Characterization of Non-Starch Polysaccharides from Hull-less Barley

BY

JOANNE M. STORSLEY

A Thesis
Submitted to the Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Food Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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DEDICATION

To my husband, Leroy, for his patience and continual encouragement during this challenging time in my life. I couldn't have done it without you.

ACKNOWLEDGMENTS

I would like to express my gratefulness to Dr. M.S. Izydorczyk for allowing me the privilege of working under her supervision. Her dedication to the pursuit of excellence, marked by genuine concern for her students, was a real inspiration to me. Also, thanks to Dr. A.W. MacGregor and Dr. M. Eskin for use of their resources, and participation on my thesis examining committee.

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LIST OF ABBREVIATIONS

Ara*f*	L-arabinofuranosyl
BSA	bovine serum albumin
^{13}C-NMR	carbon-13 nuclear magnetic resonance
CV	coefficient of variation
D/M	ratio of di-substituted to mono-substituted xylose residues
DMSO	dimethyl sulfoxide
DP	degree of polymerization
G′	storage modulus
G″	loss modulus
Glc*p*	glucopyranosyl
HDL	high density lipoprotein
HPAEC-PAD	high performance anion exchange chromatography with pulsed amperometric detection
HPLC	high performance liquid chromatography
HPSEC	high performance size exclusion chromatography
ID	internal diameter
LDL	low density lipoprotein
MALLS	multi-angle laser light scattering
M_w	weight average molecular weight(s)
M_w/M_n	polydispersity index (ratio of weight average molecular weight to number average molecular weight)
NSP	non-starch polysaccharide(s)

R_g	root mean square radius of gyration
RI	refractive index
RT	room temperature
SCFA	short chain fatty acids
SD	standard deviation
TFA	trifluoroacetic acid
w/w	weight by weight
Xyl*p*	xylopyranosyl
U-Xyl*p*	un-substituted xylopyranosyl residues
2-Xyl*p*	mono-substituted xylopyranosyl residues at the 2- position
3-Xyl*p*	mono-substituted xylopyranosyl residues at the 3-position
2,3-Xyl*p*	di-substituted xylopyranosyl residues

ABSTRACT

In the selection of a hull-less barley variety for the purpose of incorporating its fiber component (β-glucans and arabinoxylans) into foods, it is necessary to assess the content, molecular characteristics, and physicochemical properties of these polymers within various cultivars. The following study examined the content and solubility of β-glucans in several genotypes of hull-less barley, and assessed various thermal, physical, and enzymic treatments as potential strategies for increasing the content and viscosity of soluble β-glucans. In addition, detailed analysis of the molecular and structural characteristics of β-glucans and arabinoxylans from selected hull-less barleys was carried out.

Twenty-nine registered and experimental genotypes of hull-less barley (normal, high amylose, waxy, and zero amylose waxy lines) were analyzed for moisture, protein, amylose, 100 kernel weight, starch, β-glucan (total and soluble), β-glucanase activity, and slurry viscosity. Significant differences in total β-glucan were observed among the groups, with average values of 7.49%, 6.86%, 6.30%, and 4.38% for high amylose, waxy, zero amylose waxy, and normal barley, respectively. The extractability of β-glucans in high amylose barley was relatively low (20.6% to 29.7%) compared to that of normal (29.8% to 44.3%), zero amylose waxy (34.0% to 52.5%), and waxy (36.7% to 52.7%) barley genotypes. The viscosity of barley flour slurries was affected by the content of soluble β-glucans, β-glucanase activity, and molecular weight of β-glucans.

Hydrothermal treatments (autoclaving and steaming) of barley had no effect on extractability of β-glucans, but prevented enzymic hydrolysis, and thereby substantially improved their molecular weight. The addition of enzymes (protease and esterase) during

extraction and/or physical treatments (sonication) increased the extractability of β-glucans from barley.

Beta-glucans and arabinoxylans isolated from eight hull-less barley varieties were characterized in detail. The polysaccharides were extracted sequentially with water at 45 °C, water at 95 °C, $Ba(OH)_2$, water, and NaOH, yielding five fractions (WE45, WE95, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH). Monosaccharide analysis confirmed that β-glucans and arabinoxylans were the major polysaccharide components; β-glucan accounted for 76.02 to 93.28% of polysaccharides in WE45 and WE95, while arabinoxylans constituted 96.20 to 99.71% in the $Ba(OH)_2$ fraction; fractions $Ba(OH)_2/H_2O$, and NaOH contained mixtures of the two polymers. Significant inter-varietal differences ($p \leq 0.05$) in the proportions of β-glucans and arabinoxylans were observed among each of the five fractions.

Molecular and structural assessment of water-extractable β-glucans indicated differences between WE45 and WE95 fractions and among varieties. Higher weight average molecular weights (M_w), higher β-(1→4) to β-(1→3) linkage ratios measured by ^{13}C-NMR, and greater amounts of cellulosic regions analyzed by lichenase, of β-glucans in the WE95 fraction were generally observed compared to WE45. However, inter-varietal differences in these parameters were also found. Viscoelastic behaviour of WE45 and WE95 fractions show that β-glucans derived from certain barley cultivars may have better physiological and functional properties than those from others.

As with β-glucans, differences in the structure of arabinoxylans from the various fractions and varieties were observed. Arabinoxylans from WE45 and WE95 had significantly lower xylose to arabinose (Xyl*p*/Ara*f*) ratios (1.47 and 1.52) than those from

$Ba(OH)_2$ (1.70), indicating a greater degree of branching in more readily soluble fractions. The small varietal differences in molecular weight (M_w), root mean square radius (R_g), and polydispersity (M_w/M_n) among $Ba(OH)_2$ fractions were found to be correlated with the substitution pattern of the xylan backbone. When the $Ba(OH)_2$ fractions were sub-fractionated with ammonium sulphate, separation of arabinoxylans based on Xyl*p*/Ara*f*, M_w, M_w/M_n, and R_g was achieved; however, polydispersity indices remained high, pointing to the extreme heterogeneity of these polysaccharides.

FOREWORD

The work presented in Part One of this thesis was published in the Journal of Agricultural and Food Chemistry under the following citation:

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1. INTRODUCTION

Barley, like other cereal grains, is made up of many distinct constituents, each possessing varying degrees of complexity in structure and function. Some of the minor barley components, particularly the non-starch polysaccharides, have begun to attract increasing scientific attention over the last two decades. The current interest in barley and oat non-starch polysaccharides (NSP) stems from evidence for reduction of serum cholesterol and glucose levels, as well as the associated risks for chronic diseases, when the grains are consumed in the human diet (Yokohama et al., 1997; Hecker et al., 1998; Kalra and Jood, 2000). These physiological effects are thought to be specifically due to the presence of beta(β)-glucans, a group of NSP and the primary components of soluble dietary fiber in both barley and oats (Newman and Newman, 1991). Beta(β)-glucans have the capacity to form highly viscous solutions; it has been hypothesized that, upon ingestion, they increase small intestinal viscosity, thereby reducing cholesterol and glucose absorption (Yokohama et al., 1997; Kalra and Jood, 2000). Arabinoxylans constitute another prominent group of barley non-starch polysaccharides; however, relatively little is known about these polymers within the barley grain. Wheat and rye arabinoxylans have been studied more extensively (Gruppen et al., 1993; Rouau and Moreau, 1993; Schooneveld-Bergmans et al., 1999ab). It has indeed been demonstrated that arabinoxylans are capable of forming viscous solutions, and that the viscosity of arabinoxylans slows the rate of digestion in monogastrics (Antoniou et al., 1981). Thus it is possible that arabinoxylans may have a similar physiological effect to β-glucans upon human consumption; however, this has not yet been evidenced. The presence of β-glucans and arabinoxylans may also, in

addition to nutritional enhancement, help to improve quality parameters such as processing behaviour and stability or shelf-life within cereal-based food systems (Biliaderis et al., 1995; Gan et al., 1995; Temelli, 1997; Lee et al., 1998; Klamczynski and Czuchajowska, 1999). Although certain unique characteristics of barley starches and proteins may predispose the grain for specific uses, it is likely that the amount and properties of barley NSP will play a critical role in determining the extent of barley utilization by the food industry, because of the nutritional and functional attributes ascribed to these polymers, coupled with the growing demand for nutraceuticals/ functional foods.

The content of β-glucans and arabinoxylans in barley, as well as their solubility and molecular characteristics, can be influenced by genetic and environmental factors. In addition, solubility and molecular characteristics of β-glucans and arabinoxylans are highly dependent on the methods employed to extract or isolate them (Lee et al., 1997; Xue et al., 1997; Andersson et al., 1999; Knuckles and Chiu, 1999). The influence and interaction of these factors have made assessment of different barley varieties for their potential as a food ingredient difficult. Some of the newly developed hull-less barley genotypes with unique starch characteristics have been reported to contain much higher β-glucan levels than traditional hulled malting and feed barley varieties; however, it is not known whether the molecular characteristics and/or physicochemical behaviour of the polymers within many of the new genotypes differ. Hence, there is clearly a need to systematically evaluate the content, molecular characteristics, and physicochemical properties of β-glucans and arabinoxylans among barley cultivars to predict their suitability, and ultimately, to ensure maximum functionality, in human food applications.

The objectives of this study were to examine the content and solubility of β-glucans in several genotypes of hull-less barley, to assess various thermal, physical, and enzymic treatments as potential strategies for increasing the content and viscosity of soluble β-glucans, and to obtain detailed information on the molecular weight and structural characteristics of β-glucans and arabinoxylans isolated from hull-less barley with variable amylose content.

2. LITERATURE REVIEW

2.1. Barley

2.1.1. Classification

The three fundamental criteria by which barley may be classified are: 1) two-rowed or 6-rowed, 2) the presence or absence of the hull after harvesting, and 3) the inherent starch characteristics. The hull or hull-less characteristic is established during development and maturation of the grain. In hull-less barley, the unattached, loose husk is visibly separated from the kernel during threshing, unlike hulled barley, on which the husk remains attached (Bhatty, 1986). Either form may be two-rowed, six-rowed, aleurone colour of blue, yellow or purple, or may even have dark grain color due to pigments in the pericarp (Bhatty, 1986.). Generally, hulled barley is chosen for malting, while hull-less barley has been used in animal feeds and also has potential for incorporation into human foods. The hull in malting barley protects the germinating embryo from mechanical injury, contributes to more uniform germination, and also contributes to the flavor of malt and beer. However, the hull-less barleys tend to have higher nutritional value, including higher β-glucan contents; this is partly due to the fact that the hull, containing essentially no β-glucans and consisting mainly of cellulose, hemicellulose, and lignin, dilutes the nutrient content in hulled barley, since it constitutes 10 to 13% of the dry weight of the barley grain (Newman et al., 1989; Bhatty et al., 1975).

Barley also may be classified according to its starch characteristics. The starch type can vary from normal (~75% amylopectin, 25% amylose), to waxy (up to 100% amylopectin) to high amylose (~60% amylopectin, 40% amylose). The ratios of amylose

to amylopectin are important determinants for the technological and nutritional properties of barley starches (Gudmundsson and Eliasson, 1996).

2.1.2. Composition

Barley composition is influenced by both genetic factors and environmental conditions, and the interaction between the two (Andersson et al., 1999). In the case of β-glucan content, several studies have demonstrated that genetics have the greater influence (Stuart et al., 1988; Peterson, 1991; Miller et al., 1995). As shown in Table 1, which gives a proximate composition of barley (MacGregor and Fincher, 1993), carbohydrates constitute 79 to 87% of the grain on a dry weight basis. The most abundant carbohydrate and overall constituent in barley is starch, comprising approximately 60 to 64% of the grain. However, non-starch polysaccharides, particularly arabinoxylans and β-glucans, are also quantitatively important; β-glucan levels of the barleys represented in Table 1 range from 3.6 to 6.1%, while arabinoxylans may be present in equal or even greater amounts (4.4 to 7.8%). It is apparent, from the data in Table 1, that the relative proportions of barley components among different barleys may vary significantly. Furthermore, compositional analysis of other barleys indicates that even greater variation exists; average starch contents (U.S. barleys) as low as 57% (Åman and Newman, 1986) and β-glucan levels as high as 16% have also been reported (Newman et al., 1989; Wu et al., 1994).

Table 1. Barley composition[1] (adapted from MacGregor and Fincher, 1993).

Component	Content (%, dry weight)
Starch	60.0 - 64.0
Arabinoxylans	4.4 - 7.8
β-Glucans	3.6 - 6.1
Cellulose	1.4 - 5.0
Simple carbohydrates (glucose, fructose, sucrose, maltose)	0.4 - 2.9
Oligosaccharides (raffinose, fructosans)	0.2 - 1.8
Proteins	8.0 - 15.0
Lipids	2.0 - 3.0
Minerals	3.0 - 3.0

Note: Barley also contains small quantities of the B-complex vitamins, including thiamin (B1), riboflavin (B2), nicotinic acid, pyridoxine (B6), and pantothenic acid, biotin, folic acid, and vitamin E.

2.2. Distribution of β-Glucans and Arabinoxylans within the Barley Kernel

The localization of β-glucans and arabinoxylans in the barley grain and their interaction with other constituents is technologically important, since it affects isolation and purification procedures directed at obtaining fractions enriched in these polysaccharides, and influences commercial processing operations such as milling (Izydorczyk and Biliaderis, 2000). Figure 1 shows a longitudinal section through a barley kernel. It is composed of the seed enclosed by a multilayer fruit coat (pericarp), the seed coat (testa), and husk or hull (which is lost during harvesting in the case of hull-less barley). The bulk of the seed is made up of endosperm cells containing starch and proteins, surrounded by the

three-cell thick aleurone layer. Beta-glucans and arabinoxylans make up the majority of the cell walls of the endosperm and aleurone, where they act as a structural network. These polysaccharides are also found in the cell walls of the non-endospermic tissue, although their concentration declines due to the presence of other non-starch polysaccharides such as cellulose, xyloglucans, and glucuronoarabinoxylans (Izydorczyk and Biliaderis, 2000).

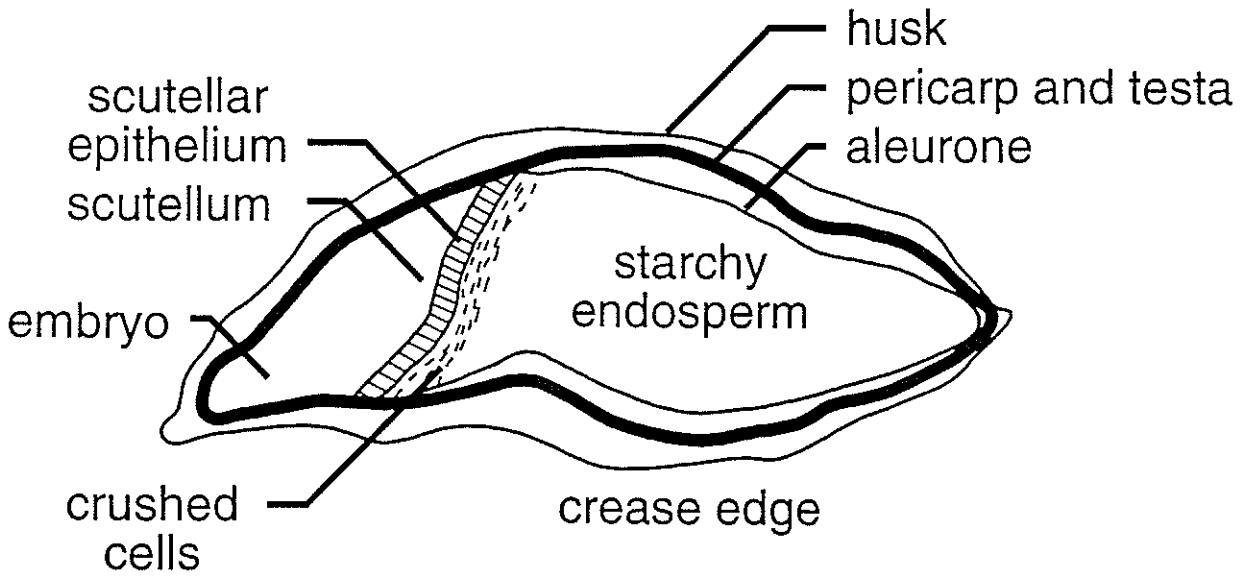


Figure 1. Longitudinal section of a barley kernel.

Generally, endosperm cell walls in barley consist of β-glucans (70%) and to a lesser extent arabinoxylans (20%), while aleurone cell walls are composed primarily of arabinoxylans

(67-71%), with smaller amounts of β-glucans (26%) (Stone and Clarke, 1993). It was thought at one time that the distribution of β-glucans and arabinoxylans within the endosperm cell walls was relatively uniform; Miller and Fulcher (1995) reported that β-glucan in barley was more uniformly distributed throughout the endosperm compared to oats, in which higher β-glucan concentrations were found in the sub-aleurone (the region just below the aleurone layer). However, more recent studies give clear evidence for both the heterogeneous distribution of β-glucans within barley endosperm tissue, as well as differences in the distribution patterns between barley varieties (Oscarsson et al., 1997; Zheng et al., 2000). According to a milling study by Zheng et al. (2000), the relative distribution of β-glucans within the endosperm varies significantly, and interestingly, is dependent on β-glucan content. In barleys with low β-glucan content, β-glucan levels were relatively higher in the sub-aleurone region than in the remainder of the endosperm, whereas barleys with average to high β-glucan content contained more β-glucan in the interior of the endosperm than in the sub-aleurone region. This data supports the findings of Bhatty et al. (1997) and Oscarsson et al. (1997), who demonstrated through Calcoflouor staining that 1) the cell walls in the endosperm of high β-glucan barley were thicker than in barleys with low β-glucan levels and that 2) the thicker cell walls extended much deeper into the endosperm of high β-glucan barley, with low β-glucan barley containing thicker cell walls only in the sub-aleurone region. The knowledge gained from these studies, i.e. that low β-glucan varieties contain less β-glucan in the inner endosperm and have thinner endosperm cell walls, may explain why low β-glucan hull-less barley is more easily roller-milled compared to high β-glucan varieties (Zheng et al., 2000).

2.3. β-Glucan Molecular Structure

Although β-glucan content and distribution within the kernel are important barley characteristics, variations in β-glucan structure will affect the physical properties of the polymers in solution, and as a consequence, will impact both their functional role within food systems and physiological properties (Wood et al., 1991). Like other plant polysaccharides, β-glucans exhibit a high degree of structural heterogeneity (Izydorczyk et al., 1998ab). They are linear polysaccharides composed entirely of D-glucopyranosyl residues (Glc*p*). However, rather than consisting exclusively of β-(1→4) linkages, as in cellulose, these polymers contain a mixture of β-(1→3) and β-(1→4) linkages (Figure 2). It is the presence of the β-(1→3)-linkages in the glucan chain that results in irregularities in molecular shape, rendering β-glucans more soluble and easier to hydrolyze than cellulose (Theander et al., 1993).

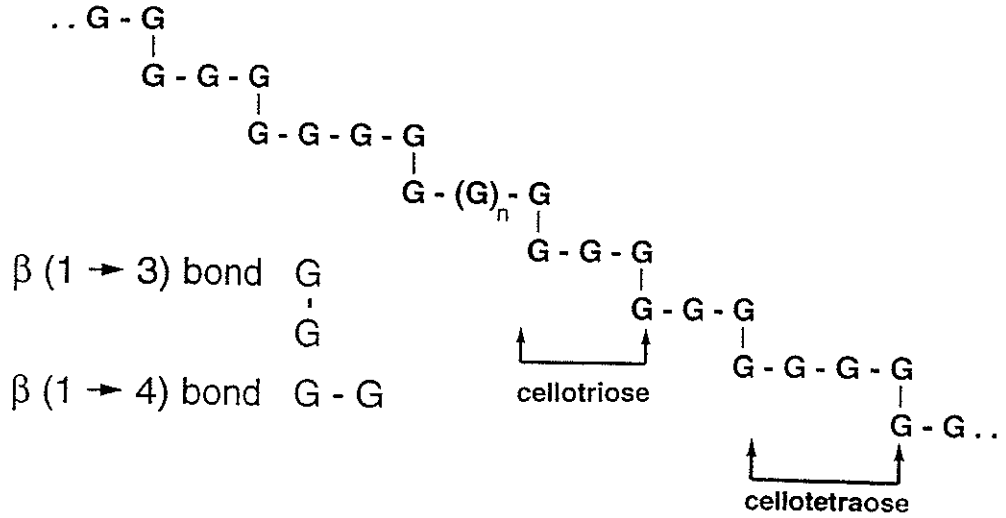


Figure 2. Generalized structure of a (1→3)(1→4)-β-D-glucan molecule.

2.3.1. Analysis of Released Oligosaccharides Following Lichenase Digestion

Structural studies have already established that (1→3)(1→4)-β-D-glucans are unbranched, that the β-(1→3) linkages occur singly, and that the majority of the β-(1→4) linkages occur in groups of two or, less frequently, three, (Woodward et al., 1988; Wood et al., 1994). Lichenase digestion, followed by quantitation of the released oligosaccharides by high performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD), is one method that has more recently been used to successfully elucidate the fine structure of β-glucans (Wood et al., 1994b; Izydorczyk et al, 1998a). Lichenase is a (1→3),(1→4)-β-D-glucan-4-glucanohydrolase, an enzyme which specifically cleaves the β-(1→4) linkage of the 3-O-substituted glucose units in the β-glucan molecule (Figure 3) (Wood et al., 1994b). The major products (85-90% yield) after digestion are 3-O-β-cellobiosyl-D-glucose and 3-O-β- cellotriosyl-D-glucose (Wood et al., 1991), indicating groups of two and three consecutive β-(1→4) linkages, respectively, in the original β-glucan molecule. Products having a higher degree of polymerization (between 5 and 11), indicative of longer strands of β-(1→4) linkages, or "cellulosic regions", have also been identified in barley and oat β-glucan, although the amounts were small (~3-5% yield) (Wood et al., 1994; Izydorczyk et al., 1998a); the presence of these cellulosic regions has been confirmed by methylation analysis and ^{13}C-NMR. However, the distribution of these regions and those of lower DP along the β-glucan chain, is unknown.

