were initiated rather than their final size. Results in the present study confirmed a trimodal distribution of wheat starch according to Bechtel et al (1990) and Raeker et al (1998). However, cutoff points for differentiating three populations of granules of present study were different from Bechtel et al (1990) and Raeker et al (1998). Bechtel et al (1990) reported cutoff points 5 and 16 μm , Raeker et al (1998) reported cutoff points 2.8 and 9.9 μm , whereas cutoff points for endosperm starch (control) found in current study were about 1.9 and 10 μm . This could be attributed to different techniques used during starch isolation, particle size determination, wheat varieties assessed, spherical equivalent, or the different methods used to calculate particle size. Starch granules smaller than 10 μm were labeled as small granules in Table 3.3. Bran starch contained significantly greater amounts of small starch granules than does its counterpart endosperm starch (Table 3.3). A representative granule size distribution graph of bran starch and endosperm starch isolated from variety D8006 is shown in Fig. 3.2. The granule size distribution graph by volume depicts the basic three populations of starch granules for both

bran starch and endosperm starch. However, the large-granule peak of bran starch shifts towards left side of the scale and peak started at smaller granule size than endosperm starch (Fig. 3.2). Also bran starch shows a much broader peak than endosperm starch for second size population (1.9-10 μm). The second peak for bran starch is more like a shoulder to the major large granule population. Normally, the numbers of A-type large granules are fewer than those of B-type small granules, and they represent the majority of the mass of the starch. A and B-type starch granules have different chemical compositions and functional properties, including different amylose and lipid contents, pasting properties, and baking qualities (Maningat and Seib 1997). Granule size and starch granule morphology affect the physicochemical properties of starch, such as pasting viscosity, gelatinization and retrogradation properties. Different sizes and shapes of the A- and B-type granules determine their commercial applications (Ao and Jane 2007). The findings from the current study suggest that the starch granules close to the wheat kernel's outer tissue developed later than starch granules located in the inner core of the wheat kernel, resulting in the presence of more small granules in the bran fraction upon milling.

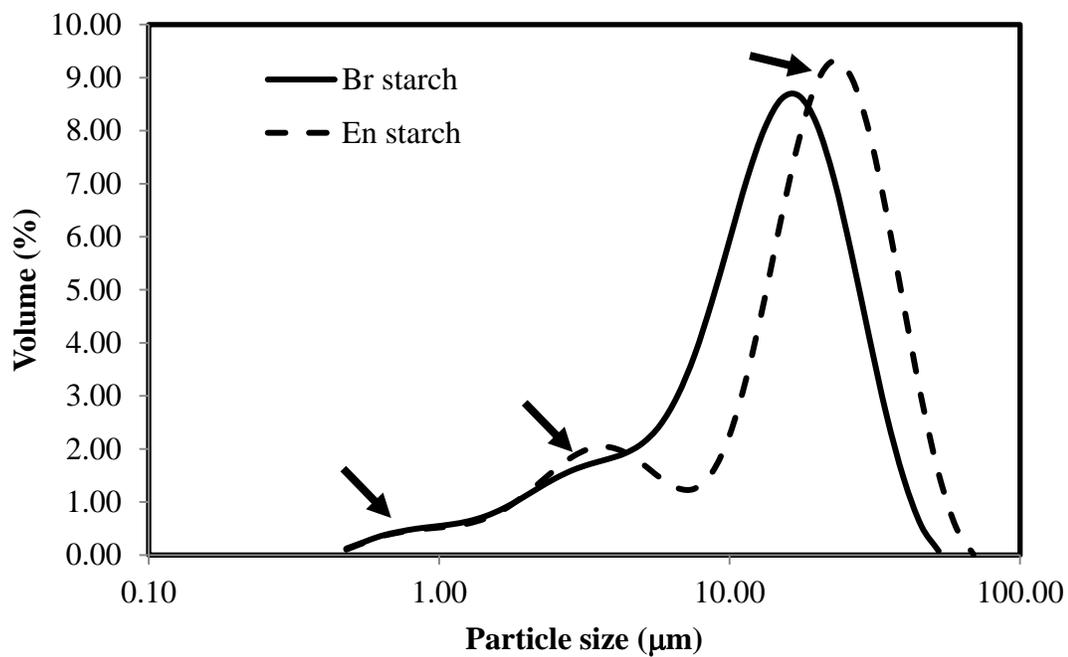


Figure 3.2. Representative granule size distribution of bran (Br) starch and endosperm (En) starch isolated from variety D 8006. Three arrows show the three populations of En starch granules [left to right: <1.9 µm, 1.9–10 µm, >10 µm].

3.4.3 Pasting properties

A typical rapid viscosity analyzer (RVA) pasting profile of bran starch compared to endosperm starch is shown in Fig.3.3. Results of the studies varieties are summarized in Table 3.4. Generally, bran starch had lower peak, setback and final viscosities than its counterpart endosperm starch. Pasting properties of starch are affected by amylose content, granule size, lipid content and branch chain-length distribution of amylopectin (Jane et al 1999). Starch rheology is mainly influenced by particle size: suspensions of large-size particles tend to be more viscous (Wong et al 1982). As shown in Table 3.3, bran starch had more small B-type granules than did endosperm starch, and was less viscous and had a lower pasting viscosity. These phenomena are also consistent with previous findings that B-type granules (small granules) significantly decreased the viscosity of the starch (Ao and Jane 2007). During heating in water, amylopectin in starch contributes to swelling of the starch granules and starch paste formation, while amylose and lipids inhibit the swelling process (Tester and Morrison 1990). Bran starch had higher amylose content than endosperm starch (Table 3.3); this was associated with a smaller degree of swelling during heating, which could explain the lower viscosity as well. It also confirms the very low level of lipid present in the samples had minimal effect on granule swelling.

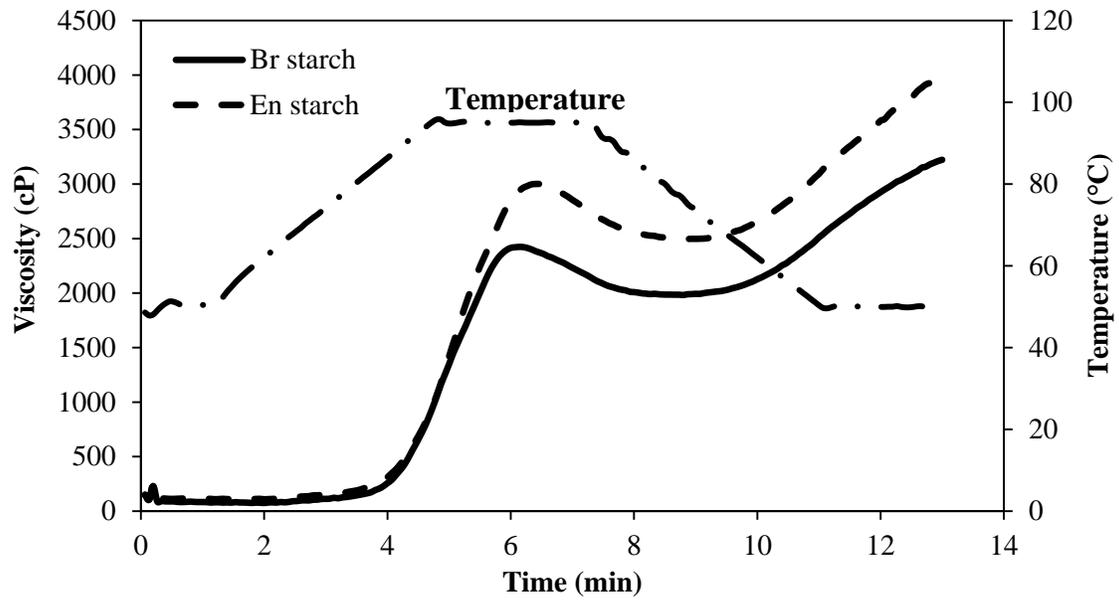


Figure 3.3. Comparison of RVA pasting curves of bran (Br) starch and endosperm (En) starch isolated from variety Aubrey.

Table 3.4 Pasting Properties of Bran Starch and Endosperm Starch Isolated from Three Varieties^x

Sample	Viscosity (cP)			
	Peak	Breakdown	Final	Setback
Br-Aubrey	2437.5	457.0	3222.5	785.0
En-Aubrey	3003.0	508.0	3986.5	983.5
Br-Caledonia	2311.5	506.9	3147.0	835.5
En-Caledonia	2522.5	454.0	3509.0	986.5
Br-D8006	2417.5	580.5	3188.0	770.5
En-D8006	3056.5	554.0	4071.0	1014.5

^x Values are means of triplicated results. Br: bran starch; En: endosperm starch.

3.4.4 Crystalline structure

Bran starch and endosperm starch both displayed typical A-type X-ray diffraction patterns, with peaks found at $2\theta = 15^\circ, 17^\circ, 18^\circ,$ and 23° (Fig.3.4). Starches with amylopectins of relatively short average branch chain lengths, such as maize, rice, wheat, tapioca, and sweet potato, display the A-type X-ray diffraction pattern with peaks at $2\theta = 15^\circ, 17^\circ, 18^\circ$ and 23° (Jane et al 1997). Other starches that have amylopectins with long branch chains, such as potato, canna, and high amylose maize, display the B-type X-ray pattern with peaks at $2\theta = 5.5^\circ, 15^\circ, 17^\circ, 22^\circ,$ and 24° . Starches with amylopectins of branch chain length in between the above two groups display the C-type X-ray pattern which is a mixture of the A- and B-type crystalline structures (Jane et al 1997). Bran starch in the present study with a higher percent of B-type granules and higher amylose content was found to have higher crystallinity than its counterpart endosperm starch (Table 3.3). Previous reports have differed on types of granules, amylose content, and starch crystallinity in starch with a high A-type granule concentration and starch with a high B-type granule concentration. Some researchers (Raeker et al 1998, Chiotelli and Lemeste 2002) indicated that A-type granule starch is more crystalline (higher in amylose content) than B-type granule starch. Others reported less crystallinity was found in small granules than in large granules (Li et al 2007). There was no statistical difference found in amylose contents of A- and B-types of granule starch (Dengate et al 1984). Xie and coworkers (2008) reported that wheat bran starch containing a higher content of B-type granules exhibited higher crystallinity and amylose content compared with commercial wheat starch. These results are in agreement with the present findings. The crystalline property of the granules of common starches arises from the organization of the amylopectin molecules within the granules, while amylose largely makes up the amorphous regions which are randomly distributed between the

amylopectin clusters (Ao and Jane 2007). But in the present study, bran starch had a higher percent B- type granules and higher amylose content, and subsequently a higher crystallinity than the counterpart endosperm starch for each of the three studied varieties (Table 3.3). These results indicate that the B-type granules isolated from wheat bran could be different in composition and properties compared to those isolated from the representative endosperm. The findings may be attributed to the amylopectin branch chain length distribution resulting in differences in crystallinity (Jane et al 1999).

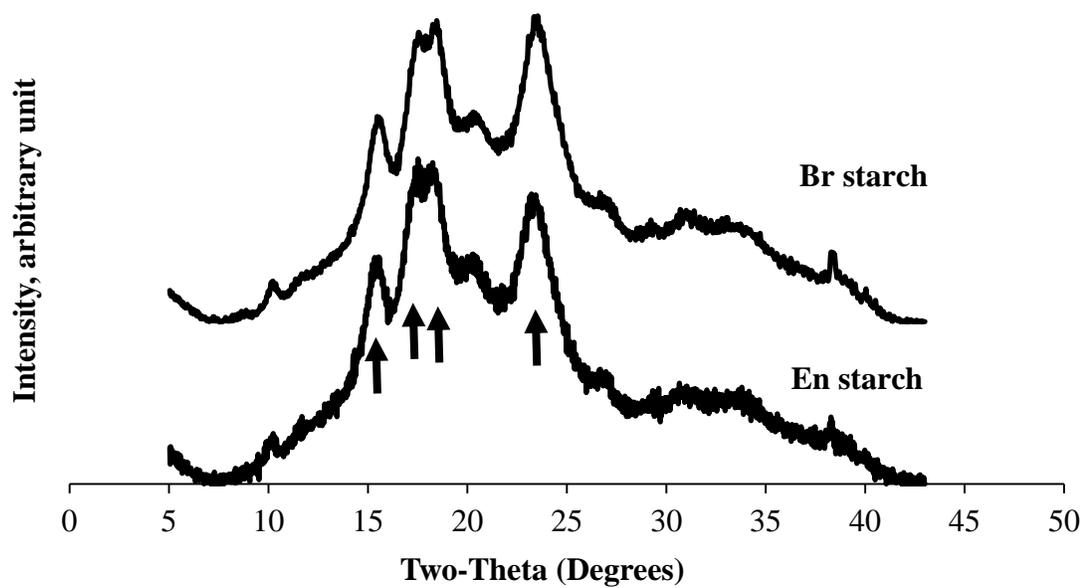


Figure 3.4. Comparison of X-ray diffraction patterns of bran (Br) starch and endosperm (En) starch. A-Type X-ray diffraction patterns with peaks at 15, 17, 18, and 23 degrees (indicated by arrows) are found.

3.4.5 Thermal properties

Gelatinization and retrogradation endotherm of studied starches are shown in Table 3.5 and 3.6. Enthalpy change (ΔH), gelatinization onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c) and gelatinization temperature ranges (T_c-T_o) were computed. Bran starch samples had broader gelatinization temperature range (lower onset temperature and higher conclusion temperature), and higher enthalpy change (ΔH) than their counterpart endosperm starch samples (Table V). These results are consistent with the observation that wheat bran starch had a higher crystallinity (Table 3.3) and required more energy for gelatinization than the endosperm starch. Bran starch was found to have a greater amount of small B-type granules in this study and showed broader ranges of gelatinization temperatures than those of endosperm starch samples. These findings are in general agreement with previous findings that small granule starch had a broader gelatinization temperature range than large granule starch (Ao and Jane 2007; Eliason and Karlson 1983).

Table 3.5 Characteristics of Gelatinization Endotherms of Bran starch and Endosperm starch
Isolated from Three Wheat Varieties^x

Sample	T _o	T _p	T _c	T _c -T _o	ΔH (J/g)
Br-Aubrey	57.2 ±0.0	62.9 ±0.4	75.7 ±0.86	18.5 ±0.8	10.3 ±0.1
En-Aubrey	58.0 ±0.1	62.6 ±0.3	72.3 ±0.78	14.3 ±0.9	8.4 ±0.7
Br-Caledonia	56.0 ±0.3	61.9 ±0.0	74.7 ±0.00	18.7 ±0.3	10.4 ±0.2
En-Caledonia	56.6 ±0.1	61.4 ±0.1	71.6 ±0.86	15.0 ±0.8	8.9 ±0.0
Br-D8006	56.2 ±0.0	61.6 ±0.2	74.6 ±0.35	18.4 ±0.3	11.3 ±0.3
En-D8006	56.7 ±0.2	61.4 ±0.1	71.8 ±0.28	15.2 ±0.4	9.2 ±0.1

^x Values are means ± standard deviation. T_o, T_p and T_c = onset, peak and conclusion temperatures (°C), respectively, of endotherms. ΔH = enthalpy change of gelatinization. Br: bran starch; En: endosperm starch

Table 3.6 Characteristics of Retrogradation Endotherms of Bran Starch and Endosperm Starch Isolated from Three Wheat Varieties after storage at 4 °C for 14 days^x

Sample	T _o	T _p	T _c	ΔH (J/g)	Retrogradation (%) ^y
Br-Aubrey	45.0 ± 0.4	54.7 ± 0.4	64.9 ± 0.8	3.8 ± 0.0	36.4
En-Aubrey	46.0 ± 0.6	56.4 ± 0.2	64.2 ± 0.8	3.1 ± 0.1	37.4
Br-Caledonia	43.9 ± 0.1	52.3 ± 0.1	59.8 ± 0.1	2.5 ± 0.2	24.0
En-Caledonia	45.9 ± 0.0	54.8 ± 1.6	62.0 ± 1.0	2.2 ± 0.0	24.5
Br-D8006	45.8 ± 2.9	53.5 ± 2.2	60.7 ± 1.9	2.4 ± 0.4	21.7
En-D8006	45.8 ± 2.0	54.0 ± 1.1	61.1 ± 0.6	3.0 ± 0.3	33.2

^x Values are means ± standard deviation. T_o, T_p and T_c = onset, peak and conclusion temperatures (°C) of endotherm. Δ H = enthalpy change of dissociation of retrograded starch. Br: bran starch; En: endosperm starch

^y % Retrogradation = 100 × (enthalpy change of retrograded starch/enthalpy change of native starch).

