Distribution and Structural Variation of Non-Starch Polysaccharides in Milling Fractions of Hull-less Barley with Variable Amylose Content and the Subsequent Evaluation of Baking Procedures for Incorporation of Barley Roller Milling Fractions Containing High Levels of Dietary Fiber into Bread

BY

MORRISON SHAWN JACOBS

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree of Master of Science

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ABSTRACT

Three hull-less barley genotypes with variable amylose contents were pearled to 10% and roller milled into two major milling fractions, flour and fiber-rich fractions (FRF), in order to determine their composition and compare the distribution and structural variation of non-starch polysaccharides (NSP) originating from their different locations in the barley kernel. Each milling fraction contained various proportions of proteins, starch, ash, and non-starch polysaccharides, mainly β–glucans, arabinoxylans and arabinogalactans. The pearling-by-products (PBP) were enriched in arabinoxylans, protein and ash and contained small amounts of starch and β–glucans. FRF were considerably enriched in β–glucans and arabinoxylans. Arabinogalactans were concentrated in the outer portion of the barley kernel. The content and solubility of NSP in various milling fractions was also dependent on the type of barley. The solubility of β–glucans was higher in PBP than in FRF. The solubility of arabinoxylans was higher in FRF than in PBP. Isolation of water- and alkali-extractable sub-fractions revealed that β–glucans and arabinoxylans exhibited structural heterogeneity derived from differences in their location within the kernel as well as from barley genotype. Substantial differences in the molecular weight of NSP in different milling fractions were also observed.

FRF from waxy and high amylose starch genotypes were evaluated as functional ingredients in pan bread using three bread making processes: a full formula mechanical development procedure; a lean formula, long fermentation straight-dough procedure; and a sponge-and-dough procedure with a 4½ hr sponge fermentation time. The addition of 20% FRF disrupted dough properties and depressed loaf volume. The impacts of FRF from waxy and high-amylose starch barley on bread characteristics were similar. Baking process strongly influenced bread quality. The full formula mechanical development procedure was poor because of

disruption at mixing resulting in no development. The long fermentation straight-dough procedure was better, the long fermentation allowed better dough development than the mechanical dough development. The sponge-and-dough process produced the best FRF-enriched bread due to the positive effect of sponge fermentation on gluten development and hydration. Pre-hydrated FRF added at the dough stage to fully developed sponge improved bread quality compared to adding un-hydrated FRF. CWRS and CWES flour produced comparable FRF-enriched sponge-and-dough bread. Addition of xylanase to the sponge-and-dough formula improved the loaf volume, appearance and crumb structure of FRF-enriched bread.

INTRODUCTION

Hull-less barley has recently attracted a lot of interest among food scientists and technologists as an excellent source of both soluble and insoluble fiber. Dietary fiber has been shown to have health benefits in human diets by improving gastro-intestinal function, lowering blood cholesterol (Newman et al, 1989; Anderson et al, 1990; Ranhotra et al, 1991; Braaten et al, 1994; Bhatty, 1999a) and blood glucose lowering (hypoglycemic) properties (Wood et al, 1994; Bhatty, 1999a). As part of its initiative to provide consumers with the information they need to make healthy nutritional choices about foods and dietary supplements, the U. S. Food and Drug Administration announced that whole grain barley and barley-containing products are allowed to claim that they reduce the risk of coronary heart disease (FDA, 2005). The two non-starch polysaccharides which account for the majority of dietary fiber in barley are mixed linkage β-(1→3, 1→4)-glucans and arabinoxylans (Henry, 1988). Research has shown that waxy and high amylose hull-less barley cultivars contain consistently higher β-glucan concentrations (6-10%) compared to normal amylose genotypes (4-6%) (Xue et al, 1991; Izydorczyk et al, 2000). The distribution of arabinoxylans and β-glucans throughout the barley kernel is not uniform, with the aleurone layer enriched in arabinoxylans and the endosperm cell walls enriched in β-glucans (Izydorczyk et al, 2003b). Although the general structure of barley β-glucans is well known (Woodward et al, 1988; Buliga et al, 1986; Izydorczyk et al, 1998a), previous research has focused mainly on β-glucans present in hulled malting barley. Additionally, little is known about structural variations of β-glucans relative to their location in the barley kernel and consequently in different milling fractions of barley. Arabinoxylans have not been investigated to the same

extent as β-glucans but they can form viscous solutions and may have favorable physiological effects.

Barley does not have a long tradition of being fractionated by milling. Nevertheless, it has been roller-milled into flour and bran, using wheat milling equipment (Bhatty, 1997; Izydorczyk et al, 2003a). Because the distribution of barley non-starch polysaccharides (NSP) is not uniform throughout the kernel, by employing both pearling and milling techniques, it is possible to obtain fractions enriched in specific functional components and/or bio-active nutrients such as β-glucans, arabinoxylans, starch, tocols, proanthocyanidins, catechins and other phenolic compounds (Slavin et al, 2000a).

Hull-less barley flour has been incorporated successfully into muffins, pancakes and biscuits (Berglund et al, 1992) and into chapattis (Sidhu et al, 1990). Barley fractions have also been incorporated into pasta and noodles (Knuckles et al, 1997; Marconi et al, 2000; Dexter et al, 2005; Hatcher et al, 2005; Izydorczyk et al, 2005; Legasse et al, 2006). The tendency for barley fractions to impart a darker grey color (Quinde et al, 2004) to pasta and noodles is a potential deterrent to traditional consumers but may be adequate or even attractive for health conscious consumers. It also appears that addition of barley fractions may have some positive effects on the texture of various food products. Hatcher et al (2005) found that texture modification of yellow alkaline noodles (YAN) enriched with hull-less barley flour was related to the starch type; YAN chewiness decreased with addition of waxy hull-less barley flour, but increased with addition of normal amylose and high amylose hulless barley flour. Baik and Czuchajowska (1997) reported that introducing 15% waxy barley flour into Japanese Udon noodles also altered their texture; the

texture profile analysis indicated that hardness, cohesiveness, springiness, gumminess and chewiness of Udon noodles decreased with the addition of barley flour.

Many researchers have tried to incorporate barley and barley fractions into bread and bread products (Dubois, 1978; Bhatty, 1986a; Swanson and Penfield, 1982, 1987, 1988; Newman et al 1990, 1998; Lai et al, 1989; Newman and Newman, 1991; Knuckles et al, 1997; Bhatty, 1986a; Cavallero et al, 2002; Gill et al, 2002a, 2002b; Mann et al, 2005; Trogh et al, 2004, 2005). Barley and barley fractions incorporated into leavened products has universally been reported to diminish bread quality, particularly loaf volume. Nevertheless, a general consensus is that it is feasible to produce barley-enriched western-style breads that have acceptable flavor, appearance and texture.

The objectives of the current study were to determine and compare the distribution of non-starch polysaccharides (NSP) in three milling fractions of hull-less barley genotypes with different starch characteristics (high amylose, normal and waxy), and to elucidate the structural variations of water soluble and insoluble non-starch polysaccharides originating from different locations in the kernel. Based on this information, a study was carried out to determine the effects of incorporating fiber rich milling fractions from pearled barley on the quality of bread. This investigation aimed to establish a method of formulating and baking a fiber-enriched bread loaf that not only has satisfactory baking quality, but also increased health benefits to consumers, thus improving market opportunities for barley producers.

LITERATURE REVIEW

Introduction

Like other cereal crops, the cultivation of barley began in the Fertile Crescent around 7000 B.C. Throughout history, barley has played an important role as a cereal crop for animal feed, malting, and human nutrition. Today, cultivated barley accounts for 12% of the world's cereal production, ranking fourth behind wheat, rice and corn (Jadhav et al, 1998).

In 2004, the world production of barley was approximately 150,000,000 t. The top barley producing countries in 2004 were Russia (18,000,000 t), Canada (13,186,000 t), Germany (12,800,000 t), Ukraine (11,200,000 t), France (11,000,000 t), Spain (10,550,000 t), Turkey (7,100,000 t), Australia (6,220,000 t), United States (6,080,000 t) and United Kingdom (5,900,000 t). Production of barley in the unified European Union-25 (61,020,000 t) and Former Soviet Union-12 (33,640,000 t) accounted for 63% of world barley production (FAOSTAT). In 2003, Canada devoted 11,600,000 acres to barley production in the provinces of Alberta, Saskatchewan, and Manitoba (Statistics Canada, 2003).

The high production of barley in Canada is essential to support the cattle and hog industries. In Canada, 70% of barley production was used as feed (Statistics Canada, 2003). Following animal feed, the second greatest use of barley is for malting purposes. When malt barley exports are considered, only 15% of Canadian barley is used for malt production. Barley for food purposes, accounts for less than 1% of Canadian production (9,000 tons).

For humans, barley offers many nutritional benefits. It has been shown that barley has serum cholesterol controlling abilities (Bourdon et al, 1999; Sundberg et al, 1995a), can aid in gastrointestinal function, and has insulin and glucose lowering effects (Cavallero et al, 2002;

Wood et al, 1994; Granfeldt et al, 1994). Barley also has cancer preventing properties and is beneficial for the general health of humans (Newman and Newman, 1991).

The barley grain is comprised of carbohydrates (80%), protein (13%), lipids (2-3%), trace vitamins and minerals (2-3%) and dietary fiber (2-3%) (Newman and Newman, 1991). Of particular interest is the portion of the grain that provides dietary fiber. Dietary fiber is the primary component responsible for viscosity of barley extracts. It has been postulated that soluble fiber increases the viscosity of food within the intestines and thus, prevents the absorption of dietary cholesterol and fat into the blood stream (Kalra and Jood, 2000; Yokohama et al, 1997). Total dietary fiber (TDF) comes from the non-starch polysaccharides and lignin that make up the cell walls (Newman and Newman, 1991). The major carbohydrate components of the cell walls are cellulose, β-glucan, and arabinoxylans. Barley grain contains some lipids with important antioxidant (e.g., tocopherols and tocotrienols) and cholesterol lowering properties (e.g., α-linoleic acid, α-D-tocotrienol). It has also been shown that barley components such as phenolic acids, phytin, vitamin E, proanthocyanidins, and catechins have antioxidant properties and may prevent formation of carcinogens (Newman and Newman, 1991). Barley is also an excellent source of B-complex vitamins, especially thiamine, pyridoxine, pantotheic acid niacin, biotin, and folacin (Newman and Newman, 1991). The minerals present in the barley grain include phosphorus, potassium, and calcium, with smaller amount of magnesium, sulphur and sodium (Briggs, 1978).

The Barley Kernel

The physical composition of the barley grain can be delineated into two regions (Figure 1). The outer part of the barley caryopsis is called the hull or husk and is formed by the lemma

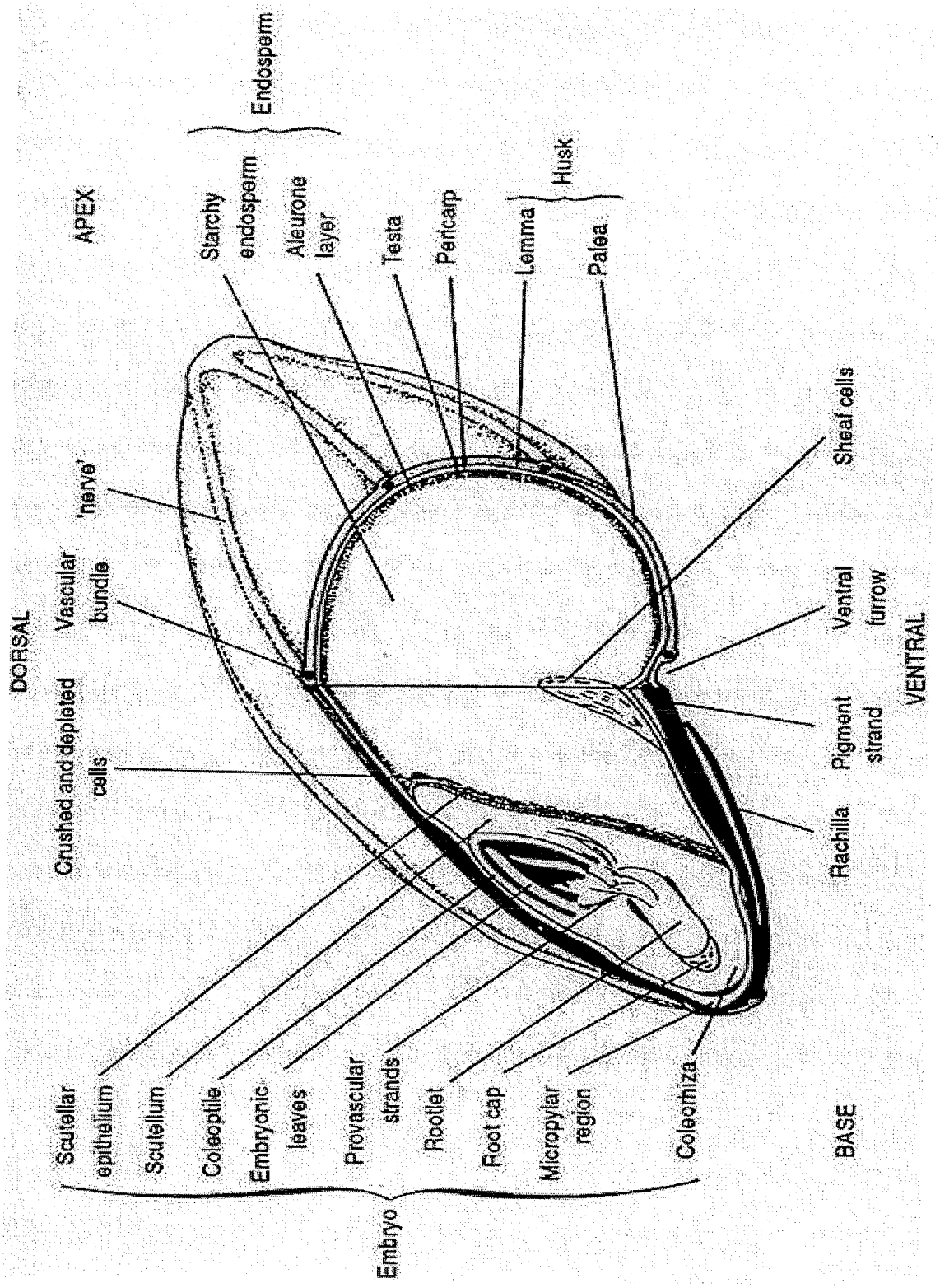

Figure 1. View of barley cross section (Briggs, 1978)

and palea. The inner part of the grain has several components and layers. Working from the outermost stratum of the caryopsis, the first layer is the pericarp. The pericarp layer is composed of cells that originally formed the ovary wall of the developing seed. The pericarp acts as a protective cover for the caryopsis. The husk and pericarp consist primarily of cellulose, hemicellulose, lignins and lignans. The husk contains the highest amount of minerals followed by the embryo and the endosperm. The next layer of cells is referred to as the integuments. These layers of cells form the seed coat. Beneath the integuments lies the aleurone layer, the outermost layer of the endosperm. The aleurone layer contains proteins and enzymes that aid in the digestion of the endosperm by the growing embryo. The thick, unlignified walls of the aleurone layer are composed mainly of arabinoxylans (67-71%) β-glucans (26%) and phenolic acids. This tissue also contains proteins (17-20%), lipids, minerals, phytin and sugars (Stone, 1996). The starchy endosperm makes up about 75% of the entire barley grain. Starch, which constitutes about 80% of the endosperm, exists in a granular form with two distinct populations of large and small granules.

Starch is composed of two polysaccharides, amylose and amylopectin. Both polysaccharides consist of polymers of α-D-glucopyranosyl residues. In amylose the glucopyranosyl residues form mostly-unbranched chains of α-(1→4)-D-glucopyranosyl units, whereas amylopectin is formed by non-random α-(1→6) branching of the amylose-type α-(1→4)-D- glucopyranosyl structure. Normally amylopectin is 70-80% w/w of granule starch and amylose is 20-30% w/w of granule starch. However, barley varieties have been developed with altered starch composition and include cultivars with high amylose and waxy (100% amylopectin) starch composition. The altered starch varieties have been reported to have higher

β-glucan than varieties with normal amylose to amylopectin ratios (Bhatty, 1999a; Izydorczyk et al, 2000). The starchy endosperm also contains a relatively high amount of proteins, some lipids, and a small amount of minerals. The cell walls of starchy endosperm are built up mainly of β-(1→3, 1→4)-glucans (70%), and arabinoxylans (20%) and smaller amounts of proteins, β (1→3)-glucans and other polysaccharides containing galactose, mannose and uronic acids. Lastly, the embryo which lies within the barley grain contains a large amount of proteins (34%), lipids (14-17%), minerals (5-10%), and sugars.

The molecular characteristics and amount of β-glucan and arabinoxylans can be strongly influenced by environmental and genotypic conditions. Generally, β-glucan concentration is highly correlated with protein concentration (Fastnaught, 1995). Also, a genotype with higher concentration of starch will generally have a lower concentration of β-glucans and arabinoxylans. Therefore, waxy and high amylose genotypes have more β-glucans than non-waxy genotypes (Bhatty, 1999b).

β-Glucan

A major component of the cell walls in the endosperm is β-glucan, a polymer built up of D-glucopyranosyl residues (Figure 2). In barley, β-glucans make up 76% of the cell wall of the endosperm and 26% of the cell walls of the aleurone layer (Stone, 1996). β-Glucan is also found in low quantities in the husk, and the outer layers of the barley grain. β-Glucan is composed of glucopyranosyl residues joined via β(1→3) and β(1→4) linkages. Within β-glucan, about 30% of the glucopyranosyl residues are joined by β(1→3) glycosidic links and the other 70% by β(1→4) glycosidic links. These linkages are not arranged at random. Two or three contiguous

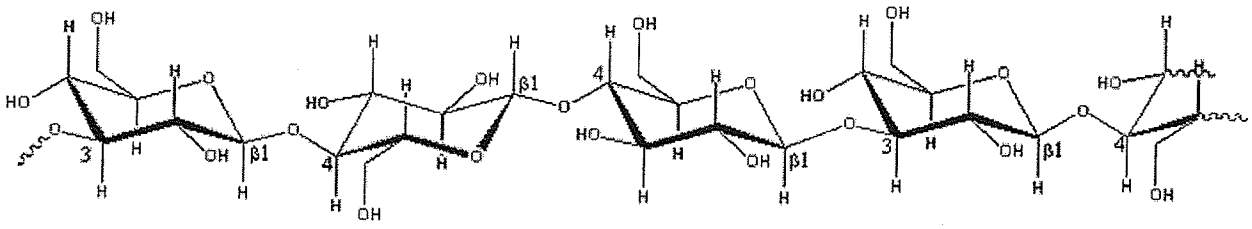

Figure 2. The structure of β-glucan showing the D-glucopyranosyl residues that makes up the polymer.

(1→4) linked β-glucosyl residues separated by a single (1→3) linkage are the most prevalent. Hydrolysis of the native polymer reveals that ~90% of the polysaccharide chain consists mainly of cellotriosyl and cellotetrosyl units connected by single β(1→3) linkages. The remaining structure contains longer consecutive β-(1→4)-linked D-glucopyranosyl units (cellulose-like fragments) (Aman and Graham, 1987). The fine molecular structure of β-glucans has been investigated using specific enzymes which partially hydrolyze the polymer. Lichenase, (1→3)(1→4) β-D-glucan-4-glucanohydrolase cleaves the linkages of the 3-O-subsituted glucose units in the β-glucan (Figure 3). Oligosaccharides produced after hydrolysis of β-glucans with lichenase can be analyzed by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) or matrix-assisted laser desorption/ionization mass spectroscopy (MALDI-MS) (Jiang and Vasanathan, 2000). HPAEC-PAD is the most common technique, but it is not sensitive for oligosaccharides which have a high degree of polymerization. Recently, the MALDI-MS, has shown some promise as a rapid and sensitive technique for quantification of oligosaccharides (Jiang and Vasanthan, 2000).