The molar ratio of tri- to tetrasaccharides (DP 3/DP 4) has been used as a numerical fingerprint for comparing structure among β-glucans. This ratio has been found to differ substantially among various cereals; Wood and colleagues (1991; 1994) found a

consistently higher ratio of tri- to tetrasaccharides in β-glucans from barley (2.8-3.3) compared to those from oats (2.1-2.4). Beta-glucans of wheat and rye, occurring in lesser amounts than in barley or oats, were reported to have even higher ratios (3.0-3.2 for rye, and 3.3-3.8 for wheat) (Wood, 1993; Wood et al., 1994b). More recent studies using HPAEC-PAD have demonstrated that barley β-glucans may have a much wider range in the molar ratio of tri- to tetrasaccharides than reported by Wood et al. (1991; 1994b); DP 3/DP 4 ratios in β-glucans isolated from malting barley by Izydorczyk et al. (1998ab) ranged from 1.76 to 2.43. Generally, higher ratios of tri- to tetrasaccharides have corresponded with decreased solubility/extractability (Izydorczyk et al., 1998a). It has been suggested that long blocks of consecutive cellotriosyl residues might be responsible for the insolubility of barley β-glucans (Woodward et al., 1988). Since it has been established that a helix of three consecutive cellotriosyl residues would form a crystalline structure in β-glucan molecules, it is plausible that a higher content of these cellotriosyl fragments may contribute some conformational regularity, and hence may render β-glucans less soluble (Tvaroska et al., 1983; Izydorczyk et al., 1998a). It remains to be proven, however, whether differences in DP 3/DP 4 ratios are large enough to significantly affect β-glucan conformation and/or solubility.

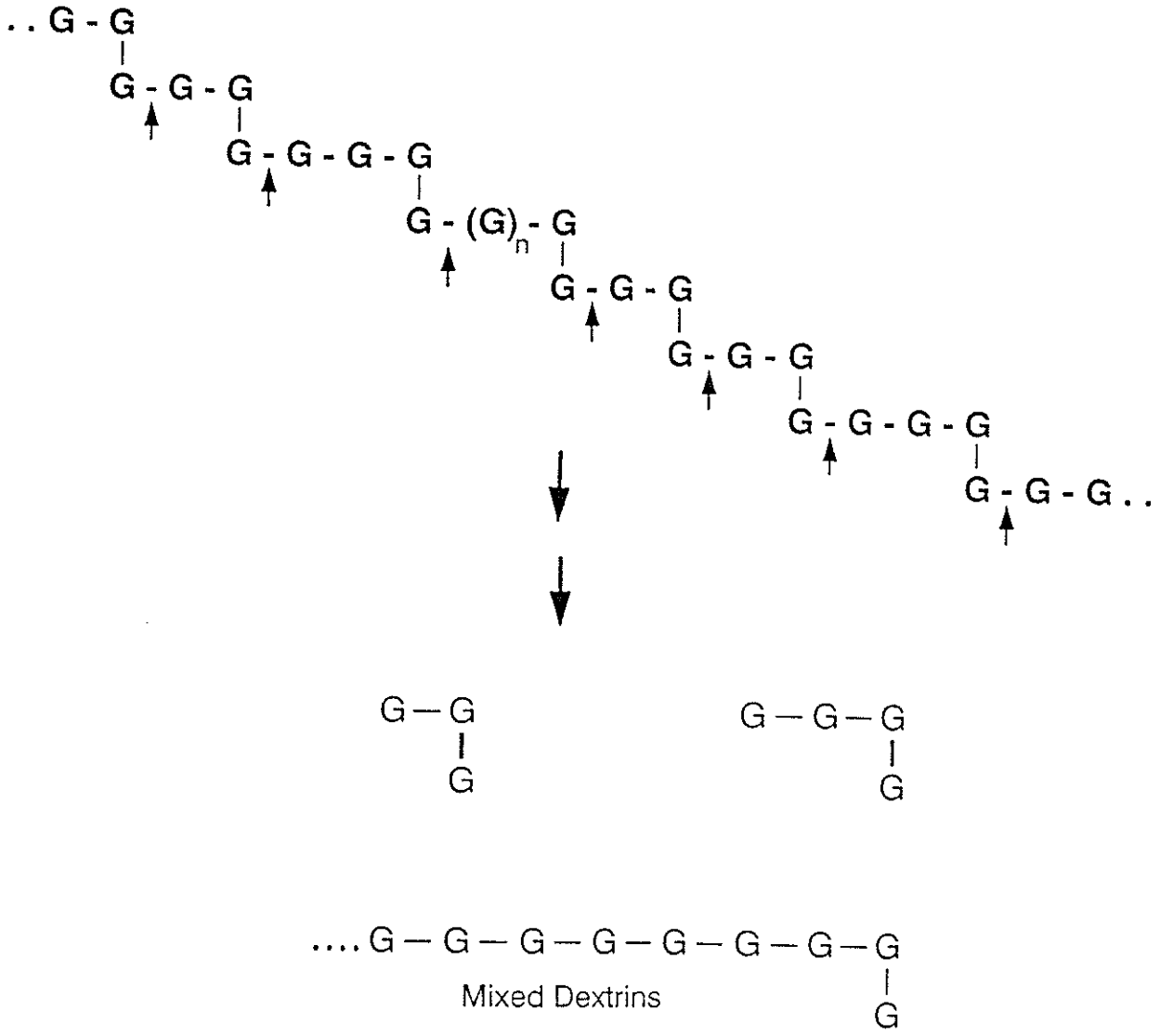


Figure 3. Action of lichenase on mixed-linkage β-glucan (Megazyme Int. Ireland Ltd.).

2.3.2. Carbon-13 Nuclear Magnetic Resonance

Carbon-13 Nuclear Magnetic Resonance (^{13}C-NMR) is another method often employed in the determination of β-glucan structure, and has been recognized as one of the most efficient methods for configurational and conformational investigations in carbohydrate chemistry (Breitmaier and Voelter, 1987). An example of a ^{13}C-NMR spectrum for β-glucan is presented in Figure 4. The resonance at ~103.9 ppm is due to C-1 of 4-O-substituted glucose (Glc*p*) residues engaged in β-(1→3) linkages, whereas the doublet at 102.5 ppm is from 3-O- and 4-O- substituted Glc*p* residues engaged in β-(1→4) linkages (Izydorczyk and MacGregor, 2000). Through integration of the two peaks at 103.9 and 102.5, the ratio of β-(1→4) to β-(1→3) linkages can be calculated. The cellulosic regions and β-(1→3) linkages are represented by the down field resonance at 79.4 ppm, indicating consecutive β-(1→4) linkages, and the resonances at 79.6 and 79.7 ppm, which are assigned to C-4 of Glc*p* residues flanked on either the reducing or non-reducing end by a β-(1→3) linkage (Izydorczyk and MacGregor, 2000). The relative proportions of cellulosic regions and β-(1→3) linkages may therefore be determined by using the ratios of the integrals at 79.4, 79.6 and 79.7 ppm from the NMR spectra of various β-glucan samples. This method in combination with lichenase digestion/HPAEC-PAD, allows for a reliable and in depth assessment of β-glucan structure.

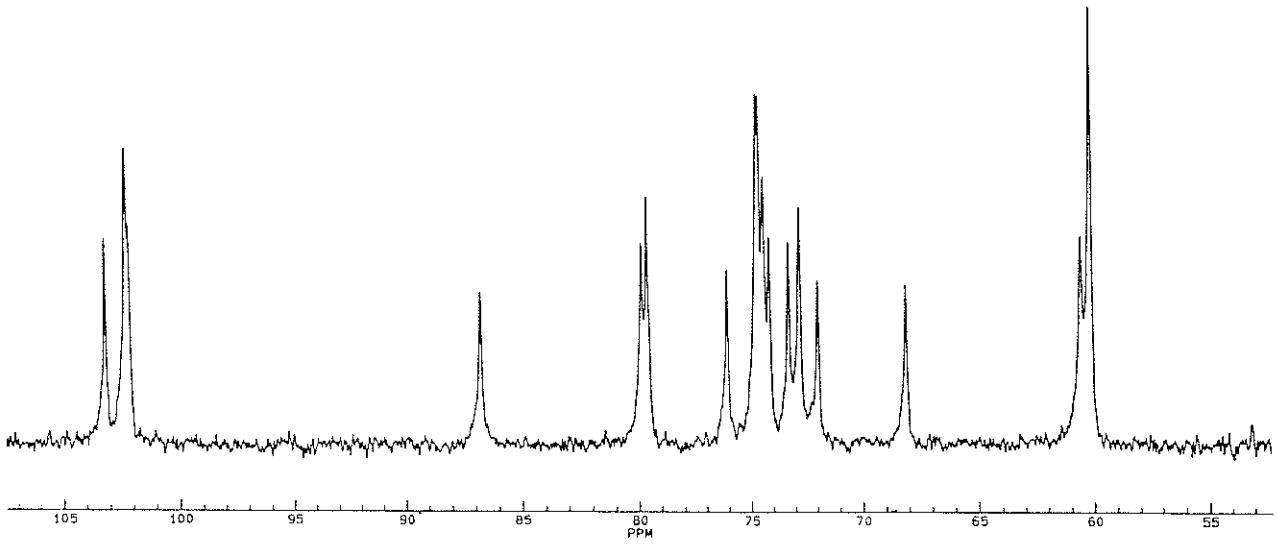


Figure 4. ^{13}C-NMR spectra of barley β-glucan (Izydorczyk and Mac Gregor, 2000).

2.4. Arabinoxylan Molecular Structure

Arabinoxylans are built from the pentose sugars arabinose and xylose; hence the name arabinoxylan, or pentosan. They are composed of a linear backbone of β-(1→4)-linked D-xylopyranosyl residues (Xyl*p*), to which α-L-arabinofuranosyl units (Ara*f*) are attached (Izydorczyk and Biliaderis, 1995) as shown in Figure 5. Although most of the arabinosyl residues in arabinoxylans occur as monomeric substituents linked either α (1→2) or α (1→3), side-chains may also consist of two arabinosyl residues linked α (1→2) and α (1→3). A small portion of side-chains consisting of three arabinosyl units linked via α (1→2), α (1→3) and α (1→5) linkages have also been reported in wheat and rye arabinoxylans (Izydorczyk and Biliaderis, 1995).

In addition to pentose residues, the arabinoxylan structure may contain ferulic acid, covalently linked via ester linkages to O-5 atoms of the arabinosyl substituents (Pomeranz, 1988; Ishii 1991; Bartolomé et al., 1997); such linkages of ferulic acid to arabinoxylan have been found in barley, as well as wheat aleurone and endosperm. As a result, arabinoxylan chains in cell walls may be cross-linked with one another through dehydrodiferulic acid bridges and/or cross-linked with other cell wall polymers via ether or ester linkages. Various ferulic acid dimers have been identified in plant cell walls, which give evidence for this phenomenon (Ralph et al., 1994).

Ferulic acid accounts for 0.14% of dry matter in the barley grain (Nordkvist et al., 1984). Approximately 75% of this is bound to the aleurone and husk of the kernel, and about 10% is present in the endosperm. Ferulic acid associated with arabinoxylans has been considered to be responsible for the unique ability of arabinoxylans to form three-dimensional networks, either gels or viscous solutions (Izydorczyk and Biliaderis, 1995). The presence and relative amounts of ferulic acid have been thought to affect the shape and solubility of the arabinoxylan chain (Pomeranz, 1988). Ferulic acid dimers have been detected in wheat arabinoxylans (Dervilly et al., 2000), suggesting that arabinoxylans may be partially coupled in their native form; therefore, the level of such dimers, if present in barley arabinoxylans, may be another major parameter to explain variations in arabinoxylan macromolecular characteristics.

Although arabinoxylans from various cereals and plant tissues are similar in general molecular structure, they may differ substantially in their fine structural features, and consequently, in their physicochemical properties. Major structural differences are found

in the ratio of arabinose to xylose, the relative proportions and sequence of the various glycosidic linkages, and the presence of other substituents such as feruloyl residues (Izydorczyk and Biliaderis, 1995). These fine structural features will affect the conformation and hence the capacity of arabinoxylans to interact with each other or other molecules (Izydorczyk and Biliaderis, 1995).

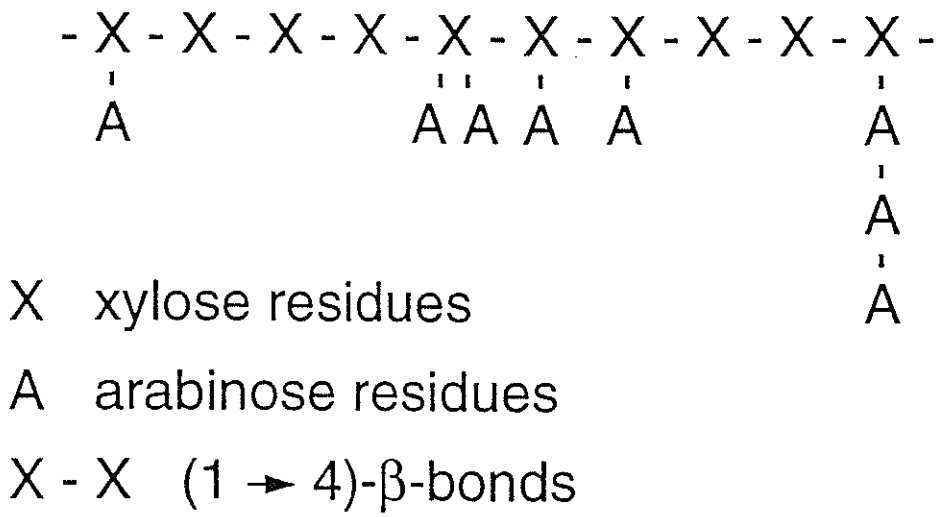


Figure 5. Generalized structure of an arabinoxylan molecule.

2.5. Isolation Procedures

2.5.1. β-Glucans

Numerous techniques for the isolation of β-glucans from barley and oats have been documented in the literature. Dry milling and sieving (Knuckles et al., 1992; Wu et al., 1994; Wood et al., 1989b) and water or solvent extraction at various temperatures and pH (Wood et al., 1989b; Bhatty, 1993; Saulnier et al., 1994) have been used extensively for the

preparation of β-glucan concentrates. Key steps in the extraction procedures are the inactivation of endogenous β-glucanases from the plant material, and the removal of contaminating constituents, particularly starch and protein. Endogenous β-glucanases cleave β-glucans, reducing their molecular size and hence their viscosity (Burkus and Temelli, 1998). Common practices for β-glucanase inactivation include refluxing the flour or grist with ethanol (>70%) prior to extraction, and immediately following extraction, heating of the extract (95 °C, 5 min) (Wood et al., 1989b; Izydorczyk et al., 1998ab). Much of the starch and protein in extracts can be removed by treatment with α-amylases and proteases, followed by dialysis against distilled water, or precipitation with ethanol. Izydorczyk et al. (1998a) were able to obtain β-glucan extracts from refluxed malting barley grist with >90% purity using water as the solvent (sequential treatment at 40 °C followed by 65 °C) and purification through both enzyme treatment (α-amylases and proteases) and dialysis. This procedure was successful in obtaining highly pure water-soluble β-glucans, which is partially due to the fact that most arabinoxylans are not soluble in water. Subsequent alkali treatment (saturated barium hydroxide ($Ba(OH)_2$ fraction), water ($Ba(OH)_2$ /H_2O fraction), and 1M sodium hydroxide (NaOH fraction), resulted in the isolation of mixtures of β-glucans and arabinoxylans, with the exception of the $Ba(OH)_2$ fraction containing almost pure arabinoxylan (Izydorczyk et al., 1998b). The $Ba(OH)_2/H_2O$ and NaOH fractions were further fractionated by stepwise ammonium sulphate ($(NH_4)_2SO_4$) precipitation at 30%, 50%, 65% and 100% saturation. This method was effective in separating the two polysaccharide populations in the $Ba(OH)_2/H_2O$ fraction, as the sub-fraction precipitated at 30% $(NH_4)_2SO_4$ saturation contained almost pure β-glucans.

However, the β-glucans in the NaOH fraction could not be separated from the arabinoxylans, even at a low saturation level of the salt (Izydorczyk et al., 1998b). Knuckles et al. (1997) experienced the same phenomenon, attempting to remove total β-glucan from hull-less barley via a single extraction with 1N NaOH for 1h at 65°C. Removal of 100% total barley β-glucan was achieved; however, relatively large amounts of arabinoxylans were also solubilized. Another concern with total extraction of β-glucan by $NaOH/NaBH_4$ is that it appears to be accompanied by degradation of the molecule, as evidenced by Beer and coworkers (1997).

Although obtaining pure and unhydrolyzed β-glucans may not be feasible or in some cases even desirable in the large scale isolation of these polysaccharides, it is necessary for their characterization and ultimate understanding of their physicochemical properties. Therefore, the chosen isolation procedure for β-glucans will depend on their intended use.

2.5.2. Arabinoxylans

Early interest in the arabinoxylans, or pentosans, of wheat, led to the development of a variety of procedures for their isolation and purification. Pence et al. (1950) obtained a soluble pentosan preparation by heating an aqueous flour extract to 90-95°C, and then treating the heated extract with activated clay to remove proteins. Perlin (1951) was able to further separate the water-soluble fraction into pentosan-rich and hexosan-rich fractions by acteylation and fractional precipitation (Pomeranz, 1988). Some advances in analytical techniques were made; however, a failure to agree on a particular procedure for purification may have hindered the establishment of their true chemical nature (Pomeranz, 1988).

A large portion of barley arabinoxylans cannot be extracted from cell wall material with water. Separation of the water-soluble arabinoxylans from the insoluble ones can be accomplished via aqueous extraction (Izydorczyk and Biliaderis, 1995); however, it is the removal of contaminating water soluble proteins, starch (α-glucans) and β-glucans which can be difficult. Extraction of ground barley with saturated $Ba(OH)_2$ containing 0.26M sodium borohydride ($NaBH_4$)has been found to be selective for arabinoxylans, though the mechanism for its selectivity is not clear (Schooneveld-Bergmans et al., 1999b). Bivalent cations, such as barium (Ba^{2+}) have been considered responsible for the unextractability of β-glucans (Gruppen et al., 1991), while the hydroxyl ions are believed to cause swelling of cellulose, disruption of hydrogen bonds between cellulose and the desired NSP, and hydrolysis of ester linkages between phenolic acids (including ferulic acid) and arabinoxylans. Addition of sodium borohydride ($NaBH_4$) prevents alkaline peeling of β-glucans, and may also be jointly responsible for rendering β-glucans insoluble with this type of extraction (Schooneveld-Bergmans et al., 1999b). Despite the high selectivity of $Ba(OH)_2$ extraction for arabinoxylans, not all arabinoxylans are recovered; furthermore, significant amounts of β-glucan still remain in the residue (Gruppen et al., 1991; Izydorczyk et al., 1998b). Following extraction with $Ba(OH)_2$, the residue may be subjected to an additional extraction with water, and subsequently, 1 M NaOH (Izydorczyk et al., 1998b). In separating arabinoxylans into smaller populations for characterization purposes, step-wise ammonium sulfate ($(NH_4)_2SO_4$) precipitation of the polymers has been successfully employed. Izydorczyk and Biliaderis (1992) discovered that with increasing salt concentration, there was an increase in the ratio of Ara*f*/Xyl*p* and in the relative amount

of doubly substituted xylose residues; at the same time, ferulic acid content and intrinsic viscosity measurements of the isolated fractions decreased.

2.6. Molecular Weight

Because β-glucans and arabinoxylans are structurally polydisperse, it follows that they should also exhibit a wide range of molecular weights. The physical properties of the two carbohydrate polymers are reported to vary depending on the source and variety of barley (Bhatty 1987). Discrepancies in molecular weight estimates of β-glucans from different sources may stem from variations in cell wall thickness (thicker cell walls are more resistant to the extraction of higher molecular weight polymers), extraction and isolation methods (solvent and temperature will affect solubilization), aggregation phenomena (affected by structural features and solvent quality), and depolymerization during extraction (resulting from either endogenous or microbial β-glucanases) (Izydorczyk and Biliaderis, 2000). Several researchers have studied the molecular size profiles of β-glucans and arabinoxylans using high performance size exclusion chromatography (HPSEC) and other chromatographic methods (Izydorczyk and Biliaderis, 1992; Beer et al., 1997; Doehlert et al., 1997; Izydorczyk et al., 1998ab; Schooneveld-Bergmans et al., 1999ab), comparing elution times to those of standards with known molecular weight. However, these methods only give information on relative molecular weights. Further detail on the molecular characteristics of β-glucans and arabinoxylans, such as the weight and number average molecular weights (M_w and M_n, respectively), radii of gyration (R_g), and polydispersity (M_w/M_n) can be obtained through HPSEC in conjunction with multi-angle

laser light scattering (MALLS).