For the retrogradation study, DSC-gelatinized starches were retrograded for 14 days at 4 °C. Thermal property results are shown in Table 3.6. This can be considered as long-term storage, and both amylose and amylopectin recrystallize and contribute to the retrogradation process. During native starch retrogradation, both amylose and amylopectin eventually play their parts: swollen amylopectin-enriched granules with interpenetrating amylose gel matrix form a composite gel network (Miles et al 1985). However, there is short-term development of gel structure via amylose crystallization, and long-term reordering of amylopectin which is a much slower process involving recrystallization of the outer branches (DP 15) of amylopectin (Miles et al 1985; Ring et al 1987). A previous study found that after 15 days storage at 5 °C, native waxy starch retrogradation enthalpy reaches a near-constant final value (Liu et al 2010). In the present study, during storage with conditions of 14 days at a low temperature (4 °C), amylopectin was expected to recrystallize extensively and contribute to the retrogradation. During long-term storage, the recrystallization of amylopectin causes increasing rigidity of the swollen granules, thereby reinforcing the continuous amylose phase (Liu et al 2010). It is known that amylose molecules and long-branch chains of amylopectin retrograde quickly (Li et al 2007). The retrogradation values (%) of the bran starch samples studied were lower than those of their counterpart endosperm starch samples. This could be attributed to the fact that bran starch had greater quantities of small B-type granules, which have fewer long branch chains (DP > 25) and more short branch chains (DP 6-12) than endosperm starch. The trend for retrogradation among different varieties and types of starch (Table 3.6) followed the same trend of setback viscosity measured via RVA (Table 3.4), since setback is an index of retrogradation tendency in starch paste (Ancona et al 2001). Bran starch of Br-D8006, with the highest percent small granules

(Table 3.3), had the lowest setback viscosity and lowest retrogradation degree (%) of all bran and endosperm starch samples studied (Table 3.4 and 3.6).

3.4.6 Amylopectin branch chain length

In order to compare the amylopectin molecules between bran starch samples and their respective endosperm starch samples, the distributions of the amylopectin chain length of bran starch and endosperm starch from the three varieties were measured (Table 3.7). The averaged distribution of amylopectins side chain lengths of the three varieties is shown in histogram in Fig. 3.5. The average amylopectin molecule of bran starch had more branch chains of DP 6-12 and fewer branch chains of DP 25-36 than did the amylopectin of the counterpart endosperm starch. Bran starch of Caledonia and D8006 had fewer long B₂ chains (DP > 37) than their counterpart endosperm starches (Table 3.7). Details of the chain length differences are shown in the differential histograms of the chain-length distribution between bran starch and endosperm starch for three different varieties (Fig.3.6A-C). Bran starch contained more short branch chains for varieties Aubrey (DP 6-20) and Caledonia (DP 9-19) than did the counterpart endosperm starch samples. Variety D8006 bran starch had more short chains of DP 6-20 than its counterpart endosperm starch. These results showed that bran starch generally consists of more A (DP: 6-12) chains than endosperm starch for the studied varieties. According to the retrogradation results (Table 3.6), bran starch of variety D8006 was found to have the lower retrogradation (%) than bran starches of the other two studied varieties, correspondingly, had wider range of short branch chains in which bran starch had more distribution than their counterpart endosperm starch. These results confirm that amylopectin side chain length distribution play an important role in retrogradation in bran starch. Bran starch samples in the present study was found to have more

short branch chains of DP 6-12 (Table 3.7) and tended to have lower degree of retrogradation (%) than their respective endosperm starch (Table 3.6), which is in agreement with previous reported results that retrogradation rates of starches were inversely correlated with the proportion of short chains (Shi and Seib 1992).

Table 3.7 Branch Chain Length Distribution of Amylopectin from Bran Starch and Endosperm Starch Isolated from Three Varieties^x

Sample	Distribution (%)			
	DP 6-12	DP 13-24	DP 25-36	DP \geq 37
Br-Aubrey	22.0 \pm 1.2	48.2 \pm 1.5	16.7 \pm 0.8	11.0 \pm 1.0
En-Aubrey	21.4 \pm 0.9	49.2 \pm 0.5	19.5 \pm 0.3	10.9 \pm 0.8
Br-Caledonia	21.3 \pm 0.0	49.8 \pm 0.4	19.3 \pm 0.5	9.9 \pm 0.6
En-Caledonia	20.0 \pm 0.7	49.3 \pm 0.3	20.8 \pm 0.5	10.8 \pm 0.3
Br-D8006	21.3 \pm 0.5	49.5 \pm 0.8	19.2 \pm 0.2	8.5 \pm 0.2
En-D8006	20.2 \pm 0.7	49.5 \pm 0.7	20.7 \pm 0.9	9.1 \pm 0.6

^x Values are means \pm standard deviation. Br: bran starch; En: endosperm starch

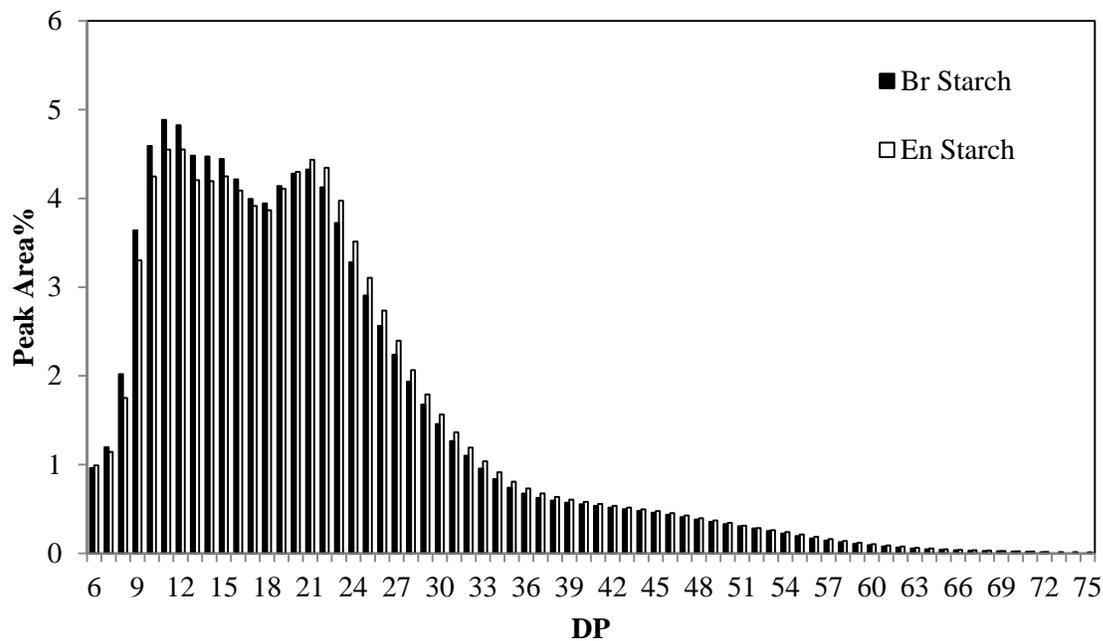


Figure 3.5. Averaged amylopectin branch chain-length distributions of bran starches and the counterpart endosperm starches from three different wheat varieties using a high-performance anion-exchange chromatography system. DP: degree of polymerization. Br: bran starch; En: endosperm starch.

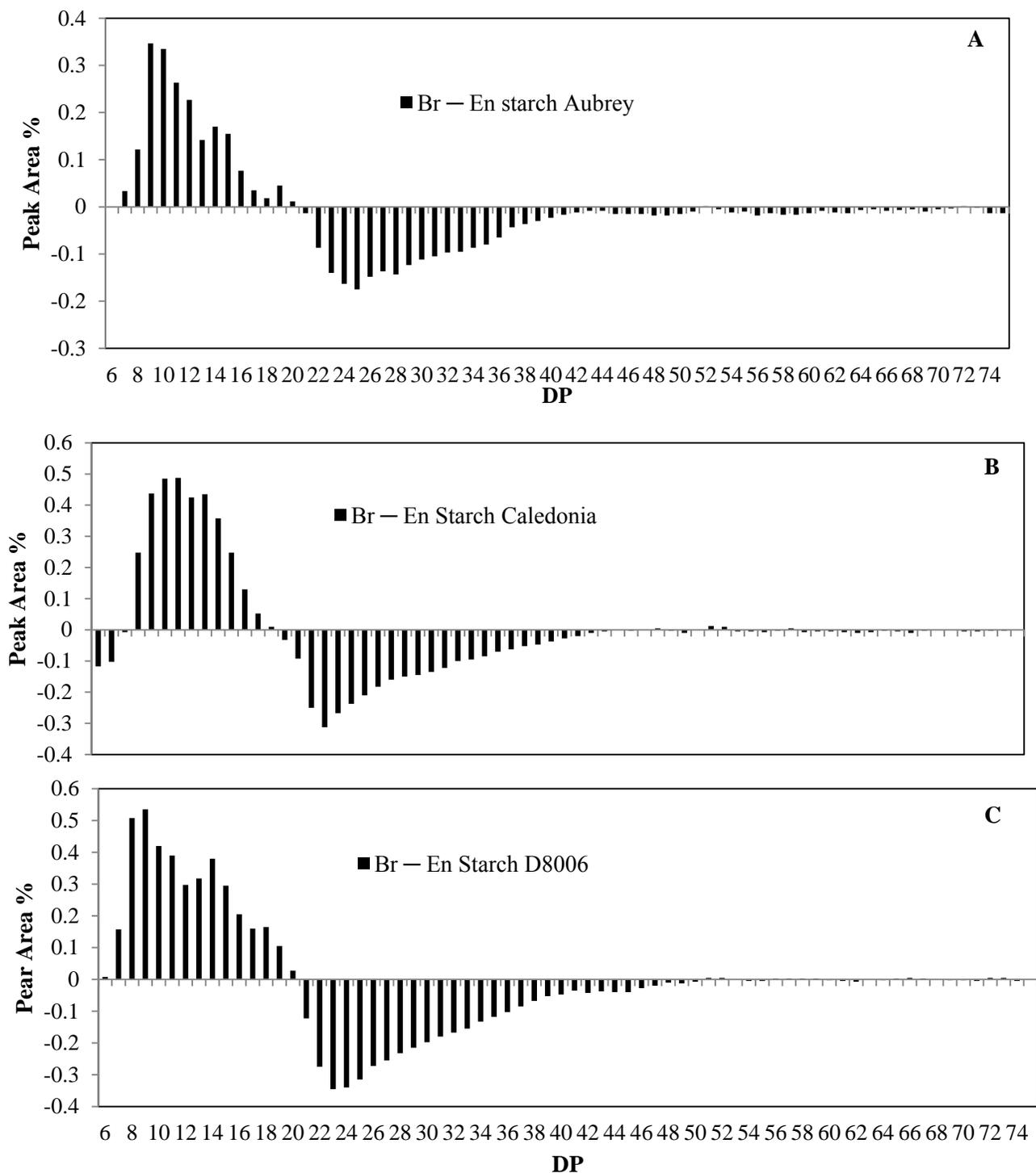
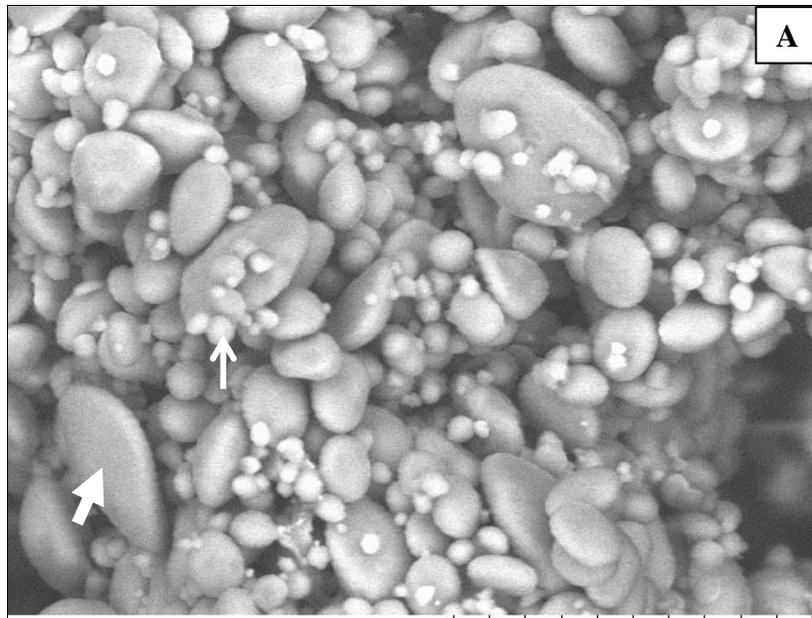


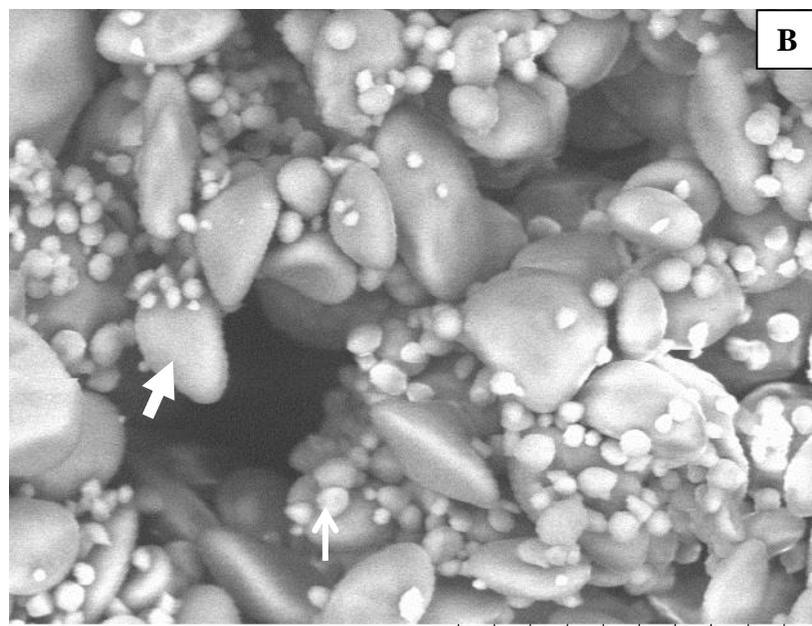
Figure 3.6. Differential histograms of amylopectin branch chain-length distributions for bran starch and endosperm starch isolated from wheat varieties (A) Aubrey, (B) Caledonia, and (C) D8006. DP: degree of polymerization; Br: bran starch; En: endosperm starch.

3.4.7 Starch granule morphology

The isolated bran starch and its counterpart endosperm starch granules had bimodal size distributions (Fig.3.2). Representative SEM images of variety D8006 (Fig.3.7) confirmed this finding, and also confirmed that small sized starch granules were not lost during isolation steps. The A-type starch granules displayed a disk shape with diameters of 10-30 μm , and the B-type granules displayed a spherical shape with diameters of about 2 μm . The morphology of the starch was in agreement with published results (Jane et al 1997; Van de Velde et al 2002). Small granules in bran starch were roughly spherical or polygonal in shape with diameters smaller than 10 μm ; large granules in bran starch were disc-like and lenticular with size greater than 10 μm (Fig.3.7). The scanning electron micrographs of the starch samples generally showed a greater proportion of small granules in bran starch than in endosperm starch. All granules seen in SEM images had smooth surfaces and intact structure which indicates the isolation procedure did not change the morphology of the starch granules.



50 μm



50 μm

Figure 3.7. Scanning electron micrographs of wheat bran starch (A) and its counterpart endosperm starch isolated from variety D8006 (B). Scale bar=50μm. → : B-type starch (small) granule; → : A-type starch (large) granule.

3.5 CONCLUSIONS

Isolation methods by alkaline extraction were developed to obtain bran starch and endosperm starch from milled bran fraction and milled flour fraction respectively. A higher starch recovery was obtained from endosperm flour than from milled bran. Protein contents for all isolated samples were less than 0.36%. Starch damage content was less than 2.5%, which indicates that the grinding step during isolation did not cause further starch damage. The findings of current study showed that the structures and properties of bran starch samples of three studied varieties and their counterpart endosperm are distinct. Bran starch was found to have higher percent small granules, higher amylose content, higher crystallinity, broader gelatinization temperature range, higher enthalpy of gelatinization, lower retrogradation, and lower pasting peak and setback viscosity than its counterpart endosperm starch. A-type X-ray diffraction patterns were found for both bran starch and endosperm starch. Bran starch of variety Aubrey had the highest crystallinity (21.75%) and gelatinization temperature (62.9 °C). Bran starch of variety D 8006 had the highest percent B-type granules and lowest retrogradation (21.7%). The bran starch consisted of amylopectin that had more short branch chains than did the counterpart endosperm starch. Bran starch had broader gelatinization temperature range which is related to presence of a greater proportion of small granules. Bran starch had lower degree of retrogradation than endosperm starch may be due to a higher percent of short branch chains and less long-branch chains. SEM images revealed small and large starch granules present in bran starch after starch isolation and a greater proportion of small granules in bran starch. Bran starch had lower peak, breakdown and setback viscosities than endosperm starch, which may be due to the greater proportion of small granules present in bran starch considering there were no significant differences in lipid content among the different starches. This may be also because

that higher amylose content is associated with less granule swelling. Amylopectin molecules of bran starch had more branch chains of DP 6-12 and fewer branch chains of DP 25-36 than did the amylopectin of the counterpart endosperm starch, which may explain the lower degree of retrogradation of bran starch compared to endosperm starch. Results showed that the structures and properties of bran starches and their counterpart endosperm starches were distinct, which suggests that considering their commercial end-use applications should be different.