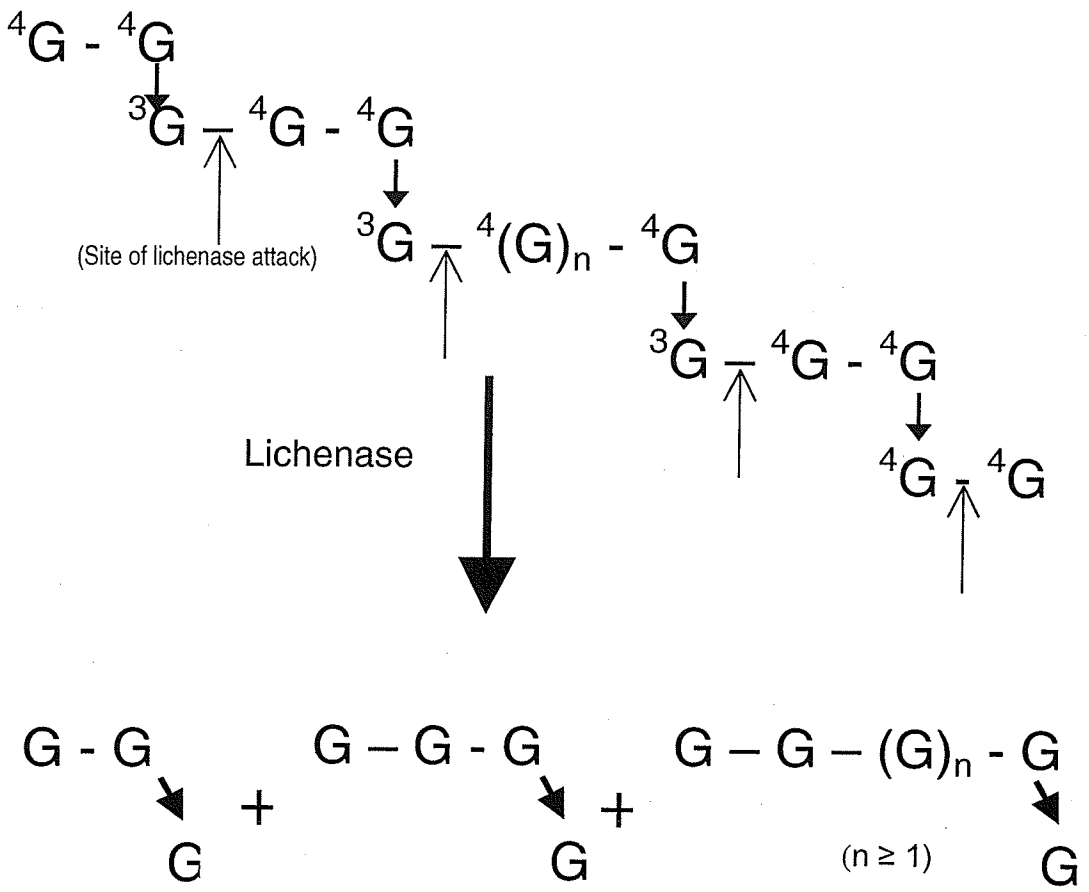

Figure 3. The effect of lichenase on mixed linkage β-glucan. (Adapted from Jiang and Vasanthan, 2000)

When purified barley β-glucans are examined by HPAEC-PAD after lichenase treatment the released oligosaccharides, 3-O-β-cellobiosyl-D-glucose (DP3) and 3-O-β-cellotriosyl-D-glucose (DP4), are the main building blocks of the β-(1→4)-linked polysaccharide in the original chain accounting for ~90% of the polysaccharide structure. The remaining structure contains four to fifteen consecutively (1→4)-linked D-glucopyranosyl units, indicative of the cellulose-like regions in the β-glucan (Wood et al, 1994, Izydorczyk et al, 1998b). The molar ratio of DP3 to

DP4 has been found to differ substantially among various cereals when analyzed by HPAEC-PAD; Wood et al (1991 and 1994) reported a higher ratio of DP3 to DP4 in β-glucans purified from whole barley (2.8-3.3) compared to that from purified oats (2.1-2.4). When whole barley was milled to obtain fractions containing bran, shorts and flour which represent the pericarp, aleurone and starchy endosperm respectively the ratio of tri- and tetrasaccharides ranged from 1.51 to 1.77, with shorts having lower values than flour and bran (Andersson et al, 2004). It is generally believed that the ratio of tri- to tetrasaccharides affects the gelation and viscoelastic properties of β-glucans. Higher ratios of tri- to tetrasaccharides have previously been associated with decreased solubility or extractability of β-glucans (Izydorczyk et al, 1998a). It has been postulated that since a helix of three consecutive cellotriosyl residues would form a crystalline structure, a higher content of these cellotriosyl fragments might contribute to greater conformational regularity, translating into lower β-glucan solubility (Tvaroska et al, 1983; Izawa et al, 1993; Izydorczyk et al, 1998a).

The apparent molecular weight estimates of isolated mixed-linkage β-glucan fractions vary from $2.0x10^4$ to $40.0x10^6$ (Fincher and Stone, 1986). The apparent discrepancies of the molecular weight estimates of β-glucans from different sources may be attributed to variation in cell wall structures. This phenomenon was observed by Andersson et al (2004) who reported β-glucans had slightly lower molecular weight in white flour compared to shorts milling fractions. Variation among barley cultivars has also been observed. Storsley et al (2003) reported the average molecular weight of β-glucans isolated from high amylose, waxy, and normal hull-less barley genotypes ranged from 0.22×10^6 to $5.95x10^6$ g/mol with higher molecular weights observed in high amylose and waxy barleys compared to the normal barley genotype.

Arabinoxylans

The other structural polysaccharides of interest to this research are arabinoxylans. In the barley grain, arabinoxylans account for 60-70% of the aleurone cell walls and for 20-40% of the endosperm cell walls. These percentages fluctuate due to environmental and genetic factors (Izydorczyk and Biliaderis, 1993).

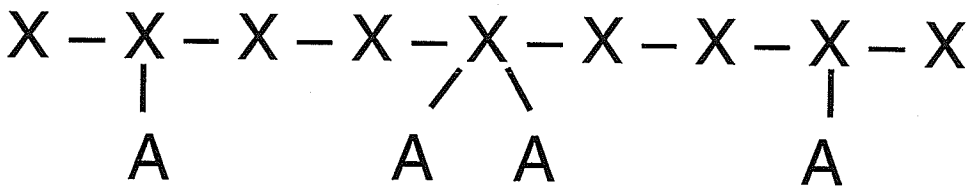

Figure 4. General structure of arabinoxylans

Arabinoxylans are composed of a linear (1→4)-β-D-xylan (Xyl*p*) backbone with α-L-arabinofuranose (Ara*f*) residues attached to the xylose units at the C(O)2, C(O)3 and/or at both C(O)2,3 positions (Figure 4) (Briggs et al, 1981; Izydorczyk and Biliaderis, 1993). These arrangements result in four structural elements in the molecular structure of arabinoxylans: mono-substituted Xyl*p* at O-2, or O-3, di-, and un-substituted Xyl*p* (Figure 5). In most cases, the Ara*f* units are attached as single units, although short chains of Ara*f* have also been found. The distribution of Ara*f* along the xylan backbone is non-random (Izydorczyk and Biliaderis, 1994). Regions that are sparsely substituted may be binding sites for polymer-polymer interaction. Arabinoxylans present in the outer layers of barley (husk and bran) also contain glucuronic acid (and its 4-methyl ester) residues in addition to arabinose and xylose residues (MacGregor and Fincher, 1993). Some Ara*f* units carry esterified feruloyl residues (FA) at C(O)5 (Mueller-

Harley and Hartley, 1986), which allow them to cross-link to other Ara*f* via dehydrodiferulic acid bridges. Arabinoxylans can potentially cross-link with other cell wall polymers by ferulate dimerization via photochemical or free radical coupling reactions of ferulate-polysaccharide esters (Izydorczyk and Biliaderis, 1994).

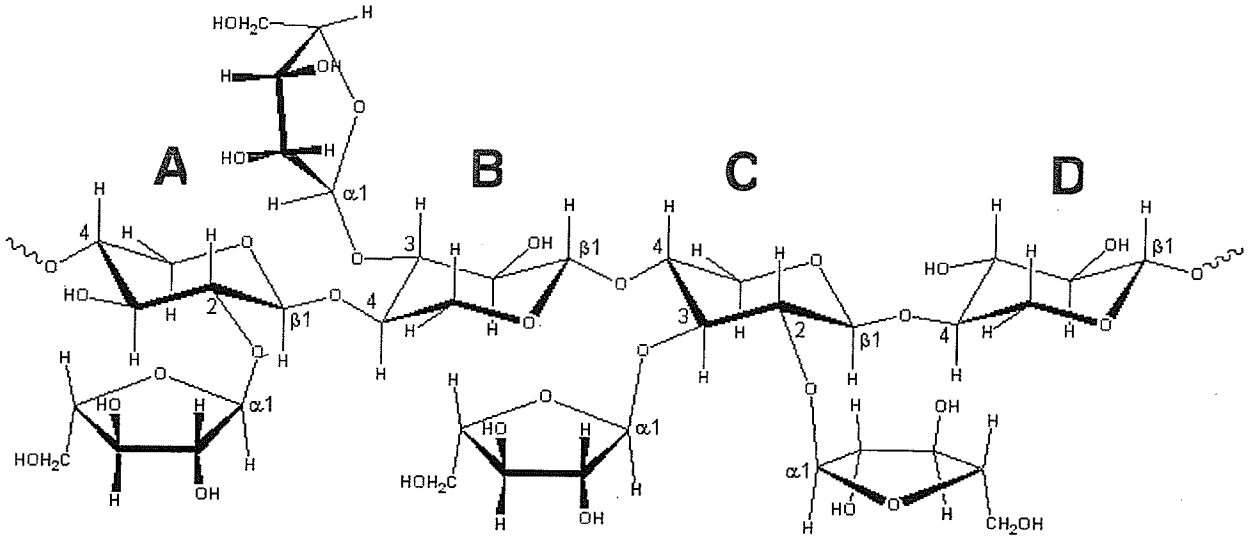

Figure 5. Structural elements present in arabinoxylans: (A) monosubstituted Xylp at O-2; (B) monosubstituted Xylp at O-3; (C) disubstituted Xylp at O-2,3; (D) unsubstituted Xylp

The number of arabinose side units along the xylan chain affects the solubility of arabinoxylans and is indicative of the degree of branching. Depending on the origin of arabinoxylans, the ratio of Ara/Xyl may vary from 0.3 to 1.1 (Izydorczyk et al, 2003b). Different ratios of Ara/Xyl units do not reveal the relative amounts of mono-, di-, and unsubstituted xylose residues nor their sequence in the polysaccharide chain. Water- soluble arabinoxylans from barley contain between 47 and 65% unsubstituted, 20-25% monosubstituted, and 19-26% disubstituted xylose residues (Oscarsson et al, 1996; Izydorczyk et al 1998a; Dervilly et al, 2002). The presence, amount and distribution of arabinosyl residues along the

xylan backbone affects the solubility and potential of arabinoxylans to interact with each other or with other polysaccharides.

The molecular weight, chain conformation, and molecular structure affect the viscosity and gel formation potential of arabinoxylans in solution. Size exclusion chromatograms of arabinoxylans from barley indicate a very broad distribution of molecular weights (Izydorczyk, et al, 2003b). Arabinoxylans exhibit a very high viscosity in aqueous solutions because of their high molecular weight and locally stiff, semi-flexible random coil conformation. Arabinoxylan solutions can form hydrogels through dimerization of ferulic acid substituents which covalently cross link in the presence of free-radical generating agents (Izydorczyk and Biliaderis, 1993; Carvajal-Millan et al, 2005). Additionally, the amount and distribution of arabinose residues along the xylan backbone also affects their gelation capacity (Izydorczyk and Biliaderis, 1993).

Milling Barley

Barley is a lesser used food grain than oats and wheat because of its brittleness and shattering when processed for food in the same manner as oats or wheat. It is desirable to increase the amount of barley in food products because it provides potential health benefits. Barley grain usually needs to be abraded or milled into flour to be used for human consumption. Abraded or pearled barley can be used directly in soups, stews, and porridges, although it is often subjected to further processing such as milling, grinding or flaking before incorporation into food products. Barley flour has potential uses in baked goods, instant foods, pastas, and extruded snacks (Bhatty 1987, 1996; Newman et al, 1990; Berglund et al, 1992), or it can also be fractionated into starch and non-starch carbohydrates and used as ingredients in various food and non-food applications (Klamczynski et al, 1998).

Common methods for milling barley include roller milling, hammer or pin milling followed by sieving and/or air classification. Pearling is often conducted before milling to improve the color of barley flour and/or change the composition of milling fractions. Pearling is one of the oldest practices of processing barley and involves abrasive scouring that gradually removes hull, pericarp, seed coat, aleurone and sub-aleurone layers, and embryo. Since pearling removes the components present in the outer layers, the composition of pearled barley differs from that of whole barley. Generally, the amount of vitamins and insoluble fiber decreases, but the amount of soluble fiber and β-glucans increases. The content of fat, protein, tannin, and phosphorous increases with pearling rates up to about 15-30% decortication and decreases thereafter (Pedersen et al, 1989; Sundberg and Aman, 1994; Bhatty 1996, 1997).

Although roller milling of barley does not have a long tradition, barley has been roller milled into flour and bran using wheat milling equipment. The process involves several steps of breaking the grain, sizing and reducing the particles using corrugated and smooth rolls (Izydorczyk et al, 2003a). Shorts duster passages, which utilize impact to separate starchy endosperm from endosperm cell walls, appear to facilitate flour release. The number and sequence of passages in a mill affects the yield and composition of milling fractions. The main fractions obtained after roller milling of barley are flour, bran and shorts. However, the composition of the latter two fractions differs substantially from those obtained by milling wheat. For barley samples pearled to ≥20%, the ' bran' fraction is derived mainly from the sub-aleurone and endosperm layers rather than from the more outer layers as in wheat. In addition, the fraction designated as 'shorts' (fine bran) in wheat milling, originates mainly from the endosperm cell walls, regardless whether barley was pearled before milling; this fraction is rich in β-glucans

and arabinoxylans and was, therefore, designated as fiber rich fraction (FRF) (Izydorczyk et al, 2003a). The other important factors affecting the flour yield are the interrelated contents of starch and β-glucans in the barley grain. After milling, hulless barley (HB) genotypes with high β-glucan give lower flour yields than low β-glucan genotypes of HB under the same conditions (Bhatty, 1999b). Bhatty (1997) showed that dry milling of HB with regular starch content results in a greater flour yield (58%) than dry milling of HB waxy starch genotype (42%). Also, the high β-glucan barley genotypes yield higher amounts of 'bran' and 'fiber-rich fraction' than normal barley (Izydorczyk et al, 2003a). Bhatty (1999b) postulated that the presence of β-glucans in the cell walls resists particle size reduction during the milling processes and therefore results in lower flour yields. Pre-processing practices, such as tempering and pearling, may affect the yield, particle size distribution and composition of milling fractions (Izydorczyk et al, 2003a). The flour that results from roller milling of barley contains 2-5% β-glucans. By sieving the ground barley, the β-glucan content can be increased to about 25% (Sundberg and Aman, 1994).

β-Glucan-enriched fractions can also be obtained when barley is pin or hammer milled and fractionated by air classifier and sifters. This method is optimized when the grain moisture is at 8% prior to air classification. Three products can be generated: protein rich fines (15-18 um), isolated starch granules (15-35um), and a coarse fraction (33-45um) (Jadhav et al, 1998). This method provides an effective means for producing protein-rich, starch-rich and β-glucan-rich fractions from barley and, therefore, maybe favored by food processors that have specific ingredient needs. Research has shown that using air classification followed by stack sieving, to

increase the yield of coarse fractions, improves the quality and quantity of the end products in terms of β-glucan content (Aman and Newman, 1986; Sundberg and Aman, 1994).

Dry milling of barley (e.g., by an abrasion mill) followed by sieving is another method for fractionation of barley grain and obtaining fractions with different composition. The process involves an initial grind of the grain followed by separation using progressively smaller sieves. The resulting fractions, especially those with particles >147 μm may contain, depending on the variety, between 18.6 to 22.5% total β-glucan, which amount to 2.7 to 4.3 fold increase over the original contents. This method, however, yields relatively small amounts of the β–glucan enriched fractions (2.1-4.5%) in comparison with other fractionation methods (Andersson et al, 2004).

Bread Making

Millions of people rely on bread as a staple to meet daily dietary requirements. The ingredients, the production of the bread, and the way the bread is consumed vary from culture to culture. Numerous cereals are used to produce bread, however, wheat is the best suited as its gluten properties contribute to the production of loaves that expand to high volumes. The basic principle of the breadmaking process is the same regardless of the method used to produce the bread. The simplest bread making procedure is a straight-dough system (Finney, 1984). In such a system, all the formula ingredients are mixed into developed dough that is then allowed to ferment. The fermentation time may vary quite widely in time (Preston et al, 1982).

A short-time baking procedure requires mixing all of the formula ingredients at high speed until fully developed. After the mixing step, the procedure is essentially a no-time or no bulk fermentation straight-dough system (Preston et al, 1982). The most popular baking process

in the U.S. is the sponge-and-dough procedure (Cauvain, 1998). In this process, part of the flour (approximately 2/3), part of the water, and the yeast are mixed just enough to form a loose dough, or sponge. The sponge is allowed to ferment up to 5h. Then it is combined with the rest of the formula ingredients and mixed into developed dough.

The bread making process can be divided into three basic operations: mixing or dough formation, fermentation, and baking. The mixing process involves combining the ingredients to develop the gluten, which requires hydration of the proteins in the flour and the application of energy through the process of kneading. Wheat gluten, composed of the proteins gliadin and glutenin, is responsible for the viscoelasticity of kneaded dough, which allows it to be leavened, as well as for the "chewiness" of baked products like bagels. Gluten, rich in charged, polar, and non-polar amino acids, is developed through formation of hydrogen bonds, hydrophobic interactions, ionic bonds, and van der Waals bonds during dough development. The elasticity of gluten results from formation of glutenin subunits into macropolymers by covalent disulfide bonds (Grosch, 1986). There are two forms of glutenin subunits, high molecular weight (HMW) and low molecular weight (LMW). Bread making qualities of wheat are directly impacted by the variation of the HMW subunits. The gluten matrix consists of HMW and LMW subunits cross-linked by disulfide bonds, while gliadin monomers are incorporated through hydrogen bonding with glutamine and hydrophobic bonding (Cauvain, 1998). The development of a gluten network allows formation of a viscoelastic dough matrix that can retain the carbon dioxide gas produced during fermentation. Gas retention in dough is, therefore, closely linked with the degree of dough development and can be affected by a large number of ingredients and processing parameters (Cauvain, 2000). Another important aspect of dough mixing is the incorporation of air gases, particularly nitrogen. As dough becomes cohesive, it starts to incorporate nitrogen and

thus decrease in density (Hoseney, 1985, 1994). The nitrogen gas trapped during mixing provides the nuclei for subsequent gas expansion, or leavening of the dough (Hoseney, 1984b). The production of a defined cellular structure in the baked bread depends entirely on the creation and retention of gas bubbles in the dough during mixing (Hoseney, 1994). The number and sizes of gas bubbles in the dough at the end of mixing are strongly influenced by the mechanism of dough formation and the mixing conditions in a particular machine (Cauvain et al, 1999). As the level of energy per kg dough in the mixer increase, up to an amount that does not induce overmixing, bread volume increases and with the increase in bread volume comes a reduction in cell size, increased cell uniformity and improved crumb softness (Cauvain, 2000).

The choice of flour used will impact loaf volume and crumb structure characteristics. Wheat flour is a versatile ingredient because wheat protein levels and gluten properties may vary and therefore wheat flour can be incorporated into may different food products. Wheat flour low in protein is generally more suited for products such as baguettes, cookies, cakes. Wheat flour high in protein is best suited for products with high loaf volume because it is suitable to support extended structures (Schofield, 1986). The level and quality of the gluten-forming proteins depends heavily on the wheat cultivar, agricultural practices, and environmental effects. In general, flour with higher protein content produces a gluten network with an improved ability to trap carbon dioxide gas and as a result larger bread volumes are observed (Cauvain, 2000). However, protein content is not associated with the quality of the protein, which also will influence the final product quality (Cauvain, 2000).

Desirable changes resulting from 'optimum' dough development are related to the ability of the dough to retain gas bubbles and permit the uniform expansion of the dough under the influence of carbon dioxide gas from yeast fermentation of sugars during proofing and baking.

As carbon dioxide is produced, the pH decreases and the aqueous phase of the dough becomes saturated with highly soluble carbon dioxide which in turn reduces the gluten molecules during proofing and baking (Cauvain, 2000). After the aqueous phase has become saturated, newly formed carbon dioxide cannot form new bubbles, thus the gas enters the pre-existing nitrogen air bubbles, produced during mixing, and increases the pressure inside the bubble. The viscoelastic properties of the dough allows the bubble to expand to equalize the pressure causing the total volume of the dough mass to increase, ie. the dough is leavened (Hoseney, 1984a).

After bulk fermentation, the dough is divided, molded, proofed, and baked. The surface of the dough that is exposed to the oven atmosphere skins over and forms a crust almost immediately when the dough is placed into the oven. Browning of the crust is the result of a Maillard reaction. Sucrose, a non-reducing sugar, is converted by invertase present in the yeast to reducing sugars, glucose and fructose, that brown readily (Hoseney, 1994). The dough expands in size rapidly, called oven-spring, as the internal temperature of the dough gradually increases to 100°C. The oven-spring is caused by the additive effects: yeast becoming more active and producing carbon dioxide, the carbon dioxide increasing in volume upon heating and becoming less soluble, and the ethanol-water mixtures being vaporized (Hoseney, 1984b, 1994). The transformation of dough into an elastic bread crumb is a continuous process. It starts at 65°C when starch gelatinization begins. Extensive thermal denaturation of gluten at 75°C causes the rupturing of the gluten network. The dough continues to become more elastic and less extensible up to 95°C (Marston and Wannan, 1983; Hoseney, 1994). As dough is heated, the membranes surrounding the gas cells must withstand the reduction in extensibility and the buildup of pressure within the gas cell without breaking. Loaf volume depends on the ability of the dough to expand without loss of carbon dioxide. When the pressure inside the gas cells

become large enough the gas cell walls rupture, transforming the dough from a foam structure in which the gas cells are self-contained into the gas-continuous sponge structure in which the gas cells are interconnected.

Fiber in Bread Making

Whole grain products and breads that contain fibrous material have been available to consumers for decades (Dubois, 1978; Swanson and Penfield, 1988; Newman and Newman, 1991; Slavin et al, 2000a). Consumer awareness of increasing fiber in their diets has lead to interest in the development of fiber-added baked products.