Currently, information on the molecular characteristics of barley β-glucans and arabinoxylans using HPSEC/MALLS is rather limited. Knuckles and coworkers (1997), using this technique, reported weight average molecular weights (M_w) of 0.44×10^6 to 2.34×10^6 g/mol in β-glucans from four cultivars of hull-less barley. Molecular weight differences in β-glucans among barley varieties were apparent, although it was suggested that the differences could reflect storage history whereby enzymatic degradation differed (Knuckles et al., 1997). Surprisingly, β-glucans extracted with water at 23 °C had higher molecular weights than those extracted with water at 100 °C. It was suggested by the researchers that there may have been β-glucanase activity during the additional extraction steps at the higher temperature (Knuckles et al., 1997); other workers have demonstrated a general increase in molecular weight and/or size with increase in aqueous extraction temperature (Izydorczyk et al., 1998a). Compared to the results of Knuckles and colleagues (1997), substantially lower weight average molecular weights (2.1×10^5 to 5.7×10^5) were reported by Gómez et al. (1997a) for a combination of commercial oat and barley β-glucans and those obtained via aqueous extraction of a single barley cultivar at 65 °C. So far, no information of the M_w of arabinoxylans from barley has been reported using HPSEC-MALLS. In a study by Dervilly et al. (2000), arabinoxylans from a single wheat variety were reported to have M_w values of 3.07×10^5 to 5.90×10^5, depending on the fraction obtained by graded ethanol precipitation. The reported molecular weights of barley β-glucans have differed dramatically, and although these differences may be partially a reflection of the heterogenous nature of the polysaccharides both within and between barley

cultivars, it is quite certain that some differences also have arisen from discrepancies in extraction conditions and/or sample preparation. Several authors have also reported that light scattering results can be misleading because of association of β-glucan molecules (Vårum et al., 1992; Gómez et al., 1997a), especially when light scattering is used in the batch mode as opposed to a chromatographic line, and/or at higher temperatures; such factors can contribute significantly to irreproducibility of results.

2.7. Viscosity

The high viscosity of barley β-glucans and arabinoxylans has great technological as well as nutritional significance. It is the same factor responsible for causing problems with absorption and digestion of nutrients in broiler diets, and has also been implicated in poor lautering performance and severe filtration problems in the brewhouse (Edney et al., 1991). Yet this characteristic is what confers great potential for the use of barley in human food and nutritional applications.

Beta-glucans have the ability to form viscous aqueous solutions, because of their conformation, interactive properties, and high molecular weight (Izydorczyk and Biliaderis, 2000). When in solution, β-glucans tend to adopt a partially stiff, wormlike cylindrical conformation (Gómez et al., 1997b). Randomly spaced β-(1→3) linkages interrupt the linearity of the β-(1→4)-linked segments, imparting an asymmetric shape, and hence, solubility to the β-glucan molecules. Gómez and coworkers (1997b) found that barley β-glucan appears to form what they termed "structured solutions", an aggregation mechanism involving temporary links between short chain fragments. The degree to which this

phenomenon occurred seemed to increase at high temperatures (70°C compared to 25°C), and was stress sensitive, as it relaxed upon shearing.

The use of various heat treatments in an attempt to increase the viscosity and/or solubility of non-starch polysaccharides from oats has been explored (Doehlert et al., 1997; Zhang et al., 1997). In a study by Doehlert et al. (1997), the effects of roasting and steaming of whole oat grain, and the combination of these treatments, on the viscosity of slurries and soluble extracts produced from flour of the treated grain were examined. As is observed in Table 2, steamed samples were found to have the highest apparent flour slurry viscosities, although an increase in those of roasted samples was observed, relative to the control. Steaming also enhanced the apparent viscosities of previously roasted samples, making them equivalent to those samples exposed only to the steam treatment. In contrast, roasting samples after steaming partially reversed the increased apparent viscosity (Doehlert et al., 1997). Like the flour slurries, soluble extracts of steamed samples also had higher relative viscosities than did those of raw samples. This was coupled with the appearance of low molecular weight species and disappearance of high molecular weight species in raw and roasted samples, pointing to the action of enzymes degrading large molecular weight β-glucans in raw and roasted oat flours. Thus, steaming appeared to increase β-glucan viscosity through inactivation of β-glucanase; such a hydrothermal treatment may have large scale applications in the pretreatment not only of oats, but also of barley for food purposes, and deserves further investigation.

Table 2. Effect of heat treatments of three oat cultivars on apparent viscosity (mPa s) of their respective flour slurries (6.5g flour in 21.5 g water), 1 h after addition of water (n = 3) (adapted from Doehlert et al., 1997).

Treatment	Apparent Viscosity (mPa s)			
	Robert	Steele	Marion	average
raw	880	1310	2780	1656
steamed	2056	5170	6900	4708
roasted	1336	2173	3993	2501
steamed/roasted	1513	4240	4323	3359
roasted/steamed	1826	5470	8303	5200
least significant difference ($\alpha = 0.05$)	239	591	1601	531

The apparent viscosity of aqueous solutions of β-glucans and arabinoxylans is governed by molecular size, concentration, and magnitude of shear rates applied to the solution. Additionally, differences in fine structure and/or conformation will have an effect on their viscoelastic behaviour. At low shear rates, they maintain a constant viscosity; this is considered to be the Newtonian region of their mechanical spectrum. At increasing shear rates, however, they demonstrate shear thinning (pseudoplastic region), the onset and magnitude of which depends on concentration and molecular weight of the polymers. Figure 6 (a) and (b) show the steady shear flow curves and mechanical spectra, respectively, of solutions of a purified wheat arabinoxylan fraction at different polymer concentrations. As can be observed in Figure 6 (a), shear thinning was exhibited even for arabinoxylan

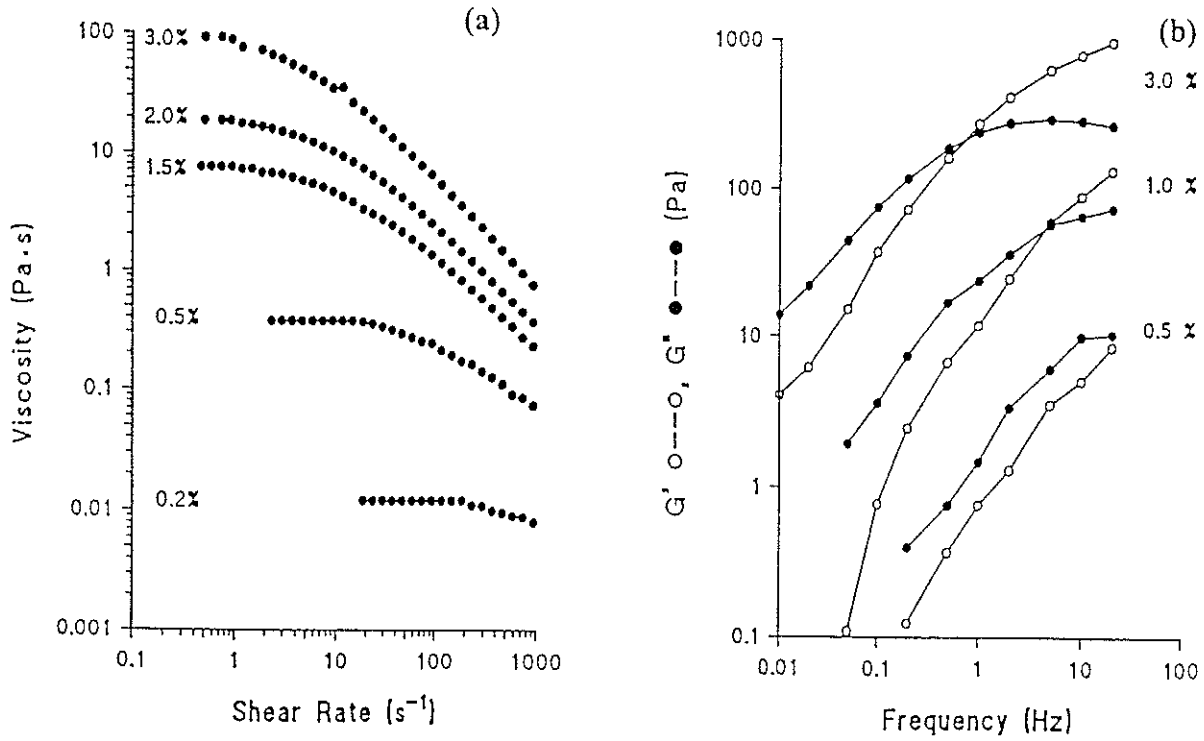


Figure 6. Steady shear flow curves (a) and mechanical spectra (b) for solutions of a purified fraction of wheat arabinoxylan at different concentrations (Izydorczyk and Biliaderis, 2000).

concentrations as low as 0.2% (w/v) and the shear rate at which shear thinning occurred increased with decreasing concentration (Izydorczyk and Biliaderis, 2000). Arabinoxylan concentration also had an effect on the mechanical spectra (Figure 6b); a change in viscoelastic behaviour was observed with increasing polymer concentration, from that of a viscous solution (G''>G'at all frequencies) to that of a weakly elastic solution (G'>G''

at high frequencies) (Izydorczyk and Biliaderis, 2000). Moreover, molecular weight affected the viscoelastic properties of the arabinoxylan preparations; the shear rate at which shear thinning occurred and the frequency at which G′and G′′ crossed both increased with decreasing molecular weight of the arabinoxylan polymers; such viscoelastic responses are typical of macromolecular solutions with topological entanglements (Izydorczyk and Biliaderis, 2000).

Because of their high viscosity in solution at relatively moderate to low concentrations, both β-glucans and arabinoxylans may find applications in formulated food systems as viscosity enhancers or emulsion stabilizers. Future efforts should be directed towards examination of the viscoelastic behaviour of β-glucans and arabinoxylans in hull-less barley, if the widespread use of these polysaccharides in food formulations is to be considered.

2.8. Functional Roles of Non-Starch Polysaccharides in Bread Making

Research concerning the role of arabinoxylans and β-glucans of wheat (often collectively termed pentosans) in bread making, has led to confusing results. Early studies on the effects of pentosans on bread showed that the water-extractable portion of wheat flour contributed to loaf volume, but the effects of carbohydrate components could not be separated from those of soluble protein (Pence et al., 1950; Pomeranz, 1988). Pence et al. (1950) identified the factor contributing to the improved baking performance of reconstituted loaves as the crude albumin fraction, with the contribution of pentosans being limited. However, tests by Cawley (1964) showed a significantly positive effect of

pentosans in baking when both pentosan- and protein-degrading enzymes were used. Subsequent studies (Jelaca and Hlynka, 1972) introduced even more ambiguity than clarity into our knowledge of the functional role of wheat flour pentosans in bread doughs. Different purities of arabinoxylan/β-glucan preparations, and varying bread making procedures, have contributed to this uncertainty regarding their functional role (Courtin and Delcour, 1998). Moreover, differences in experimental design have made interpretation of the findings even more difficult . Three basic approaches taken in the elucidation of arabinoxylan functionality include: relationships between arabinoxylan supplementation and dough/bread properties, reconstitution, and enzymatic modifications (Izydorczyk and Biliaderis, 2000). The conclusions of such studies have sometimes been contradictory, with some predicting negative, and others, positive impacts on bread quality and dough characteristics. In the majority of supplementation studies, however, addition of arabinoxylans to bread formulations has resulted in increased water (farinograph) absorption of the dough, enhanced loaf volume, and decreased crumb firmness (Cawley, 1964; D'Appolonia et al., 1970; Biliaderis et al., 1995); the latter two effects are considered desirable as they contribute to increased perception of freshness and/or quality. Interestingly, a study by Biliaderis et al. (1995) showed a definite correlation between loaf volume and both the molecular weight of arabinoxylans, as well as the concentration of arabinoxylans in the dough system (Figure 7). The researchers concluded from their results that there seems to be an optimum concentration for maximum loaf volume, and that a higher than optimum concentration results in decreased loaf volume, thought to be caused by viscosity build-up which would thereby decrease loaf expansion. Higher molecular

weight arabinoxylans also produced greater improvements in loaf volume at their optimum concentration. These observations may explain why in some studies the addition of arabinoxylans caused detrimental effects on bread properties; the level of addition was

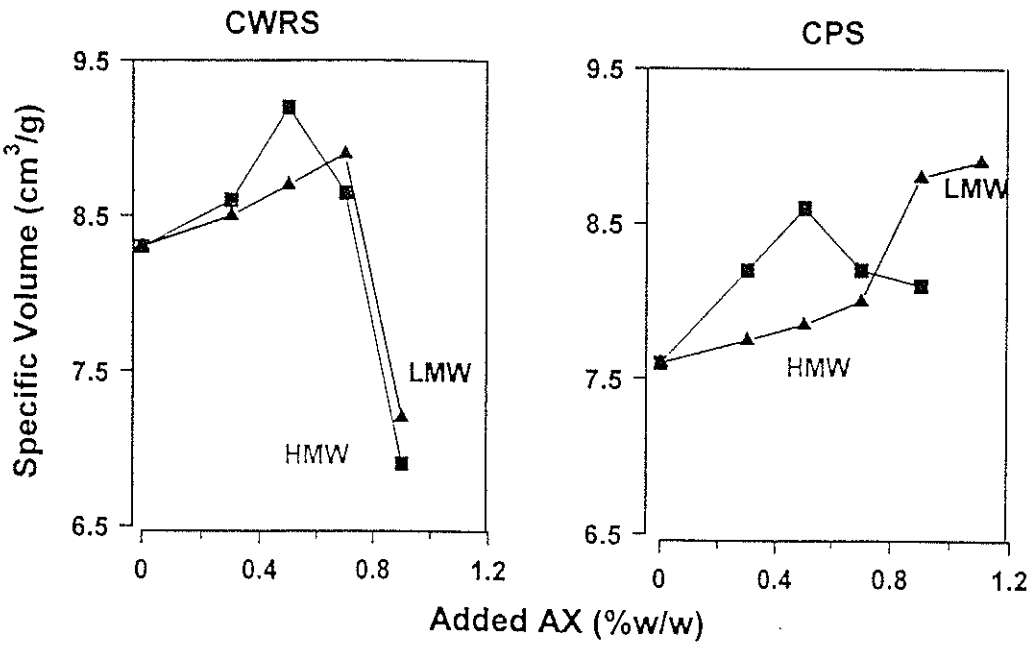


Figure 7. Effect of added arabinoxylans on loaf volume of bread made with Canadian Western Red Spring (CWRS) and Canadian Prairie Spring (CPS) flours (Biliaderis et al., 1995).

likely not the optimum. As for crumb firmness values, higher moisture content of the bread crumb may be one mechanism by which crumb firmness is decreased; the same study by Biliaderis and colleagues (1995) showed that crumb firmness values coincided with the values for moisture content. Hence, the large hydration capacity of non-starch

polysaccharides may serve to inhibit firming, one of the major characteristics of staling in bread.

Supplementing bread with wheat arabinoxylans and/or β-glucans (pentosans) has been demonstrated to elicit changes in farinograph absorption, loaf volume, and crumb firmness. Some of these changes (such as increased dough development time and difficulty in dough handling) may not be desirable. However, most supplementation studies have shown improvements in loaf volume and inhibition of crumb firmness. Pentosans have also been shown to cause improvements in the crumb grain (Wang et al., 1998). The magnitude of these many changes depends on factors such as source, purity, solubility, molecular weight, and level of addition. Thus far, studies have focused on the effects of wheat pentosans on bread quality and have ignored barley as a rich source of non-starch polysaccharides (Izydorczyk and Biliaderis, 2000). This is likely due to the fact that barley has not been extensively used in bread formulations. However, with the growing interest in functional foods, the need to establish the role of barley β-glucans and arabinoxylans in bread and other food systems will become increasingly apparent.

2.9. Physiological Effects of β-Glucans and Arabinoxylans

The unique inherent properties of barley β-glucans and arabinoxylans, such as their molecular structure, partial solubility, and viscoelastic behaviour, may impart nutritional benefits when consumed in the diet, in addition to functionality within cereal-based food systems. These polymers are considered part of dietary fibre as they are resistant to digestion by the alimentary enzymes of humans (Slavin, 1987). The insoluble portion of

dietary fiber is noted for increasing faecal bulk and thereby exerting beneficial effects on the gastrointestinal tract (Jenkins et al., 1985). The soluble portion of dietary fiber, on the other hand, has been implicated in the lowering of glycaemic responses (Jenkins et al., 1995), reduction in serum cholesterol levels (Hecker et al., 1998), and reduced risk of coronary heart disease (Pietinen et al., 1996) in both animal and clinical trials. The postulated mechanism by which soluble fiber may exert some or all such effects is through increasing viscosity of the gut contents and in effect slowing glucose and/or cholesterol absorption (Wang et al., 1992). Furthermore, soluble fiber may contribute to the health of the gastrointestinal (GI) tract by another mechanism. It has been demonstrated that soluble dietary fiber is more readily fermented into short-chain fatty acids (SCFA) by colonic microorganisms than insoluble dietary fiber (Karpinnen et al., 2000). These SCFA may have important implications for human health; for example, butyric acid is metabolized by the epithelial cells of the colon which has been said to help maintain a healthy mucosa (Cummings and Englyst, 1995).

Much of the research on the health benefits of barley has focused specifically on the hypocholesterolaemic effect of β-glucans. A recent study by Kalra and Jood (2000) found significant ($p<0.05$) reductions in total- and LDL-cholesterol and triglyceride, as well as significant elevation in the level of HDL-cholesterol in the serum of rats fed a particular barley diet (Table 3). The flours of three barley cultivars, Dolma (hull-less), DL-88 (hull-less) and BH-331 (hulled), containing 6.23, 4.60, and 2.18% total β-glucan and 5.39, 2.06, and 1.08% soluble β-glucan contents, respectively, were incorporated into rat diets; a casein diet was also fed as a control. After a 40 day feeding trial, the Dolma diet, richest

in total and soluble β-glucan, had the most pronounced hypocholesterolaemic effect. The authors concluded that total and soluble β-glucan appeared to be strong predictors of the cholesterol-lowering effect both in the serum and livers of rats.

Table 3. Effects of barley β-glucan diets on total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides of serum of rats (adapted from Kalra and Jood, 2000).

Dietary Group	Total Cholesterol (mg/100mL)	LDL-cholesterol (mg/100mL)	HDL-cholesterol (mg/100mL)	Triglycerides
Dolma	143 ± 3.1	63 ± 1.1	54.3 ± 1.6	126 ± 2.5
DL-88	180 ± 4.0	103 ± 2.0	49.4 ± 1.8	139 ± 2.5
BH-331	213 ± 3.8	137 ± 2.8	46.8 ± 1.4	148 ± 1.8
Control (casein)	236 ± 3.2	163 ± 2.6	40.5 ± 1.6	159 ± 1.8

2.10. Future Prospects

Because of the functional and nutritional advantages of barley β-glucans and arabinoxylans, barley has potential for much wider use than at present. Barley flour and/or milling fractions can be successfully incorporated into breads, other baked products such as muffins and biscuits, and even noodles and pasta, with acceptable sensory quality (Newman et al., 1989; Bhatty, 1986). Barley is also an excellent material for extrusion into

high-fiber cereals (Jadhav et al., 1998). Development of functional foods by supplementing with purified barley β-glucan and arabinoxylan fractions could provide further opportunities for introducing new food uses of barley (Jadhav et al., 1998; Newman et al., 1989). Two products currently being produced for supplementation purposes include Barleytrim, a water-soluble mixture of amylodextrins and β-glucans used for β-glucan enrichment in fruit juices (Inglett and Grisamore, 1991), and Barley* Complete 25 (Zumbro Inc., MN), a barley flour hydrolyzed by its natural enzymes and said to provide the benefits of soluble fiber (β-glucans) at 7% and insoluble fiber at 12%, used in nutritional beverages and as a natural sweetener (Jadhav et al., 1998). The potential also exists for barley β-glucans and arabinoxylans to be used as fat replacers; one example of such a product (already on the market) is known as Oatrim, a low dextrose-equivalent maltodextrin containing 1 to 10% β-glucan (dry basis). Oatrim is produced via mild enzymatic hydrolysis of oats or oat bran with heat stable α-amylase, separation of the solubilized material containing starch hydrolysate and β-glucans by centrifugation, and drying of the hydrolysate into a fine powder. Oatrim is stable, tasteless, odorless, thermally stable, and is easy to use in low calorie formulations as a substitute for saturated fats in frozen desserts and other dairy products (Inglett and Grisamore, 1991). Aqueous gels produced from Oatrim contain one-ninth the calories of fat, while the concentration of soluble β-glucans is comparable to that of oat bran, and is therefore a significant source of soluble dietary fibre.

Further research will be necessary to assess the effects of processing on solubility, molecular characteristics, viscosity, and physiological effects of β-glucans and arabinoxylans as they exist in the cereal based products and/or functional foods they are a

part of. However, in order to optimize human health benefits, initial selection of cultivars with high content, solubility, and viscosity of β-glucans is important, and necessitates the need to first determine which barley cultivars best meet these criteria.

3. MATERIALS AND METHODS

This section is a compilation of the materials and methods employed in Parts One and Two; Part One is referred to as the Variation in β-glucan Content Study, and Part Two, the Molecular Characterization Study.

3.1. Material

3.1.1. Variation In β-glucan Content Study

Twenty-nine samples of registered varieties and experimental genotypes of hull-less barley were assessed. Varieties Falcon, Condor, CDC Gainer, Bear, AC Hawkeye, and AC Bacon, obtained from James Farms Ltd. (Winnipeg, MB), were grown in 1998 in Manitoba, Canada. The remaining genotypes were grown in 1997 at the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada. All four lines of zero amylose samples are full sister lines from the same cross SB85750 x Azhull. Waxy SR93139 and SR93135 are full sister lines from the cross SB85738 x SB88490 and SB93965 is a half sister to both being from the cross SB88579/SB85738. High amylose 92-55-06-04 and 92-55-06-54 are full sisters lines from the cross SB86106/Glacier. SB94897 and SB94893 are full sister lines from the cross SB88488/Glacier and thus all are half sisters. Before analysis, grain samples were ground in a cyclone mill (Udy Corp. Fort Collins, CO) to pass through a 0.5 mm screen.