LITERATURE CITED

LITERATURE CITED

- AACC International. 2000. *Approved Methods of Analysis*, 10th Ed. AACC International: St. Paul, MN.
- Ancona, D. A. B., Guerro, L. A. C., Matos, R. I. C., and Ortiz, G. A. 2001. Physicochemical and functional characterization of baby lima bean (*Phaseolus lunatus*) starch. *Starch* 53:219-226.
- Ao, Z., and Jane, J. 2007. Characterization and modeling of the A- and B-granule starches of wheat, triticale, and barley. *Carbohydrate Polymers* 67:46-55.
- Bechtel, D. B., Zayas, I., Kaleikau, L., and Pomeranz, Y. 1990. Size distribution of wheat starch granules during endosperm development. *Cereal Chem.* 67:59-63.
- Chiotelli, E., and LeMeste, M. 2002. Effect of small and large wheat starch granules on thermo-mechanical behavior of starch. *Cereal Chem.* 79:286-293.
- Dengate, H., and Meredith, P. 1984. Variation in size distribution of starch granules from wheat grain. *J. Cereal Sci.* 2:83-90.
- Eliasson, A. C., and Karlson, R. 1983. Gelatinization properties of different size classes of wheat starch granules measured with differential scanning calorimetry. *Starch* 35:130-133.
- Evers, A. D., Greenwood, C. T., Muir, D. D., and Venables, C. 1974. Studies on the biosynthesis of starch granules: A comparison of the properties of the small and the large granules. *Starch* 26:42-46.
- Gidley, M. J., and Bulpin, P. V. 1987. Crystallization of maltooligosaccharide as models of the crystalline forms of starch: Minimum chain-length requirement for the formation of double helices. *Carbohydr. Res.* 161:301-304.
- Gudmundsson, M., and Eliasson, A. C. 1990. Retrogradation of amylopectin and the effects of amylose and added surfactants/emulsifier. *Carbohydr. Polym.* 13:295-315.
- Hizukuri, S. 1985. Relationship between the distribution of the chain length of amylopectin and the crystalline structure of starch granules. *Carbohydr. Res.* 141:295-206.
- Jane, J., Shen, L., Lim, S., Kasemsuwan, T., and Nip, W. K. 1992. Physical and chemical studies of taro starches and flours. *Cereal Chem.* 69:528-535.
- Jane, J., Wong, K., and McPherson, E. 1997. Branch-structure difference in starches of A- and B-type X-ray patterns revealed by their Naegeli dextrans. *Carbohydr. Res.* 300: 219-227.

- Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A.E., Wong, K. S., Radosavljevic, M., and Kasemsuwan, T. 1999. Effects of amylopectin branch chain length and amylose content on the gelatinization and pasting properties of starch. *Cereal Chem.* 76:629-637.
- Karlsson, R., Olered, R., and Eliasson, A. C. 1983. Changes in starch granule size distribution and starch gelatinization properties during development and maturation of wheat, barley and rye. *Starch* 35:335-340.
- Li, L., Blanco, M., and Jane, J. 2007. Physicochemical properties of endosperm and pericarp starches during maize development. *Carbohydr. Polym.* 67:630-639.
- Liu, H., Yu, L., Tong, Z., and Chen, L. 2010. Retrogradation of waxy corn starch studied by DSC. *Starch* 62:524-529.
- Liu, Y., and Ng, P. K. W. 2012. Physicochemical properties of bran starches and endosperm starches of soft wheat grown in Michigan. AACC international annual meeting. Hollywood, FL.
- Lopez-Ahumada, G. A., Ramirez-Wong, B., Torres-Chavez, P. I., Bello-Perez, L. A., Figueroa-Cardenas, J. D., Garzon-Tiznado, J. A., and Gomez-Aldapa, C. A. 2010. Physicochemical characteristics of starch from bread wheat (*Triticum aestivum*) with “yellow berry”. *Starch* 62: 517-523.
- Miles, M. J., Morris, V. J., Orford, P. D., and Ring, S. G. 1985. The roles of amylose and amylopectin in the gelatinisation and retrogradation of starch. *Carbohydr. Res.* 135:271-281.
- Mistry, A. H., Schmidt, S. J., Eckhoff, S. R., and Sutherland, J. W. 1992. Alkali extraction of starch from corn flour. *Starch* 44:284-288.
- Parker, M. L. 1985. The relationship between A-type and B-type starch granules in the developing endosperm of wheat. *J. Cereal Sci.* 3:271-278.
- Perez, S., and Bertoft, E. 2010. The molecular structures of starch components and their contribution to the architecture of starch granules: A comprehensive review. *Starch/Starke* 62:389-420.
- Raeker, M. O., Gaines, C. S., Finney, P. L., and Donelson, T. 1998. Granule size distribution and chemical composition of starches from 12 soft wheat cultivars. *Cereal Chem.* 75:721-728.
- Ring, S. G., and Colonna, P. 1987. The gelation and crystallisation of amylopectin. *Carbohydr. Res.* 162:277-293.
- Seal, C. J., and Brownlee, I. A. 2010. Whole grains and health, evidence from observational and intervention studies. *Cereal Chem.* 87:167-174.
- Shi, Y. C., and Seib, P. A. 1992. The structure of four waxy starches related to gelatinization and retrogradation. *Carbohydr. Res.* 227:131-145.

- Shi, Y. C., and Seib, P. A. 1995. Fine structure of maize starches from four wx-containing genotypes of the W64A inbred line in relation to gelatinization and retrogradation. *Carbohydr. Polym.* 26:141-147.
- Soulaka, A. B., and Morrison, W. R. 1985. The amylose and lipid contents, dimensions, and gelatinization characteristics of some wheat starches and their A- and B-granule fractions. *J. Sci. Food Agric.* 36:709-718.
- Van De Velde, F., Van Riel, J., and Tromp, R. H. 2002. Visualisation of starch granule morphologies using confocal scanning laser microscopy (CSLM). *J. Sci. Food Agric.* 82:1528-1536.
- Verwimp, T., Vandeputte, G. E., Marrant, K., and Delcour, J. A. 2004. Isolation and characterization of rye starch. *J. Cereal Sci.* 39:85-90.
- Whistler, R. L., and Bemiller, J. N. 1997 Chapter 6 starch. Pages 117-152 in: *Carbohydrate Chemistry for Food Scientist*. AACC International: St. Paul, MN.
- Wong, R. B. K., and Lelievre, J. 1982. Rheological characteristics of wheat starch measured under steady shear conditions. *J. Appl. Polym. Sci.* 27:1433-1440.
- Xie, X., Cui, S. W., Li, W., and Tsao, R. 2008. Isolation and characterization of wheat bran starch. *Food Research International* 41:882-887.
- Yuan, R. C., Thompson, D. B., and Boyer, C. D. 1993. Fine structure of amylopectin in relation to gelatinization and retrogradation behavior of maize starches from three wx-containing genotypes in two inbred lines. *Cereal Chem.* 70:81-89.

**CHAPTER 4 RELATIONSHIP BETWEEN BRAN CHARACTERISTICS AND BRAN
STARCH OF SELECTED SOFT WHEATS GROWN IN MICHIGAN**

4.1 ABSTRACT

The aim of wheat dry milling is to separate the kernel's starchy endosperm from the outer layers, which are collectively called the bran. Wheat bran is a multi-layer system consisting of pericarp, seed coats, and the aleurone layer with some attached remnants of starchy endosperm. Bran starch, the starch attached to the bran, exhibits significantly different characteristics than its counterpart endosperm starch. However, its relationship to bran tissue has not been studied. The aims of this study were to investigate differences among chosen wheat varieties in their bran starch quantity, bran particle size, and bran thickness, and to investigate the relationship between bran characteristics and bran starch content. Wheat grain samples were milled to obtain the milled bran fraction. Bran particles larger than 2 mm were obtained by sizing with a US10 screen. Bran starch content was determined by Megazyme kit and bran thickness was measured after conditioning the bran. Non-cellulosic sugar profile in wheat bran was investigated by derivitizing sugar to sugar alditol acetates that were then injected into GC-MS. Total and free ferulic acid contents were determined by HPLC. Microstructure of outer layers of kernels and milled bran particles was examined on SEM.

Results showed that bran starch content was negatively correlated with percent large bran particles (> 2 mm). The neutral saccharide profile of the wheat bran was dominated by arabinose, xylose, and glucose, whereas mannose and galactose were present in small amounts. Variety D8006 had lower levels of arabinose and xylose in year 2009 than two other crop years studied. Bran thickness was found to have a positive correlation with bran starch content. Bound ferulic acid (BFA) and BFA to xylose ratio showed positive correlations with percent large bran particles, and therefore negative correlations with bran starch content. SEM images revealed that outer layers of wheat bran were deformed and the aleurone layer was no longer apparent after

milling. Milled bran particles were about twice as thick as intact outer layers of a wheat kernel in the present study. Bran characteristics can explain the variation seen in bran starch content and percent large bran particles of various wheat varieties. BFA to xylose ratio and bran thickness could both play roles in the mechanical properties of bran, and therefore change the percent of large bran particle produced during milling. The information on studied bran characteristics could be helpful for breeders to gain more precise control over the milling quality of wheat varieties with regard to their specific food applications.

4.2 INTRODUCTION

A wheat kernel is a multilayer system. Besides the embryo, from the center to the periphery of the grain, the wheat kernel consists of the endosperm, aleurone layer, the seed coats (composed of the nucellar epidermis and the testa), and the pericarp (composed of the tube cells, the cross cells, the hypodermis, and the epidermis). The aleurone layer, seed coats and pericarp are wheat outer layers, and collectively called the wheat bran. Wheat grains normally contain 14.5% (w/w) bran, 83% (w/w) endosperm, and 2.5% (w/w) germ. Wheat flour is the major product from a dry milling process and is fully used by the grain industry. However, most commercial wheat bran is sold as animal feed at a very low price. In order to improve the commercial value of wheat bran, bran utilization needs to be maximized and developed.

Previous research has confirmed that wheat bran contains a number of high-value components, such as phenolic compounds, starches, water-soluble and water-insoluble dietary fibers, and proteins (Peng et al 1999). Cereal dietary fiber, non-starch polysaccharides and resistant starch, are the major health-beneficial carbohydrates in cereal whole grain food products, and mainly found in the bran fraction. Sufficient consumption of dietary fiber has a

protective effect against development of diet-related disorders, such as cardiovascular disease and type II diabetes, and also against cancers, such as digestive-tract cancer, hormone-related cancers and pancreatic cancer (Seal and Brownlee 2010). Wheat bran is a major source of cereal dietary fiber which is believed to be beneficial for human health. In the milled bran fraction, starch remains one of the main components. Bran starch is the starch adherent to bran tissue after a dry milling process. Bran starch comprises about 20% (w/w) of the milled wheat bran fraction, and the physicochemical properties of bran starch have been compared to its counterpart endosperm starch (Liu and Ng 2012). However, the relationships between bran starch and bran characteristics have not been investigated for soft wheat grown in Michigan.

Softness equivalent is a measurement that indicates friability of the endosperm of the kernel and is an indicator of break flour yield and ease of separation between endosperm and outer layer tissues (Anonymous 2011). If milled flour has smaller particle size and greater break flour yield, it will have a greater softness equivalent. In other words, high friability of a wheat kernel's endosperm indicates better milling efficiency and reduced energy requirements to recover flour (Anonymous 2011). On the other hand, high milling efficiency with low bran contamination of the resulting flour requires bran with low friability (high extensibility). Peyron et al (2002a) found that polymers in bran tissue with low friability can slide and reorient during material deformation. Reducing this polymer mobility in the cell wall network could result in a reduction in extensibility and an increase in bran friability.

The physical properties of wheat bran tissues and the characteristics of the interface layer between these tissues may influence their behavior during milling and grinding. Bran friability affects the extent of particle size reduction obtained in grinding. Bran particle size is especially related to tissue extensibility (Peyron et al 2002a). A strong positive correlation between

extensibility of the wheat grain outer layers and the proportion of larger size coarse bran (> 2mm) was reported by Greffeuille and coworkers (2006). The large bran particles (LBP) are easily separated from recovered fine flour during the milling process, and assure low bran contamination. Since bran starch content is tightly related to the bran fractionation from endosperm during the milling process (Peyron et al 2002c), physical properties of wheat bran are expected to affect milling quality and therefore impact bran particle size and bran starch quantity and type.

In aleurone walls, where arabinoxylans (AX) account for 70% (w/w) of the polysaccharide material, the degree of AX cross-linking controlled by phenolic acids in the aleurone layer significantly influences the strength and extensibility of the bran (Renger and Steihart 2000; Bunzel et al 2000). Thus, the degree of AX cross-linking is one of the determinants of bran friability and must be taken into account in explaining bran physical properties (Peyron et al 2002a). The cross-linking of cell wall AX occurs as a result of a reaction (ferulic acid dimerization) that has an oxidative mechanism, probably mediated by endogenous peroxidases or phenol oxidases in wheat bran (Fry 2000 and Peyron et al 2001). Bran thickness, its biochemical composition and the degree of AX cross-linking in the cell walls have been proposed to be the major factors controlling the physical properties of bran (Peyron et al 2002a).

A previous study confirmed that ferulic acid predominated among the phenolic acids found in different varieties of wheat and barley (Klepacka and Fornal 2006). Parker et al (2005) found that ferulic acid accounted for approximately 95% (w/w) of the cell wall-bound esterified phenolics in mature wheat (*Triticum aestivum* L. cv. Avalon). Phenolic acids present in the cell wall are thought to play an important part in the cross-linking of polysaccharides with other cell-wall components, including lignin through ester and ether bonds, and also in the cross-linking of

polysaccharide chains such as AX. It was suggested that the ferulic acid released by concentrations of NaOH of up to 2N are esterified to the terminal arabinosyl units of AX (Parker et al 2005; Dupont and Selvendran 1987; Rombouts and Thibault 1986) and that the ferulic acid released by 4 N NaOH is probably ether-linked with the matrix polymers and/or with lignin (Dupont and Selvendran 1987). Based on these findings, the concentration of NaOH used in the process of hydrolysis for ferulic acid analysis determines which type of bound ferulic acid will be measured. In order to compare among studied wheat varieties and growing environments, bound ferulic acid (BFA) to xylose ratio was used to indicate degree of AX cross-linking in wheat bran.

Although the relationships of biochemical composition and bran thickness to bran mechanical properties are known, and the relationships between bran particle size and its mechanical properties have been reported, the relationship between bran characteristics and bran starch is not clear. Furthermore, the relationship between bran particle size and bran starch content in soft white wheat has not been investigated. The aims of this study were to investigate: (1) Soft wheat varietal and environmental differences on bran starch quantity, bran particle size, bran thickness and bran chemical composition, (2) the relationship between bran characteristics and bran starch quantity, and (3) the differences in microstructure of the wheat kernels and milled bran among chosen varieties.

4.3 MATERIALS AND METHODS

4.3.1 Wheat samples

Soft wheat grain samples of varieties Aubrey, Caledonia, and D8006 harvested in the year of 2009, 2010 and 2011 were used for this study. These varieties were selected to represent

the spectrum of softness equivalence of 16 varieties of previously studied soft wheat grown in Michigan (Liu et al 2011).

4.3.2 Milling process and particle sizing

All soft wheat grain samples were tempered to 14.5% grain moisture for 18 hr prior to milling. Wheat samples were milled using a Buhler laboratory mill MLU-202 (Buhler, Inc, Uzwil, Switzerland) according to AACCI Method 26-10A. After milling, eight fractions were obtained: three break stream flours, three reduction stream flours, bran, and shorts. Large bran particles (LBP: bran particles larger than 2 mm) were collected using a mechanical shaker (Great Western Manufacturing. Co., Inc. Kansas, USA) equipped with a US 10 (2 mm mesh) stainless steel screen and weighed. The whole milled bran fraction from each milling was used for particle sizing, considering small particles tend to settle and migrate to the bottom of the container through the voids between large pieces of bran particles. If only part of the milled bran fraction had been sized, the results of percent LBP and bran starch content might have been shifted due to non-homogeneous blending of bran particles and starch particles.