The addition of high fiber material can cause problems in bread production. Any addition which causes a dilution of gluten alters the standard bread ingredients and changes the bread quality characteristics such as volume, texture and shelf life. Addition of more than 10% of any fibrous material produces changes in quality characteristics of the bread and processing methodology (Dubois, 1978; Knuckles et al, 1997; Newman et al 1998; Bhatty, 1986a; Trogh et al, 2005).

The gas retention and loaf volume characteristics are most affected by fiber inclusion because of a weakening in cell structure caused by the addition of fibrous material which can break the gluten strands reducing gas retention (Lai et al, 1989). Loaf volume is negatively affected by the addition of fiber materials when using bran, cellulose and oat hulls (Pomeranz et al, 1977; Bhatty, 1986a; Knuckles et al, 1997). Pomeranz et al (1977) reported that an addition of 5% fibrous material decreased loaf volume to an expected level from dilutions of functional gluten proteins. However at levels above 7%, the decrease was much more than could be explained by this dilution and it was suggested that this larger decrease was due to reduced ability to retain gas.

Straight dough, pre-ferment and sponge and dough processes can be used to produce fiber enriched breads (Dubois, 1978; Swanson and Penfield, 1988). However, when including a fibrous material the production methodology has to be modified. The selection of the baking method should be based on the ability of fully hydrated wheat gluten to carry the addition of fiber-rich material (Swanson and Penfield, 1988; Trogh et al, 2004, 2005). Additionally, there are numerous elements that need to be adjusted in the bread formulation. Two of the most important adjustments are flour choice and water absorption. Flour needs to contain a high level of strong gluten to carry the 'dead weight' in the bread with increased fiber; it was suggested that a high protein spring wheat flour or a strong spring wheat-winter wheat flour blend would work well (Dubois, 1978). The addition of fiber and cellulose in breads results in an increase in the water absorption. The availability of water is very important in the overall bread structure since water is used to completely hydrate the gluten. A bread formula having a total fiber content of 20% or more will require absorption of about 120% flour weight basis (Dubois, 1978; Nelson, 2001).

Barley as a Fibrous Material in Bread

Increasing fiber component in food products has become a trend for consumers to overcome numerous health problems such as hypertension, diabetes and hyperlipidemia (Newman et al, 1989, Newman and Newman, 1991). Barley, a good source of soluble fiber, has shown some promising evidence for health benefits (Jenkins et al, 1988; Newman et al, 1989, Newman and Newman, 1991), and is suitable for use in many products such as breakfast cereals, pasta and baking products (Liljeberg et al, 1994; Prentice et al, 1979; Yvone et al, 1994). Incorporation of barley grain in bread lowers the glycemic response (hypoglycemia) compared with normal bread. This is done by decreasing the rapid digestion and absorption that result in

high glucose and insulin in the blood (Jenkins et al, 1988; Urooj et al, 1998). Specifically, fractions containing high levels of β-glucans contribute the hypocholesterolemic, (Hudson et al, 1992) lowering serum cholesterol and anticarcinogenic properties (Jadhav et al, 1998) caused by increasing intestinal viscosity as a result of the hydrophilic nature of β-glucans and arabinoxylans (Bhatty, 1992; Rosemary et al, 1989; Wood et al, 1989). Urooj et al. (1998) found that whole barley bread induced a significant decrease in blood glucose which is important to persons suffering from diabetes (Jadhav et al, 1998). The therapeutic advantage of β-glucans and arabinoxylans in barley should promote value added processing and wider use of this grain in foods other than just breads.

In general, partial or complete replacements of wheat flour by barley flour in baked goods resulted in smaller loaf volume, higher gumminess, increased moisture content, and poorer crumb structure than in bread made from 100% wheat flour (Swanson and Penfield, 1988; Chaudhary and Weder, 1990; Newman et al, 1990; Berglung et al, 1992). Previous research has shown that pearled barley and flour from whole grain barley can be incorporated into formulated breads with overall flavor, appearance and texture comparable with breads prepared with refined wheat flour (Urooj et al, 1998). However, increasing the percentage of barley flour can result in a significant decrease in tenderness (Swanson and Penfield, 1982). Swanson and Penfield (1988) reported a decrease in loaf size by 5-6% when 20% barley flour was used in breads. In quick breads, replacement of 20% wheat flour by either waxy or non-waxy barley flour resulted in softer crumb of fresh bread and stored (48h) bread (Klamczynski and Czuchajowska, 1999). Bhatty (1999b) pointed out that barley genotypes with variable amylose/amylopectin ratios may affect the quality characteristics such as crumb softness and staling of dough and bread.

Effect of Components of Barley on Incorporation and Bread Characteristics

Wheat flour non-starch polysaccharides, such as arabinoxylans, are important in bread making. These polysaccharides constitute 2-3% of white wheat flour (Meuser and Suckow, 1986). When the components of whole barley, starch and non-starch polysaccharides, such as β-glucans (4-8%) and arabinoxylan (3-7%) (MacGregor and Fincher, 1993), are added to wheat flour they change the mixing properties. Wheat arabinoxylans affect the dough development and, therefore, influence loaf volume, and crumb and crust characteristics of the bread (MacGregor and Fincher, 1993). Yin and Walker (1992) suggested that faster dough development upon addition of arabinoxylans might be attributed to weak secondary bonds forming between the polysaccharides and gluten. Westerlund et al (1990) investigated the changes in the molecular structure of arabinoxylans during baking; they observed that arabinoxylans isolated from bread crumb and crust had a higher proportion of disubstituted xylopyranosyl residues than arabinoxylans isolated from the flour and dough. These changes were thought to be due to increased solubilization of highly substituted arabinoxylans during baking, assuming that arabinoxylans that were originally insoluble have a more highly substituted structure than their soluble counterparts (Westerlund et al, 1990). Water-soluble polysaccharides also have a positive effect on loaf volume (D'Appolonia et al, 1970; Jelaca and Hlynka, 1971, 1972; Michniewicz et al, 1992)

Izydorczyk et al (2001) observed that with the addition of barley flour and/or isolated and purified β-glucans and arabinoxylans added to wheat flour resulted in significant increases in peak dough resistance, mixing stability, and work input compared to control wheat flours. It was reported that the effects exerted by arabinoxylans on the weaker flours were more pronounced

than on the stronger flours (Courtin and Delcour, 2002; Izydorczyk et al, 2001). Izydorczyk et al (2001) proposed that a high content of total and soluble β-glucans in waxy or zero amylose waxy barley might improve dough strength because the presence of the β-glucans might counteract the negative effects associated with dilution of wheat gluten. β-Glucans and arabinoxylans are high molecular weight polymers that are partially soluble in water and, therefore have been shown to impart high viscosity to aqueous solutions. The benefit of increased viscosity from the addition of high molecular weight β-glucans and arabinoxylans to stabilize gas cells is seen in early baking stages when high pressure is generated inside the gas cells (MacGregor and Fincher, 1993; Izydorczyk and Biliaderis, 1994; Biliaderis et al, 1995). In solution, both β-glucans and arabinoxylans bind large quantities of water causing increased viscosity. It has been observed that high molecular weight arabinoxylans increased the farinograph water absorption to a greater extent than their low molecular weight counterparts (Biliaderis et al, 1995). In this manner water-soluble arabinoxylans and β-glucans are also thought to compete with other flour constituents for available moisture which negatively affects gluten development and increase gluten's resistance to extensibility (Michniewicz et al, 1991: Wang et al, 2004). By sequestering water in the dough, β-glucans and arabinoxylans form elastic networks that might contribute to the overall elasticity and strength of the dough. However, with respect to arabinoxylans there is an optimum amount that can be added to the dough system and levels exceeding the optimum may be detrimental to the dough system by causing an excessive increase in the viscosity of the dough system and consequently limiting or decreasing the volume of the final baked products (McCleary, 1986; Biliaderis et al, 1995).

Adding barley β-glucan enriched milling fractions to bread increases the dietary fiber content of the bread, which decreases the total flour content, lowering the caloric content (Knuckles et al, 1997). For every 1% increase in barley content there is a 0.5% increase in water content of bread and this is due to the strong interaction of β-glucans with the water causing an increased need of water for dough development, and greater retention of water after baking (Knuckles et al, 1997).

Enzymes in Bread Making

Enzymes in breadmaking are used to optimize dough properties (Barrett, 1975; Kruger and Lineback, 1987; Kulp, 1993) as well improve the quality of fresh and stored baked products (Collar and Armero, 1996). For example, xylanases are used as dough conditioning enzymes that improve dough handling, consistency, fermentation stability, oven rise, bread volume and crumb structure (Martinez-Anaya and Jimenez, 1997; Qi Si, 1997; Courtin and Delcour, 2002; Wang et al, 2004). It was also reported that pentosanases increased gluten coagulation in diluted dough systems (Weegels and Hamer, 1992) and gluten strength and elasticity (Qi Si, 1997). A study by Rouau et al. (1994) investigated the role of crude pentosanases and found that at an optimal level the enzymes improved uniformity in crumb structure and loaf volume. However, when an excess of the enzyme was added, the dough characteristics deteriorated. The use of xylanase in combination with peroxidase prevented extensive degradation of arabinoxylans by cross-linking them into larger aggregates (Hilhorst et al, 2002). Enzymes such as amylase and/or pentosanase/xylanase significantly shortened fermentation time without affecting pH or machinability of doughs, resulting in an improved volume, a greater intense aroma, softer texture and delayed firming (Martinez-Anaya and Jimenez, 1997).

Partial enzymatic hydrolysis of the fiber fraction in fiber-enriched breads improves bread quality characteristics. Laurikaninen et al. (1998) investigated the effects of several enzymes including hemicellulolytic culture hemicellulases, xylanase and fermizyme (α-amylase with standardized levels of hemicellulase activity) on the quality of fiber rich breads. The results of Laurikaninen et al (1998) study indicated a decrease in dough stability, but an increase in softening with the addition of each enzyme. The hemicellulolytic culture hemicellulases had the largest effect. Incorporation of all enzymes also improved the loaf volume, produced a softer crumb and retarded the staling of the bread. The enzyme mixtures used by Laurikaninen et al. (1998) were more efficient than individual endoxylanases in reversing the negative effects of water-insoluble arabinoxylans (Courtin and Delcour, 2002).

Evaluation of Bread Properties

Broadly there are three groups of attributes which are taken into account during non-sensory evaluation of bread properties: external characteristics such as loaf volume, symmetry, degree of break, color and crust; internal characteristics which consider the size, number and distribution of cell in the crumb, the crumb color, and any major quality defects; and finally, texture which assesses the mechanical properties such as firmness, resiliency as well as eating quality (Cauvain, 2000).

The process by which bread quality is determined still relies heavily on subjective assessment (Cauvain, 1998) except for bread volume and crumb texture. In bread crumb scoring, subjective scores for external appearance, crumb structure, and crumb color are commonly used (Kilborn and Tipples, 1991) Crumb appearance and texture are evaluated based on fineness (open versus closed cells), uniformity, cell shape, cell wall thickness and color that is indicative of the degree of oxidation (Kilborn and Tipples, 1981; Pyler, 1988). The traditional

method for crumb grain scoring is qualitative and subjective (Scanlon and Zghal, 2001). Over the last number of years there has been a push to develop newer techniques such as digital and video image analysis that are consistent and reliable, and can be automated for the high through-put nature of the modern commercial bakery.

Crumb firmness, as measured by the force required to compress a slice of bread to achieve a pre-selected compression, i.e. indentation, is the most common method to measure crumb physical texture. Deformation of a crumb specimen between parallel plates in a uniaxial compression test can also be used to measure the mechanical properties of the bread crumb (Scanlon and Zghal, 2001). A slice of bread presents a non-homogeneous surface to the testing instrument, therefore, the best results are achieved when the largest possible flat surface on the compressing probe is used. However, the probe size can not exceed the size of the bread slice or encroach on the areas close to the crust, as they will have a disproportionately large effect on the compressibility test (Spies, 1990). The measurements obtained under constant load correlate to subjective estimates of staleness and show that the crumb firmness increases over time. The crumb firmness obtained depends on several factors including: cell structure uniformity, cell wall size and thickness, and uniformity and sustainability of rise, i.e. loaf volume (Eliasson and Larsson, 1993; Scanlon and Zghal, 2001). The lower the specific loaf volume, the higher is the force required to deform the samples, and hence the higher the firmness of the bread sample (Eliasson and Larsson, 1993). As for, cell wall thickness and cell distribution with regard to influencing crumb firmness; finer, thin walled, uniformly-sized cells yield a softer and more elastic texture than do coarse, open and thick walled cell structures (Pyler, 1988). Additionally, with increasing crumb moisture content an increase in firmness and decrease crumb elasticity has been observed (Spies, 1990) All of these factors, when combined, give an indication of

tenderness, springiness, and moistness of the bread slice (Spies, 1990; Eliasson and Larsson, 1993; Scanlon and Zghal, 2001).

CHAPTER 1

Distribution and Structural Variation of Non-Starch Polysaccharides in Milling Fractions of Hull-less Barley with Variable Amylose Content

ABSTRACT

Three hull-less barley genotypes containing starches with variable amylose content (23.8%, normal; 4.3%, waxy; 41.8%, high amylose barley) were pearled to remove 10% (by weight) to remove of the outer layers and then roller milled to produce pearling by-products (PBP), flour and fiber-rich fractions (FRF). PBP were enriched in arabinoxylans, protein and ash and contained small amounts of starch and β–glucans. FRF were considerably enriched in β–glucans and arabinoxylans. The solubility of β–glucans was higher in PBP than in FRF. The solubility of arabinoxylans was higher in FRF than in PBP. Small amounts of arabinogalactans detected in barley were concentrated in the outer portion of the barley kernel. The content and solubility of non-starch polysaccharides (NSP) in various milling fractions were also dependent on the type of barley. In order to obtain more detailed information about the content and molecular structure of non-starch polysaccharides, each milling fraction was sequentially extracted with water, $Ba(OH)_2$, again with water, and finally with NaOH. These extractions resulted in four sub-fractions, designated WE, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH, respectively. The extracts were analyzed for monosaccharide and linkage composition and molecular weight. β-Glucans and arabinoxylans exhibited structural heterogeneity derived from differences in their location within the kernel as well as from the genetic origin of barley. The WE arabinoxylans from FRF and flour had a substantially lower degree of branching than those from PBP. The WE arabinoxylans from FRF of high amylose and normal barley contained more unsubstituted Xyl*p* residues but less doubly substituted Xylp and singly substituted Xylp at O-2 than their counterparts from PBP. The WE arabinoxylans from FRF of waxy barley had a relatively high content of doubly, but very little singly substituted Xylp residues. In all three barley genotypes,

the ratio of tri- to tetrasaccharides in β–glucans from PBP was higher than from flour and FRF. Substantial differences in the molecular weight of NSP in different milling fractions were also observed.

INTRODUCTION

Hull-less barley has recently attracted a lot of interest among food scientists and technologists as an excellent source of both soluble and insoluble fiber. The two non-starch polysaccharides which account for the majority of dietary fiber in barley are mixed linkage β–(1→3, 1→4)-glucans and arabinoxylans (Henry, 1988), but other polysaccharides containing galactose, glucose, mannose, and uronic acids may also be present. The amount of β–glucans in barley normally varies from 4 to 8 % and that of arabinoxylans from 3 to 7% (MacGregor and Fincher, 1993). Both oat and barley β–glucans, in particular, have been implicated in serum cholesterol and blood glucose reduction, and are thought to have some anticarcinogenic properties (Jadhav et al 1998).

The content of β–glucans in barley is influenced by both genetic and environmental factors (MacGregor and Fincher, 1993). According to the current model for biosynthesis of mixed linkage β–(1→3, 1→4)-glucans (Buckeridge, 2004), the β–(1→3, 1→4)-glucan synthase is that of a core like synthase that makes cellobiosyl and even-numbered cellodextrin units, and a distinct glycosyl transferase adds a third glycosyl residue to complete the cellotriosyl and higher odd-numbered units. Further investigation of the synthase activity in vitro showed that the cellodextrin unit distribution is altered drastically depending on the UDP-Glc concentration. The suboptimal UDP-Glc concentrations favor the synthesis of longer cellodextrin units in β–glucan, particularly the cellotetrosyl unit, whereas at the highest UDP-Glc concentrations tested, the cellotriose units were predominant of the total polymer synthesized (Buckeridge, 2004). The presence of waxy or high amylose genes in barley appears to affect the carbohydrate metabolism in the grain, and waxy and high amylose barleys are consistently higher in β–glucans than

normal genotypes (Xue et al 1997; Izydorczyk et al 2000). Barley arabinoxylans have not been investigated to the same extent as β–glucans, and factors affecting their content are not clearly elucidated. The distribution of both polymers throughout the barley kernel is not uniform. It is generally agreed that the cell walls of the aleurone layer are composed mainly of arabinoxylans (67-71%) and β–glucans (26%) whereas the cell walls of the starchy endosperm are mainly built up from β–glucans (70%) and contain smaller amounts of arabinoxylans (20%) (Fincher and Stone, 1986; Bacic and Stone 1981). Although the general structure of barley β–glucans is well known (Woodward et al, 1988; Buliga et al 1986; Izydorczyk et al 1998a) much more attention has been given to β–glucans present in hulled malting barley varieties than to those present in hull-less genotypes. Furthermore, little is known about structural variation of β–glucans relative to their location in the barley kernel. The structural complexity of β–glucans and arabinoxylans, their spatial organization in the cell walls and their predisposition for interactions with other cell wall components render them partially insoluble in water. Because of specific physiological effects associated with water-soluble β–glucans, there has been some interest in optimizing procedures for water extractability of these polymers from barley via controlling the temperature and/or pH of aqueous solvents (Temeli 1997). Recently, hydrothermal, enzymic, and physical treatments of barley grain have been evaluated for the purpose of weakening the intermolecular associations within the cell walls and thus increasing the yield of extractable β–glucans (Izydorczyk et al, 2000). Extraction of β–glucans from whole barley grain remains, however, an expensive and time consuming process.

Unlike wheat, barley does not have a long tradition of being fractionated by milling. Nevertheless, several studies have been reported recently in which hull-less barley has been

experimentally milled and fractionated into several streams with variable content of starch, protein, and dietary fiber (Bhatty 1987, 1992, 1993, 1997, 1999a; Newman and Newman, 1991; Wang et al 1993; Sundberg and Aman 1994; Danielson et al 1996; Klamczynski and Czuchajowska 1999; Kiryluk et al 2000). Several research groups milled barley by an abrasion mill and sieved the ground material through a series of sieves to obtain fractions with a high β–glucan content (Knuckles et al 1992; Sundberg and Aman 1994; Yoon et al 1995; Knuckles and Chiu 1995; Lee et al 1997) This new interest in fractionation of barley stems from current demand for grain fractions with unique composition and functionality. Because the distribution of various barley components is not uniform throughout the kernel, grain fractionation may indeed prove to be an efficient way to obtain products enriched in specific functional components and/or bio-active nutrients.

The objectives of the current study were to determine and compare the distribution of non-starch polysaccharides (NSP) in three milling fractions of hull-less barley genotypes with different starch characteristics (high amylose, normal, and waxy), and also to gain a better insight into the structural variation of water soluble and insoluble non-starch polysaccharides originating from their different locations in the kernel. Its is believed that such information will not only increase our knowledge about the structure of NSP in barley, but will also be useful for developing more effective methods of extracting NSP with specific composition and structural characteristics, and for formulating barley milling fractions targeted for specific applications.

MATERIALS AND METHODS

Barley Samples

Three hull-less barley genotypes with variable starch characteristics: normal (cv. Falcon), waxy (cv. CDC Candle), and high amylose (CDC 92-55-06-48) were obtained from field trials in

western Canada during the 2000 crop year. The barley samples were tempered to 14% moisture over a 65h period and subsequently pearled (Satake, Type TM) to obtain a yield of 90% of pearled grain and 10% of pearling by-products (PBP).

Milling

Milling took place in a climate-controlled room (21°C and 60% RH) with a mill equipped with 25cm diameter rollers described by Black et al (1980). A short flow milling procedure was developed that allowed efficient separation of pearled barley into bran-rich by- product, flour, and a fiber-rich fraction (FRF) (Fig.6). Initially the pearled barley samples were given four break passages through corrugated rolls (dull-to-dull roll orientation; differential 2:1 for first three passages and 1.75:1 for fourth passage). Following the fourth break, the ground product was sieved to yield flour, and coarse (> 600μm) and intermediate (> 183μm) fractions. The coarse fraction was passed through a shorts duster (Buhler, Uzvil, Switzerland), and the product passing through the shorts duster screen was sieved on a 183μm sieve to give flour and a coarser fraction (> 183μm), which was combined with the product tailing over the shorts duster screen to yield a bran-

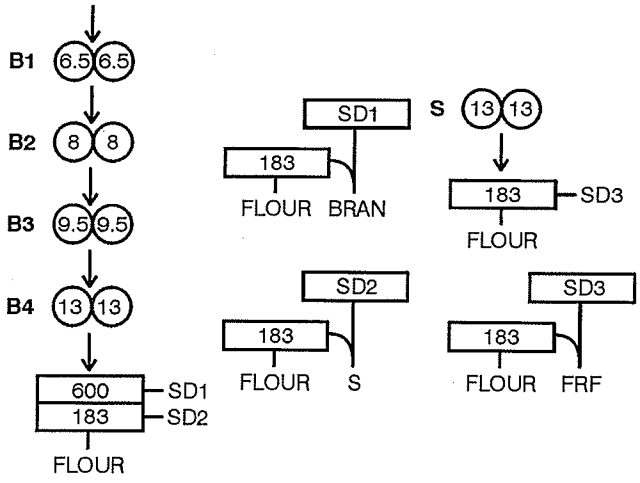

Figure 6. Barley milling flow. B: break; S: sizing; SD: shorts duster; FRF: fiber-rich fraction. Numbers on break rolls indicate corrugations per cm. Numbers on sieves indicate aperture in microns.

rich product. This minor component was not processed further. The intermediate fraction was passed through the shorts duster separately and fractionated into flour and residual intermediate material. The residual >183μm material was given a sizing passage, sieved, and the remaining >183μm fraction was passed through the shorts duster and sieved again. The four break flours were combined with the flour from the sizing and two shorts duster passages to yield the flour fraction. The >183μm material remaining following the last shorts duster passage, which contained a high proportion of endosperm cell wall remnants, was designated as FRF.