3.1.2. Molecular Characterization Study

From the 29 registered varieties and experimental genotypes of hull-less barley used in the treatment study, eight lines were selected, two from each of the four barley starch

types: Falcon (normal), CDC Dawn (normal), 92-55-06-54 (high amylose), 92-55-06-48 (high amylose), CDC Candle (waxy), SR93139 (waxy), SB94792 (zero amylose waxy), and CDC Alamo (newly registered and formerly known as SB94794; zero amylose waxy). Before analysis, grain samples were ground in a cyclone mill (Udy Corp. Fort Collins, CO) to pass a 0.5 mm screen. The ground barley (meal) was refluxed with 85% ethanol for 45 minutes to eliminate endogenous enzyme activity, filtered (Whatmann No. 4), washed with 95% ethanol, and oven-dried (40°C, 15 h). The meal was then ground in a mortar and pestle, and stored in sealed plastic containers in a desiccator at room temperature (RT).

3.2. Treatments

Heat treatments were applied to the barley grain prior to grinding. Grain was steamed for 20 min in a vegetable steamer over boiling water. Roasting consisted of placing 100 g of grain in a baking pan (25 x 25 cm) and roasting in a convection oven at 105 °C for 30 min. Autoclaving was performed at 121°C and 15 psi for 30 min in open containers. The layers of grain were not thicker than 1 cm. After heat treatments, the grain was left exposed to the atmosphere (RT) to allow moisture equilibration, and subsequently ground in the Udy mill.

3.3. Extraction and Purification Procedures

3.3.1. Variation In β-glucan Content Study

Ground barley samples, control and heat treated, were extracted (30 min) at 25 °C using deionized water (1:10 ratio). Esterase (65 U/g barley) and proteinase K (30 U/g

barley) were added to some; other samples were sonicated prior to extraction. After centrifugation (9000 rpm, 20 min), supernatants were heated for 5 min at 95 °C, filtered, and subsequently treated with α-amylase (40 U/ml extract) and proteinase K (10 U/ml extract) for 24 h. After extensive dialysis, the samples were lyophilized and used for the determination of β-glucan molecular weight.

3.3.2. Molecular Characterization Study

A schematic flow diagram of the extraction/fractionation procedure is given in Figure 3.1. Non-starch polysaccharides were sequentially extracted using the method of Izydorczyk et al. (1998 ab) with some modifications. The inactivated barley meal (150 g) was mixed (Sorvall Omni Mixer, speed #3) with deionized water (1200 mL) at 45 °C (30 min). Separation of the aqueous extract from the insoluble residue was achieved via centrifugation (9000 rpm, 15 min); the extraction procedure was repeated using 600 mL water and mixing (Sorvall Omni Mixer, speed #3) for 15 min. Immediately following centrifugation, each extract was heated to 95 °C for 5 min with continuous stirring (magnetic stirrer/hotplate) to ensure inactivation of endogenous enzymes, then cooled to RT. The supernatants were combined, and the denatured proteins removed by filtration (Whatmann No. 4) using celite (20 g/L) as a filter aid. Residual proteins were removed by adsorption on Vega clay (Pembina Mountain Clay, Winnipeg, MB); the extract was stirred (magnetic stirrer) with the clay (10 g/L) for 20 min., then centrifuged (9000 rpm, 15 min) and the pH adjusted to 6.5 with 0.1 *N* NaOH, prior to addition of 1700 U/L porcine pancreatic α-amylase (EC 3.2.1.1, type I-A, Sigma, St. Louis MO). The extract was incubated with the enzyme in a 35 °C water bath for 15 h, or until free of starch as judged

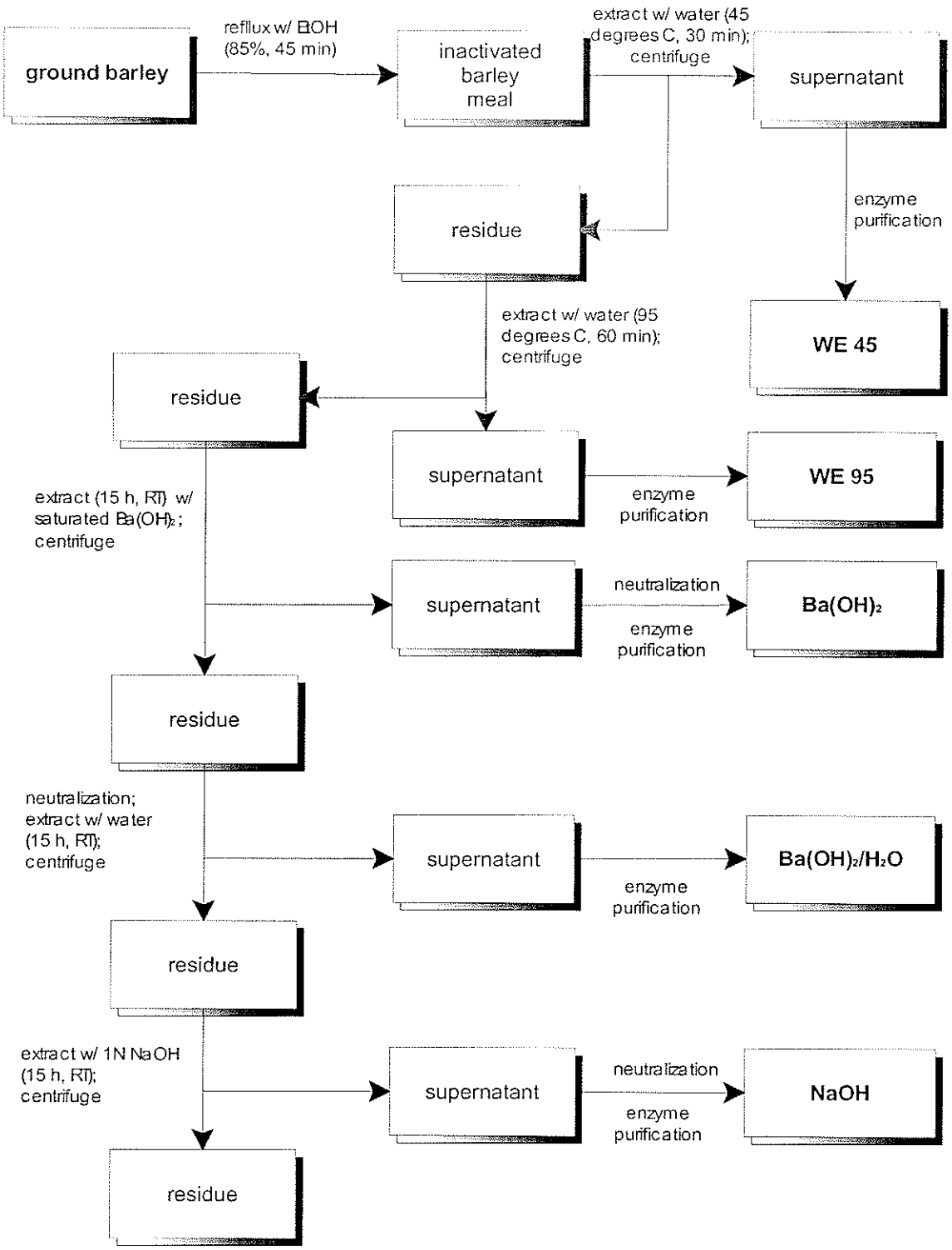


Figure 3.1. Schematic flow of the sequential extraction procedure.

by the iodine test. The extract was heated (95°C, 5 min) to inactivate the enzyme, centrifuged (9000 rpm, 15 min.), and then dialysed against distilled water (12000 - 14000 mwco, 4°C), until the dialysate was free of sugars (phenol-sulphuric acid test). Finally, the extract was lyophilized, and the fraction designated as WE45.

The insoluble residue of the meal was suspended in 1000 mL deionized water containing thermostable α-amylase (1500 U/L, Megazyme). The suspension was mixed (Sorvall Omni Mixer, speed #3) at 95°C for 60 min (maintained by a boiling water bath) and centrifuged (9000 rpm, 15 min). The residue was then subjected to a second 15 min extraction with 500 mL deionized water at 95°C. The combined extracts were purified in the manner described for the 45°C extract, and lyophilized. However, despite these purification steps, residual α-glucans originating from starch were detected in the ^{13}C-NMR spectra (resonance at 100 ppm), and therefore, this fraction was subjected to further purification via ethanol precipitation. The lyophilized material was dissolved in 300 mL deionized water, and ethanol (95%) was added (5100 mL) to obtain an ethanol concentration of 90%. The solution was allowed to sit at 4°C for 48 h. The precipitate was then separated by filtration (Whatmann No. 4), and dissolved in 400 mL deionized water using heating (70°C) and stirring, in order to evaporate any traces of ethanol. The solution was lyophilized and the material identified as the WE95 fraction.

Isolation of alkali-extractable polysaccharides was carried out on the residue remaining after sequential extraction with water at 45 and 95°C. The residue was suspended in a saturated $Ba(OH)_2$ solution (750 mL) containing 1% $NaBH_4$ to prevent alkaline degradation of the polysaccharides, stirred for 15 h at RT (magnetic stirrer), and

centrifuged (9000 rpm, 15 min). The residue was extracted again with the same solvent (250 mL, 3 h). The combined extracts were adjusted to pH 7 with glacial acetic acid, adding a few drops of octanol to prevent foaming, and allowed to sit for 15 h (RT). Porcine pancreatic α-amylase (EC 3.2.1.1, type I-A, Sigma, St. Louis MO) and proteinase K (Boehringer Mannheim, Canada) were added, and the solution incubated at 35 °C for a further 15 h. The extract was free of starch as judged by the iodine test. The enzymes were then inactivated by heat (95 °C, 5 min), and the extract centrifuged (9000 rpm, 15 min). The solution was dialyzed and lyophilized in the same manner as for WE45 and WE95, and the resulting material referred to as the $Ba(OH)_2$ fraction.

Deionized water (500 mL) was added to the insoluble residue, and the suspension was neutralized (pH 7) with glacial acetic acid, stirred for 15 h at RT (magnetic stirrer), and centrifuged (9000 rpm, 15 min). After decanting the supernatant, the residue was re-extracted with another 500 mL deionized water (RT) for 3 h and centrifuged (9000 rpm, 15 min). The extracts were combined and purified as described for the $Ba(OH)_2$ fraction; this was designated the $Ba(OH)_2/H_2O$ fraction.

The final fraction, referred to as the NaOH fraction, was obtained by suspension of the residue left after all other extractions, in 1 M NaOH (500 mL) containing 1% $NaBH_4$. The suspension was stirred for 15 h at RT and centrifuged (9000 rpm, 15 min). The residue was re-extracted using 250 mL of the solvent and stirring for 3 h, followed by centrifugation (9000 rpm, 15 min). The neutralization and purification steps were carried out as described for the $Ba(OH)_2$ and $Ba(OH)_2/H_2O$ fractions.

3.3.3. Sub-fractionation of $Ba(OH)_2$-extractable Material (Arabinoxylans)

A portion of the $Ba(OH)_2$ material was further fractionated using an ammonium sulphate precipitation technique described by Izydorczyk et al. (1997). Sub-fractions were collected at 30, 40, 50, 65, and 100% saturation of the salt, purified by dialysis, lyophilized, and were designated as $Ba(OH)_2$-30, $Ba(OH)_2$-40, $Ba(OH)_2$-50, $Ba(OH)_2$-65, and $Ba(OH)_2$-100, respectively.

3.4. Chemical Analyses

Moisture content of each barley was determined according to AACC Method 44.15A. Protein content was measured using AACC Method 46.13 (N x 5.7), while that of the five water- and alkali-extractable fractions, as well as the $Ba(OH)_2$ sub-fractions, was measured using the Lowry method for determination of soluble protein (Lowry et al., 1951), using bovine serum albumin (BSA) as a standard (Sigma Chemical Co.). Starch was isolated and purified by modifying previously published methods (McDonald & Stark, 1988; South and Morrison, 1990; and Sulaiman and Morrison, 1990), and amylose content in these starch samples was determined by potentiometric titration (Schoch, 1964). Starch content was determined enzymatically using the Megazyme kit for total starch assay (Megazyme International Ireland, Ltd). The mixed-linkage β-glucan assay kit (Megazyme International Ireland, Ltd) was used for total and soluble β-glucan determinations. Soluble β-glucan was determined after extraction (30 min) of ground barley with water at 25 °C using a Burrell wrist action shaker (Burrell Corp., Pittsburgh, PE). The activity of β-glucanase in barley samples was assessed by measuring the rate of decrease in relative

viscosity of control β-glucan solutions after addition of barley extracts. Barley extracts were obtained after extraction of 1 g barley samples with water (9 mL) at 25 °C for 30 min in the Burrell shaker and subsequent centrifugation of the suspension for 20 min at 9000 rpm. The assay involved addition of 2.0 mL of barley extracts to a control solution of purified β-glucans (0.1% w/v) in a Ubbelohde viscometer (International Research Glassware, Kenilworth NJ) and monitoring the changes of relative viscosity over a 4-hour period at 25°C. The activity of β-glucanase in barley samples is reported as the change of the relative viscosity of control β-glucan solutions per minute upon addition of barley extracts.

3.5. Monosaccharide Composition

The monosaccharides present in WE45, WE95, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, NaOH and sub-fractions of $Ba(OH)_2$ were determined by gas-liquid chromatography of alditol acetates. Fractions (50 - 100 mg) were hydrolyzed (in duplicate) in 1 M H_2SO_4 for 2 h at 100 °C. The component monosaccharides released during hydrolysis were derivatized to alditol acetates using the method of Englyst et al. (1982). Gas chromatography was performed on a Varian 3400 GC instrument; the derivatized samples were injected (Varian 3800 Auto Sampler) onto a Supelco SP-2330 column (30m × 0.25mm ID, 0.20μm thickness) and analyzed using Varian Star Chromatography Software (Version 4.02). Column conditions were: initial temperature 185°C, held 1 minute, then increase temperature by 12.5 °C/min. for 4 minutes (final temperature 235°C), hold for 15 minutes. Erythritol (Sigma Chemical Co., Ltd.) was used as the internal standard.

3.6. Ferulic Acid Content

The content of *cis* and *trans* ferulic acid in WE45, WE95, and $Ba(OH)_2$ fractions was performed according to the method described by Izydorczyk et al. (1991) for quantification of phenolic acids in wheat pentosans. Fractions (100 - 150 mg) were treated with 8 mL of 2N NaOH (containing 0.5 mg *p*-hydroxybenzoic acid as the internal standard) under nitrogen in the dark for 4 h (35 °C). The hydrolysate was acidified to pH 2.0 with 6N HCl, and extracted twice with hexane (HPLC grade) at a hexane to water phase ratio of 1:1. The free phenolic acids, contained in the water layer, were then extracted twice with equal volumes of diethyl ether-ethyl acetate (1:1, HPLC grades), and once with the same solvent at a solvent to aqueous phase ratio of 1:2. The three extracts were combined, dehydrated with anhydrous sodium sulfate, transferred to clean tubes, and evaporated to dryness under vacuum at 30 °C. The residue was dissolved in 1 mL of 50% methanol (HPLC grade), and analyzed using a modified method of Hagerman and Nicholson (1982) with a Supercoil LC-18 column (33 × 46 mm, 3 μm diameter particles, 37 °C, Supelco Canada Ltd., Oakville, ON) and an absorbance detector (Model 490, Waters Associates, Milford, MA) set at 280 nm. Gradient elution was performed as follows. Solvent A consisted of 10% (v/v) aqueous methanol plus 1 mM TFA, and solvent B, 80% (v/v) aqueous methanol plus 1 mM TFA. The run was programmed isocratically for 5 min with 100% solvent A at 1 mL/min followed by a 10 min linear gradient to 70 % solvent A and 30% solvent B, and finally maintained at this level for 4 min. Output signals were collected and integrated by Millennium software. Hydroxybenzoic acid and other phenolic acids used as standards were purchased from Sigma Chemical Company. The *cis* isomer of ferulic

acid was obtained via exposure of *trans* ferulic acid to UV light for 2 h (Hartley and Jones, 1976).

3.7. Molecular Weight

3.7.1. Variation In β-glucan Content Study

Molecular weights of purified β-glucan preparations were estimated by high-performance size exclusion chromatography. A Jordi Gel DVB sulphonated mixed bed column (10 x 250 mm, 5 μm particle size, Jordi associates, Bellingham MA) and Waters 510 pump, 712 WISP sample injector, and a 410 differential refractometer (Waters) were used. Samples were dissolved in 20% (v/v) DMSO (3 mg/mL) by heating and stirring, and filtered through a Whatmann GF/A glass fiber filter. Samples were eluted isocratically for 35 min with 0.7 mL/min 20% DMSO at 35 °C. A Shodex standard P-82 kit containing pullulan standards with molecular weights from 5.8 x 10^3 to 1660.0 x 10^3 was used to calibrate the column.

3.7.2. Molecular Characterization Study

The molecular characteristics of polysaccharides obtained by sequential extraction (WE45, WE95, $Ba(OH)_2$, and $Ba(OH)_2$ sub-fractions), were examined using a HPSEC-MALLS-RI system. Calibration constants of the refractive index (RI) and multi-angle laser light scattering (MALLS) detectors were determined by the method of You et al. (1999). Bovine serum albumin (monomer; Sigma Chemical Co.) was used in the normalization of the photo diodes surrounding the scattering cell. The high performance size exclusion chromatography (HPSEC) system consisted of a pump (Waters 510), and injection valve

(Model 7010, Rheodyne) with a 200 μL sample loop, a guard column (TSK PWH, Tosoh Corporation), a TSK G5000 PW size exclusion column (7.8 × 600 mm, Tosoh Corporation), and MALLS (Dawn DSP, Wyatt Technology) and RI (Waters 410) detectors. In the analysis of the alkali-extractable fractions, an ultraviolet (UV) detector was also connected to the system. The columns were kept at room temperature. The flow rate of the mobile phase (0.15M $NaNO_3$ containing 0.02% NaN_3), which was filtered through 0.2μm followed by 0.1μm cellulose acetate membranes, was 0.4 mL/min. The calculation of weight average molecular weight (Mw), root mean square radius (Rg) and polydispersity index (Mw/Mn) were performed by Astra 4.72 software (Wyatt Technology), based on the Berry plot with a third-order polynomial fit (You et al., 1999). From the results of previous work, values of dn/dc, defined as the proportional change in refractive index with change in polymer concentration, were assumed to be 0.145 for β-glucans (Knuckles et al.,1997), and 0.146 for arabinoxylans(Dervilly et al., 2000).

3.8. ^{13}C-NMR Spectroscopy

Natural abundance proton decoupled ^{13}C-nuclear magnetic resonance spectroscopy (125.8 MHZ) was performed at 90 °C on a Bruker AMX 500 spectrometer. Fractions WE45 and WE95 (2 % w/v) were dissolved in deuterated DMSO (DMSO-d_6) by heating and stirring at 90°C for 1 to 3 h. Sweep width was 153 ppm, pulse width 70 degrees, and recycle time, 3.85 seconds. Spectra were processed with resolution enhancement (Spinworks Software, Version 0.9), and chemical shifts (δ) were expressed in parts per million (ppm) down field from tetramethylsilane.

3.9. Analysis of Oligosaccharides Released by Lichenase

β-Glucans from WE45 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. To prevent the formation of a precipitate during lichenase digestion, it was necessary to dissolve WE95 fractions in 1 mL DMSO (90%) prior to addition of phosphate buffer (4 mL) and lichenase. After digestion, samples were heated to 95°C for 15 min to inactivate the enzyme, and then centrifuged (10000 × g, 10 min). Oligosaccharides released by lichenase from WE45 and WE95 β-glucans 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). Eluent A consisted of 150 mM NaOH, and eluent B, 150 mM NaOH containing 300 mM sodium acetate. Samples eluted for 1 min with 65% A and 35% B, then from 1 to 9 min over a gradient to 50% B, and from 9 to 22 min over a gradient to 100% B. The run time was 35 min at a flow rate of 1 mL/min (RT). Data was 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 oligosacccharides combined) for a given sample.

3.10. ^{1}H-NMR Spectroscopy

The $Ba(OH)_2$ fractions were dissolved in deuterium oxide (D_2O) at a concentration of 0.7% (10 mg in 1.5 mL D_2O). Proton(^{1}H)-NMR spectra of the fractions were recorded at 300 MHZ (85°C) on a Bruker DPX 300. Spectra were processed with resolution enhancement (Spinworks Software, Version 0.9), and chemical shifts (δ) were expressed in parts per million (ppm) down field from D_2O. The proportions of un-substituted (U-Xyl*p*), mono-substituted (2-Xyl*p*, 3-Xyl*p*) and di-substituted (2,3-Xyl*p*) xylose residues in arabinoxylans from each fraction were calculated by combining the ^{1}H-NMR spectral data with the xylose to arabinose ratios obtained from monosaccharide analysis (Appendix I), following the method of Roels et al. (1999).