For each milled bran sample, a 100 g portion at a time was weighed and then screened on the shaker for 10 sec, overs were collected as LBP, throughs were collected separately from LBP as bran particles less than 2 mm, and this was repeated until all milled bran portions from each sample were sifted. Sum of the weights of LBP collected from all siftings of the bran portion of one milled wheat sample were used to calculate percent LBP of the milled bran fraction (total weight of LBP and bran particles less than 2 mm) for that sample.

4.3.3 Bran thickness

Thicknesses of LBP (> 2 mm) collected from the previous step were measured. The moisture content of LBP was first adjusted to 17% by conditioning in saturated NaCl solution for 24 h at 30 °C according to Peyron et al (2002a). The thicknesses of three randomly selected pieces of bran tissue from the LBP bran fraction of each wheat sample were measured using a micrometer (Peyron et al 2002a). Thickness values from five micrometer readings of each of the three selected bran particles were recorded. Averaged values ($5 \times 3 = 15$ values per wheat sample) were reported.

4.3.4 Total starch content

For each wheat sample, a portion of each collected LBP fraction was ground into powder using a Cyclone Sample Mill (UDY Corp., Fort Collins, Co) equipped with a 0.5 mm screen. Finely ground bran (100 mg) was weighed to determine bran starch content in duplicate using a Megazyme Total Starch Kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland).

4.3.5 Non-cellulosic pentosan content in wheat bran

Arabinoxylan and non-cellulosic pentose contents of ground LBP of studied wheat samples (obtained in section 4.3.4) were estimated by gas chromatography-mass spectrometry (GC-MS) of methylated sugar derivatives as follows:

4.3.5.1 Defatting

For each studied wheat sample, a ground LBP sample (100 mg in each screw capped tube) was defatted with hexane (5 ml) for 1 hr at 35 °C with rotary shaking (40 rev/min). After

centrifugation (5 min, $3800 \times g$, $20\text{ }^{\circ}\text{C}$), the defatted pellets were dried for 24 h at room temperature (Brillouet and Mercier 1981).

4.3.5.2 Destarching [adapted from total starch Megazyme kit (Megazyme International Ireland Ltd. Co., Wicklow, Ireland)]

The dried defatted bran pellets from the defatting step were weighed into glass tubes (100 mg in each tube). In the last step, the mixture of glucose solution and bran particles were centrifuged at $5440 \times g$ for 10 min. Supernatant was discarded. Four ml of 99% ethanol was added to each tube, followed by centrifugation ($5440 \times g$, 10 min). The precipitate was washed twice with 8 ml of 50% ethanol. Bran sample was centrifuged, and freeze dried overnight.

4.3.5.3 Derivatization

Alditol acetates were obtained based on methods of Melton and Smith (2001), Delcour et al (1999) and Englyst et al (1994). All analyses were done in triplicate. Twenty mg of defatted destarched bran from each bran sample were hydrolyzed by 5 ml 2 M trifluoroacetic acid in the presence of nitrogen at $110\text{ }^{\circ}\text{C}$ for 2 hr in glass tubes. To 3 ml of filtered hydrolysates (using a glass syringe fitted with a stainless steel 13-mm filter unit and a $0.45\text{ }\mu\text{m}$ PTFE filter), 1 ml of internal standard (1 mg of β -D allose in 1 ml 50% saturated benzoic acid) was added. The tubes were placed in ice-water and 1 ml of $\text{NH}_3 \cdot \text{H}_2\text{O}$ (25% NH_3 in H_2O) was added. Alkalinity of the mixture was tested using a pH indicator strip. Forty μl of 2-octanol and 0.2 ml of 2 M NH_3 containing NaBH_4 (200 mg NaBH_4/ml 2 M NH_3) were added. The tubes were capped and vortexed followed by incubation in a $40\text{ }^{\circ}\text{C}$ water bath for 1 hr, after which 0.4 ml glacial acetic

acid was added to each tube. To 0.5 ml aliquots of this last mixture, 0.5 ml 1-methylimidazole and 5 ml acetic anhydride were added. After 10 min, the solution in each tube was mixed with 0.9 ml ethanol, left for 5 min, and then mixed with 10 ml distilled water. 0.5 mL (0.04%, w/w) bromophenol blue was added to each tube dye the hydrophilic layer. The tubes were placed in an ice-water bath, and 5 ml of 7.5 M KOH was added twice within a 5-minute period. One ml ethyl acetate was then added to extract alditol acetates. The tubes were mixed by inverting 20 times and were left for phase separation for 30 min in the ice water bath. The upper phase was transferred to a small glass test tube. Sufficient anhydrous sodium sulfate was added into the small glass tube until white particles were observed in the tube. The clear phase was collected into a GC vial.

Separation of the alditol acetates was on a SP-2380 fused silica capillary column (30 m length, 0.25 mm ID, 0.2 μm film thickness), in the split mode (Supelco, Bellefonte, PA). The GC-MS system consisted of a Clarus 680 Gas Chromatograph and Clarus 600S Mass Spectrometer (Perkin Elmer, Inc. Shelton, Connecticut, US). The GC temperature program was set to the following sequence: (1) 2 min at an initial temperature of 80 $^{\circ}\text{C}$, (2) increasing to 200 $^{\circ}\text{C}$ at a rate of 45 $^{\circ}\text{C min}^{-1}$, (3) increasing to 240 $^{\circ}\text{C}$ at a rate of 5 $^{\circ}\text{C min}^{-1}$, and (4) holding at 240 $^{\circ}\text{C}$ for 7.3 min. The GC injector temperature was set at 250 $^{\circ}\text{C}$. The split flow rate and helium carrier gas flow rate were at 10 ml min^{-1} and 1 ml min^{-1} , respectively.

A retention time chromatogram of alditol acetates of each neutral sugar standard was constructed. Sugar composition was identified by comparing retention time to the retention time map and by mass spectra of each alditol acetate of each sugar standard. Total non-cellulosic sugars were calculated by the equation noted in Maes and Delcour (2002): non-cellulosic sugars = $0.88 \times (\% \text{ Ara} + \% \text{ Xyl}) + 0.9 \times (\% \text{ Man} + \% \text{ Gal} + \% \text{ Glu})$; arabinoxylan was calculated as:

arabinoxylan = $0.88 \times (\% \text{ Xyl} + \% \text{ Ara} - 0.7 \times \% \text{ Gal})$. [Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: non-cellulosic glucose]

4.3.6 Bound ferulic acid

The remaining ground LBP samples from section 4.3.4 were used for bound ferulic acid analyses, in duplicate, utilizing a modified method based on Hartman et al (2005). Bound ferulic acid was obtained by calculating the difference between total and free ferulic acid. Free and total ferulic acid of ground LBP samples were determined as follows:

4.3.6.1 Preparation for free ferulic acid

Ground bran (250 ± 10 mg) was weighed into a 20 ml screw-topped tube. Two ml of hydrochloric acid (0.1 M) and 0.5 ml internal standard solution 1000 ppm o-coumaric acid were added.

4.3.6.2 Preparation for total ferulic acid

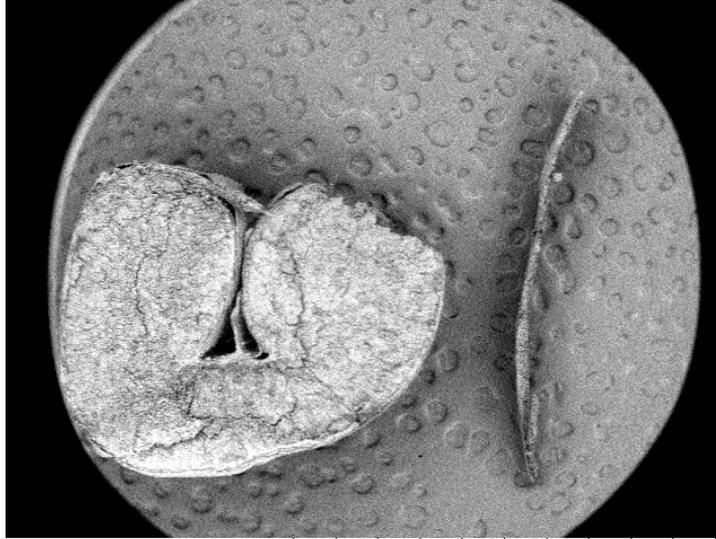
Ground bran ($40 \text{ mg} \pm 1 \text{ mg}$) of each sample was saponified by 2ml of 2N NaOH in the presence of nitrogen, vortexed for 30 sec and centrifuged at $510 \times g$ for 5 sec. Samples were mixed on a multi-tube vortex mixer for 52 rad/sec and mixed for 18 hours. Then solutions were neutralized by 0.5 ml 4 N hydrochloric acid and internal standard was added.

The free and total ferulic acid in their prepared mixtures, obtained from the procedures above, were extracted using 3 ml ethyl acetate three times by centrifugation at $800 \times g$ for 10 min. The ethyl acetate phases were collected in test tubes and evaporated to dryness in the presence of nitrogen. The dry extract was dissolved in 1 ml methanol; the dissolved extract was injected onto the HPLC system consisting of a Waters Alliance 2690 separation module and a

Waters 996 photodiode array detector (Waters Corp., Milford, MA) equipped with a Hyper Clone HPLC column (250 × 4.6 mm, 5-micron; Phenomenex, Torrance, CA) and eluted at 0.8 ml/min with the following gradient profile: 35-55% B for 15 min, 55-95% B for 15 min; 95-35% B for 3 min and then 35% B for 2 min (solvent B: 0.1% trifluoroacetic acid in methanol; solvent A: 0.1% trifluoroacetic acid in distilled water). Detection was done at 310 nm.

4.3.7 Microstructure of wheat bran by scanning electron microscope

For each wheat variety studied, scanning electron micrographs of milled wheat bran were compared with wheat grain micrographs with a Hitachi tabletop microscope TM 3000 (Hitachi Corp. Tokyo, Japan) with principle of under low-vacuum observation. A scalpel was used to dissect wheat grain and bran particles. For wheat grain dissection, two kernels were randomly selected for each wheat sample; the apical extremity of the grain was eliminated and a 2 mm-thick disc in cross-section of the grain was obtained from each kernel. For each wheat sample, two milled LBP were randomly selected, and full thickness strips of bran, about 2 mm in width, were cut off of the large bran particles using a scalpel. Each bran strip was affixed onto a piece of conducting double-sided tape which was attached to the specimen stub. Each bran strip was affixed on one cut edge, with the parallel longitudinal cut strip edge facing upward. A transverse section of counterpart wheat grain sample (control) was placed beside it on the tape (see Fig.4.1). The specimen stub was placed on the specimen height gauge to adjust its height. Hitachi TM 3000 application software was used for observation and data recording. Visual observation was conducted with × 600, × 800 and × 1000 magnifications. The scale bar indicating 100 μm is noted in each image.



4 mm

Figure 4.1. SEM image of transverse cross section disc of wheat kernel (left) and milled wheat bran strip (right) from the same sample, placed side by side for comparison.

4.3.8 Statistical analyses

A completely randomized design was used for all experiments. The data was analyzed using two-way analysis of variance (ANOVA) in SAS (SAS, Inc, 2006). When statistically significant differences ($P < 0.05$) were found among crop year and variety for a particular parameter studied, comparisons were conducted using the Least Significant Difference (LSD) *t*-test procedure. Linear correlation was used to investigate relationships between variables. A multiple regression model was used to explain variation of bran starch content and percent large bran particles.

4.4 RESULTS AND DISCUSSION

4.4.1 Relationship between bran particle size and bran starch content

Percent bran particles larger than 2 mm (LBP) and bran starch content of these LBP of studied samples varieties are listed in Table 4.1 and Table 4.2. There were statistically significant differences among varieties for percent LBP and for bran starch content. A negative correlation ($r = 0.939$) was found between percent LBP and bran starch content for LBP of studied bran samples, which was in agreement with previous finding of Liu et al (2011), who reported an R^2 value of 0.8637. Variety Aubrey had the lowest percent of LBP and the highest LBP bran starch content among the three studied varieties, while variety Caledonia had the highest proportion of LBP in its milled bran fraction and the lowest bran starch present in LBP. Varieties Aubrey and Caledonia had the highest percent LBP in 2010 while variety D8006 had the highest percent LBP in 2009 (Table 4.1). Varieties Aubrey and Caledonia had the lowest bran starch contents in 2011, but variety D8006 had the lowest bran starch content in 2010 (Table 4.2). Varieties Aubrey and D8006 had generally similar bran starch contents in LBP for 2009 and 2011, however, bran

starch content of variety Caledonia gradually decreased from 2009 to 2011. Thus, it appears that environment had different effects on the studied parameters of different soft wheat varieties. The findings could be useful for breeders to study particular environmental factors that would result in differences in wheat bran particle size and bran starch content in LBP for a specific wheat variety.

Table 4.1 Large Bran Particles (% of total milled bran fraction, w/w) of Three Soft Wheat Varieties Grown in Three Crop Years

	Aubrey	Caledonia	D8006
2009	25.50	32.30	30.23
2010	30.78	34.91	29.80
2011	21.67	33.56	29.93
Average ^x	25.98c	33.59a	29.99b

^x Values in this row marked with the same letters are not significantly different from each other (P > 0.05).

Table 4.2 Bran Starch Content (% w/w)^x in Large Bran Particles of Three Soft Wheat Varieties Grown in Three Crop Years

	Aubrey	Caledonia	D8006
2009	17.85 ± 0.17	16.34 ± 0.05	16.04 ± 0.10
2010	18.73 ± 1.25	15.00 ± 0.10	14.94 ± 0.09
2011	17.54 ± 0.85	14.87 ± 0.52	16.65 ± 0.27
Average ^y	18.04c	15.40a	15.88b

^x Values are means ± standard deviation.

^y Values in this row marked with the same letters are not significantly different from each other (P > 0.05).

Bran characteristics were confirmed to relate with bran particle size upon milling. Abecassis et al (1993) reported that differences in large particle distribution after milling were related to bran mechanical properties. Friability of outer layer bran tissues is an important factor in milling because more friable outer tissues of a wheat sample result in greater incorporation of fine bran particles in the relevant flours. Large bran particles are more easily separated from endosperm particles and thus reduce bran contamination in flours (Greffeuille et al 2006). In the present study, lower bran starch content found in wheat bran contained higher percent LBP is aligned with Greffeuille et al's finding, indicating a clean separation between bran tissue and endosperm particles, since starch is mainly an endosperm component in wheat grain. The information about the environmental conditions that are associated with low bran starch content in LBP will be valuable to study the environmental effect on dissociation between wheat outer layers and endosperm.

4.4.2 Bran thickness and bran starch content

Milled wheat bran particles of varieties Aubrey, Caledonia and D8006 were significantly different in bran thickness (Table 4.3). The results showed that bran thickness of LBP of Aubrey was highest among the three varieties studied. Variety Aubrey was found to have the highest bran starch content in LBP (Table 4.2). Caledonia had the lowest value of bran thickness (Table 4.3) and the lowest bran starch content in LBP (Table 4.2). These relationships will be investigated later to explain the variation of bran starch content in different varieties. Differences in bran thickness were found among crop years as well; LBP of varieties Aubrey and D8006 grown in 2011 were 20-30 μm thinner than LBP of these two varieties in 2009 and 2010. In a previous study, it was found that production of proportionately more wheat LBP required low

toughness but high extensibility of the bran tissue (Peyron et al 2002a). In the current study, soft wheat bran thickness was found to be negatively correlated with percent large bran particles. Thin bran tissue in the present study was associated with a higher percent of LBP upon milling, and this bran tissue could thus possess lower tissue toughness and stiffness. Lucas et al (1995) revealed that intrinsic toughness of plant tissue is positively correlated with outer layer tissue thickness. The outer layers of the wheat samples in the present study will be discussed in section 4.4.6 based on observations in SEM micrographs.

Table 4.3 Bran Thickness (mm)^x of Large Bran Particles of Three Soft Wheat Varieties Grown in Three Crop Years

	Aubrey	Caledonia	D8006
2009	0.138 ±0.000	0.114 ±0.002	0.118 ±0.012
2010	0.131 ±0.003	0.092 ±0.001	0.127 ±0.003
2011	0.111 ±0.010	0.100 ±0.001	0.098 ±0.009
Average ^y	0.126c	0.102a	0.114b

^x Values are means ± standard deviation.

^y Values marked with the same letter in this row are not significantly different from each other (P > 0.05). Comparisons were conducted among the averaged values of three varieties across crop years.