Isolation of water- and alkali-extractable sub-fractions

In order to inactivate the endogenous enzymes, the PBP, FRF and flour were refluxed in 80% ethanol for 1h. The residue was filtered and washed with 95% ethanol, then allowed to evaporate overnight at room temperature (RT). The samples were subsequently vacuum dried (40°C). Isolation of water soluble and insoluble non-starch polysaccharides from various fractions of barley was based on the method of Izydorczyk et al (1998a and 1998b). Each milling fraction was sequentially extracted with water, $Ba(OH)_2$, again with water, and finally with NaOH. These extractions resulted in four sub-fractions, designated WE, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH, respectively.

Analytical methods

Protein (%N x 6.25) was determined by combustion nitrogen analysis (CNA) using a LECO Model FP-428 CNA analyzer (Leco Corp. St. Joseph, MI) calibrated against ethylenediaminetetraacetic acid (EDTA). Ash content was performed according to AACC (2000) Approved Method 08-01. The results are expressed on a 14% moisture basis.

Total β–glucan and starch contents in PBP, flour and FRF were determined enzymatically using Megazyme kits (Megazyme International Inc., Ireland) according to AACC (2000) Approved Methods 32-23 and 76.13, respectively. To determine soluble β–glucan, 1g of material was added to10mL of water and mechanically shaken in an incubator (40°C) for 1h. The samples were centrifuged (5000 x g, 20 min) and aliquots (1 mL) were taken for analysis. The Megazyme method was resumed with the addition of sodium phosphate buffer. Arabinoxylan (AX) content was determined colorimetrically according to the method by Douglas (1981) using D-xylose as a standard. Water-soluble AX was determined by suspending 1.0g of milling fractions in 20mL of water. The samples were mechanically shaken for 1h, centrifuged (5000 x g, 20min) and aliquots were taken for analysis. Protein content of water- and alkali-extractable materials was determined according to the Lowry method (Lowry et al 1951) using bovine serum albumin (BSA) as a standard.

Phenolic acids were liberated from the water-extractable sub-fractions by treating the material (5 mg) in 2mL 2M NaOH for 30min at 35°C in the dark. Subsequently the samples were acidified to pH 2 with HCl and extracted twice with hexane at a hexane-to-water phase ratio of 1:1. Free phenolic acids in the water layer were then extracted three times with ethyl acetate (at a solvent-to-aqueous phase ratio of 1:1). The phenolic acids in the ethyl acetate phase were quantified by determining the absorbance at 320nm. Ferulic acid was used as a standard and the results are expressed as mg ferulic acid/ g arabinoxylans in the sub-fractions. All analyses were conducted in duplicate with CV < 4%.

The monosaccharide composition of water- and alkali-extractable sub-fractions was determined via gas-liquid chromatography of alditol acetates. Fractions (50 - 100 mg) were

hydrolyzed in 1 M H_2SO_4 for 2 h at 100°C. Derivatization of the component monosaccharides released during hydrolysis was accomplished using the method of Englyst and Cummings (1984). The derivatized samples were injected onto a Supelco SP-2330 column (30 cm × 0.25 mm ID) with a temperature gradient of 185 to 235°C; injector and detector temperatures were 250°C. Erythritol (Sigma Chemical Co., Ltd.) was used as the internal standard. The amount of arabinogalactans in the isolated sub-fractions was calculated from the monosaccharide analysis, using the arabinose-galactose ratio of 0.7.

^{1}H-NMR spectra of the water- and alkali extractable fractions from the pearling by-product, flour and FRF were dissolved in D_2O (1.0% w/v) were recorded on a Bruker AM 300 FT spectrometer at 85°C (Bruker Spectrospin Canada, Milton, Ont., Canada). Approximately 256 pulses were collected with a pulse retention time of 6.3s at a pulse angle of 68.6° (external reference of sodium-3-trimethylsilyltetradeuteriopropionate was used).

β–Glucans in water- and alkali-extractable sub- fractions (10 mg) were dissolved in 5 mL phosphate buffer (0.01 M, pH 6.5) and digested (in duplicate) with (1→3)(1→4)–β–D-glucan-4-glucanohydrolase, known as lichenase (4 U/mL, Megazyme) for 20 h at 40°C. After digestion, samples were heated to 95°C for 15 min to inactivate the enzyme, and then centrifuged (10 000 x g, 10 min). Oligosaccharides released by lichenase were analyzed by high performance anion exchange chromatography (HPAEC) using a Waters pump, 715 WISP sample injector (Waters Associates, Milford MA), a Danaus CarboPac PA1 column (4 × 250 mm) with a PA1 guard column, and a pulsed amperometric detector (PAD-2; Danaus Canada Ltd., Etobicoke, ON). The running conditions were adapted from Izydorczyk et al (1998a) and Wood et al. (1994). Data were processed using Waters Millennium chromatography software (Version 3.2). The

oligosaccharide composition (% mol) was calculated based on the percent of the total peak area (peak area of all oligosaccharides combined) for a given sample.

Molecular Weight Determination

Weight-average molecular weights of polysaccharides were estimated by using high-performance size-exclusion chromatography with online multi-angle light-scattering detection (HPSEC-MALS). The HPSEC-MALLS system comprised 3TSK-gel packed columns (G5000, 60 cm; G3000, 30 cm; and G2500, 30 cm; Tosoh Corporation), a DAWN DSP laser-light-scattering detector (Wyatt Technology, USA), refractive index and UV detectors (Waters 410 and Waters 490, respectively). Samples were dissolved in water by heating in a boiling water-bath and filtered through 0.1mm Acrodisc glass fiber syringe filters (25mm, PALL Gelman Laboratory USA). Solutions (0.2mL, 2mg/mL) were injected and eluted at 0.4mL/min with 0.15M sodium nitrate and 0.02% sodium azide as a mobile phase. The calculations of weight average molecular weights were performed using Astra 4.72 software (Wyatt Technology), based on the Berry plot with a first-order polynomial fit. The refractive indices increment (dn/dc) of 0.145 and 0.146 were used for β–glucans and arabinoxylans, respectively.

RESULTS AND DISCUSSION

Yield and composition of milling fractions

Three hull-less barley genotypes containing starches with variable amylose content [4.3% (waxy), 23.8% (normal), and 41.8% (high amylose)] were used in this study. In agreement with previous reports (Xue et al 1997; Izydorczyk et al 2000), barley genotypes with anomalous starch composition had much higher β–glucan but lower starch contents than normal barley sample (Table 1). The samples were pearled to 10% and then roller milled to produce two major

fractions, flour, and a fiber-rich fraction (FRF). According to Bhatty (1997), pearling of hull-less barley to remove 10% (by weight) of the outer layers of the barley grain facilitates almost complete removal of the pericarp and testa, and a single layer of the aleurone. PBP were, therefore, enriched in components present in the outer tissues of the barley grain. They contained especially large amounts of arabinoxylans, protein, and ash, but only small amounts of starch and β–glucans as compared to the whole grain (Table 1). Proteins, arabinoxylans, β–glucans, starch, and ash amounted to less than 60% of the pearling by-products, making it reasonable to assume that insoluble hollocellulose, hemicellulose, and lignins constituted most of the remaining 40% portion. PBP have also been reported to contain such micro nutrients as tocols, proanthocyanidins, catechins and other phenolic compounds (Slavin et al 2000b).

PBP from normal and waxy barley contained higher amounts of arabinoxylans than those from high amylose barley (Table 1). The content of β–glucans in pearling by-products differed little among the three barley samples. Different starch contents in PBP may indicate possible differences in the physical properties of kernels in various genotypes, such as seed coat thickness, kernel size, shape and plumpness, and/or hardness.

The yields of flour and FRF obtained after roller milling of pearled barley differed significantly among the samples. In general, high amylose and waxy barley samples yielded

Table 1. Yield and composition of pearling by-product (PBP), flour, fiber-rich fractions (FRF) milling fractions from normal, waxy, and high amylose barley.

Barley Genotype/ Milling Fraction	Yield[a] (%)	Proteins[b] (%)	Ash[b] (%)	Starch[b] (%)	Total β-glucan (%)	Water Soluble β-glucan (%)		Total Arabinoxylan (%)	Water Soluble Arabinoxylan (%)	
High Amylose		11.9	1.76	57.9±0.5	6.11±0.10	2.00±0.20		5.28±0.70	0.92±0.05	
PBP	10.0±0.0	18.9	6.37	6.9±0.1	2.40±0.04	1.43±0.27	(60)[c]	14.27±0.72	1.42±0.03	(10)
Flour	53.2±0.4	12.1	1.17	80.3±1.0	3.77±0.13	1.99±0.03	(53)	2.87±0.12	0.61±0.03	(21)
FRF	28.9±0.6	12.2	1.72	50.0±0.3	13.37±0.06	2.75±0.13	(21)	8.03±0.31	1.57±0.09	(20)
Normal		10.9	1.78	67.0±0.5	3.43±0.10	1.67±0.10		4.70±0.15	0.52±0.05	
PBP	10.0±0.0	19.0	7.14	11.8±0.8	2.10±0.10	1.30±0.14	(62)	17.21±0.18	1.00±0.00	(6)
Flour	67.7±0.5	10.0	0.93	84.0±1.1	2.80±0.10	1.35±0.06	(48)	2.16±0.02	0.37±0.01	(17)
FRF	16.3±0.1	13.9	2.66	54.9±0.9	8.10±0.10	3.83±0.01	(47)	9.31±0.31	1.04±0.01	(11)
Waxy		11.3	1.63	63.7±0.6	5.73±0.10	3.17±0.15		4.56±0.10	0.48±0.05	
PBP	10.0±0.0	21.1	7.12	9.8±0.1	2.18±0.07	2.05±0.08	(94)	18.47±0.19	0.93±0.02	(5)
Flour	59.4±0.4	10.7	0.97	82.9±0.3	3.33±0.16	2.14±0.08	(64)	1.68±0.07	0.29±0.00	(17)
FRF	23.3±0.8	12.3	1.49	57.9±0.5	15.17±0.04	7.26±0.13	(48)	7.38±0.41	0.94±0.03	(13)

[a] Yield based on total amount of barley prior to pearling and milling; PBP, flour, and FRF amount to less than 100% because of bran fraction constituting ~2% and some losses during processing.
[b] Analyses conducted in duplicate with CV < 4%.
[c] Numbers in brackets express the amount of water soluble β–glucans and arabinoxylans as % of the total.

substantially more FRF but less flour than normal barley. It was concluded that the content of β–glucans and starch in barley clearly affects the relative yield of fractions that can be obtained after roller milling. This agrees with the findings of Bhatty (1999b). It also appears that roller milling quite effectively separates endosperm cell wall material from the other endosperm components. The flour fractions contained more than 80% of starch and only small amounts of non-starch polysaccharides. FRFs, on the other hand, were enriched in cell wall material as evident by substantial amounts of β-glucans and arabinoxylans. FRF from the high amylose and waxy samples contained much higher amounts of β–glucans than those from normal barley; this was consistent with differences in total β–glucan content among the different barley genotypes. The content of arabinoxylans in FRFs differed only slightly among genotypes, indicating that these polysaccharides were not affected to the same degree as β–glucans by the presence of waxy or high amylose genes in barley.

The proportion of soluble β–glucans in different milling fractions varied substantially: soluble β–glucans were present in greater proportion in PBP, followed by those in flour and FRFs (Table 1). This trend was observed for all three barley genotypes. Overall, the solubility of β–glucans was greater in all three fractions of waxy barley than in those of normal or high amylose barleys.

The solubility of arabinoxylans in all milling fractions was generally very low compared to solubility of β–glucans (Table 1). Also, the solubility of arabinoxylans in high amylose milling fractions was greater than in normal and waxy samples, contrary to the solubility trends observed for β–glucan.

Composition of water- and alkali-extractable sub-fractions

Differences in the yield of each non-starch polysaccharide sub-fraction (Table 2) indicate differences in the content and relative solubility of non-starch polysaccharides in the milling fractions. Each milling fraction contained more polysaccharides extractable with alkali [($Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH combined] than with water. The water insolubility of barley non-starch polysaccharides may arise from their intrinsic characteristics and/or from their interactions (both covalent and non-covalent) with other grain components (Izydorczyk et al 1998 a).

Protein and ferulic acid contents in water- and alkali-extractable sub-fractions are also presented in Table 2. The WE sub-fractions from FRF contained the least amount of proteins, followed by those from flour and pearling by-products. This was observed for all three barley genotypes. The high amount of proteins in the WE sub-fractions obtained from PBP may have resulted from the higher amount of soluble proteins in the aleurone cells. Despite extensive purification, including proteinase digestion, most of the alkali-extractable sub-fractions, contained much higher amounts of proteins than the WE sub-fractions. These results indicate the effectiveness of alkali in solubilizing barley proteins. The $Ba(OH)_2$ sub-fractions, especially those extracted from flours, contained the highest amount of proteins.

Arabinose, xylose, glucose and small amounts of galactose residues were found in the water- and alkali-extractable sub-fractions. These findings, confirmed by H-NMR analysis, showed that arabinoxylans, β–glucans and arabinogalactans constituted the bulk of non-starch polysaccharides present in all milling fractions of barley. The relative amount of these three polysaccharides depended, however, on the milling fraction and barley genotype.

Table 2. Yield and composition of water-extractable (WE) and alkali-extractable sub-fractions from different pearling by-product (PBP), flour, and fiber-rich fraction (FRF) milling fractions of high amylose, normal, and waxy barley.

Barley Genotype/ Milling Fraction		Yield		Protein	Ferulic Acid
		g/100g milling fraction	g/100g barley	(%)	(mg/g AX)
High Amylose Barley					
PBP					
	WE	2.68	0.27	8.5±0.1	0.48
	$Ba(OH)_2$	12.52	1.25	17.8±0.2	
	$Ba(OH)_2/H_2O$	3.28	0.33	17.1±0.3	
	NaOH	9.80	0.98	20.5±0.1	
Flour					
	WE	1.64	0.87	2.8±0.1	0.11
	$Ba(OH)_2$	1.92	1.02	42.7±0.4	
	$Ba(OH)_2/H_2O$	2.28	1.21	6.3±0.2	
	NaOH	1.32	0.70	18.8±0.4	
FRF					
	WE	7.52	2.18	0.5±0.0	0.13
	$Ba(OH)_2$	5.00	1.45	19.1±0.3	
	$Ba(OH)_2/H_2O$	8.68	2.51	1.6±0.0	
	NaOH	8.76	2.53	11.0±0.3	
Normal Barley					
PBP					
	WE	2.04	0.20	8.7±0.2	0.50
	$Ba(OH)_2$	10.72	1.07	14.6±0.2	
	$Ba(OH)_2/H_2O$	3.64	0.36	11.5±0.1	
	NaOH	6.16	0.62	15.7±0.2	
Flour					
	WE	1.36	0.92	3.7±0.1	0.12
	$Ba(OH)_2$	3.64	2.46	43.2±0.2	
	$Ba(OH)_2/H_2O$	1.60	1.08	1.9±0.1	
	NaOH	1.36	0.92	7.0±0.1	
FRF					
	WE	4.64	0.76	0.7±0.0	0.14
	$Ba(OH)_2$	2.96	0.48	19.8±0.1	
	$Ba(OH)_2/H_2O$	5.92	0.97	2.0±0.12	
	NaOH	6.00	0.98	10.1±0.2	
Waxy Barley					
PBP					
	WE	2.08	0.21	8.7±0.1	0.96
	$Ba(OH)_2$	9.04	0.90	14.6±0.1	
	$Ba(OH)_2/H_2O$	4.00	0.40	11.5±0.2	
	NaOH	9.64	0.96	15.7±0.1	
Flour					
	WE	1.64	0.97	3.7±0.1	0.14
	$Ba(OH)_2$	2.40	1.42	43.2±0.4	
	$Ba(OH)_2/H_2O$	0.88	0.52	1.9±0.2	
	NaOH	1.76	1.04	7.0±0.0	
FRF					
	WE	7.44	1.73	0.7±0.0	0.17
	$Ba(OH)_2$	3.92	0.91	19.8±0.0	
	$Ba(OH)_2/H_2O$	3.76	0.88	2.0±0.1	
	NaOH	8.64	2.01	10.1±0.5	

The WE sub-fractions from flour and FRFs in all barley genotypes contained more β–glucans than arabinoxylans; the waxy barley WE sub-fractions contained the highest amounts of β–glucans (Figure 7). The WE sub-fractions from PBP in high amylose and normal barley had more arabinoxylans than β–glucans. These results were expected, considering that PBP had very high arabinoxylan content (Table 1). The WE sub-fractions from pearling by-products in waxy barley contained, however, more β–glucans than arabinoxylans.

The $Ba(OH)_2$ sub-fractions from all three milling streams were enriched in arabinoxylans. Barium hydroxide has been shown in the past to preferentially extract arabinoxylans from cell wall materials of wheat, barley and sorghum (Bergmans et al 1996). In this study, however, it was observed that the effectiveness of $Ba(OH)_2$ to extract specifically arabinoxylans was affected by the amount and solubility of β–glucans in the materials used for extraction. For example, the $Ba(OH)_2$ sub-fraction from β–glucan-enriched waxy FRF (Table 1) contained, in addition to arabinoxylans (81.8%), about 16% of β–glucans. In contrast, the $Ba(OH)_2$ sub-fraction from β–glucan-deficient PBP contained more than 97% of arabinoxylans and no β–glucans. The other two combined alkali sub-fractions, $Ba(OH)_2/H_2O$ and NaOH, from PBP also contained more arabinoxylans than β–glucans. In flour and FRFs, on the other hand, the $Ba(OH)_2/H_2O$ and NaOH sub-fractions contained more β–glucans than arabinoxylans. This observation held for all three barley genotypes, and again was consistent with the content of these non-starch polysaccharides in the material used for extraction.

The water- and all alkali-extractable sub-fractions from PBP contained more arabinoxylans than β–glucans, with the exception of waxy barley. On the other hand, the water-

and the $Ba(OH)_2/H_2O$ and NaOH-extractable sub-fractions from flour and FRFs contained more β–glucans than arabinoxylans. All $Ba(OH)_2$ sub-fractions contained more than 90% arabinoxylans.

All the WE sub-fractions contained some ferulic acid (Table 2). It is known that ferulic acid residues esterified to arabinose residues in arabinoxylans may be responsible for formation of covalent linkages among arabinoxylan chains or for interaction of arabinoxylans with other cell wall components (Izydorczyk et al 1998a). Arabinoxylans present in the WE sub-fractions from PBP contained substantially higher amounts of ferulic acid than arabinoxylans from flour and FRFs. This was observed for all three barley genotypes. Interestingly, however, arabinoxylans present in the WE sub-fractions from PBP of waxy barley contained the highest amount of ferulic acid. This fact may partially explain poor water-extractability of arabinoxylans from PBP of waxy barley; the amount of arabinoxylans in the WE sub-fraction of waxy PBP was the lowest among three barley genotypes (Figure 7).

Arabinogalactans (AG), although only minor components among the non-starch polysaccharides, were especially concentrated in the outer portion of the barley kernel (Figure 7). Our results indicate that the PBP contain more arabinogalactans than the endosperm fractions. The WE sub-fractions from PBP in high amylose and waxy barley contained the highest amount of arabinogalactans. Interestingly, in flour more arabinogalactans were water-insoluble; among the alkali-extractable sub-fractions the $Ba(OH)_2$ ones were enriched in arabinogalactans. These differences in solubility of arabinogalactans from the outer and inner portion of the barley kernel may indicate differences in their molecular structure. This, however, remains to be investigated.