3.11. Viscosity Measurements

3.11.1. Variation In β-glucan Content Study

Viscosities of barley slurries were determined in a Bohlin VOR rheometer (Bohlin Reologi, Edison, NJ) equipped with a concentric cylinder geometry. Ground barley samples were suspended in deionized water (1:6 barley to water ratio) and the development of viscosity was followed for 90 min at 25°C at a shear rate of 147 s^{-1}. To evaluate the effects of various enzymes on viscosity of barley slurries, the following enzymes were added to the suspensions prior to viscosity measurements: lichenase, xylanase, α-amylase (Megazyme), protease, proteinase K, amyloglucosidase, and esterase (Boehringer Mannheim, Canada). To evaluate the effects of ultrasonic disruption on extractability of β-glucans and viscosity of barley slurries, the barley water suspensions were sonicated for 3

min using a high intensity ultrasonic processor equipped with a 3 mm probe (Sonics and Materials Inc., Danbury, CO)

3.11.2. Molecular Characterization Study

The viscoelastic properties of WE45 and WE95 fractions were assessed using a Bohlin VOR rheometer (Bohlin Reologi, Edison, NJ) equipped with a concentric cylinder geometry. Fractions were dissolved in deionized water at a carbohydrate concentration of 2% (adjusted for protein content), and the solutions were placed in a C-14 cylinder with a 16.91 g cm torque element. Steady shear flow behaviour was assessed by measuring the viscosity of the solutions at 15°C over shear rates of 1.02×10^{-1}/s to 1.46×10^{2} /s (measurement interval of 10 s). To examine the effect of temperature on viscosity, the same solutions were cooled to 5°C while remaining in the cylinder, and the viscosity measured at a constant shear rate of 2.31/s while heating the sample to 50°C and cooling back to 5°C at (increments of 1.5°C/min; measurement interval of 30 s).

3.12. Statistical Analyses

All statistical analyses were executed using SAS statistical software (release 6.12; SAS Institute Inc., Cary , NC). Analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine significant differences.

Experiments (with the exception of extractions) were carried out at least in duplicate. Replicated results are reported as means.

4. PART ONE:

Variation in Total and Soluble β-Glucan Content in Hull-less Barley:

Effects of Thermal, Physical, and Enzymic Treatments

4.1. Results and Discussion

4.1.1. Chemical Composition

Several genotypes of hull-less barley, with four distinct compositions of the two starch polysaccharides amylose and amylopectin, were investigated in the present study. The amylose content of barley starches with normal starch composition varied between 23.8 and 27.1%, while in high amylose varieties amylose content ranged between 37.3 and 41.8%. Waxy barleys contained only 3.8 to 6.0% amylose. Amylose was entirely absent in the zero amylose waxy type barley (Table 4.1).

The starch content of barley with normal starch polymer distribution was significantly greater than that for barley with atypical amylose to amylopectin ratios. On average, normal barley genotypes had significantly greater starch content (60.7% w/w) than high amylose (52.9% w/w), waxy (54.0% w/w), and zero amylose waxy (53.9 % w/w) varieties.

The 100 kernel weights of various barley samples are presented in Table 4.1. On average, the 100 kernel weight of the zero amylose waxy barleys was largest (4.35g) and significantly different from that of waxy (3.78g) and normal barley (3.86g). The 100 kernel weight of high amylose samples was not significantly different from any other group. There were also significant differences in the 100 kernel weight within each type of barley (Table 4.1). The greatest differences were among normal varieties with values ranging from 3.19 to 5.05g. Waxy barleys, despite the relatively large number of samples, exhibited narrower variation, from 3.31 to 4.23g.

Significant differences were observed in total β-glucan content among samples.

Table 4.1. Chemical composition of barley samples.

Sample	Amylose (% w/w)	Starch (% w/w)	Protein (% w/w)	Weight /100 kernels (g)	Total β-Glucans (% w/w)	Total β-Glucans (mg/kernel)	Soluble β-Glucans (% w/w)
Normal starch							
Falcon	23.8	61.3 ± 0.3a[1]	11.6 ± 0.1	4.07 ± 0.06b	3.64 ± 0.03c	1.48	1.65 ± 0.07bc
SB90354	24.3	58.9 ± 2.1a	12.5 ± 0.1	5.05 ± 0.06a	5.38 ± 0.03b	2.72	1.68 ± 0.04bc
CDC Dawn	25.8	61.9 ± 1.5a	11.6 ± 0.1	3.93 ± 0.07c	3.88 ± 0.02d	1.52	1.16 ± 0.08e
SR93102	27.1	59.6 ± 0.4a	11.9 ± 0.1	3.79 ± 0.02d	6.28 ± 0.11a	2.38	1.28 ± 0.11de
CDC Silky	24.6	61.1 ± 1.6a	13.0 ± 0.3	3.19 ± 0.04e	5.17 ± 0.10bc	1.65	1.30 ± 0.08de
Condor	24.6	61.5 ± 0.7a	12.2 ± 0.4	3.22 ± 0.01e	5.14 ± 0.03c	1.65	1.78 ± 0.11ab
CDC Gainer	25.8	60.2 ± 0.4a	12.5 ± 0.3	3.75 ± 0.06d	3.45 ± 0.21ef	1.29	1.48 ± 0.11cd
Bear	26.0	61.4 ± 0.6a	11.9 ± 0.4	4.05 ± 0.06b	3.30 ± 0.14f	1.34	1.82 ± 0.18ab
AC Hawkeye	25.5	61.0 ± 1.4a	12.3 ± 0.3	3.81 ± 0.06d	3.58 ± 0.10e	1.36	1.90 ± 0.13ab
AC Bacon	24.5	60.7 ± 1.1a	12.0 ± 0.5	3.78 ± 0.02d	3.96 ± 0.08d	1.50	1.96 ± 0.08a
Average	**25.2 ± 1.0**	**60.7 ± 1.0a**	**12.2 ± 0.4**	**3.86 ± 0.51b**	**4.38 ± 1.02c**	**1.69 ± 0.48**	**1.60 ± 0.28b**
High amylose							
92-55-06-54	41.4	53.3 ± 0.4ab	11.7 ± 0.6	4.24 ± 0.05c	7.04 ± 0.03c	2.98	2.02 ± 0.03a
92-55-06-04	39.8	54.7 ± 0.1a	12.5 ± 0.1	3.47 ± 0.01d	8.23 ± 0.32a	2.86	1.78 ± 0.11a
92-55-06-48	41.8	54.0 ± 1.0ab	11.8 ± 0.3	3.26 ± 0.02e	7.96 ± 0.27ab	2.60	2.00 ± 0.14a
SB94893	39.1	49.6 ± 3.2b	14.4 ± 0.1	4.55 ± 0.06b	7.36 ± 0.27bc	3.35	1.52 ± 0.03b
SB94897	37.3	52.8 ± 1.6ab	12.7 ± 0.1	4.75 ± 0.08a	6.86 ± 0.28c	3.26	1.82 ± 0.10a
Average	**39.9 ± 1.8**	**52.9 ± 2.0b**	**12.6 ± 1.1**	**4.05 ± 0.66ab**	**7.49 ± 0.59a**	**3.01 ± 0.30**	**1.83 ± 0.20b**
Waxy							
CDC Candle	4.3	56.9 ± 0.8a	11.3 ± 0.5	3.99 ± 0.07b	6.89 ± 0.27b	2.75	2.53 ± 0.04f
SB94917	4.2	53.0 ± 3.0abc	13.1 ± 0.1	3.62 ± 0.07ef	8.07 ± 0.10a	2.92	2.22 ± 0.06g
SB93965	3.9	55.6 ± 0.8a	13.1 ± 0.1	4.23 ± 0.02a	6.76 ± 0.23bc	2.86	3.32 ± 0.09ab
SR93135	4.8	54.8 ± 0.5ab	12.8 ± 0.2	3.58 ± 0.04ef	7.01 ± 0.16b	2.51	3.55 ± 0.14a
SR93139	5.9	55.2 ± 0.8a	12.8 ± 0.2	3.31 ± 0.15g	7.20 ± 0.28b	2.38	3.21 ± 0.08bc
SH96054	3.8	50.8 ± 2.6bc	13.7 ± 0.1	3.45 ± 0.06fg	6.96 ± 0.03b	2.40	3.06 ± 0.08bcd
SH96076	4.0	53.3 ± 0.8abc	12.5 ± 0.1	3.79 ± 0.09cd	6.95 ± 0.11b	2.63	2.64 ± 0.06ef
SH96090	4.2	50.0 ± 2.0c	12.9 ± 0.1	3.65 ± 0.03cd	5.52 ± 0.54d	2.02	2.91 ± 0.16cde
SH96093	5.5	54.2 ± 0.4ab	12.7 ± 0.1	3.85 ± 0.03bc	6.25 ± 0.35c	2.41	2.80 ± 0.28def
SB95134	3.8	56.1 ± 2.3a	13.4 ± 0.1	4.31 ± 0.01a	6.99 ± 0.06b	3.01	3.07 ± 0.07bcd
Average	**4.4 ± 0.7**	**54.0 ± 2.2b**	**12.8 ± 0.6**	**3.78 ± 0.32b**	**6.86 ± 0.65b**	**2.59 ± 0.30**	**2.93 ± 0.40a**
Zero amylose waxy							
SB94785	0	56.6 ± 0.3a	12.2 ± 0.6	4.78 ± 0.03a	5.83 ± 0.14c	2.79	3.07 ± 0.10a
SB94783	0	52.4 ± 0.3b	13.0 ± 0.1	4.64 ± 0.04a	6.32 ± 0.03b	2.93	2.16 ± 0.21b
SB94792	0	53.7 ± 0.6b	13.6 ± 0.1	3.34 ± 0.09b	6.95 ± 0.07a	2.32	2.84 ± 0.01a
CDC Alamo	0	52.8 ± 1.7b	13.8 ± 0.5	4.64 ± 0.04a	6.11 ± 0.01b	2.84	2.77 ± 0.04a
Average	**0**	**53.9 ± 1.9b**	**13.2 ± 0.7**	**4.35 ± 0.68a**	**6.30 ± 0.48b**	**2.72 ± 0.27**	**2.71 ± 0.39a**

[1] For each group of barley samples, values followed by the same letter (column) are not significantly different ($p \leq 0.05$).

High amylose barley samples ranked first, with an average β-glucan content of 7.49% (w/w), followed by waxy barley (6.86%), zero amylose waxy barley (6.30%), and normal barley (4.38%). The greatest variations in β-glucan content were found within the normal barleys, with values ranging from 3.30% to 6.28%. These results indicate that it is possible to identify normal barley varieties with relatively high β-glucan content. The waxy barleys also displayed a relatively large range of β-glucan, from 5.52 to 8.07%. Narrower ranges of total β-glucan in high amylose and zero amylose waxy barleys might be due to a lower number of samples within these two groups as compared to the larger number of samples of normal and waxy barley. The concentrations of total β-glucan for the 29 varieties of hull-less barley, obtained in this study, represent a slightly wider range of values (3.30 to 8.23% w/w) than normally reported (4 to 7% w/w; MacGregor and Fincher, 1993). The values did not, however, exceed the most extreme cases ever reported: 2% and up to 13.2% for wild barley (Henry and Brown, 1987), and as high as 16% in U.S. barleys (Newman et al., 1989; Wu et al., 1994).

When the total β-glucan content was expressed in milligrams per kernel, the ranking of the samples remained the same. No clear relationship was found between the β-glucan content and 100 kernel weight. When taken as a group, the high amylose barleys, with their significantly higher total β-glucan content than the normal barleys, did not have significantly different weight of 100 kernels. Moreover, there were no significant differences in the β-glucan content between the waxy and zero amylose waxy samples, but the 100 kernel weights were significantly different between these two groups. These results indicate, therefore, that the differences in total β-glucan content among the four groups of

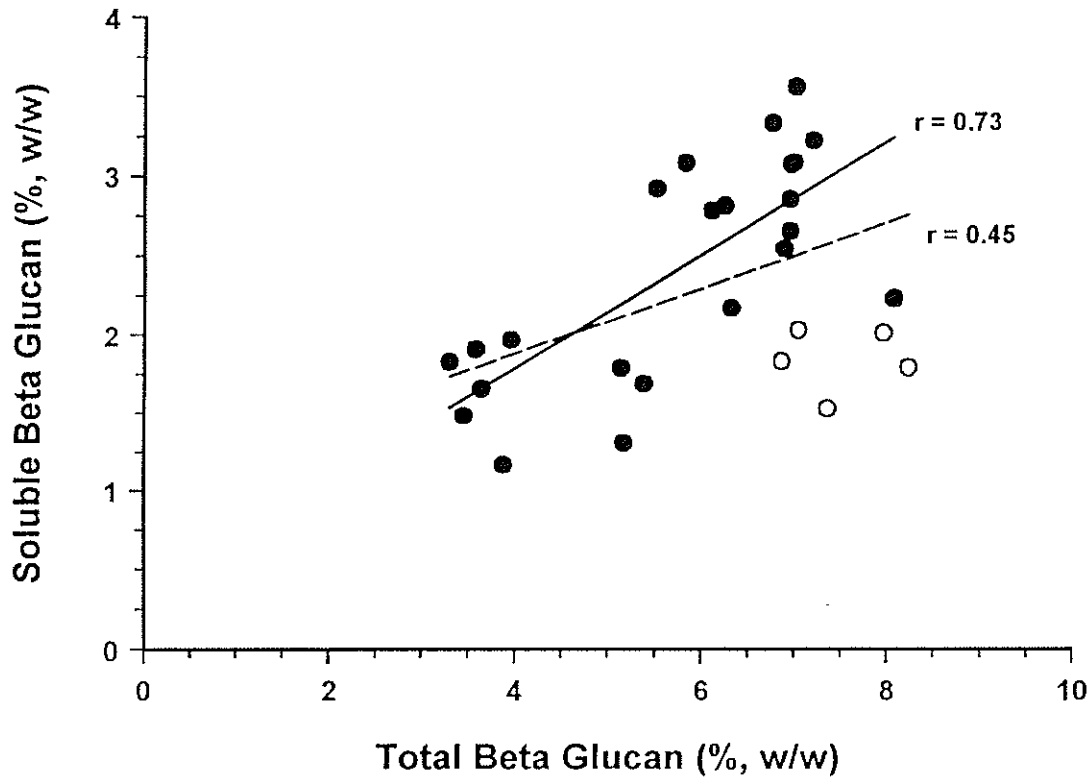
hull-less barley is likely due to genetic differences rather than differences in size and/or amount of endosperm in barley kernels.

An inverse relationship between total β-glucan content and starch content was observed. Generally, barley varieties with the anomalous amylose to amylopectin ratios had lower starch but a higher β-glucan content than the varieties with normal starch. These results agree with findings from previous studies (Andersson et al., 1999 ; Bhatty, 1999). Interestingly, despite the significantly higher starch content in barley with normal starch polymer distribution, the 100 kernel weight of this group was not significantly higher than that of groups with anomalous starch.

Although high total β-glucan content is indicative of high dietary fiber content in barley, it is important to recognize that it is the soluble component of β-glucan that is responsible for the beneficial ability of β-glucans to lower serum cholesterol and glucose levels (Wood et al., 1994; Jenkins et al., 1995; Kahlon and Chow, 1997; Yokoyama et al., 1997). Therefore, it may not be enough to determine the content of total β-glucan in the grain when searching for the most suitable barley for human consumption. The content of soluble β-glucans found in the present investigation ranged from 1.16% to 3.55% (w/w), indicating 20.6% to 52.5% extractability. Marked differences in β-glucan extractability were observed among the four types of barley analyzed. Clearly, the β-glucans in waxy and zero amylose waxy barleys exhibited the highest extractability, ranging from 36.7% to 52.7%, and from 34.0% to 52.5%, respectively. The extractability of β-glucans in normal barleys varied between 29.8% and 44.3% and in high amylose barleys between 20.6% and 29.7%. Because of the unexpectedly low solubility of these polymers in the high amylose

barley, the correlation between the soluble and total β-glucans was poor (r = 0.45); Figure 4.1). However, when the high amylose samples were excluded from consideration, the correlation improved (r = 0.73). These results indicate that high amylose barley cultivars, despite their high content of total β-glucans, might not be the best source of soluble polysaccharides. Conclusions must, however, be drawn with caution. Because the solubility of β-glucans is dependent on extraction parameters, such as solvent type, temperature, and duration of extraction, substantial differences in solubility might be expected with changes of any of these parameters. It is prudent to expect, therefore, that if the extraction conditions in our studies were changed, the relative ranking of extractability of β-glucans among the four groups of barley might have been different. Solubility of β-glucans in oat and barley has been investigated in the past by many researchers, but the methodologies used were quite different, and direct comparison of results is difficult. Also, most previous investigations dealt with hulled, malting barley rather than hull-less genotypes. Recently Anderson et al. (1999) and Oscarsson et al. (1996), who extracted barley for 2 h at 38 °C, also reported relatively low extractability of β-glucans in two Swedish high amylose naked barleys (35.1% for cultivar Hashonucier and 36.7% for cultivar Glacier). On the other hand, Xue et al. (1991), who examined the solubility of β-glucans in waxy and normal barley isogenic lines, reported that waxy varieties, in contrast to the present study, had significantly lower amounts of soluble β-glucans. Bhatty et al. (1991) used acidic buffers and one-hour extraction at 40 °C, and reported an average of 44.7% extractability of β-glucans from 13 Canadian hull-less barley genotypes.

Figure 4.1. Relationship between the content of water-soluble and total β-glucans in hull-less barleys. Open circles indicate the high amylose varieties, filled circles indicate the normal, waxy, and zero amylose waxy varieties. Dashed line indicates the linear regression line obtained for all barley varieties, solid line indicates the linear regression line obtained when high amylose varieties are excluded from calculations.


Soluble Beta Glucan (%, w/w)
Total Beta Glucan (%, w/w)
r = 0.73
r = 0.45
0
1
2
3
4
0
2
4
6
8
10

4.1.2. Viscosity of Barley Slurries

It has recently been postulated that some biological benefits of β-glucans associated with reduction of plasma cholesterol and of postprandial serum glucose levels in humans and animals stem from their solubility in water and capacity to form highly viscous solutions (Schneeman, 1998). Viscosity is also one of the most important physical characteristics of food components affecting their functionality in food systems. Although viscosity properties of barley extracts containing β-glucans have been previously studied (Aastrup, 1979; Bhatty et al., 1991; Perez-Vendrell et al., 1996), those studies were prompted by problems caused by barley β-glucans in brewing and animal nutrition rather than by the potential benefits of β-glucans in the human diet. Traditionally, the determination of extract viscosity of barley was performed in acidic solutions to avoid interferences associated with endogenous β-glucanases. Good relationships between the viscosity of acid extracts and the amount of soluble and total β-glucans in barley have been generally obtained, and therefore, the viscosity of barley extracts is often routinely used as a predictor of β-glucan content. Aastrup (1979) reported that the logarithm of viscosity of acid extracts of malting barley was highly correlated with the extractable β-glucan content ($r = 0.99$) and with the total β-glucan content ($r = 0.94$). Bhatty et al. (1991) examined 13 varieties of hull-less barley and also found a significant logarithmic relationship between acid extract viscosity of barley flour and its total β-glucan content ($r = 0.94$).

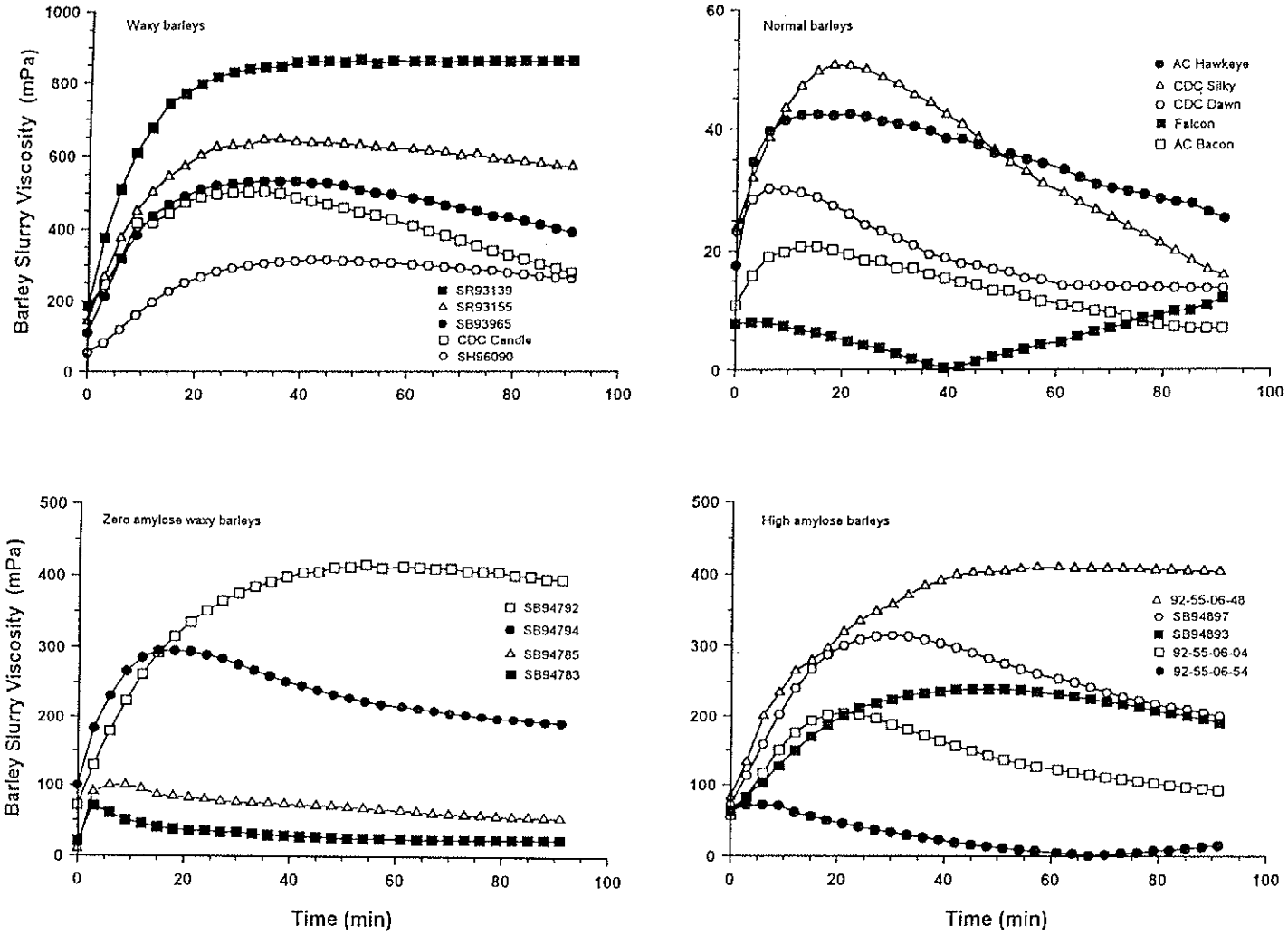
In our studies, we have monitored the changes in viscosity of barley flour slurries during a two hour period of mixing at a constant shear rate (147 s^{-1}). The samples varied not only in the maximum attainable viscosity but also in the stability of the viscosity during

measurements (Figure 4.2). Some samples exhibited a steady evolution of viscosity within the initial 20 to 30 minute period, followed by a pseudo plateau region with very little change in viscosity (e.g. SR93139, SB94792, 92-55-06-48). These samples also attained relatively high final viscosity values. Other samples showed a similar initial rise in viscosity, but a decrease of viscosity thereafter.