4.4.3 Noncellulosic sugar profile of wheat bran

A number of studies have underlined the involvement of cell wall biochemical composition and structure to explain the mechanical properties of plant tissues (Darley et al 2001; Shopfer et al 2001). Noncellulosic sugar compositions of the studied wheat bran samples are reported in Tables 4.4 and 4.5. The arabinose to xylose ratio (Ara/Xyl) is used to characterize the structure of AX, indicating the substitution pattern of the xylose backbone by arabinose residues. It is important to investigate the biochemical components in bran of the studied wheat varieties and relationships of these components to bran starch and percent LBP. The degree of arabinoxylan cross-linking was reported to significantly influence bran extensibility and therefore a promising marker of bran friability (Peyron et al 2002b).

The neutral saccharide profile of the wheat bran in the present study was dominated by arabinose, xylose and glucose, while mannose and galactose were minor. This finding is in agreement with published results (Maes and Delour 2002). The distributions of sugars in bran samples from the same wheat variety varied among the three crop years. Variety Caledonia had a drop in arabinose content in year 2011. Variety D8006 had much lower levels of arabinose and xylose in year 2009 than the other years, with total noncellulosic sugars of D8006 in year 2009 being the lowest among the three years (Table 4.4). These findings are useful for breeders to investigate environmental effects on distributions of functional neutral sugars in wheat bran samples.

The amounts of arabinose and glucose and the ratio of arabinose to xylose were found to be statistically significantly different among the three varieties (Table 4.5). Variety D8006 had the lowest amount of arabinose and highest amount of glucose for each studied crop year. The ratio of arabinose to xylose for D8006 was lower than the other two varieties for each crop year,

which indicates that the AX in the bran of variety D8006 were less branched than AX in the bran of varieties Aubrey and Caledonia. When comparing results of studied neutral sugar contents with percent LBP among the three varieties, there was no linear relationship between AX content and percent LBP or between Ara/Xyl and percent LBP (data not shown).

Table 4.4 Noncellulosic Sugar Composition of Large Bran Particles of Three Soft Wheat Varieties Grown in Three Crop Years^x

	Ara (g/100g)	Xyl (g/100g)	Man (g/100g)	Gal (g/100g)	Glu (g/100g)	AX (g/100g)	Ara/Xyl	Total non-cellulosic sugars (g/100g)
2009								
Aubrey	14.90 ± 0.20	24.21 ± 1.17	0.11 ± 0.01	0.63 ± 0.03	2.86 ± 0.03	33.48 ± 1.56	0.59 ± 0.00	39.46 ± 1.57
Caledonia	13.87 ± 0.29	23.35 ± 0.10	0.20 ± 0.01	0.78 ± 0.00	3.06 ± 0.04	32.35 ± 0.45	0.59 ± 0.01	36.07 ± 0.13
D8006	10.85 ± 0.14	21.81 ± 0.10	0.32 ± 0.06	0.59 ± 0.09	4.05 ± 0.13	28.38 ± 0.15	0.50 ± 0.00	33.19 ± 0.22
2010								
Aubrey	13.60 ± 1.19	25.25 ± 0.19	0.20 ± 0.02	0.55 ± 0.00	3.37 ± 0.06	33.85 ± 1.21	0.54 ± 0.04	37.90 ± 1.24
Caledonia	13.55 ± 0.61	24.19 ± 0.45	0.23 ± 0.00	0.67 ± 0.02	3.41 ± 0.09	32.80 ± 0.94	0.56 ± 0.01	37.09 ± 0.83
D8006	12.31 ± 0.64	24.33 ± 1.36	0.21 ± 0.00	0.74 ± 0.13	3.76 ± 0.16	33.10 ± 1.68	0.51 ± 0.00	36.02 ± 1.09
2011								
Aubrey	14.97 ± 1.21	22.87 ± 1.55	0.26 ± 0.07	0.44 ± 0.02	2.60 ± 0.55	31.77 ± 2.07	0.59 ± 0.00	35.01 ± 2.53
Caledonia	12.55 ± 0.42	23.16 ± 0.83	0.15 ± 0.01	0.50 ± 0.02	3.08 ± 0.25	31.09 ± 1.09	0.54 ± 0.00	34.83 ± 1.34
D8006	12.14 ± 0.12	24.18 ± 0.37	0.26 ± 0.01	0.68 ± 0.01	4.19 ± 0.19	31.53 ± 0.42	0.50 ± 0.00	36.57 ± 0.58

^x Values are means ± standard deviation. Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose; AX (arabinoxylans) = $0.88 \times (\% \text{ Xyl} + \% \text{ Ara} - 0.7 \times \% \text{ Gal})$; Ara/Xyl = the arabinose to xylose ratio. Total non-cellulosic sugars = $0.88 \times (\% \text{ Ara} + \% \text{ Xyl}) + 0.9 \times (\% \text{ Man} + \% \text{ Gal} + \% \text{ Glu})$

Table 4.5 Comparison of Noncellulosic Sugar Composition of Large Bran Particles of Three Soft Wheat Varieties^x

	Ara (g/100g)	Xyl (g/100g)	Man (g/100g)	Gal (g/100g)	Glu (g/100g)	AX (g/100g)	Ara/Xyl	Total non- cellulosic sugars
Aubrey	14.49 ± 0.77b	24.11 ± 1.19a	0.19 ± 0.08a	0.54 ± 0.10a	2.94 ± 0.39a	33.03 ± 1.11a	0.57 ± 0.03b	37.46 ± 2.26a
Caledonia	13.32 ± 0.69b	23.57 ± 0.55a	0.19 ± 0.04a	0.65 ± 0.14a	3.18 ± 0.20a	32.08 ± 0.89a	0.56 ± 0.03b	36.00 ± 1.13a
D8006	11.77 ± 0.80a	23.44 ± 1.41a	0.26 ± 0.06a	0.67 ± 0.08a	4.00 ± 0.22b	31.00 ± 2.40a	0.50 ± 0.01a	35.26 ± 1.81a

^x Values are means across three crop years ± standard deviation; within a column, means followed by the same letter are not significantly different ($P > 0.05$). Ara: arabinose; Xyl: xylose; Man: mannose; Gal: galactose; Glu: glucose; AX (arabinoxylans) = $0.88 \times (\% \text{ Xyl} + \% \text{ Ara} - 0.7 \times \% \text{ Gal})$; Ara/Xyl = the arabinose to xylose ratio; Total non-cellulosic sugars = $0.88 \times (\% \text{ Ara} + \% \text{ Xyl}) + 0.9 \times (\% \text{ Man} + \% \text{ Gal} + \% \text{ Glu})$

4.4.4 Bound ferulic acid

Bound ferulic acid is believed to be directly associated with AX cross linking, and therefore related to bran physical properties. The amounts of bound ferulic acid (BFA) in large bran particles are listed in Table 4.6. Results were obtained by calculating the difference between measured total and free ferulic acid contents for each sample. Free ferulic acid contents were very low in all samples, from 5-11 ppm (data not shown). The concentration of NaOH used in hydrolysis for BFA determination is varied depending on which type of BFA is expected to be released; 2 N NaOH can release BFA esterified to arabinosyl units of AX, while 4 N NaOH can release BFA ester-linked with matrix polymers and/or with lignin (Parker et al 2005). In the present study, 2 N NaOH was used to release bound ferulic acid in bran, therefore results were indicative of ferulic acid esterified to arabinoxylans. This type of BFA mostly functions to regulate the degree of arabinoxylan cross-linking. There were significant differences in BFA content among the different varieties and crop years. Variety Aubrey did not show significant differences among the crop years, but varieties Caledonia and D8006 had significantly lower amounts of BFA in year 2011 than the prior two years. Within each year, variety Aubrey always had the lowest BFA value. Correspondingly, bran of Aubrey had the lowest percent LBP, the thickest bran among the LBP, and the highest bran starch content.

Table 4.6 Bound Ferulic Acid (ppm) in Large Bran Particles of Three Different Soft Wheat Varieties Grown in Three Crop Years^x

	Aubrey	Caledonia	D8006
2009	3053.50Aa	3343.5Bb	3539.93Bb
2010	3048.46Aa	3301.16Bb	3373.28Bb
2011	2895.43Aa	3040.08Aa	3257.78Ab
Average ^y	2999.13a	3228.25b	3390.33c

^x Values are means of duplicated measurements. Means within rows followed by the same lowercase letter are not significantly different ($P > 0.05$); means within columns followed by the same uppercase letter are not significantly different ($P > 0.05$).

^y Values are the average across three crop years. Means within the row followed by the same lowercase letter are not significantly different ($P > 0.05$).

The ratios of BFA content to xylose content of all bran samples are shown in Table 4.7. The same trend for the average BFA/xylose ratios among the three varieties was found as for the average BFA content (Table 4.6), i.e., lowest for Aubrey and highest for D8006. The average BFA/xylose ratio was significantly different among the studied varieties. In the present study, the ratio of BFA to xylose was used as an indicator of the level of AX cross-linking (according to Peyron et al 2002a). BFA contents obtained in this study indicate the amount of BFA esterified to AX molecules (Parker et al 2005), therefore the higher the BFA to xylose ratio, the greater the degree of AX cross-linking, and the higher the extensibility of the bran (Peyron et al 2002a). BFA/xylose ratios were also looked at in relation to bran starch contents in LBP for the three varieties, and a negative correlation was found between ratio of BFA to xylose and bran starch content ($P = 0.0508$). This result can be explained by (a) bran starch content and percent LBP, which were found to be negatively related, and (b) the ratio of BFA to xylose, which was negatively related with bran starch content after milling. However, the positive correlation between BFA to xylose ratio and percent LBP was not statistically significant ($P > 0.1$, Table 4.8). These findings are consistent with previously reported results, in which reduced ferulic acid dimer and AX cross-linking in wheat bran resulted in an increase in material friability and a subsequently a decrease in extensibility of bran strips, and the proportion of large bran particles from milling (Peyron et al 2002b). In the present study, bran of variety Aubrey in all crop years might have had lower cross-linking of AX, thus bran was more friable and easier to break into smaller particles during milling, thereby yielding a smaller proportion of LBP.

Table 4.7 Bound Ferulic Acid to Xylose Ratio in Large Bran Particles of Three Different Varieties of Soft Wheat Grown in Three Crop Years^x

	Aubrey	Caledonia	D8006
2009	0.0126Aa	0.0143Ab	0.0162Bc
2010	0.0121Aa	0.0136Ab	0.0139Ab
2011	0.0127Aa	0.0131Aa	0.0135Aa
Average ^y	0.0124a	0.0137b	0.0145c

^x Values are means of three measurements. Means within rows followed by the same lowercase letter are not significantly different ($P > 0.05$); means within columns followed by the same uppercase letter are not significantly different ($P > 0.05$)

^y Values are averaged across three crop years. Means within the row followed by the same lowercase letter are not significantly different ($P > 0.05$).

4.4.5 Relationship among bran biochemical composition, bran thickness and bran starch content

Several parameters evaluated in the current study had high possibilities for linear relationships between each other (Table 4.8), although the coefficients of determination (r) were less than 0.5 (data not shown). Bran starch was found to have a significant positive correlation with bran thickness, and a negative correlation with BFA content and ratio of BFA to xylose. Percent LBP was negatively correlated with bran thickness, and positively correlated with BFA content ($P < 0.1$). According to Lucas et al (1995), toughness of outer layer tissues was positively correlated with its thickness until the thickness reaches a plateau at about 1 mm. In the present study, the bran tissue with low thickness (much thinner than 1 mm) could have had low toughness, and therefore tend to break into larger particles and remain attached with less bran starch. A negative correlation ($r = 0.949$) between bran starch and LBP was found, which may be attributed to easy dissociation between bran inner tissues and starchy endosperm tissue. Percent LBP was found to correlate with BFA content, which might indicate there was enhanced bran extensibility due to the elevated bound ferulic acid. Variety Aubrey had a much lower percent of LBP and also had lower BFA content than the other two varieties.

Table 4.8 The P Values For Sets of Two Variables With Linear Correlations^x

	Bran starch (%)	Bran thickness (mm)	BFA (ppm)	BFA/Xylose	LBP (%)
Bran starch (%)	—	(+) 0.0283	(-) 0.0488	(-) 0.0508	(-) 0.0002
LBP (%)	(-) 0.0002	(-) 0.0900	(+) 0.0562	(+) NS	—

^x LBP: large bran particles; BFA: bound ferulic acid; (+): positive correlation; (-): negative correlation; NS: not significant ($P > 0.1$).

With knowing how BFA and milled bran thickness are related to percent large bran particles and bran starch content of LBP, it would be useful to summarize the relationships among the parameters by establishing predicting equations via multi-regression analysis:

$$\% \text{ Bran Starch in LBP} = 43.19 \times \text{Bran thickness (P = 0.0299)} - 0.003 \times \text{BFA (P = 0.0440)} + 0.035 \times \text{Xylose content (P = 0.896)} + 19.34 \text{ (P = 0.0251)}$$

$$\% \text{ LBP} = (-124.2) \times \text{Bran thickness (P = 0.0224)} + 0.01 \times \text{BFA (P = 0.0154)} + 1.35 \times \text{Xylose content (P = 0.068)} - 17.338 \text{ (P = 0.4208)}$$

Considering the low number of examined samples ($n = 9$), these regressions require further validation with more samples. However, both equations can significantly predict variation of dependent variables ($P < 0.05$). The equation for bran starch content of LBP can explain about 47% of the variation in bran starch content of LBP using bran thickness and BFA and xylose contents; and the equation for percent LBP can explain about 50% of the variation in percent LBP with knowing bran thickness, and BFA and xylose contents. It will be necessary to identify and/or other contributing variables into the model to better explain the variation seen in bran starch content and proportion of LBP in the bran fraction of milled wheat varieties.

4.4.6 Microstructure of outer layers of wheat kernel, milled bran and bran starch

Scanning electron micrographs of milled wheat bran and wheat grain kernel (Figs.4.2 and 4.3) were examined, and wheat grain transverse cross-sections of all wheat varieties were noted to have identical histological composition. For all wheat kernels studied, the multilayer configuration of the outer layers could be easily distinguished (Fig.4.2A), including the pericarp, seed coats, and single-cell thick aleurone layer. In the starchy endosperm region of the wheat kernel, starch granules of different sizes were densely embedded in the protein matrix (Fig.4.2B). For the milled bran strip, no clear distinction between different layers could be seen (Fig.4.2C).

Outer tissues were peeled off of the endosperm tissue during the milling process, and were deformed and compressed with portions of endosperm tissue (Fig.4.2C); the single-cell aleurone layer was no longer visible. Bran starch granules were randomly distributed on or in among the pericarp-seed coats tissues of the bran. During imaging evaluations of bran strip samples of different varieties, a few bran strips were found to have detached outer pericarp layers, as shown in Fig.4.2D. This dissociation of pericarp-seed coats layers from the underlying bran layers might be due to differences in mechanical properties of these specific outer tissue layers and the biochemical composition of their interfaces (Antoine et al 2003).

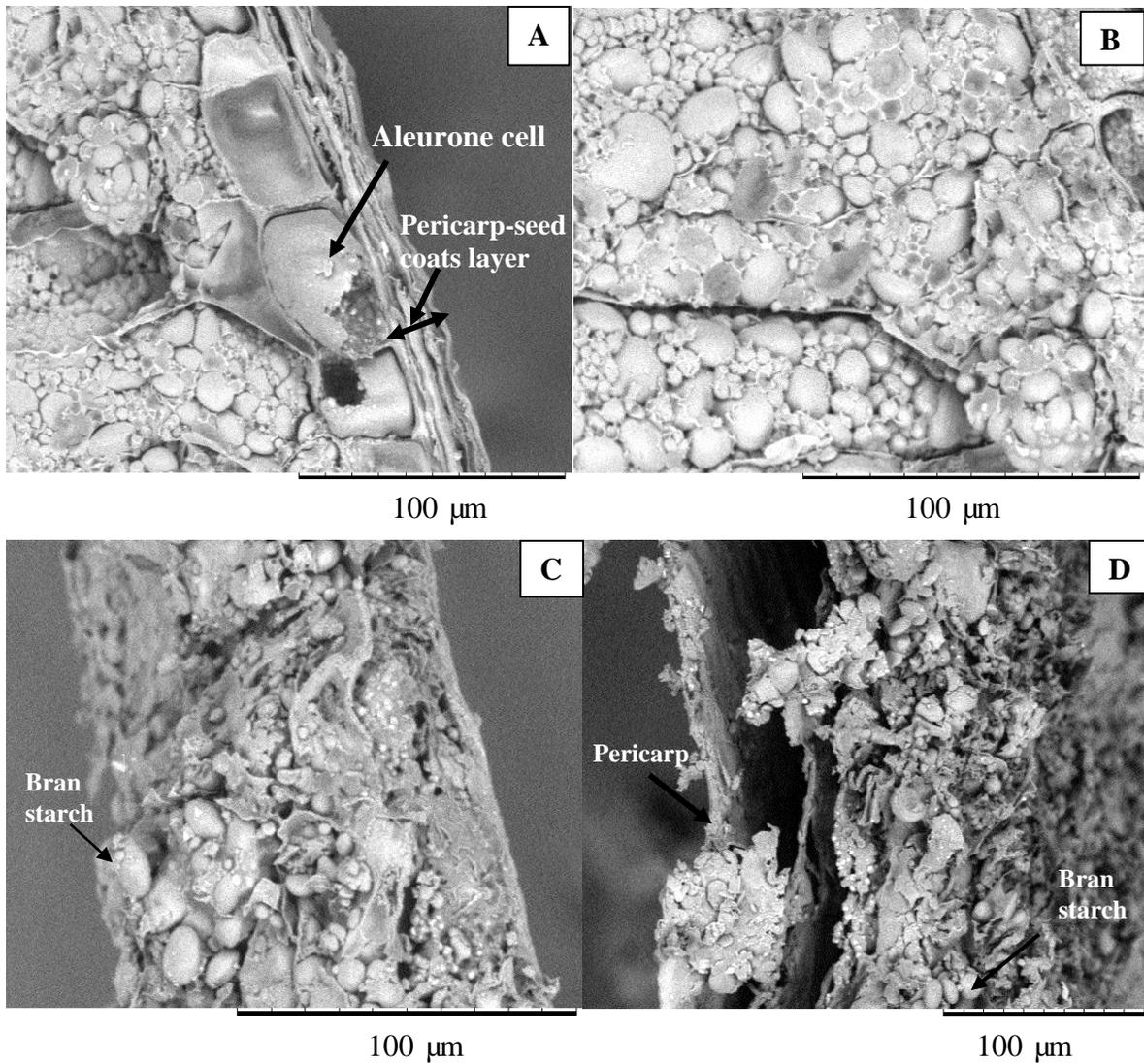


Figure 4.2. SEM images of representative (A) wheat outer layers of transverse cross section disc of wheat kernel (variety D8006); (B) wheat endosperm of transverse cross section disk of wheat kernel (variety D8006); (C) cross section of milled bran strip (variety Aubrey); and (D) cross section of milled bran strip demonstrating peeled pericarp (variety D8006).