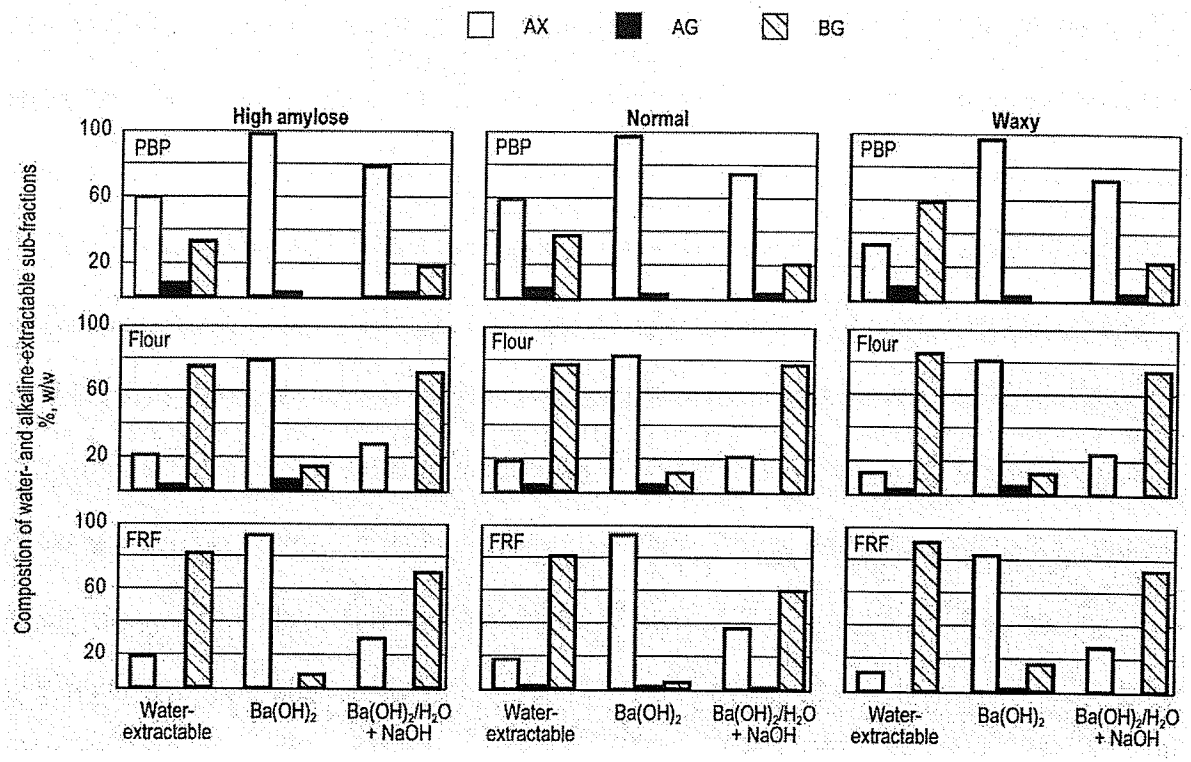

Figure 7. Composition of water- and alkali-extractable sub-fractions in pearling by-products (PBP), flour and fiber-rich fraction (FRF) of high amylose, normal, and waxy barleys. Arabinoxylans (AX), Arabinogalactans (AG), β-Glucans (BG).

Molecular structure of arabinoxylans

In order to unravel differences in the molecular structure of arabinoxylans originating from the different milling fractions and/or different barley genotypes, the degree of branching and modes of substitution in these polymers were investigated. Among the three milling fractions, arabinoxylans from FRFs had the lowest average degree of branching (Figure 8). This was indicated by relatively low arabinose to xylose ratio in both water- and alkali-extractable polymers. The degree of branching in arabinoxylans from flour was comparable to branching in FRFs, except for the $Ba(OH)_2$-extractable polymers which were slightly more branched. Arabinoxylans from the PBP exhibited the broadest variation in branching: arabinoxylans extracted with water, $Ba(OH)_2/H_2O$, and NaOH had a rather high degree of branching, whereas

those extracted with $Ba(OH)_2$ the lowest. Among the three barley genotypes, arabinoxylans from high amylose barley had the lowest degree of branching. This was observed for water- and alkali-soluble polymers in all three milling fractions with only one exception, namely the water-extractable arabinoxylans from flour.

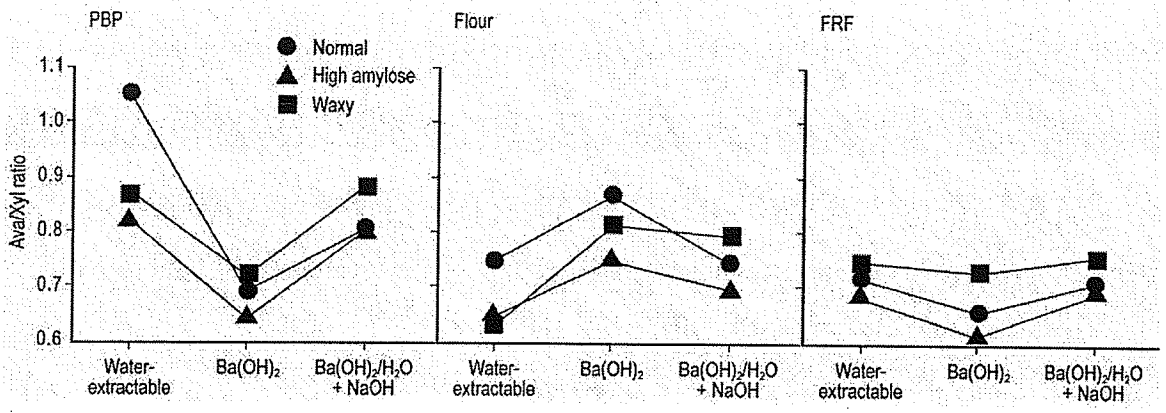

Figure 8. Ratio of arabinose (Ara) to xylose (Xyl) residues in water- and alkali-extractable arabinoxylans from pearling by-products (PBP), flour and fiber-rich fraction (FRF) of high amylose, normal, and waxy barleys.

The mode of substitution in arabinoxylans extracted from PBP and FRFs was determined by H-NMR spectroscopy. The presence of unsubstituted [→4(Xyl*p*)1→], singly [→2, 4(Xyl*p*)1→ and →3, 4(Xyl*p*)1→] and doubly [→2,3,4(Xyl*p*)1→] substituted xylose residues was detected (Table 3 and 4). The relative proportions on un-, mono-, and di-substituted xylose residues were calculated from the sugar analysis and from H-NMR spectra by integration of anomeric protons of the individual Ara*f* residues (Roels et al 1998). In general, arabinoxylans from the outer layers of the barley kernel were more substituted than those isolated from the endosperm. This was observed for all barley genotypes and is clearly shown by the higher average ratio of substituted to unsubstituted xylose residues in PBP than in FRFs (Table 3 and 4).

Table 3. Substitution pattern in arabinoxylans from water-extractable (WE) and alkali-extractable sub-fractions in pearling by-product (PBP) of high amylose, normal, and waxy barley.

Barley Genotype/ Milling Fraction/Sub-Fraction	Substitution Pattern				Sub/ Unsub	Single/ Double	2-Xyl/ 3-Xyl
	2-Xyl	3-Xyl	2,3-Xyl	U-Xyl			
High Amylose Barley							
PBP							
WE	28.6	3.3	25.7	42.4	1.4	1.2	8.6
$Ba(OH)_2$	15.0	20.3	14.4	50.3	1.0	2.4	0.7
$Ba(OH)_2/H_2O$	18.0	22.2	18.4	41.5	1.4	2.2	0.8
NaOH	8.2	37.6	22.2	32.1	2.1	2.1	0.2
average	*17.4*	*20.8*	*20.2*	*41.6*	*1.5*	*1.8*	*2.6*
Normal Barley							
PBP							
WE	20.7	15.7	35.0	35.0	2.0	1.0	1.3
$Ba(OH)_2$	11.1	21.3	17.6	49.8	1.0	1.8	0.5
$Ba(OH)_2/H_2O$	14.7	21.0	22.2	42.2	1.4	1.6	0.7
NaOH	6.6	34.9	19.5	38.9	1.6	2.1	0.2
average	*13.3*	*23.2*	*23.5*	*41.4*	*1.5*	*1.7*	*0.7*
Waxy Barley							
PBP							
WE	45.7	2.5	19.4	32.4	2.1	2.5	18.2
$Ba(OH)_2$	16.0	21.8	16.8	45.4	1.2	2.2	0.7
$Ba(OH)_2/H_2O$	27.6	21.9	17.6	32.8	2.0	2.8	1.3
NaOH	27.2	28.3	17.3	27.2	2.7	3.2	1.0
average	*29.1*	*18.7*	*17.8*	*34.6*	*2.0*	*2.7*	*5.3*

Table 4. Substitution pattern in arabinoxylans from water-extractable (WE) and alkali-extractable sub-fractions in fiber-rich fractions (FRF) of high amylose, normal, and waxy barley.

Barley Genotype/ Milling Fraction/Sub-Fraction	Substitution Pattern				Sub/ Unsub	Single/ Double	2-Xyl/ 3-Xyl
	2-Xyl	3-Xyl	2,3-Xyl	U-Xyl			
High Amylose Barley							
FRF							
WE	6.7	28.2	16.6	48.6	1.0	2.1	0.2
$Ba(OH)_2$	11.1	11.2	19.7	58.0	0.7	1.1	1.0
$Ba(OH)_2/H_2O$	36.5	4.9	14.5	44.1	1.3	1.6	7.5
NaOH	42.0	13.4	6.5	38.0	1.6	8.4	3.1
average	*24.1*	*14.4*	*14.3*	*47.2*	*1.2*	*3.3*	*3.0*
Normal Barley							
FRF							
WE	9.3	20.2	21.5	49.0	1.0	1.4	0.5
$Ba(OH)_2$	16.2	13.7	18.7	51.5	0.9	1.6	1.2
$Ba(OH)_2/H_2O$	34.6	4.8	16.0	44.6	1.2	2.4	7.3
NaOH	11.3	7.1	23.9	57.7	0.7	0.8	1.6
average	*17.8*	*11.4*	*20.0*	*50.7*	*1.0*	*1.6*	*2.6*
Waxy Barley							
FRF							
WE	8.4	5.5	30.7	55.5	0.8	0.4	1.5
$Ba(OH)_2$	8.4	14.3	25.6	51.6	0.9	0.9	0.6
$Ba(OH)_2/H_2O$	48.8	5.8	7.9	37.5	1.7	6.9	8.4
NaOH	32.1	20.6	15.0	32.3	2.1	3.5	1.6
average	*24.4*	*11.6*	*19.8*	*44.2*	*1.4*	*2.9*	*3.0*

The mode of substitution in arabinoxylans varied, however, with their solubility as well as genotypic origin. For example, the WE arabinoxylans from PBP were generally highly substituted. While those from high amylose and normal barley had almost equal amounts of singly and doubly substituted xylose residues (ratio of single/double Xyl: 1.25 and 1.04, respectively), those from waxy barley had twice as many singly than doubly substituted residues (ratio of single/double Xyl: 2.49). An interesting feature of WE arabinoxylans from pearling by-products was a very high content of Xyl*p* residues substituted at O-2 position (2-Xyl).

$Ba(OH)_2$ extractable arabinoxylans from PBP were less substituted than their water-extractable counterparts. They contained the highest amounts of unsubstituted but the lowest

amounts of doubly substituted Xyl*p* residues among all arabinoxylans extractable from the pearling by-products. This was observed for all three barley genotypes. The remaining alkali-extractable arabinoxylans from PBP, i.e., those extracted with $Ba(OH)_2/H_2O$ and NaOH, were more substituted than those extracted with $Ba(OH)_2$.

As mentioned before, arabinoxylans from the FRFs were less substituted than those from PBP. On average, they contained higher amounts of unsubstituted xylose residues but lower amounts of doubly substituted (except for waxy barley) and 3-Xyl*p* compared to arabinoxylans from PBP. The WE arabinoxylans from FRFs were substantially different than their counterparts from PBP. They were notably less substituted with a much higher content of unsubstituted Xyl*p*. Those from high amylose and normal barley contained less doubly substituted Xyl*p* and substantially less singly substituted Xyl*p* at O-2 position than their counterparts from pearling by-products. The WE arabinoxylans from FRF of waxy barley had relatively high content of doubly, but very little singly substituted Xyl*p*.

Molecular structure of β–glucans

The molecular structure of β–glucans was investigated by examining the hydrolysis products in lichenase digests of water- and alkali-extractable material from various milling fractions. The hydrolysis products from all materials were composed mainly of tri- and tetrasaccharides (90.3- 93.5%), originating from 3-*O*-β-D-cellobiosyl-D-glucose and 3-*O*-β-D-cellotriosyl-D-glucose units, respectively, in the intact β-glucan molecule (Table 5). Longer oligomers (DP≥5) with more than 2 consecutive β–(1 →4) linkages were also detected although in smaller amounts. These oligomers are indicative of the cellulose-like regions in the β–glucan chains (Izydorczyk et al 1998a) and may greatly affect the properties of these polymers. The

most striking differences were observed in the average ratio of tri- to tetrasaccharides fragments in β–glucans from PBP compared to those from flour and FRFs. For all three barley genotypes, the ratio of DP3 to DP4 oligosaccharides was higher in β–glucans from PBP than in flour and FRFs. PBP, composed mainly from the outer layers of the barley grain, contained relatively small amounts of β–glucans compared to the FRFs and flour. β–Glucans in PBP generated in this study probably originated predominantly from the aleurone tissue, whereas β–glucans in FRFs and flour originated from the endosperm cell walls. Only very small differences were observed between β–glucans from flours and FRFs. β–Glucans from flour of high amylose and waxy barleys had slightly higher DP3:DP4 ration than those from FRFs. The opposite trend, however, was observed for normal barley. β–Glucans from flour in all three barleys had slightly higher amounts of DP≥5 fragments than those from FRFs. In general, β–glucans from flour and FRFs of normal barley had lower ratio of DP3:DP4 and a slightly higher content of DP≥5 oligosaccharides than β–glucans of high amylose and waxy barleys.

For all milling fractions examined, and all barley genotypes, the ratio of DP3:DP4 increased with the decreasing solubility of these polymers (the WE sub-fractions being the most soluble and the NaOH ones the least). These results confirm previous observations. Higher ratios of tri- to tetrasaccharides have previously been associated with decreased solubility or extractability of β–glucans (Izydorczyk et al., 1998a). It has been postulated that since a helix of three consecutive cellotriosyl residues would form a crystalline structure, a higher content of these cellotriosyl fragments might contribute to greater conformational regularity, translating into lower β–glucan solubility (Tvaroska et al., 1983; Izawa et al 1993; Izydorczyk et al., 1998a). As a consequence, the increases in the ratio of tri- to tetrasaccharides between water- and alkali-

Table 5. Composition of oligosaccharides (mol %) released by lichenase from water-extractable (WE) and alkali-extractable β-glucans in pearling by-product (PBP), fiber-rich fractions (FRF) and flour milling fractions of barley samples.

Barley Genotype/ DP[a]	PBP				Flour				FRF			
	WE	$Ba(OH)_2$	$Ba(OH)_2$/H2O	NaOH	WE	$Ba(OH)_2$	$Ba(OH)_2$/H2O	NaOH	WE	$Ba(OH)_2$	$Ba(OH)_2$/H2O	NaOH
High Amylose												
3+4	91.9	-	91.7	91.9	91.5	92.1	90.4	91.1	91.1	92.3	90.9	90.6
5 to 9	8.1	-	8.1	7.9	8.5	7.9	9.1	8.4	8.9	7.7	9.1	9.1
>9	0.2	-	0.2	0.0	0.2	0.0	0.5	0.3	0.3	0.0	0.5	0.4
3:4	2.7	-	2.9	3.1	2.3	2.2	2.4	2.5	2.2	2.2	2.4	2.4
Normal												
3+4	91.5	-	91.7	93.0	90.8	92.1	90.3	90.3	90.9	93.5	90.5	91.4
5 to 9	8.5	-	8.1	7.2	9.2	7.9	9.7	9.7	9.1	6.6	9.5	8.6
>9	0.2	-	0.0	0.0	0.3	0.0	0.9	0.7	0.3	0.0	0.6	0.0
3:4	2.5	-	3.0	3.1	2.1	2.2	2.1	2.1	2.1	2.1	2.2	2.5
Waxy												
3+4	92.3	-	91.8	92.0	91.8	92.5	91.1	90.8	91.7	92.7	91.3	90.9
5 to 9	7.7	-	8.0	8.1	8.2	7.7	8.8	9.2	8.3	7.3	8.7	8.8
>9	0.0	-	0.0	0.2	0.0	0.0	0.6	0.4	0.0	0.0	0.5	0.2
3:4	2.6	-	3.0	3.1	2.3	2.3	2.4	2.4	2.3	2.3	2.3	2.31

[a] DP, degree of polymerization

extractable sub-fractions, along with the increase in cellulosic regions of DP≥9, lead to decreased solubility of these polymers.

Molecular weight of non-starch polysaccharides in various milling fractions

In order to obtain some information about the molecular weight of non-starch polysaccharides in various milling fractions of the three barley genotypes, the water- and $Ba(OH)_2$- extractable sub-fractions were subjected to size exclusion chromatography. Elution profiles of water-extractable sub-fractions obtained from PBP, flour, and FRFs of high amylose barley are shown in Figure 9. In agreement with the multi-component character of some of these sub-fractions (Table 2 and Figure 7), the elution profiles indicated the presence of one or more polymeric species. The $Ba(OH)_2$- extractable sub-fractions (Figure 10) clearly contained two well separated polymeric populations; one eluting between 50 and 75 minutes and another between 80 and 100 minutes. The WE sub-fractions also contained the second peak, but its size was relatively small. The response from the UV detector indicated the presence of UV-absorbing material, especially in the second peak. In addition, the relative size of this peak, as estimated from the RI detector, corresponded well with the amount of proteins present in the $Ba(OH)_2$- and water-extractable sub-fractions. It is, therefore, reasonable to assume that the first peak in the elution profile of the water- and $Ba(OH)_2$- extractable sub-fractions represented mainly the carbohydrate material, whereas the second peak reflected the proteinaceous material present in these sub-fractions. The average molecular weight of the species eluting in the second peak was around 40,000 g/mol.

The carbohydrate composition of the water- and $Ba(OH)_2$- extractable sub-fractions from all three milling fractions indicated the presence of arabinoxylans, β–glucans, and

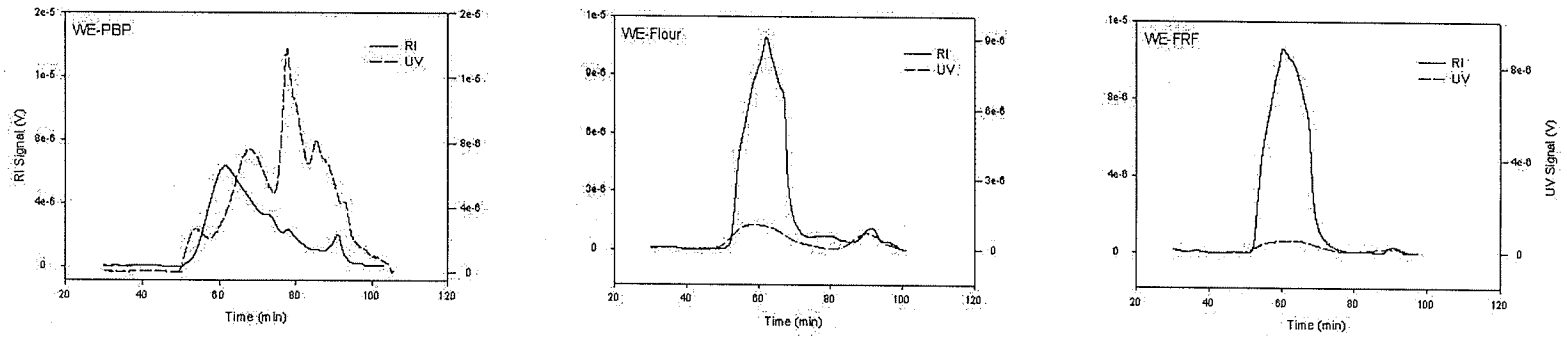

Figure 9. The high-performance size-exclusion chromatography (HPSEC) with online multi-angle light-scattering (MALS) detection coupled with refractive index (RI) and ultra-violet (UV) detection profiles of water-extractable (WE) sub-fractions from pearling by-products (PBP), flour and fiber-rich fraction (FRF) of high amylose barley.

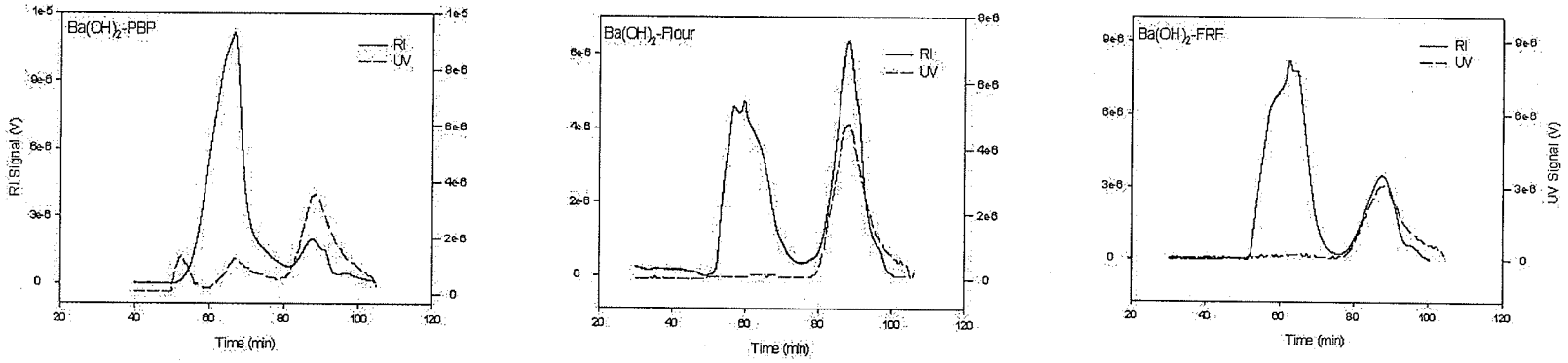

Figure 10. The high-performance size-exclusion chromatography (HPSEC) with online multi-angle light-scattering detection (MALS) coupled with refractive index (RI) and ultra-violet (UV) detection profiles of $Ba(OH)_2$-extractable sub-fractions from pearling by-products (PBP), flour and fiber-rich fraction (FRF) of high amylose barley.

arabinogalactans in various proportions, depending on the milling fraction and solvent used for extraction (Figure 7). The WE sub-fraction from PBP was probably the most heterogeneous and contained, in the case of high amylose barley, 60% of arabinoxylans, 33% of β–glucans, and 8% of arabinogalactans. Although the peak eluting between 50 and 75 min (Figure 9) clearly showed signs of multi-component character, no clear separation of the species was obtained. The average molecular weight of this peak cannot, therefore, be assigned with any certainty to any particular non-starch polysaccharide. Instead, it indicates an average M_w of all non-starch polysaccharides present in this sub-fraction. The WE sub-fractions from flour and FRF were less heterogeneous in composition and contained mostly β–glucans (>75%). The first, high-molecular weight peak in the elution profile of these samples was indicative, therefore, of the molecular weight and distribution of mainly β–glucans. The first peak in the elution profile of the $Ba(OH)_2$- extractable sub-fractions, on the other hand, indicated the molecular size of arabinoxylans, which constituted the majority of these sub-fractions. The average M_w of the first peak in the elution profiles of the water- and $Ba(OH)_2$- extractable sub-fractions obtained from the three milling fractions of the different barley genotypes are compared in Table 6. The average M_w of water- and $Ba(OH)_2$-extractable polysaccharides from PBP of high amylose barley was much higher than those from PBP of normal and waxy barley.