Preliminary studies have shown that starch granules and β-glucans were the two main contributors to overall barley flour slurry viscosity as the addition of both starch and β-glucan degrading enzymes drastically reduced viscosity (results not shown). The addition of the β-glucan degrading enzyme lichenase can cause an immediate decline in viscosity, whereas the action of α-amylase is somewhat hindered by the relatively slow enzymic hydrolysis of starch granules. Protease and xylanase had no effect on the viscosity of barley slurries, and it was concluded that proteins and arabinoxylans contribute little to the overall viscosity of barley flour slurries. The initial increase in viscosity during continuous mixing of the slurry was, therefore, ascribed mainly to the solubilization of β-glucans, with the subsequent viscosity decline attributed to the degradation of β-glucans by endogenous β-glucanases. Because α-amylase could also contribute to the decline of slurry viscosity, its effect was eliminated by the addition of silver nitrate ($AgNO_3$). Only samples which showed a rapid decrease in viscosity were somewhat affected by the addition of $AgNO_3$. Despite the slight elevation of viscosity values in the presence of $AgNO_3$, the general profile of viscosity development and decline remained the same for the samples with (results not shown) and without $AgNO_3$.

In an attempt to explain the differences in the maximum attainable viscosity, the

Figure 4.2. Development of viscosity of barley flour slurries (1:6 barley to water ratio) at 25 °C at a constant shear rate ($147s^{-1}$).


Waxy barleys
Barley Slurry Viscosity (mPa)
SR93139
SR93155
SB93965
CDC Candle
SH96090
Normal barleys
AC Hawkeye
CDC Silky
CDC Dawn
Falcon
AC Bacon
Zero amylose waxy barleys
SB94792
SB94794
SB94785
SB94783
High amylose barleys
92-55-06-48
SB94897
SB94893
92-55-06-04
92-55-06-54
Time (min)

content of total and soluble β-glucans in the samples was examined (Figure 4.3). A better correlation was obtained between slurry viscosity and the amount of soluble β-glucans (r= 0.70) than between viscosity and total β-glucans (r= 0.49; Figure 4.3). These results suggest, however, that factors other than β-glucan content might also affect the barley flour slurry viscosity. This was especially apparent for the high amylose barley samples, which, despite the relatively low content of extractable β-glucans, on average, attained much higher viscosities than the normal barley samples with only slightly lower β-glucan content. Additionally, the highest viscosity exhibited by one of the waxy samples, SR93139, a cultivar specifically selected for this trait (Rossnagel, University of Saskatchewan, personal communication), cannot be explained exclusively by its level of soluble β-glucan. Other waxy samples contained comparable or even higher amounts of this polymer but attained much lower viscosity values.

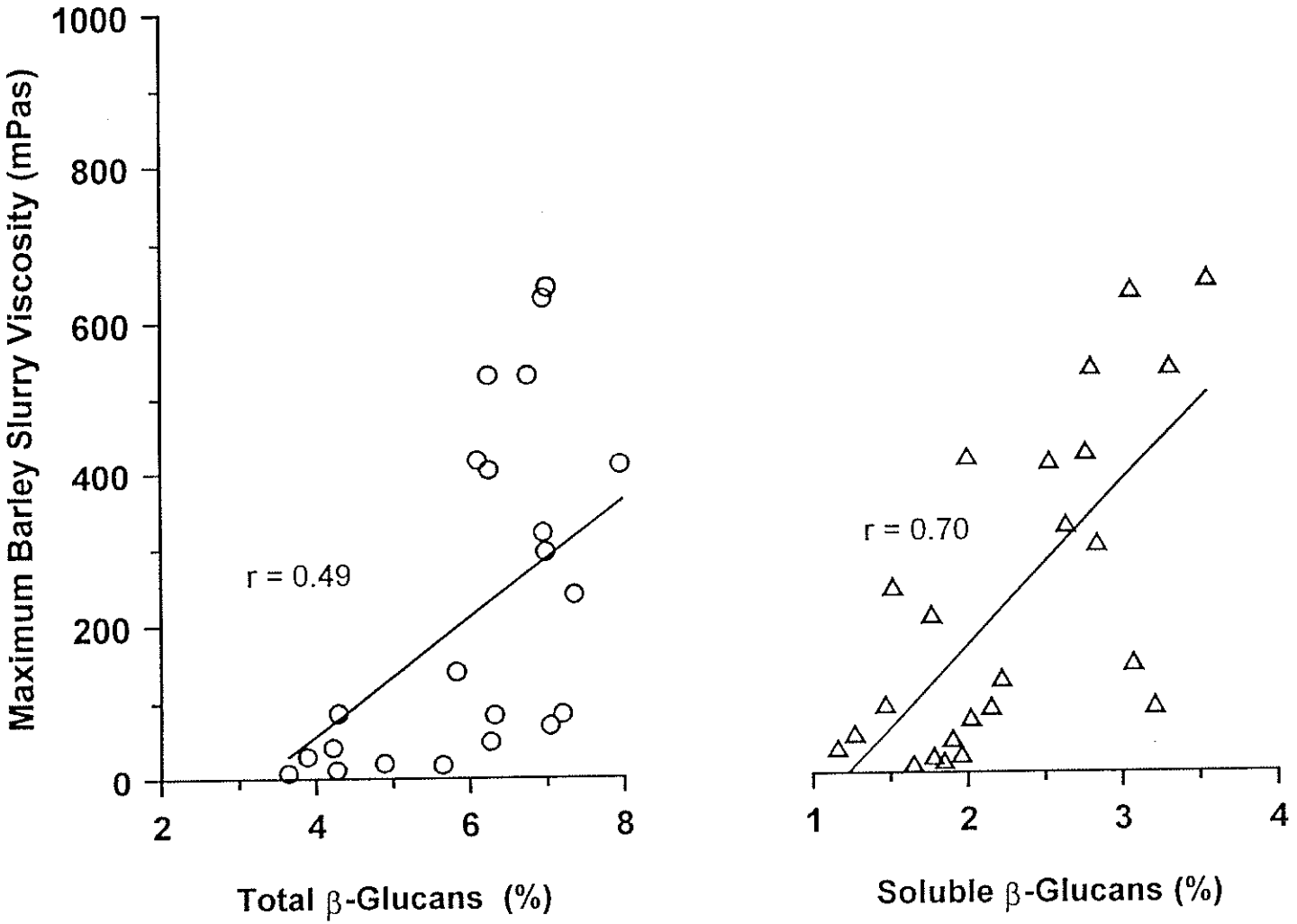
The presence of β-glucan degrading enzymes could substantially affect the viscosity and stability of barley slurries. Two isoenzymes of (1→3)(1→4)-β-D-glucan 4-glucanohydrolase (referred to here as β-glucanase) have been found responsible for the degradation of barley β-glucan as judged by their ability to rapidly depolymerize the polysaccharide (Woodward and Fincher, 1982). The enzymes are abundant in the endosperm of germinating barley. However, very low β-glucanase activity is expected in barley before germination, and little scientific data on the levels of β-glucanase in mature barley grains are available. Also, most tests suitable for detection of β-glucanase activity in malt might not be appropriate for detection of the very low levels of these enzymes in barley. Ellis et al. (1997) examined two malting varieties of barley grown in Scotland and

Table 4.2. Maximum attainable viscosity of barley slurries and β-glucanase activity in barley grains.

Sample	Maximum Viscosity of Barley Slurry[a] (mPas)	β-Glucanase Activity[b]
Normal starch genotypes		
Falcon	8 ± 1	0.0150
SB90354	140 ± 5	0.0073
CDC Dawn	30 ± 2	0.0077
SR93102	50 ± 3	0.0077
CDC Silky	50 ± 2	0.0074
Condor	20 ± 3	0.0073
CDC Gainer	89 ± 5	0.0073
Bear	12 ± 2	0.0077
AC Hawkeye	42 ± 3	0.0077
AC Bacon	20 ± 3	0.0067
High amylose genotypes		
92-55-06-54	71 ± 5	0.0065
92-55-06-04	203 ± 8	0.0063
92-55-06-48	410 ± 5	0.0036
SB94893	240 ± 8	0.0056
SB94897	313 ± 9	0.0055
Waxy genotypes		
CDC Candle	403 ± 5	0.0042
SB94917	120 ± 6	0.0039
SB93965	529 ± 8	0.0046
SR9313	644 ± 9	0.0053
SR93139	850 ± 9	0.0024
SH96054	630 ± 10	0.0045
SH96076	320 ± 8	0.0043
SH96090	313 ± 9	0.0043
SH96093	530 ± 8	0.0045
SB95134	518 ± 10	0.0045
Zero amylose waxy genotypes		
SB94785	75 ± 5	0.0065
SB94783	60 ± 6	0.0101
SB94792	416 ± 9	0.0041
CDC Alamo	295 ± 10	0.0057

[a] Maximum attainable viscosity of barley slurries (1:6 ratio of barley to water) obtained at 25°C at shear rate of $147s^{-1}$.
[b] Reported as a change of relative viscosity of control β-glucan solutions per minute upon addition of barley extracts.

Figure 4.3. Relationship between the maximum attainable viscosity of barley slurries and the content of water-soluble and total β-glucans in hull-less barleys.


Maximum Barley Slurry Viscosity (mPas)
1000
800
600
400
200
0
2
4
6
8
r = 0.49
Total β-Glucans (%)
r = 0.70
1
2
3
4
Soluble β-Glucans (%)

Spain, and reported some genetic and environmental differences in the levels of β-glucanase activity, although the values were very low compared to those reported for malt samples. Knuckles and Chiu (1999) reported insignificant differences in β-glucanase activity among 10 barley varieties grown in the United States. In both studies, β-glucanase was estimated by the method of McCleary and Shameer (1987), recommended for determination of the enzyme in malt. In our studies, β-glucanase activity in barley samples was estimated by determining the viscosity decline rate of β-glucan solutions after addition of aqueous barley extracts. This test allowed for a clear distinction between levels of activity of β-glucanase in the various samples (Table 4.2). The relatively high β-glucanase activity found in normal barley could probably explain the low viscosity values of the slurries and fast decline of viscosity upon mixing of barley flour with water. On this basis Falcon had almost twice as much β-glucanase activity as the other normal samples, and this was reflected in the extremely low viscosity of this sample. On the other hand, the very high and stable viscosities of the waxy SR93139, zero amylose waxy SB94792, and high amylose 92-55-06-48 samples corroborated well with the findings that these samples also contained the lowest levels of β-glucanase. The amount of β-glucanase activity in barley samples seems to be a very important factor affecting the overall viscosity of barley slurries. Therefore, if barley is to be incorporated into food systems, and in order to take full advantage of the physiological action of β-glucans, we should ensure that β-glucanase activity in barley is arrested and β-glucans are not degraded by enzymic activity during food preparation, processing, or storage.

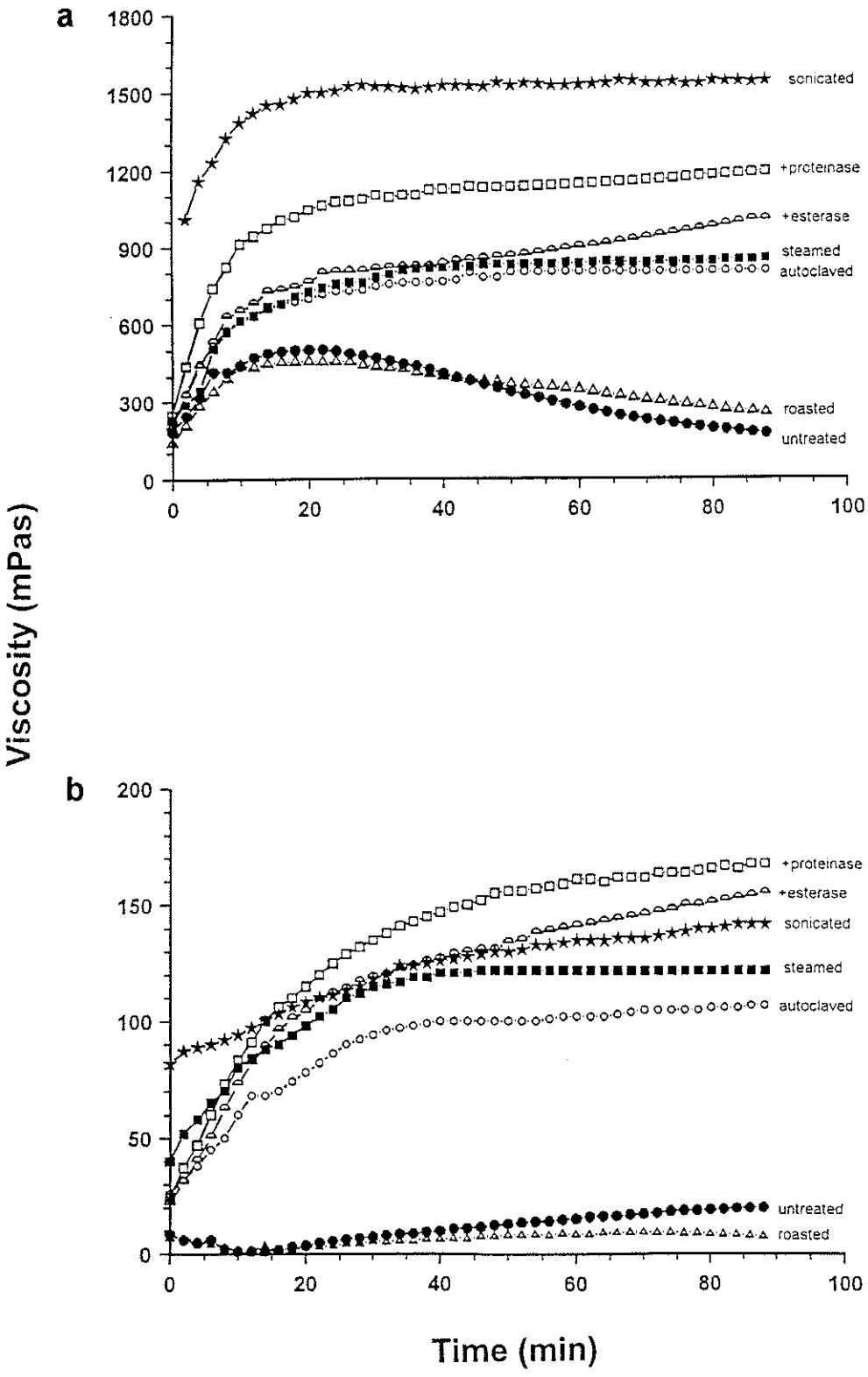
It is well known that the molecular weight of polymers is one of the principal

properties determining their viscosity. It is, therefore, possible that intrinsic differences in the molecular weight of β-glucans contributed to the differences in the viscosity of barley flour slurries. In fact, when the molecular weight of β-glucans isolated and purified from several different barleys was assessed by the size exclusion chromatography, substantial differences were observed. However, prior to the evaluation of molecular weight of isolated β-glucans, inactivation of endogenous β-glucanases must be ensured.

4.1.3. Effect of Treatments on Extractability and Viscosity of β-glucans

Since it is postulated that solubility and viscosity of β-glucans are responsible for the physiological activity of these polymers, several thermal, enzymic and physical treatments of barley grain were assessed as potential strategies for increasing the level and viscosity of β-glucans in barley extracts. The two hydrothermal treatments, autoclaving and steaming, had substantial effects on the viscosity developments of barley slurries (Figure 4.4). The samples obtained much higher maximum viscosity values than the control samples, and no detectable decline of viscosity was observed thereafter. Roasting the grain at 100 °C, on the other hand, did not result in any increase of viscosity and only slightly slowed its decline. Autoclaving of the barley grain in conjunction with either enzyme addition, esterase (3 U/mL barley slurry) or proteinase K (2 U/mL barley slurry) during mixing experiments resulted in slurries exhibiting slight but steady increases of viscosity throughout the measurement time (100 min). This observation most likely indicates a continuous solubilization of β-glucans in the presence of enzymes. Autoclaving in conjunction with a physical disruption (ultrasonication) resulted in slurries with very high

Figure 4.4. Effects of various treatments on the development of viscosity of barley slurries: (a) CDC Candle, (b) Falcon.


a
1800
1500
1200
900
600
300
0
0
20
40
60
80
100
sonicated
+proteinase
+esterase
steamed
autoclaved
roasted
untreated
b
200
150
100
50
0
+proteinase
+esterase
sonicated
steamed
autoclaved
untreated
roasted
Viscosity (mPas)
Time (min)

initial viscosities, indicating the solubilization of β-glucans prior to measurements. It should be noted that starch was not gelatinized during the heat treatments as assessed by differential scanning calorimetry and rapid viscoanalysis tests (results not shown). The changes in the slurry viscosity must be, therefore, attributed to changes in the amount and/or characteristics of β-glucans.

Interestingly, when the amount of soluble β-glucan was assessed in the treated samples, it became evident that the two hydrothermal treatments did not increase the amount of extractable polymers (Figure 4.5). The increase of the viscosity resulted rather from the much higher molecular weights of β-glucans extracted from the treated versus untreated samples, as evidenced by the profiles obtained from size exclusion chromatography (Figure 4.6). These results indicate, therefore, that although hydrothermal treatments did not significantly affect the solubility of β-glucans, they had a positive effect on the molecular weight of β-glucans. This improvement was likely due to inhibition of β-glucanase activity and subsequent prevention of polymer degradation. The elution pattern of β-glucans from the untreated Falcon barley, with very high activity of β-glucanase, indicated the presence of a polysaccharide population with relatively small molecular weights. The autoclaved samples, on the other hand, which exhibited a definite shift towards higher molecular weights, had no β-glucanase activity. In fact, no detectable β-glucanase activity was found in any of the autoclaved or steamed samples as determined by the viscosity tests utilized in our studies. These results do not agree with the findings of Knuckles and Chiu (1999), who reported some residual β-glucanase activity in autoclaved barley samples. Discrepancies might have arisen from the use of different tests

Figure 4.5. Effects of various treatments on the solubility of β-glucans in CDC Candle (a) and Falcon (b) barleys. Treatments: (1) control; (2) autoclaved, 30 min; (3) steamed, 15 min; (4) autoclaved followed by addition of esterase; (5) autoclaved followed by addition of proteinase K; (6) autoclaved followed by sonication.