Micrographs of cross-sections of wheat kernels from three varieties grown in 2009 are depicted in Fig.4.3. The thickness (~60 μm) of the combined pericarp-seed coats layer and aleurone layer of wheat kernel of variety Aubrey (Fig.4.3A), measured in the SEM micrograph, was compared with the averaged milled bran thickness (126 μm) of variety Aubrey measured by micrometer (Table 4.3). It is interesting to find that the thickness of milled bran is about twice that of the intact outer layers of unmilled cut wheat kernel. An intact aleurone layer structure does not identifiably exist in micrographs of the milled bran strip, and the outer layers of milled bran were no longer compactly packed after milling. The additional thickness of milled bran particle, therefore, likely comes from the adherent remnant portion of starchy endosperm and the space that forms between outer layers upon milling. The moisture adjustment for milled bran tissue at 17% before measuring the thickness by micrometer in section 4.3.3 could result in swelling of bran starch and the pericarp-seed coats layer in milled LBP tissue, which could be another reason for the high milled bran thickness value.

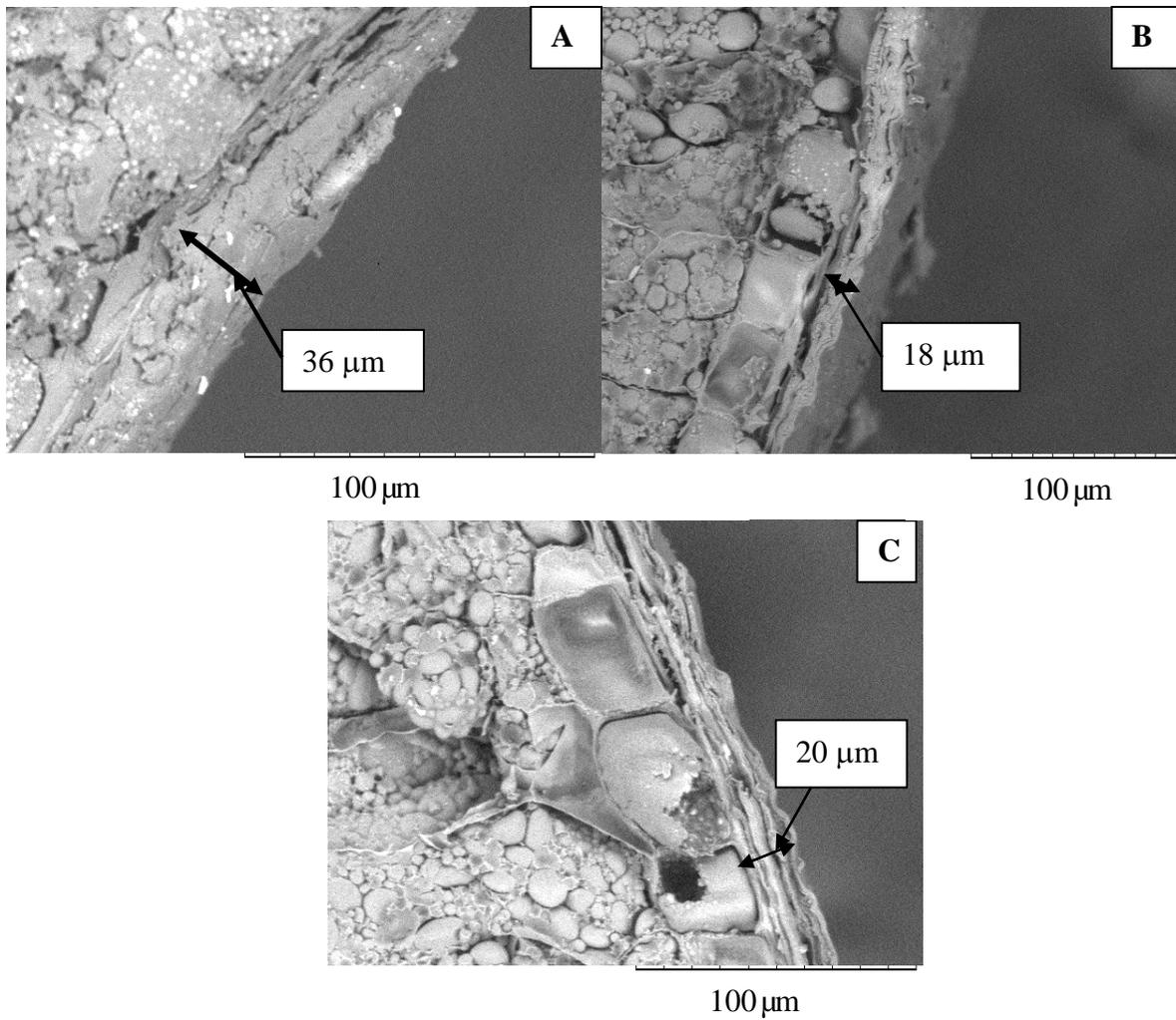


Figure 4.3. SEM images of representative wheat outer layers of transverse cross section disc of wheat variety Aubrey (A), Caledonia (B), and D8006 (C). Double-headed arrows indicate the thickness of outer layers (pericarp and seed coats layers).

As discussed in section 4.4.5, milled bran thickness measured by micrometer had a negative relationship with percent LBP. Thin bran tissues in the present study could have low toughness and low friability according to Peyron (2002a). The dependence of toughness on tissue thickness was reported in a previous study with a large array of plant materials including plant seed coat (Lucas et al 1995); as thickness increased, measured toughness increased until reaching a plateau at about 1 mm thickness. Therefore the measured thickness of outer layers of a wheat kernel would be directly related to the level of toughness exhibited by its wheat bran during milling. As previously mentioned, we found that the multiple layers of the outer tissue structures of milled bran were not observed on SEM to be intact, but could be clearly identified in micrographs of wheat kernel cross-sections. It is possible to measure the thickness of outer layers of studied varieties from the micrographs of the wheat kernel cross-sections and compare these measurements to bran thickness results. Outer layer thicknesses (of the pericarp and seed coats layers) of varieties Aubrey, Caledonia, and D8006 were about 36 μm , 18 μm , and 20 μm , respectively, based on micrograph measurements (Fig 4.3). Varieties Caledonia and D8006 had similar thicknesses for the outer layers of their wheat kernels (18 and 20 μm , respectively), and they also had similar milled bran thicknesses measured by micrometer (102 and 114 μm , respectively, Table 4.3). Observations from SEM micrographs indicated that variety Aubrey, which had the thickest milled bran tissue among the three varieties (Table 4.3), had the thickest outer layers of its wheat kernels as well. Since thicker plant tissue was reported to have higher tissue toughness, thicker milled bran tissues in the current study probably had higher toughness as well. Considering milled bran thickness is negatively related to percent LBP, from results discussed earlier, tissue toughness could negatively affect percent LBP and can be investigated

as one of the important bran physical properties that directly affect bran starch content in LBP and percent LBP in further studies.

4.5 CONCLUSIONS

A strong negative correlation between percent LBP and bran starch content was found for studied soft wheats varieties. This result confirms that a greater proportion of milled large bran particles upon milling indicating a cleaner separation between wheat outer layers and endosperm tissue, and therefore less bran starch present in milled bran particles. Significant varietal and environmental differences in bran thickness, non-cellulosic sugar distribution, BFA and BFA to xylose ratio were found. Variety Aubrey was found to have the lowest percent LBP, highest bran starch content, thickest milled bran tissue and outer layers, and the lowest BFA content and ratio of BFA to xylose. The low degree of AX cross-linking could be the reason why Aubrey had the greatest proportion of small bran particles produced upon milling and the highest bran starch content in LBP. Bran particles of variety Aubrey and D8006 grown in 2011 were 20-30 μm thinner than particles from the respective varieties grown in 2009 and 2010, which indicates that environmental conditions in 2011 could be interesting to investigate for selection of a wheat variety that has a high percent of LBP and better milling quality.

The dominant neutral saccharides (arabinose, xylose and glucose) in wheat bran can be potential indicators of wheat varieties with desired percent LBP or bran starch content in LBP. Relationship among bran thickness, bran chemical composition, and bran starch content were investigated for the first time in the present study. Bran starch content was found to positively correlate with bran thickness ($P = 0.028$), and negatively correlate with BFA ($P = 0.049$) and BFA/xylose ($P = 0.05$). Low toughness and low friability of bran could be key bran physical

properties that lead to a high percent of LBP and a low bran starch content in LBP upon wheat milling, because low bran toughness was found to associate with low tissue thickness in a previous study, and bran tissue with low friability can slide and reorient during material deformation. Increased polymer mobility in the cell wall network by high levels of AX cross-linking could result in increased extensibility and decreased friability of the bran (Greffeuille et al 2006).

Based on observations of SEM micrographs, outer wheat tissues, including the pericarp and seed coats and the single-cell-thick aleurone layer can be distinguished. Outer tissues were peeled off the endosperm tissue during the milling process, and were deformed and compressed with a portion of endosperm tissue. Bran starch granules were randomly distributed on or in between layers of the pericarp-seed coats tissues in the bran. The wheat variety with a thicker milled bran tissue measured by micrometer, was found to have thicker outer layers of its wheat kernel, measured on SEM image.

Bran thickness, non-cellulosic sugar and bound ferulic acid could be used to predict percent LBP and bran starch content in LBP. A prediction equation for bran starch content was developed and about 47% variation could be explained based on the examined bran characteristics. However further tests on a wider range of soft wheat samples are required to validate the relationships between bran starch and bran characteristics. These characteristics can be potential target traits for breeders to select for and breed new wheat varieties with enhanced physical resistance and high percent LBP or wheat varieties with desired bran starch content produced after milling. Although bran extensibility regulated by ferulic acid could be one of the main elements controlling bran mechanical properties and percent LBP, it is expected that there are other contributing variables, to be identified for improvement of the predictive equation for

bran starch content and percent LBP. Even though the viability of bran starch content and percent LBP have not been fully understood, it is confirmed that the effects of AX cross linking by ferulic acid can be a key component in studying the different effects of AX in structure and function relationships. It is also necessary to understand environmental effects on the difference in AX structure, biochemical composition and bran thickness to define possible targets for plant breeding. Potential genetic control of the variation in AX structure, biochemical composition, and bran thickness among wheat varieties can be used to produce new types of wheat with improved processing properties and desired bran starch quantity.

LITERATURE CITED

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- Anonymous 2011. Milling tests. USDA-Agriculture Rresearch Service.
<http://www.ars.usda.gov/Research/docs.htm?docid=21515>. Last modified on 03/01/2011.
- Antoine, C., Peyron, S., Mabile, F. R., Lapierre, C., Bouchet, B., Abecassis, J., and Rouau, X. 2003. Individual contribution of grain outer layers and their cell wall structure to the mechanical properties of wheat Bran. *J. Agric. Food Chem.* 51:2026-2033.
- Brillouet, J. M., and Mercier, C. 1981. Fractionation of wheat bran carbohydrates. *J. Sci. Food. Agric.* 32:243-251.
- Bunzel, M., Ralph, J., Marita, J., and Steinhart, H. 2000. Identification of 4-0-5- coupled diferulic acid from insoluble cereal fiber. *J. Agric. Food Chem.* 48:3166-3169.
- Darley, C. P., Forrester, A. M., and McQueen-Mason, S. J. 2001. The molecular basis of plant cell wall extension. *Plant Mol. Biol.* 47:179-195.
- Delcour, J. A., Van Win, H., and Grobet, P. J. 1999. Distribution and structural variation of arabinoxylans in common wheat mill streams. *J. Agri. Food Chem.* 47:271-275.
- Dupont, M. S., and Selvendran, R. R. 1987. Hemicellulosic polymers from the cell walls of beeswing wheat bran: part 1, polymers solubilised by alkali at 2 °C. *Carbohydr. Res.* 163:99-113.
- Englyst, H. N., Quigley, M. E., and Hudson, G. J. 1994. Determination of dietary fiber as non-starch polysaccharides with gas-liquid chromatographic, high-performance liquid chromatographic or spectrophotometric measurement of constituent sugars. *Analyst* 119:1497-1509.
- Fry, S. C., Willis, S. C., and Paterson, A. E. J. 2000. Intraprotoplasmic and walllocalised formation of arabinoxylan-bound diferulates and larger ferulate coupling-products in maize cell-suspension cultures. *Planta* 211:679-692.
- Greffeuille, V, Abecassis, J., Lapierre, C., and Lullien-Pellerine, V. 2006. Bran size distribution at milling and mechanical and biochemical characterization of common wheat grain outer layers: a relationship assessment. *Cereal Chem.* 83:641-646.
- Hartman. G., Piber. M., and Koehler. P. 2005. Isolation and chemical characterization of water-extractable arabinoxylans from wheat and rye during breadmaking, *Eur. Food Res. Technol.* 221:487-492.
- Klepacka, J., and Fornal, L. 2006. Ferulic acid and its position among the phenolic compounds of wheat. *Crit. Rev. Food Sci. and Nutri.* 46:639-647.

- Liu, Y., Ng, P., Lewis, J., and Souza, E. J. 2011. Relationship between bran particle size and bran starch of milled soft wheat grown in Michigan. AACC International annual meeting: Palm Springs, CA.
- Liu, Y., and Ng, P. 2012. Physicochemical properties of bran starches and endosperm starches of soft wheat grown in Michigan. AACC international annual meeting. Hollywood, FL.
- Lucas, P. W., Darwell, B. W., Lee, P. K. D., Yuen, T. D. B., and Choong, M. F. 1995. The toughness of plant cell walls. *Philosophical Transactions-Royal Society of London. Biological Sci.* 328:363-372.
- Maes, C., and Delcour, J. A. 2002. Structural characterization of water-extractable and water-unextractable arabinoxylans in wheat bran. *J. Cereal Sci.* 35:315-326.
- Melton, L. D., and Smith, B. G. 2001. Determination of neutral sugars by gas chromatography of their alditol acetates. *Current Protocols in Food Analytical Chem.* E3.2.1-E3.2.13.
- Peyron, S., Abecassis, J., Autran, J. C., and Rouau, X., 2001. Enzymatic oxidative treatments of wheat bran layers: effects on ferulic acid composition and mechanical properties. *J. Agric. Food Chem.* 49:4694-4699.
- Parker, M. L., Ng, A., and Waldron, K. W. 2005. The phenolic acid and polysaccharides composition of cell walls of bran layers of mature wheat (*Triticum aestivum* L.cv.Avalon) grains. *J. Sci. Food Agric.* 85:2539-2547.
- Peyron, S., Chaurand, M., Rouau, X., Abecassis, J., 2002a. Relationship between bran mechanical properties and milling behaviour of durum wheat (*Triticum durum* Desf.). Influence of tissue thickness and cell wall structure. *J. Cereal Sci.* 36:377-386.
- Peyron, S., Abecassis, J., Autran, J. C., Rouau, X., 2002b. Influence of UV exposure on phenolic acid content, mechanical properties of bran, and milling behavior of durum wheat (*Triticum durum* Desf.). *Cereal Chem.* 79:726-731.
- Renger, A., and Steinhart, H. 2000. Ferulic acid dehydrodimers as structural elements in cereal dietary fiber. *Eur. Food Res. Technol.* 211:422-428.
- Rombouts, F. M., and Thibault, J. F. 1986. Feruloylated pectic substances from sugar-beet pulp. *Carbohydr. Res.* 154:177-187.
- Shopfer, P., Lapierre, C., and Nolte, T. 2001. Light-controlled growth of the maize seedling mesocotyl: Mechanical cell-wall changes in the elongation zone and related changes in lignification. *Physiol Plantarum* 111:83-92.
- Waldron, K. W., Smith, A. C., Parr, A. J., Ng, A., and Parker, M. L. 1997. New approaches to understanding and controlling cell separation in relation to fruit and vegetable texture. *Trends Food Sci. Technol.* 8:213-221.