In the case of high amylose and waxy barleys, the WE sub-fractions from flour and FRFs (mostly β–glucans) had much higher M_w than the $Ba(OH)_2$-extractable sub-fractions (mostly arabinoxylans). In addition, the WE sub-fractions from flour had higher M_w than the sub-fractions from FRF. The water-extractable β–glucans (WE sub-fractions) from flour and FRFs of waxy barley had the highest M_w among the three barley genotypes.

Table 6. Weight average molecular weight of major water- and $Ba(OH)_2$ polysaccharide populations in pearling by-product (PBP), flour, and fiber-rich fraction (FRF) milling fractions of barley samples.

Milling Fraction / Extracted Sub-Fraction	Weight Average Molecular Weight x 10^{-6} (g/mol)		
	High Amylose Barley	Normal Barley	Waxy Barley
PBP			
WE	2.47	0.92	0.68
$Ba(OH)_2$	2.43	1.93	0.85
Flour			
WE	3.23	1.09	5.3
$Ba(OH)_2$	1.01	2.01	1.49
FRF			
WE	2.29	2.19	3.5
$Ba(OH)_2$	1.07	1.91	0.95

The water-extractable β–glucans (WE sub-fractions) from flour and FRFs of normal barley had the lowest M_w among the three barley genotypes. On the other hand, the $Ba(OH)_2$-extractable arabinoxylans from flour and FRFs of normal barley had the highest M_w among the three barleys.

SUMMARY AND CONCLUSIONS

Fractionation of hull-less barley grain via a combination of pearling and roller milling resulted in fractions containing various proportions of proteins, starch, ash, and non-starch polysaccharides, mainly β–glucans, arabinoxylans and arabinogalactans. In general, the FRFs, comprising the majority of the endosperm cell wall material, contained the highest amount of β–glucans, followed by straight grade flour and pearling by–products. The solubility of β–glucans, however, was inversely proportional to their levels in the fractions, with β–glucans in PBP being the most soluble and those in FRF the least. Although PBP contained the highest amount of arabinoxylans, FRF were also enriched in these polymers. The solubility of arabinoxylans was

higher in FRF than in PBP. Small amounts of arabinogalactans detected in barley appeared to be concentrated in the outer portion of the barley kernel. The content and solubility of non-starch polysaccharides in various milling fractions was also dependent on the type of barley.

Both polymers, β–glucans and arabinoxylans, exhibited structural heterogeneity derived from differences in their localization within the kernel as well as from the genetic origin of the barley. Some general trends were observed. For example, the water-extractable arabinoxylans from the endosperm cell walls (FRF and flour) had a substantially lower degree of branching than those from the outer layers (PBP). The water-extractable arabinoxylans from FRF of high amylose and normal barley contained more unsubstituted Xyl*p* residues and less doubly substituted Xyl*p* and singly substituted Xyl*p* at O-2 than their counterparts from PBP. The water-extractable arabinoxylans from FRF of waxy barley had a relatively high content of doubly, but very little singly substituted Xyl*p* residues. PBP contained relatively small amounts of β–glucans compared to FRF or flour, but the ratio of tri- to tetrasaccharides in β–glucans from PBP was higher than from flour and FRF. Substantial differences in the molecular weight of non-starch polysaccharides in different milling fractions were also observed.

While the current results are valid specifically for the particular barley cultivars studied in this project and the milling scheme used, it is reasonable to expect that certain trends observed in this study can be extrapolated to other barley cultivars. The current findings indicate also that by utilizing appropriate barley types and combining pearling and roller milling, it is possible to fractionate barley grain into fractions enriched in specific components with desirable properties and functionality.

CHAPTER 2

Evaluation of baking procedures for incorporation of barley roller milling fractions containing high levels of dietary fiber into bread

ABSTRACT

Fiber-rich fractions (FRF) obtain by roller milling waxy and high amylose starch hull-less barley genotypes were evaluated as functional ingredients in pan bread prepared from Canada Western Red Spring (CWRS) and Canada Western Extra Strong (CWES) wheat flour. Three bread processes were used: Canadian short process (CSP, a full formula mechanical development procedure), remix-to-peak (a lean formula (no fat) long fermentation straight-dough procedure) and sponge-and-dough (a 4½ hr sponge fermentation time). Addition of 20% FRF (equivalent to enrichment with 4.0 g of arabinoxylans and β–glucans per 100 g of flour) disrupted dough properties and depressed loaf volume. The impacts of FRF from waxy and high-amylose starch barley on bread were similar. Baking process strongly influenced bread quality. CSP was not suitable for making FRF-enriched bread because dough could not be properly developed. FRF-enriched remix-to-peak bread was better, especially for the stronger CWES flour. Loaf volume declined, but the bread should meet the expectation of health conscious consumers. The better bread quality compared to CSP probably was due to redistribution of water from non-starch polysaccharides to gluten during fermentation prior to remixing and final proof. The sponge-and-dough process produced the best FRF-enriched bread because of the positive effect of sponge fermentation on gluten development and hydration. FRF was added at the dough stage to fully developed dough. Pre-hydration of FRF improved bread quality. CWRS and CWES flour produced comparable FRF-enriched sponge-and-dough bread. Addition of xylanase to the sponge-and-dough formula improved the loaf volume, appearance and crumb structure of FRF-enriched bread.

INTRODUCTION

Barley is one of the most ancient crop plants, with cultivation going back to biblical times (Brennan and Cleary, 2005). Although once a staple food, barley became less popular in the twentieth century and its consumption has declined in favor of grains such as wheat, rice and maize. Nevertheless, barley grain is an excellent source of soluble and insoluble dietary fiber with clinically proven health benefits (Bhatty, 1986b, 1999b; Newman and Newman, 1991; Jadhav et al, 1998; Slavin et al, 2000a). β–Glucans, the major fiber constituents of barley, lower plasma cholesterol, reduce glycaemic index, and reduce risk of colon cancer (Jadhav et al, 1998; Brennan and Cleary, 2005). The U.S. Food and Drug Administration (FDA) has recently announced that whole grain barley and barley-containing products are allowed to carry the claim that they reduce the risk of coronary heart disease (FDA News Release, 2005). It is anticipated, therefore, that the health benefits of barley will stimulate interest among food producers and consumers in using barley for food purposes. The health benefits of other barley fiber components, which include arabinoxylans, arabinogalactans and galactomannans, have yet to be investigated.

The non-uniform distribution of barley components within the barley kernel allows fractionation into products enriched in various constituents (Izydorczyk et al, 2004). Various combinations of pearling, grinding, sieving and air classification have been used to create barley products with elevated levels of fiber (Jadhav et al, 1998; Bhatty, 1986b; Knuckles et al, 1992; Sundberg and Åman, 1994; Knuckles and Chiu, 1995; Yoon et al, 1995; Sundberg et al, 1995b; Lee et al, 1997; Marconi et al, 2000; Andersson et al, 2003; Izydorczyk, 2003a; Flores et al, 2005). Roller milling has been widely used to fractionate

barley (Bhatty 1987, 1992, 1993, 1997; Newman and Newman 1991; Danielson et al 1996; Klamcczynski and Czuchajowska 1999; Kiryluk et al 2000; Wang et al 1993). As the level of β–glucan in barley increases, the flour yield decreases (Bhatty, 1999b). However, the loss of flour yield is offset by higher yield of a fibre-rich fraction (FRF) enriched in non-starch polysaccharides from endosperm cell walls (ie β–glucans and arabinoxylans) (Izydorczyk et al, 2003a; 2003b). Barley FRF can be obtained in good yield by a simplified roller milling procedure (Izydorczyk et al, 2003a). It has potential as a functional food ingredient because of its composition, and because it is obtained by a natural chemical-free process.

Wheat is a major component of human diet in most countries because it is such a flexible food ingredient. Accordingly, a promising way to increase the food use of barley is as a functional ingredient in wheat-based products such as bread, pasta and noodles. Barley flour and other barley fractions have been incorporated into pasta, (Knuckles et al, 1992; Marconi et al, 2000; Dexter et al, 2005; Yokoyama et al, 1997) Asian noodles (Baik and Czuchajowska, 1997; Hatcher et al, 2005; Izydorczyk et al, 2005; Lagassé et al, 2006) and chemically leavened products such as muffins, pancakes and biscuits (Berglund et al, 1992). Barley flour has also been incorporated into flat bread (Sidhu et al, 1990; Başman and Köksel, 1999). All of these products exhibited some textural and visual modification to the products made from wheat flour controls, but in general were judged acceptable. In some cases barley-enriched wheat-based products exhibited improved flavour (Berglund et al, 1992; Newman et al, 1998).

Incorporation of barley and barley fractions into western pan bread has almost universally been reported to diminish bread quality, particularly loaf volume (Knuckles et al, 1997; Bhatty, 1986a; Cavallero et al, 2002; Gill et al, 2002a, 2002b; Mann et al, 2005; Swanson and Penfield, 1988; Trogh et al, 2004, 2005). Nevertheless, a general consensus is that it is feasible to produce barley-enriched western-style breads that have acceptable flavour, appearance and texture.

The loss of bread quality associated with barley enrichment is not surprising considering that the incorporation of fibre-rich ingredients into bread is known to dilute and disrupt the gluten network in dough, impairing gas retention and changing bread texture and appearance (Dubois, 1978). Dough from whole wheat flour, for example, is less cohesive than dough from white wheat four, and the specific volume of whole wheat bread is usually substantially lower than corresponding white bread (Atwell, 2004). Berglund et al (1992) suggested that if the quality of barley-enriched bread was compared to whole wheat bread, rather than white bread, barley-enriched bread would be judged more favourably.

Gill et al (2002a) attributed the lower loaf volume and firmer crumb structure of barley-enriched bread at least partially to barley components, notably β–glucans. They theorized that β–glucans tightly bind water in the dough, reducing the availability of water for development of the gluten network. This is supported by significant loaf volume depression associated with addition of small amounts of a concentrated soluble β–glucan extract from barley (Symons and Brennan, 2004). Water-unextractable arabinoxylans, which are present in elevated levels in barley, are well known to suppress bread quality

(Courtin and Delcour, 2002). Trogh et al (2004) demonstrated that adding xylanase to a barley-enriched bread formula improved bread quality, and had the additional health advantage of increasing soluble fibre content.

The incorporation of β–glucans-rich barley fractions in bread has a positive influence on human glycaemic control (Cavallero et al, 2002; Symons and Brennan, 2004; Pick et al, 1998). Andersson et al (2004) showed that the average molecular weight of barley β–glucans decreased during mixing and fermentation, and concluded that for maximum health benefits mixing and fermentation time should be keep as short as possible when baking hull-less barley bread, but they provide no data on the impact of shortening the bread process on bread palatability. There have been attempts to improve barley-enriched bread quality by modifying barley fractions by heat treatment and extrusion (Gill et al, 2002a, 2002b) and by optimizing formula for a given baking process (Swanson and Penfield, 1988; Trogh et al, 2004, 2005), but to our knowledge there have been no reports comparing the suitability of different baking processes for making barley-enriched bread. Accordingly, we initiated this investigation to determine the optimal conditions for incorporation of a FRF derived from waxy starch and high amylose starch hull-less barley cultivars (Izydorczyk et al, 2003a). Previously we have reported the successful incorporation of similar FRF products into pasta and Asian noodles (Dexter et al, 2005; Izydorczyk et al, 2005).

MATERIALS AND METHODS

Wheat flours

Wheat flours were supplied by the Canadian International Grains Institute, Winnipeg, Canada. Flours were straight-grade of approximately 75% wheat extraction, obtained by milling commercially grown samples of No 1 Canada Western Red Spring (CWRS) and No 1 Canada Western Extra Strong (CWES) wheat on the Canadian International Grains Institute pilot mill described by Holas and Tipples (1978). Protein contents (14% m.c.) were 13.6% for CWRS flour and 13.9% for CWES flour. Ash contents (d.w.b.) were 0.58% for CWRS flour and 0.71% for CWES flour.

Barley fibre-rich fractions (FRF)

Dr. Brian Rossnagel, Crop Development Centre, University of Saskatchewan, supplied samples of the Canadian hull-less barley (HB) variety CDC Candle (waxy starch, 4.3% amylose), and the experimental line CDC-92-55-06-48 (high amylose starch, 41.8% amylose). HB was conditioned to 14.5% moisture and held for 65 hr and then pearled to remove 10% by weight (Type TM, Satake, Hiroshima, Japan). Pearled barley was milled using a five-stand research mill equipped with 25-cm diameter rolls (Black et al, 1980) by the short flow outlined by Izydorczyk et al (2003b) to derive fibre-rich fractions (FRF). FRF is a coarse material coming from a shorts duster passage at the end of the mill flow, and is comprised primarily of endosperm cell wall remnants (Izydorczyk et al, 2005). The FRF samples used in the current study have been characterized in detail by Izydorczyk et al (2003b), and a summary of key components is given in Table 7.

Table 7. Composition of fiber-rich fractions from roller milling of hull-less barley [a]

Property	Hull-less barley cultivar	
	CDC-92-55-06-54 (HA)	CDC Candle (WX)
Starch (%)	50.0	58.0
Starch amylose (%)	41.8	4.3
Protein [b] (%)	11.1	11.2
Total dietary fibre (%)	27.2	27.9
Total β–glucans (%)	13.4	15.2
Soluble β–glucans (%)	2.8	7.3
Total arabinoxylans (%)	8.0	7.4
Soluble arabinoxylans (%)	1.6	0.9

[a] Expressed on dry matter basis.
[b] Expressed as N X 6.25.

Analytical methods

Protein content [N X 5.7 (wheat) X 6.25 (barley)] was determined by combustion nitrogen analysis (model FP-248 Leco Dumas CAN analyzer, St. Joseph, USA) calibrated with EDTA according to AACC Approved Method 46-30 (AACC, 2000). Ash content and total dietary fibre were determined by AACC Approved Methods 08-01 and 32-05 (AACC, 2000), respectively. Total starch and β-glucan contents were determined enzymatically using Megazyme kits (Megazyme International, Bray, Ireland) according to AACC Approved Methods 76-13 and 32-33, respectively (AACC, 2000). Amylose content of isolated, defatted starch samples was determined by potentiometric titration (Schoch, 1964). Total AX content was determined colorimetrically by the phloroglucinol method (Douglas, 1981). Soluble BG and soluble AX were determined as described by Izydorczyk et al (2003b).

Farinograph

Farinograph curves were obtained according to AACC Approved Method 54-21 (AACC, 2000).

Remix-to-peak baking

The remix-to-peak baking process is a long fermentation straight-dough procedure (Kilborn and Tipples, 1982). A lean (no fat) formula dough (see Table 8) which includes determining the optimum water by an expert baker through dough handling properties at the sheeting stage, is mixed at 135 rev/min in a GRL-200 recording dough mixer (Hlynka and Anderson, 1955). The dough is subjected to an initial fermentation of 160 min at 30°C, and then remixed to 10% past peak consistency prior to make-up and given a final proof of 55 min at 30°C and baked at 233°C for 25 min.

Canadian short process baking

The Canadian short process (CSP) is a no-time mechanical dough development baking procedure (Preston et al, 1982). A rich-formula dough (see Table 8) is mixed in a GRL-200 recording dough mixer (Hlynka and Anderson, 1955) at 165 rev/min at 30°C to 10% past peak consistency, as judged from the mixing curves. Optimum water is determined by an expert baker at sheeting. Dough is rested for 15 min at 30°C, and then sheeted, moulded and panned as described by Kilborn and Tipples (1982). After a 70- min proof at 37.5°C, baking is carried out in a heat-sink oven for 25 min at 195°C as previously described (Kilborn et al, 1990).

Table 8. Formulas for various baking methods.

Ingredient	Remix-to-peak	Canadian short process	Sponge-and-dough		
			Sponge	Dough	Total
Flour [a] (g)	100	100	140	60	200
Compressed Yeast (g)	3	3	4	----	4
Salt (g)	1	2.4	0.3	4.5	4.8
Sucrose (g)	2.5	4	----	10	10
Ammonium phosphate (g)	0.1	0.1	0.2	----	0.2
Ascorbic acid (μg)	----	150	400	----	400
Potassium bromate (μg)	15	----	------	----	----
Malt syrup (g)	0.6	0.2	0.4	0.2	0.6
Shortening (g)	----	3	----	6	6
Whey (g)	----	4	----	----	----
Skim milk powder (g)	----	----	----	4	4
Water			Variable		

[a] Total weight of flour and fiber-rich fraction (FRF), when included, on 14% moisture basis. FRF was added at the dough stage of sponge-and-dough baking.

Sponge-and-dough baking

The sponge-and-dough baking test was as described by Kilborn and Preston (1981) using the formula detailed in Table 8. The sponge was mixed in a GRL-200 recording dough mixer (Hlynka and Anderson, 1955) at 135 rev/min for 2.5 min, and then fermented for 270 min at 27°C. At the dough mixing stage, the remaining flour (60 g) and other ingredients, including optimum water to give proper dough handling properties as determined by an expert baker at sheeting, were combined with the sponge. FRF pre-soaked in water was added in place of some of the flour at the dough stage. Dough was

mixed at 135 rev/min at 30°C to 10% past peak consistency, as judged from the mixing curves. Doughs were rested at 30°C for 15 min, punched, and rested a further 15min. Doughs were then sheeted and molded, placed in baking pans as described by Kilborn and Tipples (1982), and proofed for 70 min at 37.8°C. Bread was baked in a heat-sink oven for 25 min at 195°C as previously described (Kilborn et al, 1990).

Bread evaluation

Loaf volume was determined immediately following baking by rapeseed displacement according to AACC Approved Method 72-10 (AACC, 2000), and expressed on the basis of 100 g flour.

After cooling, bread was stored at room temperature (approximately 21°C) in doubled polyethylene bags to allow moisture equilibration and bread texture stabilization. The day following baking, bread was scored for external appearance, crumb texture and crumb color as described by Preston et al (1982).

Bread crumb firmness was determined as the maximum compression force (40% compression) by pressing a 36-mm diameter cylindrical plunger into a central 25-cm thick slice of bread using a TA-XT2 Texture Analyzer (Texture Technologies Co., Scarsdale, New York, USA) according to AACC Approved Method 74-09 (AACC, 2000).

Extraction of β–glucan

Water-soluble β–glucans were extracted from ground barley grain (cv. CDC Candle) according to the methodology used by Izydorczyk et al (1998a). The ground grain was refluxed with 85% ethanol to eliminate the endogenous enzymes. Barley grist was mixed with water at 45°C for 30 min. The mixture was centrifuged and the insoluble

residue was re-extracted with water twice. The collected supernatants were brought to 95°C for 5 min., then treated with Celite and Fuller's Earth (Sigma, St. Louis MO) to remove the residual proteins. The extract was incubated with α-amylase (EC 3.2.1.1, type I-A, Sigma, St. Louis, MO) overnight, then heated to inactivate the enzyme, and centrifuged. Three volumes of ethanol were added to the purified extract and the precipitated polysaccharides were collected and dried. The purified material contained 85% β–glucans.

Statistics

All analyses and baking experiments were conducted in duplicate. All statistical analyses were performed using SAS statistical software version 8 (SAS Institute Inc., Cary, NC). Analysis of variance (ANOVA) using LSD were performed to determine significant differences. Replicated results are reported as means; coefficient of variation was less than 5 % for all tests. Differences were considered significant at $p \leq 0.05$ unless otherwise stated.