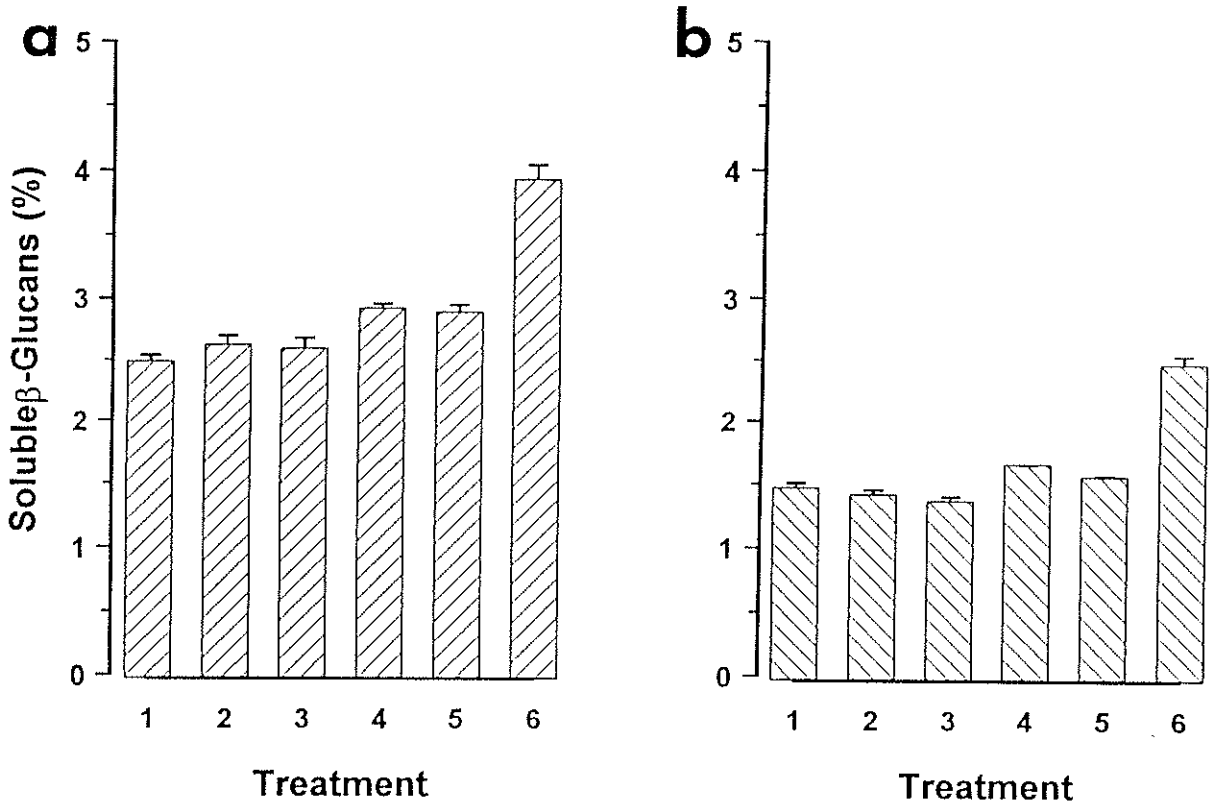
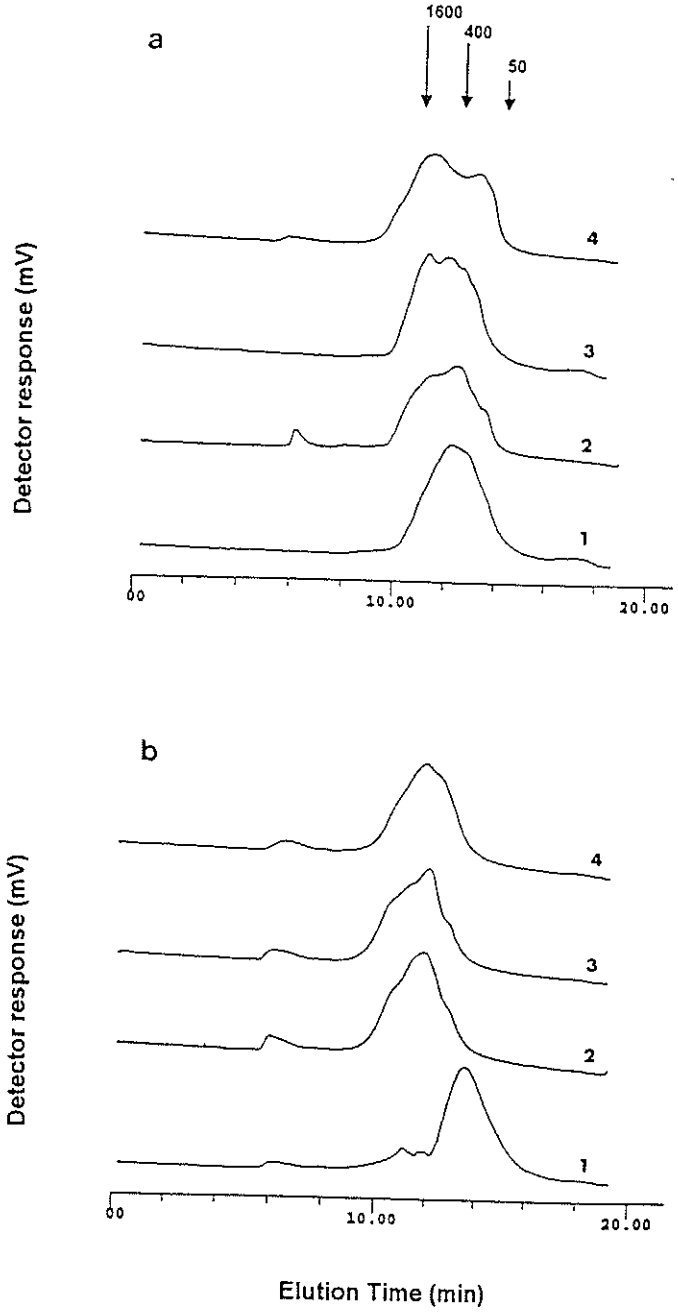

a
b
Soluble β-Glucans (%)
0
1
2
3
4
5
1
2
3
4
5
6
Treatment
Treatment

Figure 4.6. Effects of various treatments on the molecular weight of β-glucans isolated from CDC Candle (a) and Falcon (b) barley samples. (1) control; (2) autoclaved, 30 min; (3) autoclaved followed by addition of proteinase K; (4) autoclaved followed by sonication. Arrows indicate elution time of pullulan standards with various molecular weight (MW): 1600, MW 1660×10^3; 400, MW 380×10^3; and 50, MW 48×10^3.


a
1600
400
50
4
3
2
1
00
10.00
20.00
Detector response (mV)
b
4
3
2
1
00
10.00
20.00
Detector response (mV)
Elution Time (min)

to assess the activity of the enzyme in both studies, as well as from different heat treatment conditions. It appears that the presence of moisture during the heat treatments was crucial for effective inactivation of β-glucanase, as application of dry heat (roasting) did not have the same effect (Figure 4.4). It is known that heat penetration of moist air is much more rapid and occurs to a greater extent than that of dry air and might, therefore, more effectively infuse into the barley kernel, accomplishing inactivation of β-glucanase not only on the surface but also inside the kernel. Furthermore, enzymes are less heat stable in moist than in dry environments. The hydrothermal treatments of barley grains might, therefore, constitute an effective means of preventing the depolymerization of β-glucans, and should be considered as a potential pretreatment of barley before incorporation of the grain into food systems. However, various aspects and conditions of hydrothermal treatments should be investigated more thoroughly to optimize desirable effects. Hydrothermal treatments of barley for animal feed, on the other hand, should be avoided as the degradation of β-glucans in this case is desirable and might improve the feed utilization by animals. According to the study of Vukic Vranjes and Wenk (1995), a significant depression in feed efficiency, feed metabolizable energy, and fat and protein utilization resulted from feeding chickens extruded barley. The results were explained by higher amounts of soluble fiber and higher extract viscosities in heat treated versus untreated barleys. These results agree with those of the current study.

Hydrothermal treatments, aided by the presence of enzymes during extraction or by the additional physical disruption (sonication), resulted not only in improved molecular weight of β-glucans but also in increased β-glucan solubilization. The soluble β-glucan

content in the sonicated samples or in samples treated with esterase or proteinase K was significantly greater than that in the untreated, autoclaved, or steamed samples (Figure 4.5). The elution profiles of these samples also indicated the presence of an additional population of polymers, as evidenced by broader and more numerous peaks (Figure 4.6). This study has demonstrated that with the aid of appropriate enzymes and/or physical disruption of the cell wall material, it is possible to increase the pool of soluble β-glucans in the barley grain. So far, the reasons for water insolubility of β-glucans have not been fully understood. Although it has been speculated that ester linkages might be responsible for rendering a portion of β-glucans insoluble, the nature of such linkages has not yet been revealed (Bamforth et al., 1996). Also, the partial insolubility of these polymers has been attributed to the presence of non-covalent interactions between β-glucans and other cell wall components (Izydorczyk et al., 1998ab). Therefore, it is possible that the disruption of either covalent or non-covalent bonds within barley cell wall material might bring about the release and solubilization of the initially insoluble β-glucans. The improved extractability of β-glucans from barley, through proteolytic actions, also implies an association of a portion of β-glucans with proteins in the cell wall material of barley.

4.2. Summary

Large variations in the chemical and physical characteristics of a range of barley samples were found in this study. Significant differences in the content of starch, total and soluble β-glucans, β-glucanase activity, 100 kernel weight, and slurry viscosity were observed among the samples. Samples with normal starch composition had significantly higher starch but lower total β-glucan content than barley containing starches with anomalous amylose to amylopectin ratios. A relatively poor correlation between the soluble and total β-glucans ($r = 0.41$) was attributed mainly to the high amylose barley samples, which, despite the highest content of total β-glucans, exhibited very low extractability of these polymers in aqueous media. Viscosity development profiles, as measured in this study, cannot be explained by extractability and β-glucan content alone. The presence of β-glucanase in barley samples had a significant effect on the molecular weight of β-glucans in barley extracts, and thus, on viscosity development profiles.

The extractability and physical characteristics of barley β-glucans can be modified by thermal, enzymic and physical treatments of the barley grain. Hydrothermal treatments of barley had no effect on the extractability of β-glucans but prevented their fragmentation due to enzymic hydrolysis, thereby substantially improving their molecular weight and consequently, their viscosity. These treatments, therefore, might have a potential to positively affect the physiological responses to barley β-glucans in human diets. The enzymic and physical treatments were, on the other hand, more important in accounting for the increased extractability of β-glucans. Moreover, autoclaving followed by sonication proved to be the most effective of the pretreatment methods examined, as it resulted in the

greatest extractability and molecular weight of β-glucans. Considering that it is soluble dietary fiber that can induce the desirable physiological effects in humans, it is essential to investigate, in greater detail, potential strategies for improving the ratio of soluble to insoluble dietary fiber in barley, as well as the quality of the polymers themselves.

5. PART TWO:

Structure and Physico-Chemical Properties of β-Glucans and Arabinoxylans Isolated from Hull-less Barley

5.1. Results and Discussion

5.1.1. Yield

Five hemicellulosic fractions were obtained through sequential extraction of the barley meal. Consequently, after the removal of material soluble in water at 45°C, the barley residue was subjected to water extraction at 95°C; this was followed by extraction with $Ba(OH)_2$, water, and finally, NaOH. These extractions resulted in five fractions designated as WE45, WE95, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH. By performing the extractions sequentially, non-starch polysaccharides from each barley cultivar were separated based on their differential solubility, beginning with the water soluble fractions, progressing to the least soluble fractions, eventually requiring NaOH for solubilization. The yields of the purified water- and alkali-soluble fractions are given in Table 5.1.

Substantially large differences in the yields of WE45 and WE95 were observed. The percent yields of WE45 fractions (1.58 to 5.79%) were greater than those of WE95 (0.49 to 2.09%), a possible indication that the majority of water soluble non-starch polysaccharides can be extracted at 45°C. Furthermore, WE45 exhibited the highest average percent yield of the five fractions, and WE95, the lowest. These results contrast with those reported by Izydorczyk et al. (1998a) for malting barley, having achieved similar yields between fractions obtained through extraction with water at 40°C and 65°C (1.4% and 1.3%), and Saulnier et al.(1994), who reported a much higher yield for polysaccharides extracted at 90°C (12.9% compared to 1.65% extracted at 40°C). One explanation for the differences is that the NSP of hull-less barley may be more soluble than in malting barley, and thus, a greater proportion would be extracted at lower temperatures. It is also possible

Table 5.1. Yield[1] (%) of water- and alkali-extractable fractions obtained from various barley samples.

BARLEY TYPE/VARIETY	FRACTION					TOTAL
	WE45	WE95	$Ba(OH)_2$	$Ba(OH)_2/H_2O$	NaOH	
	%	%	%	%	%	%
Normal						
Falcon	1.58	0.49	3.05	1.89	4.02	**11.03**
CDC Dawn	1.73	1.37	3.89	2.20	1.70	**10.89**
High Amylose						
92-55-06-54	3.45	2.09	4.60	2.12	2.59	**14.85**
92-55-06-48	5.79	1.83	3.46	2.26	2.39	**15.73**
Waxy						
CDC Candle	4.59	1.20	3.39	1.10	1.83	**12.11**
SR93139	5.09	1.67	4.18	1.64	1.56	**14.14**
Zero Amylose Waxy						
SB94792	4.69	1.03	4.31	1.82	1.51	**13.36**
CDC Alamo	4.85	1.55	2.83	1.21	1.27	**11.71**

[1] w/w, based on barley meal used for extraction

that some losses of WE95 may have occurred in the extensive purification required for these fractions; an ethanol precipitation step was necessary to remove most of the partially degraded starch that was solubilized at the higher temperature (95 °C) and extracted along with the non-starch polysaccharides.

A wide range in the yield of each of the five fractions was observed. This was especially true in WE45, with yields ranging from 1.58% to 5.79%. Among the WE45 fractions, normal barley samples had substantially lower yields (1.58% to 1.73%) compared to high amylose, waxy, and zero amylose waxy types (3.45% to 5.79%). The differences in the yields of WE45 fractions correspond well with the differences in the content of soluble β-glucan in the same eight cultivars; waxy and zero amylose waxy samples had the highest proportion of soluble β-glucan, followed by high amylose samples 92-55-06-54 and 92-55-06-48, all of which contained significantly more soluble β-glucan than normal varieties ($p \leq 0.05$) (Table 4.1). However, the percent yield of WE45 fractions, with the exception of Falcon, was greater than the soluble β-glucan content. A partial explanation for this difference is that the extracted and purified fractions, in addition to β-glucan, may also contain some protein, arabinoxylan, and traces of α-glucan or other minor non-starch polysaccharides. Another factor for consideration is that soluble β-glucan content was measured using an extraction temperature of only 25 °C compared to 45 °C used in this study; it is possible that, particularly in the case of high amylose, waxy, and zero amylose waxy varieties, more β-glucans were extracted at 45 °C. Also, the increase in β-glucan extractability in water between 25 °C and 45 °C may have been greater than the increase in extractability between 45 °C and 95 °C, since the yield of the 45 fractions was greater. Like

WE45, the yields of WE95 fractions varied, particularly between Falcon (0.49%), and the remaining samples (1.03% to 2.09%). The fact that Falcon yielded the least water soluble material (WE45 and WE95) is not surprising, since it was found to have the lowest content of both total and soluble β-glucan (Table 4.1) of the eight barley cultivars used for the extractions.

Varietal differences in the yield of alkali-extractable fractions ($Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH) were not as distinct as for WE45 and WE95. In $Ba(OH)_2$, no trend between barley starch type and yield was evident. Among $Ba(OH)_2/H_2O$ fractions, however, a relationship between starch type and yield was observed; normal and high amylose varieties produced higher yields of $Ba(OH)_2/H_2O$-extractable material than waxy and zero amylose types. The wide range in yield among NaOH fractions was mainly due to normal barley Falcon, which gave a much greater yield (4.02%) in contrast to the remaining varieties (1.51% to 2.59%).

It is important to stress that the yield of the various fractions presented in Table 5.1 should not be considered equivalent to the yield of NSP. Further compositional analysis was required to determine the amount of NSP in each fraction and is discussed in section 5.1.2.

Total yields of water- and alkali-extractable material from each of the eight barley cultivars are also listed in Table 5.1. The largest total yields were observed in high amylose varieties 92-55-06-54 and 92-55-06-48, which produced 14.85% and 15.73% total extractable material, respectively. Waxy and zero amylose waxy samples had slightly lower yields (11.71% to 14.14%), while the lowest total yields occurred in normal barleys. This

trend is similar to that of total β-glucan content (Table 4.1); total β-glucan levels were lowest in normal, significantly higher in waxy and zero amylose waxy, and greatest in high amylose varieties ($p \leq 0.05$). One might expect, however, based on the results of total β-glucan measurements (Table 4.1), that the total yield of NSP in normal barley cultivars should be only 50% rather than the observed 80% of the total yield obtained from cultivars of the other three barley types. This incongruity may be partially accounted for by the fact that arabinoxylans, as well as some proteins and starch, were extracted along with β-glucans, contributing to total yield.

The relative distributions of the two water- and combined alkali-extractable fractions within the total yield for each barley cultivar varied (Figure 5.1). Greatest differences in the proportions of water- and alkali-extractable fractions were between normal varieties and those with anomalous starch characteristics. For instance, in normal varieties, WE45 and WE95 together made up approximately 20 to 30% of the total extractable material, whereas in high amylose, waxy and zero amylose waxy lines, the relative amount of WE45 and WE95 increased markedly (Figure 5.1). It can therefore be stated that normal barley samples contained a smaller proportion of water-extractable material (under the extraction conditions of this study) compared to high amylose, waxy, and zero amylose waxy genotypes. The variation in the total amount of water-extractable material was due primarily to variation in the proportion of WE45, since this fraction was substantially larger than WE95 for all eight cultivars.

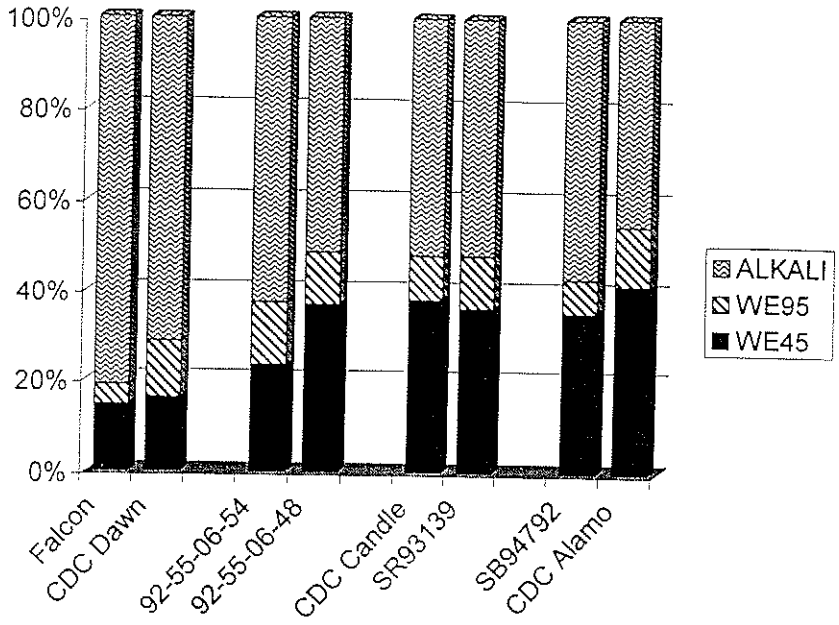


Figure 5.1. Relative distributions of the two water-extractable (WE45, WE95), and combined alkali-extractable ($Ba(OH)_2$, $Ba(OH)_2/H_2O$, NaOH) fractions within the total material recovered from each barley sample.

5.1.2. Composition

5.1.2.1. Protein Content. In spite of the purification techniques employed, protein was detected in all fractions, with the amounts depending largely on the fraction. The protein contents of fractions extracted with water and alkali are listed in Table 5.2. Average protein contents in fractions WE45 and WE95 were 2.90 and 3.85%, while $Ba(OH)_2$, $Ba(OH)_2/H_2O$ and NaOH contained an average of 29.68, 5.05, and 29.35% protein, respectively.

Protein content among samples within water-extractable fractions (WE45 and WE95) exhibited some variation, but generally remained below 5%. Levels in WE45 ranged from 1.79% to 4.65%, and in WE95, from 2.30% to 6.55%.

Table 5.2. Protein content (% w/w) in water- and alkali-extractable fractions obtained from various barley samples.

BARLEY TYPE/VARIETY	FRACTION				
	WE45[1]	WE95	$Ba(OH)_2$	$Ba(OH)_2/H_2O$	NaOH
	%	%	%	%	%
Normal					
Falcon	3.86 ± .06	2.98 ± .21	30.02 ± .43	5.14 ± .02	30.93 ± .03
CDC Dawn	3.20 ± .09	4.83 ± .06	30.34 ± .27	4.76 ± .06	26.43 ± .95
High Amylose					
92-55-06-54	4.65 ± .02	4.40 ± .08	27.49 ± .10	6.34 ± .03	30.71 ± .31
92-55-06-48	1.79 ± .02	6.55 ± .56	22.92 ± .49	3.30 ± .18	25.43 ± .52
Waxy					
CDC Candle	2.47 ± .01	2.74 ± .03	32.81 ± .33	5.09 ± .19	35.53 ± .16
SR93139	2.47 ± .02	4.04 ± .00	33.17 ± .53	5.51 ± .00	24.16 ± .08
Zero Amylose Waxy					
SB94792	2.92 ± .11	2.30 ± .03	33.62 ± .35	4.70 ± .26	31.44 ± .35
CDC Alamo	1.81 ± .01	2.99 ± .08	27.08 ± .52	5.52 ± .38	29.33 ± .21

[1] n = 2 ± SD

The material in $Ba(OH)_2/H_2O$ fractions consisted of 5.05% protein on average, slightly higher than the average protein content in WE45 and WE95. Less overall variation was observed here; minimum and maximum levels were 4.70 and 6.34%, respectively.

Protein content in $Ba(OH)_2$ and NaOH fractions was considerably greater than in the other fractions, with amounts ranging from 22.92% to 35.53%. The higher yield of the alkali-extractable fractions relative to the water-extractable ones may therefore be due in part to the increased protein content of $Ba(OH)_2$ and NaOH fractions (Table 5.1; Figure 5.1). The elevated protein levels in the $Ba(OH)_2$ and NaOH material can be explained by the fact that, with such alkali treatment, the water insoluble hordeins become solubilized and are extracted along with the polysaccharides (MacGregor and Fincher, 1993). However, levels of protein in both water- and alkali-extractable material were notably higher than those levels reported for malting barley using similar isolation and purification procedures (Izydorczyk et al., 1998ab). The reason for such differences requires further investigation

5.1.2.2. Monosaccharide Composition. Monosaccharide analysis of the isolated fractions indicated that arabinose, xylose and glucose constituted the majority of the material (Tables 5.3 and 5.4). Small amounts of galactose and mannose were found; these sugars may indicate the presence of arabinogalactans or galactomannans as minor constituents. Carbon-13 nuclear magnetic resonance (^{13}C-NMR) analysis verified that no contaminating α-glucans, or starch remnants, were present in the fractions (results not shown), and thus any

Table 5.3. Monosaccharide composition of water-extractable fractions.