Waldron, K. W., Ng, A., Parker, M. L., and Parr, A. J. 1997. Ferulic acid dehydrodimers in the cell walls of *Beta vulgaris* and their possible role in texture. *J. Sci. Food Agric.* 74:221-228.

CHAPTER 5 GENERAL CONCLUSIONS

Isolation methods by alkaline extraction were developed to obtain bran starch and endosperm starch from the milled bran fraction and milled flour fraction, respectively, of Michigan-grown soft wheat varieties. A higher starch recovery was obtained from endosperm flour than from milled bran. Protein contents for all isolated starch samples were less than 0.36%. Starch damage contents were less than 2.5%, which indicates that the grinding step during isolation did not cause further starch damage. The structures and properties of bran starch samples of the three studied varieties were distinct from those of the counterpart endosperm. Bran starch was found to have a higher percent of small granules, higher amylose content, higher crystallinity, broader gelatinization temperature range, higher enthalpy of gelatinization, lower retrogradation, and lower pasting peak and setback viscosities than its counterpart endosperm starch. A-type X-ray diffraction patterns were found for both bran starch and endosperm starch. Bran starch of variety Aubrey had the highest crystallinity (21.75%) and gelatinization temperature (62.9 °C). Bran starch of variety D8006 had the highest percent B-type granules (37.88%) and lowest retrogradation (21.7%).

The bran starch consisted of amylopectin that had more short branch-chains than did the counterpart endosperm starch. Bran starch had a broader gelatinization temperature range, which is related to the presence of a greater proportion of small granules. Bran starch had a lower degree of retrogradation than endosperm starch, and this may be due to a higher percent of short branch-chains and lower percent of long branch-chains in the amylopectin molecule structure. SEM images revealed small and large starch granules present in bran starch after starch isolation, with a greater proportion of small granules in bran starch. Bran starch had lower peak, breakdown and setback viscosities than endosperm starch, which may be due to the greater proportion of small granules present in bran starch, considering that there were no significant

differences in lipid content among the different starches. The lower viscosities may also be related to the higher amylose content of bran starch, which is associated with less granule swelling. Amylopectin molecules of bran starch had more branch chains of degree of polymerization (DP) 6-12 and fewer branch chains of DP 25-36 than did the amylopectin of the counterpart endosperm starch, which may explain the lower degree of retrogradation of bran starch compared to endosperm starch. Results showed that the structures and properties of bran starches and their counterpart endosperm starches were distinct from each other, which suggests that their commercial end-use applications could be different.

Relationships among bran thickness, bran chemical composition, and bran starch content were investigated for the first time in present research. A strong negative correlation between percent large bran particles (LBP) and bran starch content was found in the wheat varieties studied. This result confirmed that greater proportion of milled large bran particles upon milling indicates a cleaner separation between wheat outer layers and endosperm tissue therefore less bran starch present in milled bran particles. Significant varietal and environmental differences in bran thickness, non-cellulosic sugar distribution, bound ferulic acid (BFA) and BFA to xylose ratio were found. Variety Aubrey was found to have the lowest percent LBP, highest bran starch content, thickest milled bran tissue and outer layers, and lowest BFA content and ratio of BFA to xylose. Varieties Caledonia and D8006 had similar averaged values of bran starch content that were much lower than that of Aubrey. Bran particles of varieties Aubrey and D8006 grown in 2011 were 20-30 μm thinner than of those grown in 2009 and 2010. The neutral saccharide profile of the wheat bran of all three varieties was dominated by arabinose, xylose and glucose, while mannose and galactose were minor. Variety D8006 had much lower levels of arabinose

and xylose in year 2009 than in the other years, therefore the total noncellulosic sugars of D8006 in year 2009 was lowest among the three studied crop years.

Bran starch content was found to positively correlate with bran thickness, and negatively correlate with BFA and BFA to xylose ratio. The defined relationship between percent LBP and factors, including bran thickness, BFA, and xylose content, can explain about 50% of variation in the percent LBP. The defined relationship between bran starch content and studied factors can explain about 47% of variation in bran starch content of the studied wheat samples.

Based on observations on SEM micrographs, wheat outer tissues including pericarp, seed coats, and aleurone layer could be distinguished. Outer tissues were peeled off the endosperm tissue during the milling process, and deformed and compressed with portion of endosperm tissue. Milled bran tissue is about twice as thick as intact outer layers of a wheat kernel based on scanning electron micrographs. The added thickness could relate to adherent endosperm remnant part, space between outer layers formed upon milling, and swelling of milled bran tissue upon moisture adjustment to 17%. The single-cell thick aleurone layer was not apparent in the micrograph of milled bran strips. Bran starch granules were randomly distributed on or in between the pericarp-seed coats tissues in the bran. The variety with high bran thickness value was found to have thick outer layers in SEM micrographs as well.

The unique properties of bran starch found in the current study suggest that bran starch could be used for different applications in the food industry. The wider gelatinization temperature range, lower pasting peak viscosity, and lower retrogradation level compared to endosperm starch make wheat bran starch a potentially new functional ingredient. Bran starch could have more flexibility under cooking conditions with a wider range of cooking temperature. Lower pasting viscosity could make materials containing bran starch flow more easily in a

pipeline and therefore enhance processability during food production. Lower retrogradation properties of bran starch could be beneficial for shelf life extension innovation. Bran starch, with its proportionally more small granules, might be suitable for non-food applications such as making plastic film filler.

Bran starch has great potential to play the same role in the food industry that chemically modified starch does and, at the same time, maximize the utilization of the bran fraction obtained from the wheat milling process. Bran starch would be a better functional ingredient alternative over chemically modified starch, since it is naturally derived and easy to obtain from the bran through a wet milling process.

The present research established the relationships between bran starch and bran tissue, and provided some possible reasons for why bran starch content varies among wheat varieties. Results obtained provide valuable comparative information about varietal and environmental effects for breeders interested in development of new wheat varieties that provide desired bran starch quantity and type for different applications, and bran properties with enhanced physical resistance during milling. The impacts of variety and environment on studied factors that contribute to bran starch content and percent LBP should be investigated by breeders to develop new wheat varieties that will provide enhanced bran physical resistance or desired bran starch content for specific food applications. The results from this research are also helpful for millers to manipulate the milling process to produce milled bran fractions with higher percent large bran particles or desired bran starch content. Beneficial properties of bran confirmed in this research indicate that applications of bran starch can add great commercial value to the milled bran fraction, and therefore in the long run, bran utilization can eventually be improved.

CHAPTER 6 FUTURE RECOMMENDATIONS

1. Bran starch was found to have unique characteristics compared to endosperm starch. The amylose content of starch is generally proportional to the granule size and maturity of the starch, and large starch granules are usually high in amylose content. Based on findings in the current study and previous relevant research, starch isolated from wheat bran had more small granules and higher amylose content, which could be due to different composition and properties of starch granules in bran starch compared to starch isolated from the counterpart endosperm. It is recommended to isolate A-type (large starch granules) and B-type (small starch granules) starch granules from bran starch and endosperm starch, and to investigate whether small and large granules in these two type of starches are different in composition and physicochemical properties.
2. Wheat varieties studied and their environmental growing conditions were subject to availability of samples grown in the studied crop years. A wider range of soft wheat samples could be used to validate the relationships established in the current study between bran starch and percent large bran particles (LBP) and bran characteristics.
3. Although bran extensibility was confirmed to be regulated by ferulic acid, which is the main element controlling bran mechanical properties and percent LBP, it is recommended to find other contributing variables to fully predict percent LBP and bran starch content.
4. Bound ferulic acid was found to have a strong relationship with bran particle size and bran starch in the current study. Previous studies reported that ferulic acid content varies among the different histological tissues of wheat grain, with the aleurone layer having the highest concentration of ferulic acid. About 70% of the aleurone layer consists of arabinoxylans (AX) and their degree of cross-linking is the main element controlling bran mechanical properties. It is recommended to determine AX and bound ferulic acid distribution in

different wheat bran outer layer tissues (the aleurone layer and pericarp) after hand dissection. Understanding the contributions of each tissue layer to the overall mechanical properties of bran and their relationships to bran starch content is recommended.

5. Significant differences in bound ferulic acid concentrations were found among studied wheat bran samples. A positive correlation was found between bound ferulic acid and bran particle size. The majority of bound ferulic acid is in the form of dehydrodiferulic acid (DiFA), which is the direct cross linker for arabinoxylan cross-linking and largely controls the bran's extensibility. It is recommended to determine DiFA concentration in wheat bran and confirm that arabinoxylan cross-linking controls bran extensibility and affects bran starch content upon milling.

APPENDICES

APPENDIX A

SOFTNESS EQUIVALENCE AND FLOUR YIELD OF SOFT WHEAT VARIETIES GROWN IN MICHIGAN

Softness equivalence is a measurement that indicates friability of the endosperm of the kernel and is an indicator of break flour yield and ease of separation between endosperm and outer layer tissues. If milled flour has smaller particle size and greater break flour yield, the wheat grain from which this flour was milled should have greater softness equivalence. In other words, a high friability of endosperm indicates better milling efficiency and reduced energy requirements to recover flour. Means of softness equivalent values of 44 varieties grown at three locations in 2008 (Figure A-1) showed a wide spectrum ranging from 47 to 67. Flour yield ranged from 68 to 73.2% (Figure A-2). Five varieties (Table A-1) of the 44 varieties were selected for determination of relationships between bran starch and percent LBP (Appendix B) and were subject to availability of samples grown in the studied environment.

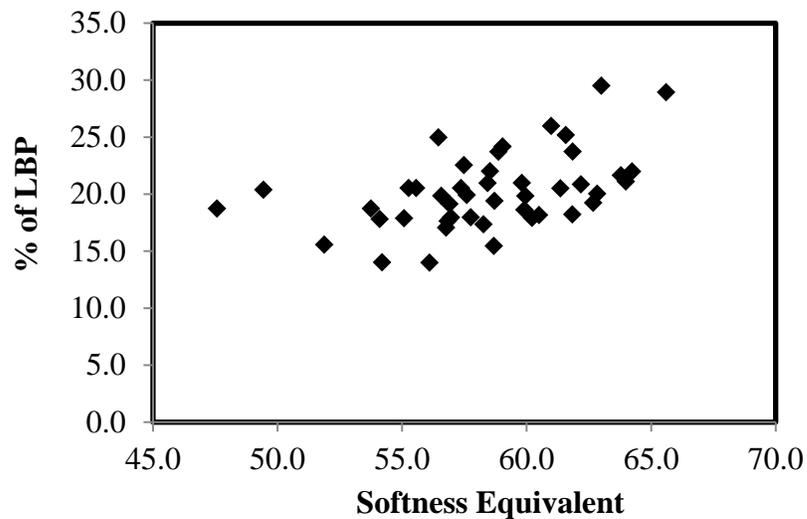


Figure A-1. Relationship between milled large bran particles (LBP) and softness equivalent value based on mean values of 44 soft wheat genotypes grown at three locations in 2008 and milled on a Buhler laboratory mill MLU-202.

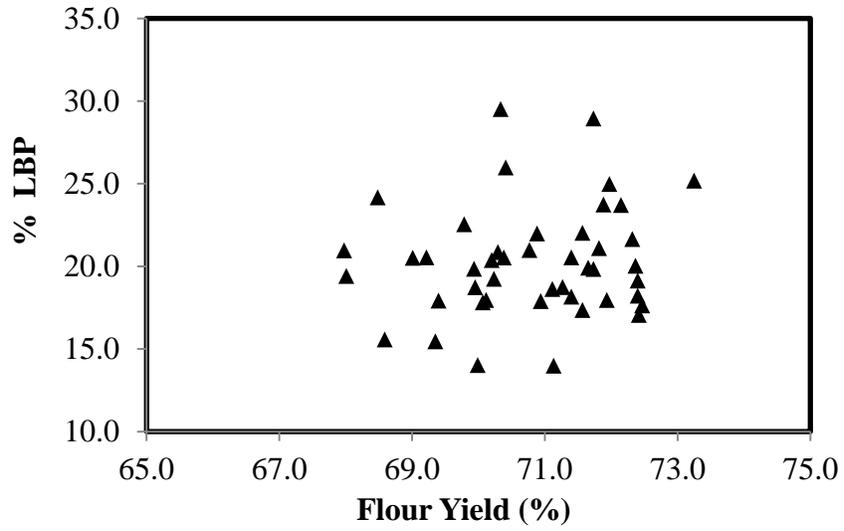


Figure A-2. Relationship between large bran particles (LBP) and flour yield based on mean values of 44 Soft Wheat Genotypes grown at three locations in 2008.

Table A-1 Means of softness equivalent and flour yield of selected soft wheat varieties grown at three locations in 2008

Variety	Softness Equivalent (%)	Flour Yield (%)
E5017	56.5	72.0
Ambassador	59.3	72.5
D8006	61.6	73.2
Aubrey	59.8	70.8
Caledonia	58.9	72.1

APPENDIX B

RELATIONSHIP BETWEEN BRAN STARCH AND PERCENT LARGE BRAN PARTICLES IN SOFT WHEAT GROWN IN MICHIGAN

ABSTRACT

Health benefits of dietary fiber in the human diet have been well-documented. Wheat bran is a major source of fiber as an ingredient in cereal-based products. During wheat milling, bran is separated from the endosperm, though a clean separation is not possible as there is always some starch adherent to the bran (namely, bran starch). The particle size of the milled bran, amount of bran starch, and composition of the bran starch could all play important roles in the processing of cereal-based foods and, in turn, quality of the end-products. The aims of this study were to investigate the relationships among bran size, bran starch content, and dietary fiber content in different Michigan soft wheat varieties, and to develop a method to isolate starch from wheat bran. Bran samples were obtained from laboratory milling of 17 soft wheat varieties that were each grown at three locations in Michigan in three crop years. Sifted bran fractions containing particles larger than 2 mm (large bran particles, LBP) were collected and weighed. Starch contents of LBP, milled flour and bran fractions, and dietary fiber content of water-washed LBP were determined by Megazyme kits. Two isolation methods were investigated for isolating both endosperm starch and bran starch. Five varieties with relatively high crop yield and milling softness equivalence were chosen from the 17 varieties. Percent LBP and bran starch content were found to be significantly different ($P = 0.0002$ and $P = 0.0019$, respectively) among the five varieties. A negative correlation was found between percent LBP and bran starch content ($r = 0.93$). No significant differences were found in soluble and insoluble fiber contents among the five varieties. Bran starch was obtained by alkaline extraction, with 84.97% of starch

recovered. These findings provide a foundation for comparing properties of bran starch with endosperm starch from the same wheat sample.

MATERIALS AND METHODS

Milled bran samples from five different varieties (E0028, Aubrey, E5017, Caledonia and D8006) of soft wheat grown in five environments (06 Midland, 06 Saginaw, 06 Sanilac, 07 Sanilac, 08 Sanilac) were used.

Sifted milled bran fractions containing large bran particles (LBP, > 2 mm) were obtained according to the method described in section 4.3.2. Bran starch content in LBP was determined in triplicate according to the method described in section 3.3.3. Soluble and insoluble fiber contents were determined using Megazyme kit methods (Megazyme International Ireland Ltd. Co., Wicklow, Ireland, 2006) in triplicate.