RESULTS AND DISCUSSION

Enrichment of remix-to-peak bread with soluble β–glucans extracted from hull-less barley

Symons and Brennan (2004) reported a significant drop in loaf volume when small amounts of concentrated barley β–glucan extract were included in a wheat bread formula. Accordingly, we decided to initiate our investigations by incorporating a highly concentrated extract of barley β–glucan into remix-to-peak bread prepared from Canada Western Red Spring (CWRS) wheat (Table 9). Optimum baking absorption increased as β–glucan content increased. Bread weight also increased, indicating that some of the extra

water is retained in the baked loaf. The negative impacts of small amounts of the β–glucan extract on bread properties were readily apparent. There was a continual decline in loaf volume, loaf appearance and crumb structure as more β–glucan extract was incorporated into the formula. Incorporation of only 1% β–glucan reduced loaf volume by 15%, demonstrating the challenge of producing high quality β–glucan-enriched western-style bread. It is important to note that the β–glucan concentrate used by us and by Symons and Brennan (2004) is solely water-extractable β–glucan and may have a less negative impact on bread quality, whereas much of the β–glucan in barley is insoluble and its impact is unknown.

Table 9. Properties of remix-to-peak bread prepared from Canada Western Red Spring wheat flour enriched with various levels of hull-less barley soluble β–glucans. [a]

	β–Glucan level (%)				
Bread property	0.00	0.25	0.50	0.75	1.00
Water absorption (%)[b]	62[e]	65[d]	69[c]	72[b]	75[a]
Bread volume (cm^3)	905[a]	865[b]	855[c]	775[d]	765[e]
Bread weight (g)	124	125	126	127	128
Bread appearance (units)	8.5[a]	8.0[b]	8.0[b]	7.8[c]	7.0[d]
Crumb structure (units)	6.0[a]	5.7[b]	5.5[c]	5.4[d]	5.0[e]
Crumb color (units)	6.0[a]	6.0[a]	6.0[a]	5.8[b]	5.8[b]

[a] Values followed by the same letter in the same row are not significantly different ($P<0.01$).

[b] Water absorption is expressed on a 14% m.b.

Selection of flours and baking processes for FRF-enrichment studies

Flours from two classes of Canadian wheat, CWRS and Canada Western Extra Strong (CWES), were chosen to determine of the impact of FRF on dough and bread properties. The CWRS class exhibits balanced dough properties (elastic and extensible),

making it ideally suited for preparation of high volume western-style pan bread. The CWES class has extraordinarily strong gluten which makes it ideal in blends with weaker flour. It is possible that the extraordinary strength of CWES could be an asset in mitigating the anticipated negative impact of enrichment with FRF.

The baking processes were chosen to reflect the full spectrum of western bread processes and formulae. The CSP process is typical of short fermentation mechanical dough processes that are popular in many countries, because good quality bread can be made from flours that are less strong than required for longer fermentation processes. The remix and the sponge-and-dough procedures are long fermentation time processes that require stronger flour to produce acceptable bread. In addition, the remix process is a lean (no fat) process that is common for hearth breads such as French bread. In contrast, the CSP and sponge-and-dough processes are rich full formula processes.

Impact of enrichment with 20% FRF on farinograph properties

Addition of 20% FRF to either CWRS flour or CWES flour had dramatic effects on farinograph mixing curves (Table 10). FRF from either barley cultivar imparted similar effects for a given base wheat flour. Dough water absorption increased by about 25% due to the hygroscopic β–glucans and arabinoxylans. The large effect on water absorption of FRF makes the farinograph curves difficult to interpret.

Table 10. Farinograph properties of Canadian Western Red Spring (CWRS) and Canadian Western Extra Strong (CWES) flour with and without enrichment with 20% fiber-rich fraction (FRF) from high amylose (HA) and waxy (WX) hull-less barley.

Treatment	Water absorption (%)[a]	Dough development time (min)	Stability (min)	Mixing tolerance index (BU)
No 1 CWRS				
Control	61.1	5 ½	24	20
20% HA FRF	85.8	9 ¾	13	15
20% WX FRF	86.6	9	14 ½	20
No 1 CWES				
Control	62.1	1 ¾	2 ½	45
20% HA FRF	85.3	16 ¼	17	20
20% WX FRF	85.7	14 ½	10	20

[a] Water absorption is expressed on a 14% m.b.

Tolerance to over-mixing, as measured by stability, was less for CWRS dough when enriched with FRF, indicative of some dough weakening. In contrast, CWES dough enriched with FRF showed a large increase in stability and a reduction in mixing tolerance index, indicative of improved tolerance to over mixing and greater dough strength. This anomaly can be explained by the extraordinarily strong gluten of CWES. CWES does not fully develop in the Farinograph, at the relatively slow speed of 63 rpm and, low work input mixer (Rao et al, 2000). The farinograph curve obtained for the CWES control dough is basically a water hydration curve. When FRF is added the dough weakens sufficiently to allow development.

Enrichment with FRF increased dough development time for both CWRS and CWES. In the case of CWRS, the extended mixing time may be due to increased

competition for water, delaying development of the gluten network as proposed by Gill et al (2002a). As discussed above, the longer dough development time for FRF-enriched CWES dough reflects, at least partially, lack of dough development of the control flour dough.

Our results appear to contradict those of Izydorczyk et al (2001), who reported that addition of whole barley and barley non-starch polysaccharides to wheat flour increased mixograph peak dough resistance, mixing stability and work input, indicative of dough strengthening. Izydorczyk et al (2001) mixed barley-enriched flour at the same water absorption as the wheat base flour despite the greater water absorbing capacity of flour enriched by barley ingredients. Mixing flours with large variations in water absorption at constant water absorption would strongly impact mixing properties, perhaps accounting for the discrepancy of their results to ours.

Enrichment of remix-to-peak bread with 20% FRF

Enrichment of remix-to-peak bread with 20% FRF from either barley cultivar imparted similar effects (Table 11, Figure 11). FRF-enrichment was accompanied by significant deterioration in loaf volume, bread appearance and crumb structure (Table 11, Figure 11). The additional strength of CWES was an asset. CWES FRF-enriched bread was clearly superior in loaf volume and bread appearance to CWRS FRF-enriched bread, although crumb structure was not better. The CWES FRF-enriched remix-to-peak bread would be judged acceptable by most consumers, based on visual appearance.

Enrichment with 20% FRF imparted a water absorption increase of over 20%. Baked bread weight was about 20 g heavier with FRF enrichment, indicating that much of the additional water was tightly bound and not lost during baking.

Table 11. Properties of remix-to-peak bread prepared from Canadian Western Red Spring (CWRS) and Canadian Western Extra Strong (CWES) flour with and without enrichment with 20% fiber-rich fraction (FRF) from high amylose (HA) and waxy (WX) hull-less barley. [a]

Treatment	Bread volume (cm^3)	Water absorption (%)[b]	Bread weight (g)	Bread appearance (units)	Crumb structure (units)	Crumb color (units)
No 1 CWRS						
No FRF	860[a]	63[c]	123	8.5[a]	6.5[a]	6.5[b]
20% HA FRF	540[c]	86[b]	147	6.0[d]	4.5[c]	4.2[d]
20% WX FRF	580[c]	86[b]	148	6.0[d]	5.2[b]	4.2[d]
No 1 CWES						
No FRF	860[a]	63[c]	125	8.5[a]	6.5[a]	6.8[a]
20% HA FRF	660[b]	86[b]	143	7.5[b]	4.5[c]	4.5[c]
20% WX FRF	650[b]	87[a]	144	6.5[c]	4.0[d]	4.0[e]

[a] Values followed by the same letter in the same column are not significantly different ($P<0.01$).

[b] Water absorption is expressed on a 14% m.b.

Firmness of FRF-enriched breads was greater than that of the corresponding control flours during storage of breads for four days (Figure 12). Relative differences in crumb firmness among breads are strongly influenced by increasing denseness as loaf volume declines. Therefore, CWES FRF-enriched breads were significantly less firm that CWRS FRF-enriched bread. It was also observed that breads enriched with waxy starch FRF were less firm than high amylose FRF-enriched bread.

Andersson et al (2004) reported that when enriching bread with barley fractions it is desirable to keep mixing and fermentation time as short as possible to minimize reduction in molecular weight of β-glucan, and to maintain its cholesterol-lowering effect. The remix-

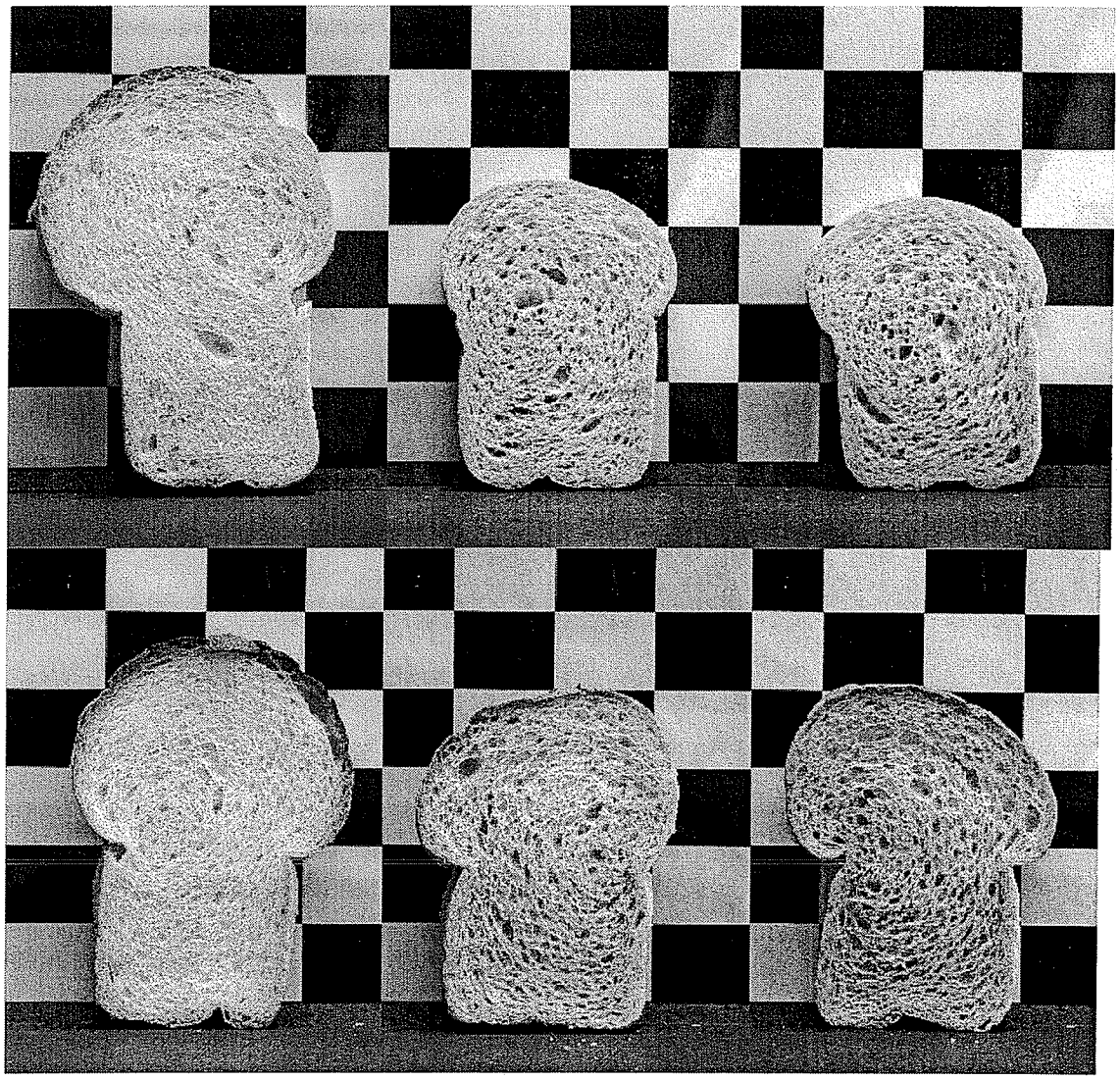

Figure 11. Remix-to-peak bread prepared from: Canadian Western Red Spring (CWRS) control flour (upper left); CWRS with 20% high amylose (HA) fiber-rich fraction (FRF) (upper middle); CWRS with 20% waxy (WX) FRF (upper right); CWES control flour (lower left); Canadian Western Extra Strong (CWES) with 20% HA FRF (lower middle); CWES with 20% WX FRF (lower right).

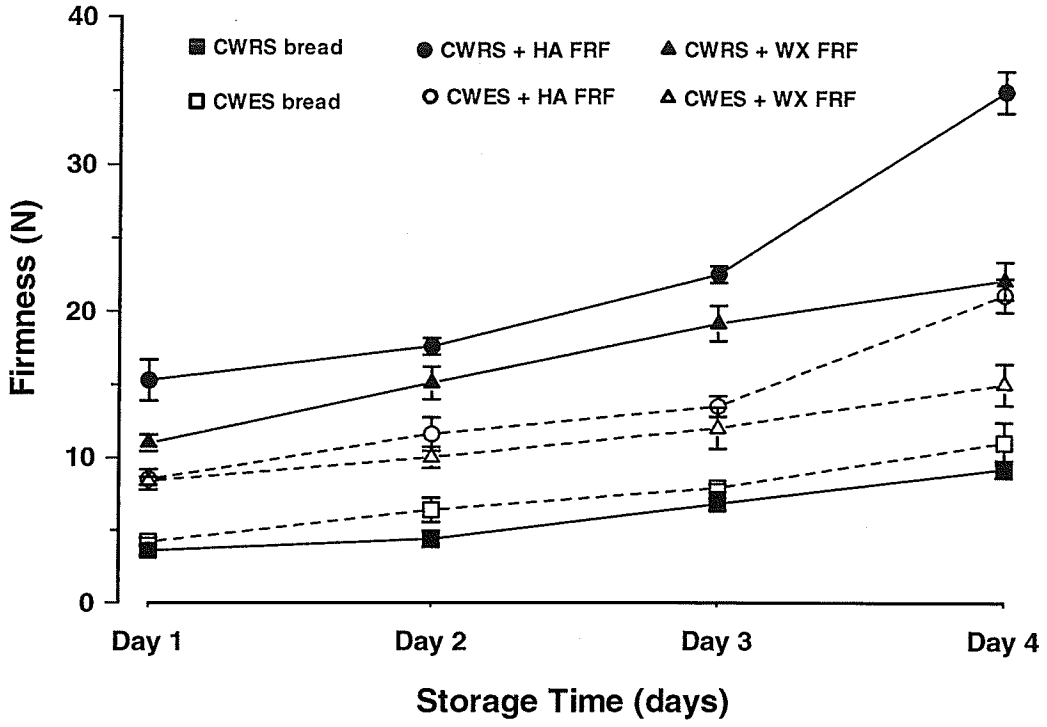

Figure 12. Crumb firmness of remix-to-peak bread prepared from Canadian Western Red Spring (CWRS) and Canadian Western Extra Strong (CWES) flours (controls) and supplemented with 20% high amylose (HA) FRF and 20% waxy (WX) FRF.appearance and crumb structure were significantly poorer, making the bread less acceptable (results not shown).

to-peak baking process is a long fermentation procedure, featuring a 160 min initial fermentation time and a 55 min final proof. Therefore, we attempted to prepare remix-to-peak bread using a 30 min initial fermentation time. However, volume of FRF-enriched bread declined about 10% compared to the full fermentation time process, and bread

Enrichment of Canadian short process bread with 20 % FRF

FRF-enriched bread was also prepared using the Canadian short process, a mechanical development process with a 15 min rest period prior to make-up and a final proof of 70 min. Initial attempts to incorporate dry FRF into Canadian short process bread

were unsuccessful. Dough was wet and pasty and would not develop. The initial remix-to-peak dough was similarly poorly developed, but developed during remixing. It is likely that during the long initial fermentation of the remix-to-peak process moisture is redistributed from the hygroscopic non-starch polysaccharides to gluten, aiding dough development during remixing. This would also account for the decline in bread quality when the initial fermentation time of the remix-to-peak process was reduced.

Subsequently, FRF-enriched bread was prepared by the Canadian short process using FRF pre-soaked in water before addition to the short process dough. However, results were still poor (Table 12, Figure 13). FRF-enriched Canadian short process bread from CWRS was superior to that from CWES, but still would be at best marginally marketable. Loaf volume was poor, crumb structure was coarse and bread was very firm (results not shown). The better performance of CWRS when enriched with FRF was consistent with shorter dough development times observed on the farinograph compared to CWES (Table 10). The CWRS FRF-enriched Canadian short process dough was apparently partially developing, whereas the CWES FRF-enriched dough remained undeveloped despite the relatively higher rate of work input of the GRL 200 mixer.

Table 12. Properties of Canadian short process bread prepared from Canadian Western Red Spring (CWRS) and Canadian Western Extra Strong (CWES) flour with and without enrichment with 20% fiber-rich fraction (FRF) from high amylose (HA) and waxy (WX) hull-less barley. FRF presoaked prior to addition to flour. [a]

Treatment	Bread volume (cm^3)	Water absorption (%)	Bread weight (g)	Bread appearance (units)	Crumb structure (units)	Crumb color (units)
No 1 CWRS						
No FRF	990 [a]	71 [d]	148	8.2 [a]	6.0 [b]	8.2 [a]
20% HA FRF	580 [c]	88 [a]	168	3.5 [c]	3.5 [c]	4.5 [c]
20% WX FRF	560 [c]	87 [b]	170	3.0 [d]	3.0 [e]	4.3 [d]
No 1 CWES						
No FRF	820 [b]	70 [e]	147	7.5 [b]	6.5 [a]	8.0 [b]
20% HA FRF	420 [d]	87 [b]	170	3.0 [d]	3.0 [e]	4.0 [e]
20% WX FRF	390 [d]	86 [c]	162	3.0 [d]	3.2 [d]	4.0 [e]

[a] Values followed by the same letter in the same column are not significantly different($P<0.01$).

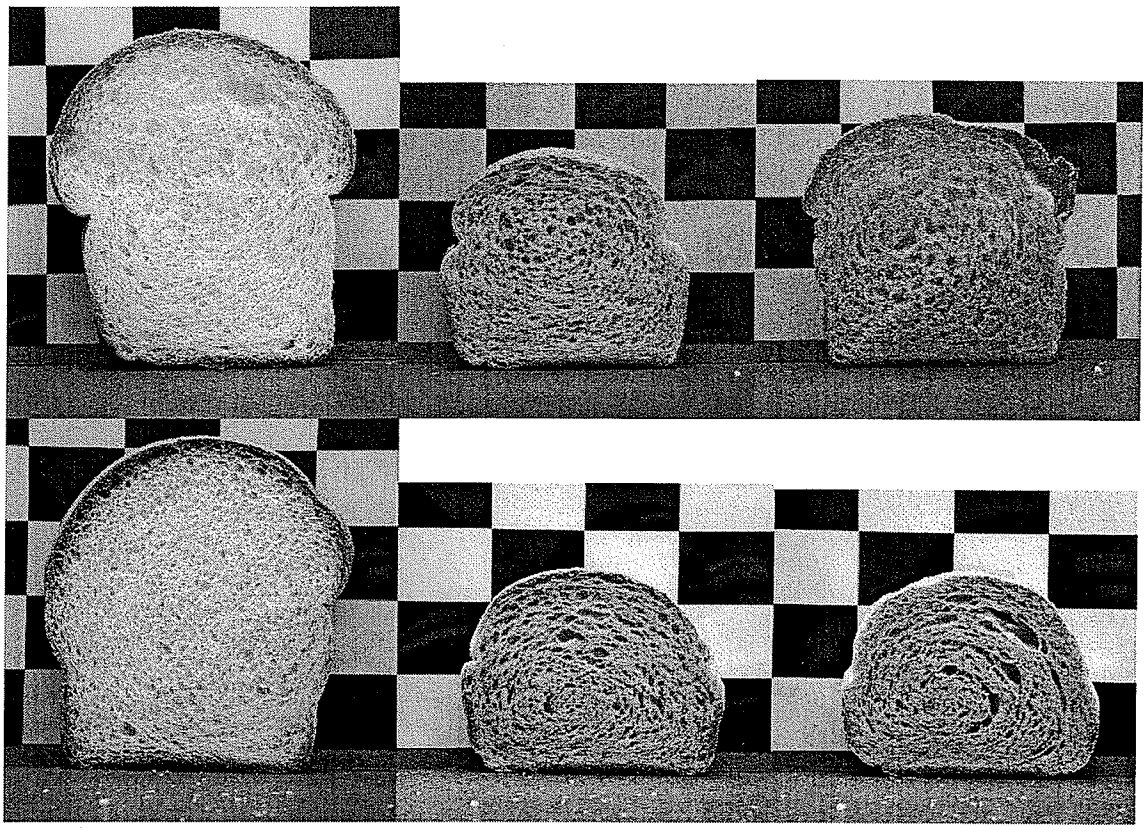

Figure 13. Canadian short process bread prepared from: Canadian Western Red Spring (CWRS) control flour (upper left); CWRS with 20% high amylose (HA) FRF (upper middle); CWRS with 20% waxy (WX) FRF (upper right); Canadian Western Extra Strong (CWES) control flour (lower left); CWES with 20% HA FRF (lower middle); CWES with 20% WX FRF (lower right).

Enrichment of sponge-and-dough bread with FRF and incorporation of xylanase

An alternative baking procedure that limits fermentation time and has potential to produce marketable FRF-enriched bread is the sponge-and-dough process. The initial long sponge fermentation would develop gluten structure prior to addition of the FRF at the dough stage. Thus FRF is present in the dough only for the 15 min rest phases after mixing and prior to make-up and the 70 min final proof, comparable to total Canadian short

process fermentation time. FRF was pre-soaked prior to addition at the dough stage in an effort to maintain gluten hydration during mixing.

The quality of the 20 % FRF-enriched sponge-and-dough bread was equal to or was better than the remix-to-peak bread (Tables 11 and 13). The CWRS FRF-enriched sponge-and-dough breads exhibited less loaf volume depression compared to the CWRS control flour than for remix-to-peak bread. The FRF-enriched CWES sponge-and-dough bread was slightly superior in volume to the CWRS bread, but the advantage was much less than observed for the remix-to-peak process.

FRF enrichment of 20% corresponds to about 4.0 g of arabinoxylans and β-glucans combined per 100 g flour. To evaluate the impact of lower FRF addition, additional experiments were conducted with CWRS flour enriched with 12% FRF, equivalent to 2.5 g of arabinoxylans and β-glucans combined per 100g flour. As expected, the resulting bread was considerably improved (Tables 13 and 14). Loaf volume was about 10% higher than for 20% FRF enrichment, with corresponding improvements in bread appearance, although bread crumb properties were not improved.

Table 13. Properties of sponge-and-dough bread prepared from Canadian Western Red Spring (CWRS) and Canadian Western Extra Strong (CWES) flour with and without enrichment with 20% fiber-rich fraction (FRF) from high amylose (HA) and waxy (WX) hull-less barley. FRF presoaked prior to addition to the dough stage [a,b].

Treatment	Bread volume (cm^3)	Water absorption (%)[c]	Bread weight (g)	Bread appearance (units)	Crumb structure (units)	Crumb color (units)
No 1 CWRS						
No FRF	1090[a]	70[c]	146	8.0[b]	6.2[b]	8.0[b]
20% HA FRF	750[bc]	81[a]	163	5.2[c]	4.4[d]	6.0[d]
20% WX FRF	735[c]	79[b]	165	5.0[d]	4.4[d]	6.0[d]
No 1 CWES						
No FRF	1100[a]	69[d]	147	8.2[a]	6.7[a]	8.2[a]
20% HA FRF	785[b]	81[a]	164	5.2[c]	4.8[c]	6.2[c]
20% WX FRF	760[bc]	79[b]	165	5.2[c]	4.4[d]	6.2[c]

[a] Bread volume and bread weight expressed on a 100 g flour weight basis.
[b] Values followed by the same letter in the same column are not significantly different ($P<0.01$).
[c] Water absorption is expressed on a 14% m.b.

Table 14. Properties of sponge-and-dough bread prepared from Canadian Western Red Spring (CWRS) flour with and without enrichment with 12% fiber-rich fraction (FRF) from high amylose (HA) and waxy (WX) hull-less barley, with and without xylanase enzyme. FRF presoaked prior to addition to at the dough stage [a,b].

Treatment	Bread volume (cm^3)	Water absorption (%)[c]	Bread weight (g)	Bread appearance (units)	Crumb structure (units)	Crumb color (units)
No 1 CWRS						
No FRF	1095[b]	70[d]	145	8.4[a]	6.2[a]	8.2[b]
12% HA FRF	855[d]	81[a]	159	6.3[e]	4.4[f]	6.2[d]
12% WX FRF	870[d]	80[b]	159	6.8[c]	4.7[e]	6.2[d]
No 1 CWRS and xylanase						
No FRF	1175[a]	69[e]	147	8.1[b]	6.0[b]	8.3[a]
12% HA FRF	965[c]	79[c]	154	6.0[f]	5.0[d]	5.9[e]
12% WX FRF	970[c]	80[b]	155	6.4[d]	5.2[c]	6.4[c]

[a] Bread volume and bread weight expressed on a 100 g flour weight basis.
[b] Values followed by the same letter in the same column are not significantly different ($P<0.01$).
[c] Water absorption is expressed on a 14% m.b.

Trogh et al (2004, 2005) have reported that bread enriched with barley fractions can be improved by xylanse addition. Accordingly, CWRS flour with and without 12% FRF enrichment was baked with addition of xylanase at the dough stage. The control bread exhibited a loaf volume increase of close to 10% (Tables 13 and 14, Figure 14). The FRF-enriched bread exhibited over a 10% increase in volume, and significant improvement in crumb structure. As noted by Trogh et al (2004, 2005), addition of xylanase has the additional health advantage of increasing soluble fiber content in the bread. Firmness of FRF-enriched sponge-and-dough bread was substantially improved compared to that of remix-to-peak bread (Figures 12 and 15). The FRF-enriched sponge-and-dough breads exhibited only slightly firmer texture than the control bread. Bread enriched with waxy starch FRF was substantially less firm during the first two days of storage than high amylose FRF-enriched bread and the differences might be attributed to different properties of starches associated with the amylose content. Although the re-crystallization of amylopectin is the main cause of firming of bread crumb, especially during the long term storage, amylose initiates starch retrogradation. The initial firmness of bread is associated with the fast gelation properties of amylose during cooling of bread (Zobel, 1992). Hayakawa et al (2004) observed a softening of bread texture with the incorporation of 5-30% of waxy wheat flour and noted that waxy wheat flour may potentially delay retrogradation. Waxy barley starches have also been reported to have very slow retrogradation kinetics (Czuchajowska et al, 1998; You and Izydorczyk, 2002). Additional improvements in bread firmness were observed with the incorporation of xylanase (Figure 15).

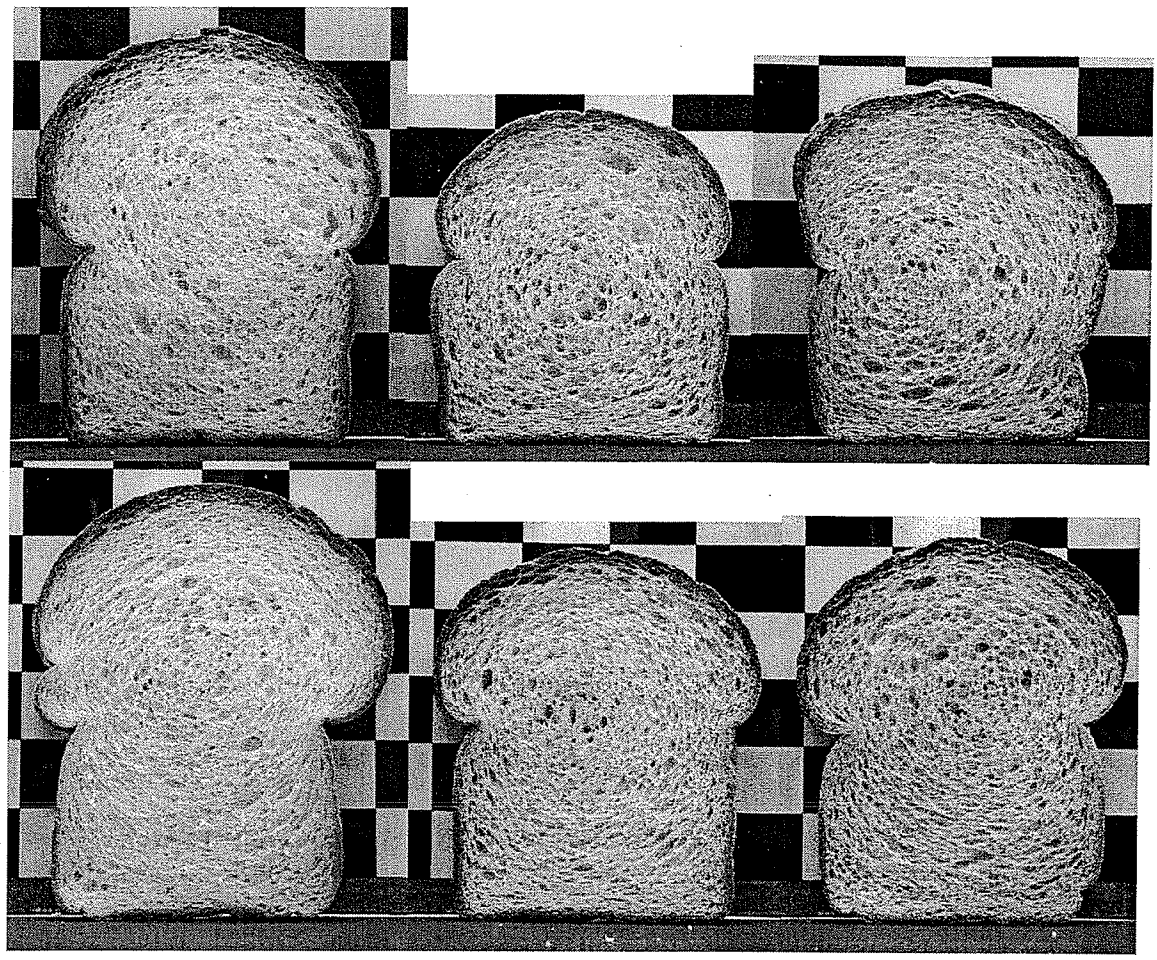

Figure 14. Sponge-and-dough bread prepared from: Canadian Western Red Spring (CWRS) control flour (upper left); CWRS with 11.5% high amylose (HA) fiber-rich fraction (FRF) (upper middle); CWRS with 11.5% waxy (WX) FRF (upper right); Canadian Western Extra Strong (CWES) control flour with xylanase (lower left); CWES with 11.5% HA FRF and xylanase (lower middle); CWES with 11.5% WX FRF and xylanase (lower right).

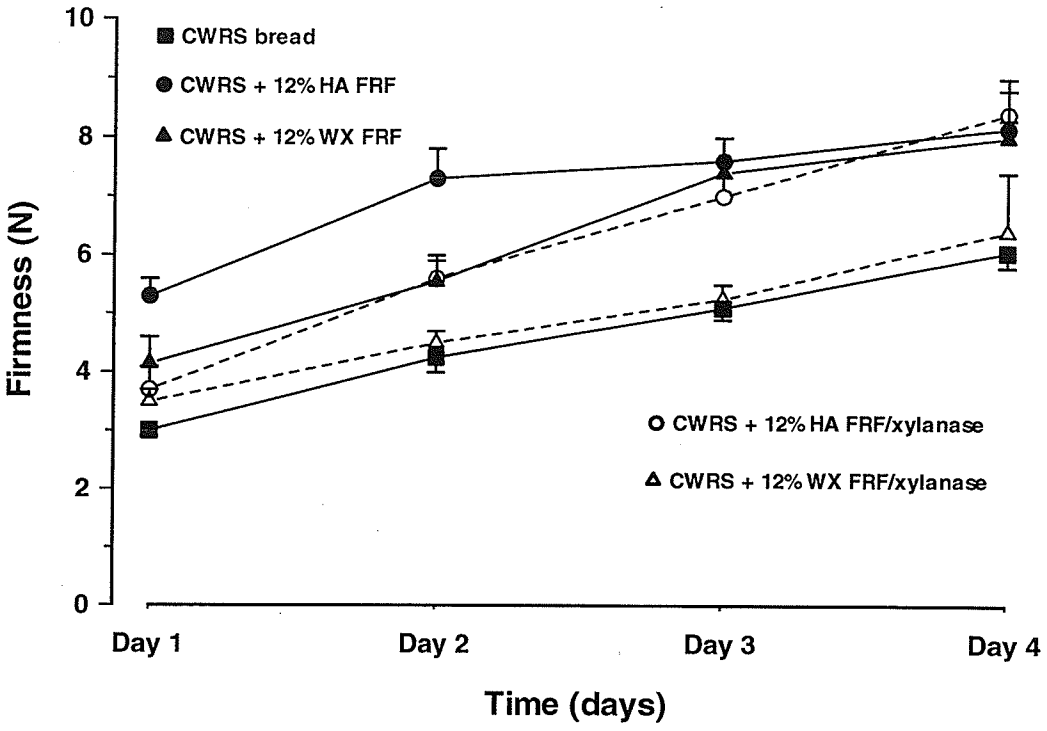

Figure 15. Crumb firmness of sponge-and-dough bread prepared from Canadian Western Red Spring (CWRS) flour (control), CWRS with 12% high amylose (HA) fiber-rich fraction (FRF), and CWRS with 12% waxy (WX) FRF with and without xylanase addition.

CONCLUSIONS

Addition of less than 1% of highly purified barley soluble β-glucans significantly depressed loaf volume and bread appearance and adversely affected crumb structure, in agreement with Symons and Brennan (2004), demonstrating the challenge in producing marketable bread from fiber-rich barley fractions. Addition of 20% FRF from barley roller milling (equivalent to enrichment with 4.0 g of arabinoxylans and β-glucans per 100 g of flour) disrupted dough properties, depressed loaf volume and adversely affected overall bread quality. The impacts of FRF from high-amylose starch barley and waxy starch barley

on baking performance, bread appearance, crumb structure, and color were essentially the same.

The method of bread production strongly influenced bread quality. The Canadian short process, a short fermentation mechanical development procedure, was not suitable for making barley-enriched bread because dough was not properly developed. A probable cause of the poor bread is that the high affinity for water of the barley non-starch polysaccharides in FRF gives gluten inadequate access to water for development. However, pre-soaking of FRF prior to incorporation gave only a modest improvement to bread quality, indicating that disruption of the gluten network by FRF β–glucans and/or other components is also a factor.

FRF-enriched bread produced by the remix-to-peak process, a long fermentation straight dough procedure, was better than bread produced by the Canadian short process. Loaf volume declined by about 25% with the addition of 20% FRF, but the bread was sufficiently good to meet the expectation of health conscious consumers. The improvement in bread quality compared to the short process may be due to the redistribution of water from non-starch polysaccharides to gluten during the long fermentation prior to remixing and final proof. This was supported by a noticeable deterioration in bread quality when the initial remix-to-peak fermentation time was reduced from 160 min to 30 min.

The sponge-and-dough process probably produced the best FRF-enriched bread because of the positive effect of sponge fermentation on gluten development and hydration. The FRF was incorporated at the dough stage, limiting exposure to fermentation to about 100 min compared to 215 min for the remix-to-peak process. The shorter exposure of FRF

to fermentation is a potential advantage because it has been reported that extended fermentation can potentially reduce the cholesterol lowering capacity of β-glucans due to degradation of β-glucans by endogenous β-glucanases (Andersson et al, 2004).

Addition of xylanase to the sponge-and-dough formula improved the loaf volume, appearance, crumb structure and firmness of FRF-enriched bread. This corroborates the findings of Trogh et al (2004), who noted that xylanase would have the additional health benefit of increasing soluble fiber content in the bread.

GENERAL SUMMARY AND CONCLUSIONS

Fractionation of hull-less barley grain via a combination of pearling and roller milling resulted in fractions containing various proportions of proteins, starch, ash, and non-starch polysaccharides. Arabinoxylans, β–glucans and arabinogalactans constituted the bulk of non-starch polysaccharides present in all milling fractions of barley as confirmed by H-NMR analysis. The FRFs, contained the highest amount of β–glucans, followed by straight grade flour and PBP. The PBP contained especially large amounts of arabinoxylans, protein, and ash, but only small amounts of starch and β–glucans. FRF were also enriched in arabinoxylans. The small amounts of arabinogalactans detected in barley appeared to be concentrated in the outer portion of the barley kernel. Differences in the yield of each NSP sub-fraction indicate differences in the content and relative solubility of non-starch polysaccharides in the milling fractions, which was also dependent on barley genotype. The WE sub-fractions from flour and FRFs in all barley genotypes contained more β–glucans than arabinoxylans; the waxy barley WE sub-fractions contained the highest amounts of β–glucans. Alkali-extractable sub-fractions from all three milling streams were enriched in arabinoxylans. The solubility of β–glucans was inversely proportional to their levels in the fractions, with β–glucans in PBP being the most soluble and those in FRF the least. The solubility of arabinoxylans was higher in FRF than in PBP. All the WE sub-fractions contained some ferulic acid. Interestingly, however, arabinoxylans present in the WE sub-fractions from PBP of waxy barley contained the highest amount of ferulic acid, which may partially explain the poor water-extractability of arabinoxylans from PBP of waxy barley.

Both polymers, β–glucans and arabinoxylans, exhibited structural heterogeneity derived from differences in their localization within the kernel as well as from barley genotype. Some general trends were observed. For example, the WE arabinoxylans from the endosperm cell walls (FRF and flour) had a substantially lower degree of branching than those from the outer layers (PBP). The WE arabinoxylans from FRF of high amylose and normal barley contained more unsubstituted Xyl*p* residues and less doubly substituted Xyl*p* and singly substituted Xyl*p* at O-2 than their counterparts from PBP which were generally highly substituted. The WE arabinoxylans from FRF of waxy barley had a relatively high content of doubly, but very little singly substituted Xyl*p* residues.

The lichenase hydrolysis products from all materials were composed mainly of tri- and tetrasaccharides (90.3- 93.5%). Longer oligomers (DP≥5) with more than two consecutive β–(1 →4) linkages were also detected, although in smaller amounts. These oligomers are indicative of the cellulose-like regions in the β–glucan chains and may greatly affect the properties of these polymers. For all milling fractions examined, and all barley genotypes, the ratio of DP3:DP4 increased with the decreasing solubility of these polymers (the WE sub-fractions being the most soluble and the NaOH ones the least). The increases in the ratio of tri- to tetrasaccharides between water- and alkali-extractable sub-fractions, along with the increase in cellulosic regions of DP≥9, lead to decreased solubility of these polymers.

Substantial differences in the M_w of non-starch polysaccharides in different milling fractions were also observed. In agreement with the multi-component character of some of these sub-fractions, the elution profiles indicated the presence of one or more polymeric

species. For the high amylose and waxy barleys, the WE sub-fractions from flour and FRFs (mostly β–glucans) had much higher M_w than the $Ba(OH)_2$-extractable sub-fractions (mostly arabinoxylans). In addition, the WE sub-fractions from flour had higher M_w than the sub-fractions from FRF. The water-extractable β–glucans (WE sub-fractions) from flour and FRFs of waxy barley had the highest M_w among the three barley genotypes. The water-extractable β–glucans (WE sub-fractions) from flour and FRFs of normal barley had the lowest M_w among the three barley genotypes. On the other hand, the $Ba(OH)_2$-extractable arabinoxylans from flour and FRFs of normal barley had the highest M_w among the three barleys.

The results from the baking study indicate that blending wheat bread flour with 20% FRF from roller milled high amylose and zero waxy barley (equivalent to enrichment with 4.0 g of arabinoxylans and β–glucans per 100 g of flour) significantly disrupted dough properties, depressed loaf volume and adversely affected overall bread quality. The impacts of FRF from high-amylose starch barley and waxy starch barley on baking performance and bread quality were essentially the same. The hygroscopic nature of β–glucans and arabinoxylans caused about 25% increase in dough water absorption with the addition of 20% FRF to either CWRS flour or CWES flour. When FRF is incorporated into CWES dough, the gluten network weakens sufficiently to allow development; CWES does not normally fully develop in the Farinograph because of the extraordinarily strong gluten in the CWES.

The findings of this study also indicated that the method of bread production strongly influenced the bread quality. 20% FRF-enriched bread produced by the remix-to-

peak process, exhibited a 25% decline in loaf volume. The additional strength of CWES produced a FRF-enriched bread that was clearly superior in loaf volume and bread appearance to CWRS FRF-enriched bread. The 20% increase in water absorption observed with FRF enrichment resulted in 20 g increase in baked bread weight, indicating that much of the additional water was tightly bound to non-starch polysaccharides and then redistributed to gluten during the long fermentation prior to remixing and final proof, and not lost during baking. This was supported by a noticeable deterioration in bread quality when the initial remix-to-peak fermentation time was reduced from 160 min to 30 min.

The CSP was not suitable for making barley-enriched bread. The dough was wet and pasty and would not properly develop, resulting in poor loaf volume, coarse crumb structure and very firm bread. The NSP in the FRF have a high affinity for water and out-compete gluten for access to water during mixing (Courtin and Delcour, 2002, Wang et al 2004). The result is inadequate water for dough development and a probable cause for the poor bread produced by the CSP. CWRS performed better than CWES as a base wheat during the CSP. This is because the CWES FRF-enriched dough did not have enough time to properly develop based on the observation with farinograph mixing tolerances of the CWES FRF-enriched dough.

The sponge-and-dough process produced a loaf that was superior for all FRF blends compared to the other baking methods tested. The FRF-enriched CWES sponge-and-dough bread was slightly superior in volume to the CWRS bread. Limiting FRF exposure to fermentation through pre-steeping and subsequent incorporation of hydrated FRF at the dough stage is an essential step within the baking protocol, providing increases in loaf volume for all blends by allowing a strong gluten network to form during sponge

fermentation without having to carry "dead weight" of FRF incorporation. When CWRS flour was enriched with 12% FRF, (equivalent to 2.5 g of arabinoxylans and β-glucans combined per 100g flour) the resulting loaf volume was about 10% higher than for 20% FRF enrichment, with corresponding improvements in bread appearance, although bread crumb properties were not improved. The addition of xylanase at the dough stage resulted in a further 10% increase in volume, and significant improvement in crumb structure.

The current findings indicate that by combining pearling and roller milling, it is possible to obtain fractions enriched in specific grain components with desirable properties and functionalities. The sponge-and-dough process was identified as the most suitable for incorporation of barley FRF, however, further optimization with the addition of vital gluten and dough conditioners should be examined. Fiber-rich fractions, obtained via a chemical free, physical fractionation of grain, are excellent functional food ingredients. In addition to their high dietary fiber content they also contain other phytochemicals (phenolics, tocols, vitamins and minerals) naturally present in the grain tissue.

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