RESULTS AND DISCUSSION

Percentage of large bran particles (LBP) (Figure B-1) and the mean of bran starch content (Fig.II-2) were each found to be significantly different ($P = 0.0002$ and $P = 0.0019$, respectively) among the five varieties. A negative correlation was found between percentage of LBP and bran starch content (Figure B-3) ($r = 0.93$). No significant differences were found in soluble and insoluble fiber contents among the five varieties (Figure B-4 and Figure B-5). These findings provide a foundation for comparing properties of bran starch with endosperm starch from the same wheat sample. Greater proportion of LBP associated with low bran starch content in LBP might due to clean separation between bran and endosperm tissue for LBP particles with less bran contamination in the flour fraction during milling. The negative correlation found between

percent LBP and bran starch content of LBP is critical knowledge for investigating the relationship between bran starch and bran characteristics, since bran particle size was confirmed to be associated with bran physical properties. A strong positive correlation between extensibility of the wheat grain outer layers and the proportion of larger size (> 2mm) coarse bran was reported by Greffeuille and coworkers (2006). Thus bran physical properties such as extensibility can be considered as a controlling factor responsible for variation in bran starch content of LBP.

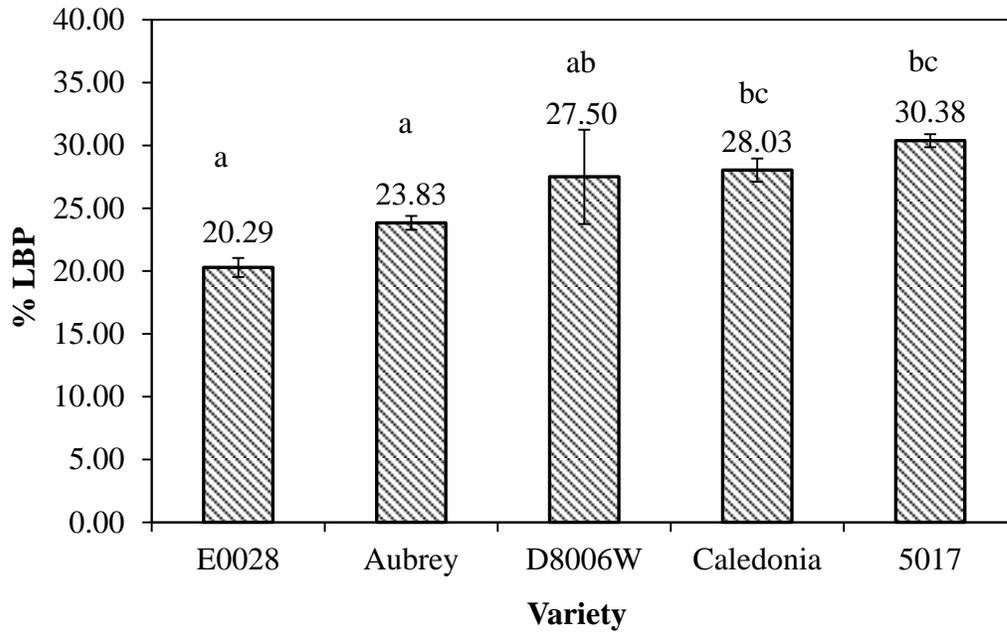


Figure B-1. Percent large bran particles (LBP) for five studied soft wheat varieties. Error bars represent standard errors. The treatments marked with the same letters are not significantly different from each other ($P > 0.05$).

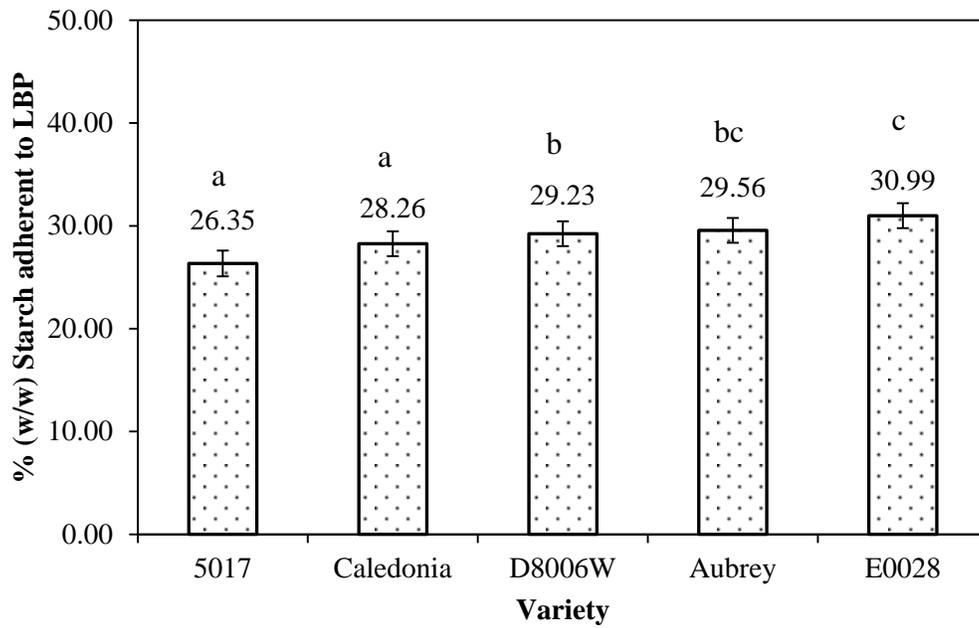


Figure B-2. Mean starch contents of large bran particles (LBP) for five milled soft wheat varieties. Error bars represent standard errors. The treatments marked with the same letters are not significantly different from each other ($P > 0.05$).

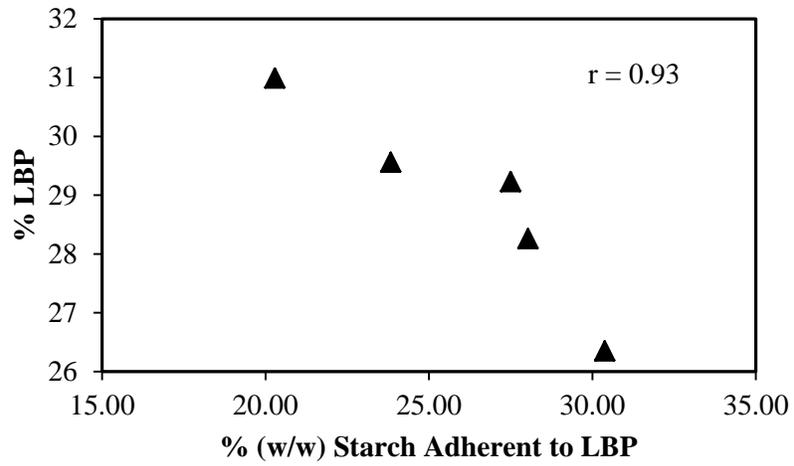


Figure B-3. Relationship between percent large bran particles (LBP) and LBP bran starch content mean of five milled soft wheat varieties.

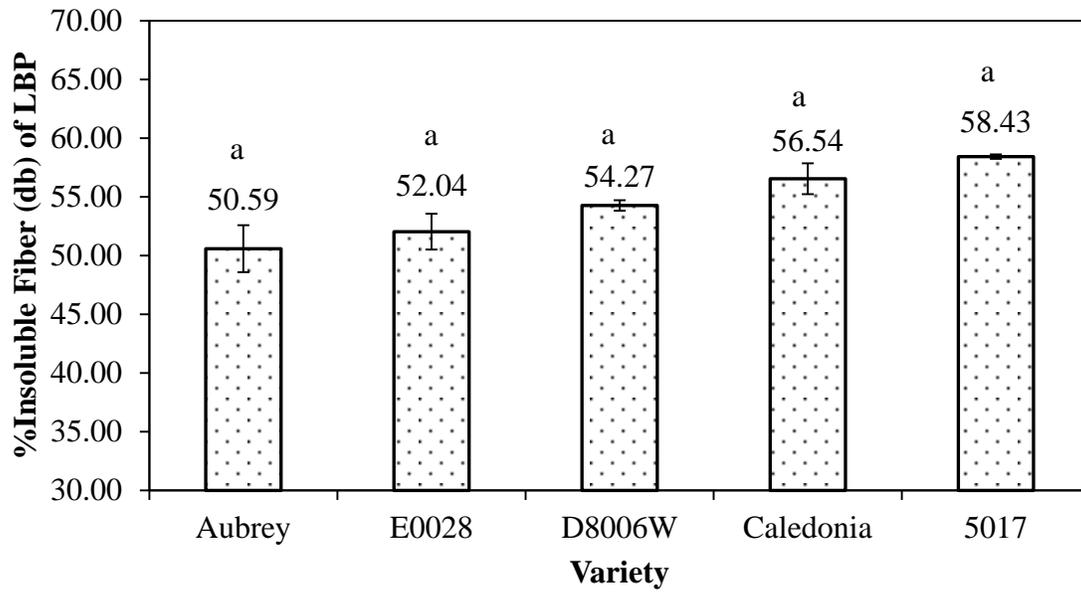


Figure B-4. Percent insoluble fiber means of large bran particles (LBP) for five studied soft wheat varieties. Error bars represent standard errors. The treatments marked with the same letters are not significantly different from each other ($P > 0.05$).

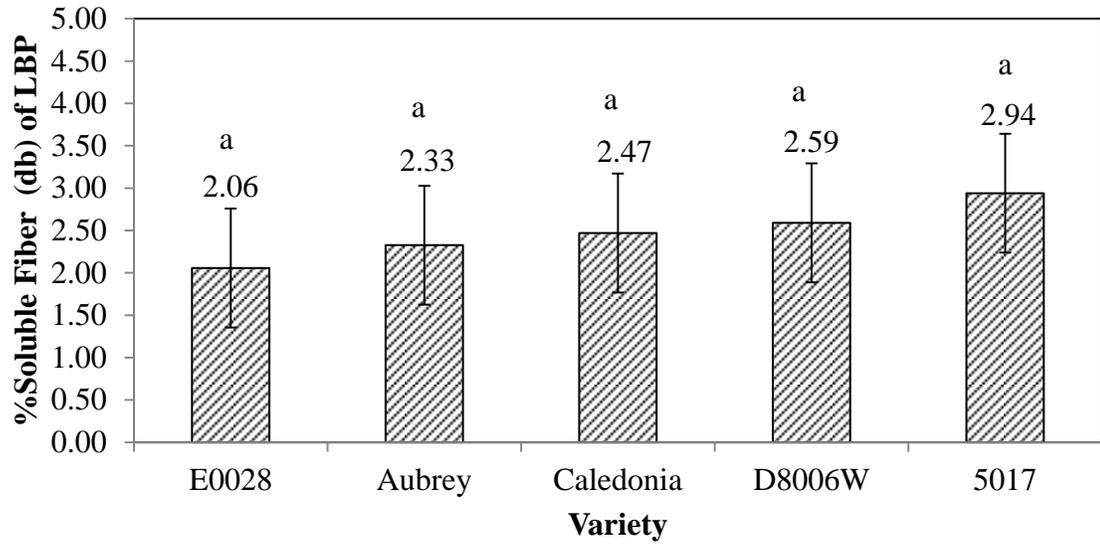


Figure B-5. Percent soluble fiber means of large bran particles (LBP) for five studied soft wheat varieties. Error bars represent standard errors. The treatments marked with the same letters are not significantly different from each other ($P > 0.05$).

APPENDIX C

EFFECT OF PRECONDITIONING GRAIN MOISTURE LEVEL ON PRODUCTS OF A DRY MILLING PROCESS

Characteristics of milled bran can be affected by the milling process. A study as designed to examine the effects of moisture level to which wheat grain is tempered prior to milling. A wheat sample was tempered up to three different moisture levels for the same period of time and milled using the same milling method. Table C-1 shows that wheat samples with different tempering moisture level had different levels of percent LBP produced during milling with higher tempering moisture resulting in higher percent LBP produced. In addition, break flour yield decreased as tempering moisture increased. Total weight of milled bran fraction was also increased by tempering at a higher moisture level.

It was noted that differences in bound ferulic acid specifically its dimer can lead to variations in the water absorption capacity of cell walls, and thus affect a tissue's mechanical properties. As a result, milling performance can be different (Peryon 2002). So biochemical composition of bran tissue is still the fundamental regulator of the milling performance when different processing conditions are considered. Information from this study provides additional reference for millers to manipulate the milling process in order to obtain desired milling quality and high percent LBP fraction.

Table C-1 Effect of Tempering Moisture Level on Percent Large Bran Particles (LBP) in the Milled Bran Fraction of Soft Wheat Variety Caledonia Grown at Allegan in 2010^x

Tempering moisture (%)	Bran yield (%)	Break flour (%)	% LBP ^y
13.5	17.15 ±0.07	35.05 ±0.64	28.34 ±1.94a
14.5	18.15 ±0.07	32.50 ±2.26	34.57 ±0.28b
15.5	19.55 ±0.21	32.10 ±0.99	37.55 ±0.15b

^x Values are means ± standard deviation.

^y Values marked with the same letters are not significantly different from each other (P > 0.05).

APPENDIX D

AMYLOSE CONTENTS OF BRAN STARCH AND ENDOSPERM STARCH ISOLATED BY DIFFERENT ISOLATION METHODS

Amylose contents were determined for isolated starches using two different isolation methods, alkaline extraction method and NaCl and toluene shaking method. Since there was not much starch yield for endosperm starch isolation using the NaCl and toluene shaking method, no results for endosperm starch isolated with this method was obtained. As shown in Table V-1, amylose contents in bran starch isolated by both alkaline extraction and NaCl and toluene shaking method were all higher than endosperm starch isolated by the alkaline method, which was in agreement with results in Table 3.3, and confirmed that bran starch contained significantly higher amylose than its counterpart endosperm starch. There was an elevated amylose content of bran starch when isolating by alkaline extraction. The alkaline method could possibly recover more amylose than the toluene shaking method, and identified the alkaline method as having an advantage over the toluene shaking method for the objectives of the research in the current study.

Table D-1 Amylose Contents of Bran Starch and Endosperm Starch, Isolated using Two Different Methods^{x,y}, from Soft Wheat Variety Caledonia Grown at Huron in 2010^z

	Amylose content (%)
Br starch ^x	17.80 ± 3.01
Br starch ^y	20.90 ± 0.69
En Starch ^x	—
En Starch ^y	13.41 ± 2.61

^x Starch isolated by 0.5 M NaCl and toluene according to Xie et al (2008).

^y Starch isolated by alkaline extraction described in section 3.3.2.

^z Values are means ± standard deviation. Br: bran; En: endosperm.

APPENDIX E

RESISTANT STARCH CONTENTS IN BRAN STARCH AND ENDOSPERM STARCH ISOLATED FROM THREE WHEAT VARIETIES

Resistant starch contents in bran starch and counterpart endosperm starch isolated from three soft wheat varieties (Aubrey, Caledonia, and D8006) grown at Lenawee in 2011 by alkaline extraction method (Figure.3.1) were determined by Megazyme kit methods (Megazyme International Ireland Ltd. Co., Wicklow, Ireland, 2006). No significant differences in resistant starch content were found among all three varieties or between counterpart starches of each variety (Table D-1). The percent resistant starch present in bran starch and endosperm starch of studied soft wheat varieties was very low, less than 0.3%. This finding was different from what has previously been reported (9.49% in bran starch) in Xie et al (2003). Since starches in the present study were all native starch, it is reasonable for them to be very low in resistant starch content. It was reported that flour (unknown wheat variety) has 3% resistant starch (Sajilata et al 2006). Resistant starch contents measured in current study were lower than this reported flour resistant starch content, and might be due to variations among wheat varieties. During isolation method development of the present research, resistant starch contents of isolated bran starch and endosperm starch were found to be less than 1% as well (Appendix V). After hydrolysis of non-resistant starch by enzymes (α -amylase and amyloglucosidase) at 37 °C for 16 hr, very little white starch was observed in the centrifuge tube. Even with increased centrifugation force to 12,900 \times g, the quantities of resistant starch isolated were still very low. There was no significant difference found between the resistant starch content of bran starch and that of endosperm starch, with only trace amount isolated from each.

Table E-1 Resistant Starch Content in Bran Starch and Endosperm Starch Isolated from Three Soft Wheat Varieties^x

Sample	Resistant Starch (%)
Br-Aubrey	0.31 ±0.00a
En-Aubrey	0.27 ±0.02a
Br-Caledonia	0.29 ±0.01a
En-Caledonia	0.30 ±0.02a
Br-D8006	0.28 ±0.02a
En-D8006	0.27 ±0.00a

^x Values are means ± standard deviation. Values marked with the same letter are not significantly different from each other ($P > 0.05$). Comparisons were conducted between Br and En starches within the same variety. Br: bran starch; En: endosperm starch.

LITERATURE CITED

LITERATURE CITED

- Greffeuille, V., Abecassis, J., Lapierre, C., and Lullien-Pellerin, V. 2006. Bran size distribution at milling and mechanical and biochemical characterization of common wheat grain outer layers: a relationship assessment. *Cereal Chem.* 83:641-646.
- Peyron, S., Chaurand, M., Rouau, X., Abecassis, J., 2002. Relationship between bran mechanical properties and milling behaviour of durum wheat (*Triticum durum Desf.*). Influence of tissue thickness and cell wall structure. *J. Cereal Sci.* 36:377-386.