Barley Type/Variety	% MOL OF CARBOHYDRATE MATERIAL[1]				
	Ara	Xyl	Glc	Gal	Man
WE45					
Normal					
Falcon	9.71 [a 3]	11.13 [a]	76.44 [d]	2.73	nd [2]
CDC Dawn	8.70 [ab]	10.84 [a]	78.85 [c]	1.63	nd
High Amylose					
92-55-06-54	9.07 [a]	11.79 [a]	76.02 [d]	1.65	1.47
92-55-06-48	7.96 [b]	12.22 [a]	79.18 [c]	nd	1.29
Waxy					
CDC Candle	5.55 [cd]	8.41 [b]	84.34 [b]	1.05	0.67
SR93139	5.80 [c]	8.86 [b]	85.35 [b]	nd	nd
Zero Amylose Waxy					
SB94792	4.98 [cd]	6.47 [c]	88.56 [a]	nd	nd
CDC Alamo	4.54 [d]	6.11 [c]	89.36 [a]	nd	nd
Average	**7.04 [D]**	**9.47 [D]**	**82.26 [B]**		
WE95					
Normal					
Falcon	6.44 [a]	9.13 [b]	82.75 [e]	nd	1.69
CDC Dawn	4.13 [d]	5.73 [d]	89.41 [c]	nd	nd
High Amylose					
92-55-06-54	5.56 [b]	8.11 [c]	84.85 [d]	nd	1.48
92-55-06-48	6.43 [a]	10.44 [a]	81.27 [e]	nd	1.88
Waxy					
CDC Candle	2.72 [e]	4.01 [e]	93.28 [a]	nd	nd
SR93139	5.12 [c]	8.74 [b]	86.15 [d]	nd	nd
Zero Amylose Waxy					
SB94792	2.88 [e]	4.52 [e]	92.60 [ab]	nd	nd
CDC Alamo	3.00 [e]	4.44 [e]	91.24 [b]	nd	1.34
Average	**4.53 [E]**	**6.89 [E]**	**87.69 [A]**		

[1] CV<5.0; [2] nd = not detected; [3] Means with different letters (column) are significantly different ($p<0.05$); capital letters = differences among fractions, small letters = differences among varieties within each fraction.

Table 5.4. Monosaccharide composition of alkali-extractable fractions.

Barley Type/Variety	% MOL OF CARBOHYDRATE MATERIAL[1]				
	Ara	Xyl	Glc	Gal	Man
$Ba(OH)_2$					
Normal					
Falcon	36.56 [c,4]	60.34 [c]	2.11 [a]	1.00	nd [2]
CDC Dawn	36.06 [c]	62.74 [b]	1.21 [b]	nd	nd
High Amylose					
92-55-06-54	35.85 [c]	63.20 [b]	0.95 [bc]	nd	nd
92-55-06-48	34.12 [d]	65.59 [a]	0.58 [cd]	nd	nd
Waxy					
CDC Candle	38.38 [ab]	61.13 [c]		0.99	nd
SR93139	37.57 [b]	61.09 [c]	0.74 [bcd]	0.97	nd
Zero Amylose Waxy					
SB94792	38.98 [a]	60.18 [c]	0.43 [bcd]	0.83	nd
CDC Alamo	37.62 [b]	60.90 [c]	0.74 [bcd]	0.74	nd
Average	**36.89** [A]	**61.90** [A]	**0.76** [E]		
$Ba(OH)_2/H_2O$					
Normal					
Falcon	27.32 [b]	34.61 [b]	36.17 [d]	2.08	nd
CDC Dawn	27.77 [b]	34.64 [b]	35.57 [d]	2.02	nd
High Amylose					
92-55-06-54	32.18 [a]	37.47 [a]	27.48 [e]	2.03	0.85
92-55-06-48	25.56 [c]	30.77 [c]	42.19 [b]	1.56	nd
Waxy					
CDC Candle	27.68 [b]	31.40 [c]	39.10 [c]	1.83	nd
SR93139	22.19 [d]	31.63 [c]	45.03 [a]	1.16	nd
Zero Amylose Waxy					
SB94792	25.21 [c]	29.76 [c]	42.97 [b]	1.91	tr [3]
CDC Alamo	25.86 [c]	31.60 [c]	40.96 [bc]	1.59	nd
Average	**26.72** [B]	**32.72** [B]	**38.65** [D]		
NaOH					
Normal					
Falcon	19.70 [b]	32.78 [b]	42.05 [g]	2.06	3.42
CDC Dawn	14.44 [e]	22.53 [f]	59.60 [b]	1.16	2.29
High Amylose					
92-55-06-54	12.66 [f]	19.98 [g]	63.85 [a]	1.24	2.28
92-55-06-48	20.90 [a]	34.53 [a]	42.77 [g]	1.42	0.79
Waxy					
CDC Candle	16.56 [d]	25.51 [d]	54.47 [d]	1.62	1.85
SR93139	17.37 [c]	31.93 [c]	48.42 [f]	1.44	0.85
Zero Amylose Waxy					
SB94792	16.20 [d]	24.54 [e]	55.49 [c]	1.87	1.91
CDC Alamo	16.68 [d]	26.18 [d]	53.17 [e]	1.76	2.21
Average	**16.81** [C]	**27.24** [C]	**52.48** [C]		

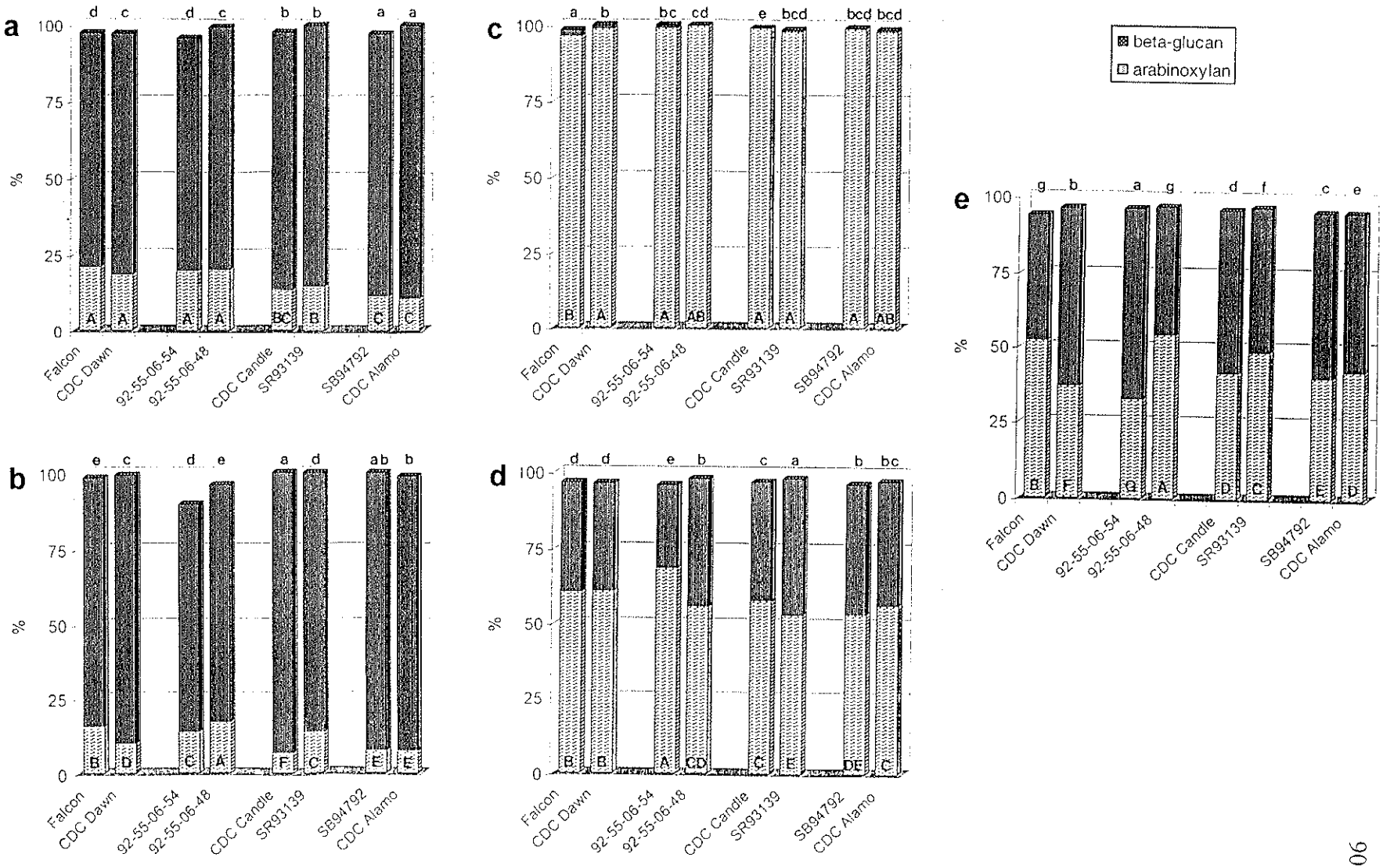
[1] CV<5.0; [2] nd = not detected; [3] tr = trace (< 0.5 %); [4] Means with different letters (column) are significantly different ($p<0.05$); capital letters = differences among fractions, small letters = differences among varieties within each fraction.

glucose detected was assumed to have originated from β-glucans. Therefore, it was concluded that arabinoxylans and β-glucans were the major non-starch polysaccharides present in the fractions.

Compositional differences among the fractions (WE45, WE95, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH) were noted. Fractions WE45 and WE95 consisted mainly of β-glucans, as was evidenced by the large amount of glucose in these fractions; a small percentage of arabinoxylans (arabinose and xylose) was also detected (Table 5.3). In contrast to WE45 and WE95, carbohydrate material obtained via extraction with saturated $Ba(OH)_2$ consisted almost entirely of arabinose and xylose; arabinoxylans comprised an average of 98.47% of the total carbohydrate content in the $Ba(OH)_2$ fraction (Table 5.4). Such observations point to the specificity of saturated $Ba(OH)_2$ solution for arabinoxylans, in agreement with the findings of Gruppen et al. (1991). $Ba(OH)_2/H_2O$ and NaOH extractable material consisted of a more heterogeneous mixture of β-glucans and arabinoxylans in comparison to the previous three fractions (Table 5.4).

Using the results obtained from monosaccharide analysis, the distribution of β-glucans and arabinoxylans and the ratio of xylose to arabinose in each sample were calculated. Corrections were made for arabinose in samples containing galactose, assuming that some arabinose is associated with galactose in the form of arabinogalactans (arabinose-to-galactose ratio of 0.7) (Dervilly et al., 2000; Loosveld et al., 1997), and that if mannose is present, a portion of galactose is also involved in galactomannans (galactose-to-mannose ratio = 0.35) (Izydorczyk and Biliaderis, 1996). Figure 5.2 (ab) shows the distribution of β-glucans and arabinoxylans in WE45 and WE95. WE45 contained an average of 15.91%

Figure 5.2. Distribution of β-glucans and arabinoxylans in WE45 (a), WE95 (b), $Ba(OH)_2$ (c), $Ba(OH)_2/H_2O$ (d), NaOH (e) fractions. Small letters (upper) = differences in β-glucan content between varieties; capital letters (lower) = differences in arabinoxylan content between varieties.


a
b
c
d
e
%
100
75
50
25
0
beta-glucan
arabinoxylan
Falcon
CDC Dawn
92-55-06-54
92-55-06-48
CDC Candle
SR93139
SB94792
CDC Alamo

arabinoxylan, whereas WE95 consisted of only 11.42%; this difference was found to be significant at $p \leq 0.05$. On the other hand, β-glucans comprised an average of 87.69% of the carbohydrate material in WE95, compared to only 82.26% of carbohydrates in WE45. When protein content was taken into account, WE95 fractions were found to be significantly purer than all other extracted fractions in this study. The ethanol precipitation step in the preparation of the WE95 fraction may have contributed to its high purity.

Some differences ($p \leq 0.05$) in the relative proportion of β-glucans and arabinoxylans among varieties within water-extractable fractions were observed (Figure 5.2). In WE45, the greatest proportion of β-glucan occurred in zero amylose varieties (88.56 to 89.36%)followed by waxy samples (84.34 to 85.35%). Normal and high amylose varieties contained significantly less β-glucan (76.44 to 79.18%) and larger proportions of arabinoxylan (18.39 to 20.90%). Within WE95 fractions, unlike their WE45 counterparts, significant inter-varietal differences in β-glucan ($p \leq 0.05$) were present; β-glucan contents of the two zero amylose waxy lines, SB94792 and CDC Alamo (92.60% and 91.24%, respectively), however, were not significantly different. Similar to WE45, WE95 fractions from zero amylose waxy varieties contained the largest amount of β-glucan.

Although not as prominent, some differences in monosaccharide composition were observed among fractions extracted with saturated $Ba(OH)_2$ (Table 5.4). Material isolated from Falcon contained significantly less arabinoxylan (96.2%), and more β-glucan (2.11%) than the other genotypes. Among $Ba(OH)_2$ fractions, those from normal barley had higher levels of β-glucan than from high amylose, waxy, and zero amylose barley samples ($p \leq 0.05$).

Inter-varietal differences in β-glucan and arabinoxylan levels within $Ba(OH)_2/H_2O$ and NaOH fractions occurred, but these did not correlate with barley starch characteristics. Interestingly, however, 92-55-06-54, containing the lowest β-glucan level among the $Ba(OH)_2$ /H_2O fractions, possessed the greatest amount among all NaOH fractions (63.85%). As the NaOH fractions constitute the most insoluble material, it could be deduced that a relatively large portion of the β-glucans in this high amylose barley has a low degree of solubility.

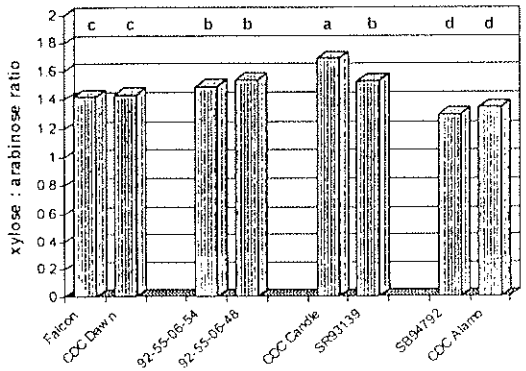
The composition of these five fractions was similar to the composition of fractions isolated from malting barley by Izydorczyk and coworkers (1998ab), using the same general extraction procedure. They found the water-extractable (40°C) material to consist of 82.5% glucose in the form of β-glucans, and 15.9% arabinose and xylose (arabinoxylans); water extraction performed at 65°C in this same study yielded material containing a higher proportion of β-glucan (93.3%) and less arabinoxylan (5.8%). The distributions of β-glucans and arabinoxylans in alkali-extractable fractions from malting barley were also similar to those of hull-less barley, although the NaOH fraction contained slightly less β-glucan (40.9%) and more arabinoxylan (58.2%) than the hull-less varieties. Therefore, the current findings suggest that the relative proportions of water- and alkali-extractable β-glucans and arabinoxylans do not differ significantly between hull-less and malting barley.

One structural characteristic that can be determined from monosaccharide composition is the degree of substitution of arabinoxylans, taken from the xylose to arabinose ratio (Xyl*p*/Ara*f*). Such a parameter is important because of its influence on the shape, solubility, and molecular characteristics of the arabinoxylan polymer. When the

ratios were examined, differences among the fractions and among varieties in each fraction were found, indicating varying degrees of substitution of the xylan backbone (Figure 5.3). Overall, ratios were highest in arabinoxylans extracted with $Ba(OH)_2$ (1.70) and NaOH (1.68), i.e. these arabinoxylans were the least substituted (Figures 5.3c,e). The next highest ratios occurred in WE45 and WE95, (1.47 and 1.52, respectively), significantly lower than $Ba(OH)_2$ and NaOH but not from one another. Finally, the lowest Xyl*p*/Ara*f* ratios , indicating the most highly substituted arabinoxylans, were generally found in $Ba(OH)_2/H_2O$ fractions, with an average Xyl*p*/Ara*f* ratio of 1.29. Significant differences among varieties within each fraction were present (Figures 5.3a to 5.3e). Arabinoxylans in the WE45 fraction of CDC Candle had the highest Xyl*p*/Ara*f* ratio (1.69), markedly greater ($p \leq 0.05$) than the arabinoxylans in WE45 from the other seven varieties (1.30 to 1.54). The general trend in both WE45 and WE95 was consistent; waxy and high amylose varieties had the higher xylose to arabinose ratios, while normal and zero amylose waxy varieties contained arabinoxylans with lower xylose to arabinose ratios. In contrast, $Ba(OH)_2$-extractable arabinoxylans with the highest Xyl*p*/Ara*f* ratios were found in normal and high amylose varieties, with the lowest ratios occurring in waxy and zero amylose genotypes. Among the $Ba(OH)_2$ fractions from normal and high amylose, those from 92-55-06-48 had a significantly higher Xyl*p*/Ara*f* ratio (1.92 ; $p \leq 0.05$). In $Ba(OH)_2/H_2O$, arabinoxylans from waxy sample SR93139 had the highest Xyl*p*/Ara*f* ratio, 1.48, while those of other barley cultivars had higher substitutions levels, reflected by lower Xyl*p*/Ara*f* (1.21 to 1.34). Finally, the NaOH fractions consisted of arabinoxylans with high Xyl*p*/Ara*f* ratios, similar to $Ba(OH)_2$; ratios ranged from 1.59 for CDC Dawn to 1.94 for SR93139. The NaOH

Figure 5.3. Ratio of xylose to arabinose in arabinoxylans from WE45 (a), WE95 (b), $Ba(OH)_2$ (c), $Ba(OH)_2/H_2O$ (d), NaOH (e) fractions. Means with different letters are significantly different ($p \leq 0.05$).

a


xylose : arabinose ratio
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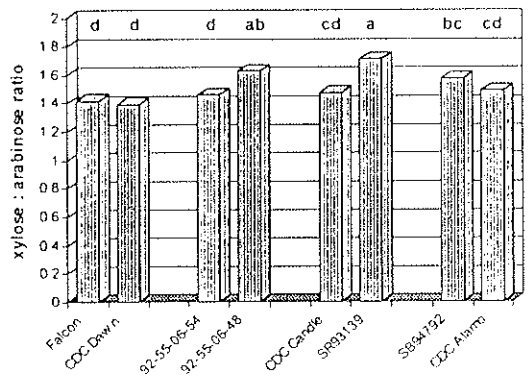
CDC Dawn
92-55-06-54
92-55-06-48
CDC Candle
SR93139
SB94792
CDC Alamo

b


xylose : arabinose ratio
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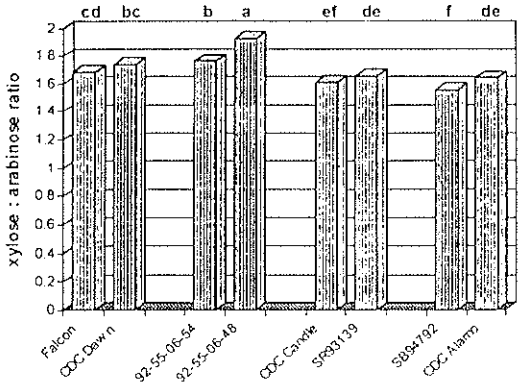
SB94792
CDC Alamo

c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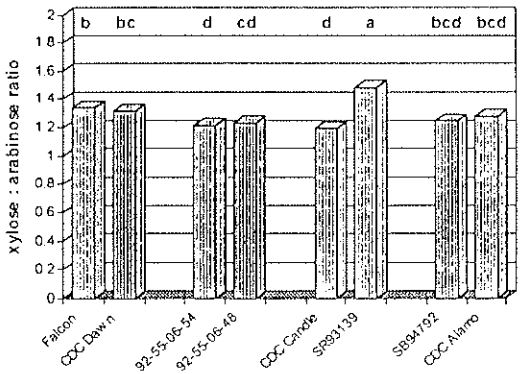
SB94792
CDC Alamo

d


xylose : arabinose ratio
b bc d cd d a bcd bcd
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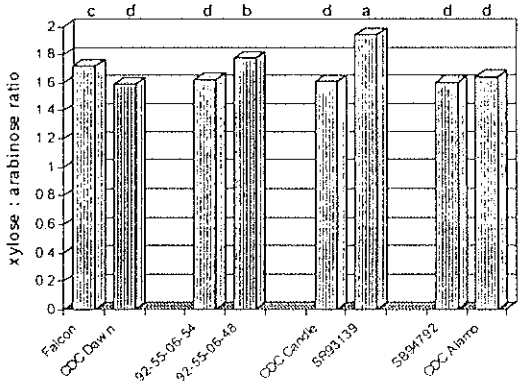
92-55-06-48
CDC Candle
SR93139
SB94792
CDC Alamo

e


xylose : arabinose ratio
c d d b d a d d
Falcon
CDC Dawn
92-55-06-54
92-55-06-48
CDC Candle
SR93139
SB94792
CDC Alamo

fraction obtained from SR93139 contained arabinoxylans with the highest Xyl*p*/Ara*f*, or the lowest degree of substitution, of all arabinoxylans analyzed.

5.1.2.3. Ferulic Acid Content. WE45 and WE95 were found to contain ferulic acid in both the *cis* and *trans* configuration (Table 5.5). Only traces of caffeic, *p*-coumaric, and sinapic acids were found in some samples (Figure 5.4). Ferulic acid content was calculated based on the amount of arabinoxylan in each isolate, and expressed in mg ferulic acid per g arabinoxylan. Ferulic acid associated with arabinoxylans has been considered to be responsible for the unique ability of arabinoxylans to form three-dimensional networks, either gels or viscous solutions (Izydorczyk and Billiaderis, 1995). The presence and relative amounts of ferulic acid have been thought to affect the shape and solubility of the arabinoxylan chain (Pomeranz, 1988). It is interesting to discover that these water-soluble arabinoxylans contain substantial amounts of ferulic acid, since this gives them the potential to undergo gelation through oxidative coupling in the presence of free-radical generating agents (H_2O_2/peroxidase, ferric chloride, ammonium persulphate, or laccase) (Geissmann and Neukom, 1973; Izydorczyk et al., 1990). Arabinoxylans in the $Ba(OH)_2$ fractions were also studied for ferulic acid content, but were found to contain only trace amounts (results not shown); it is likely that due to the harsh alkali treatment, ferulic acid was stripped away and lost during extraction and subsequent dialysis.

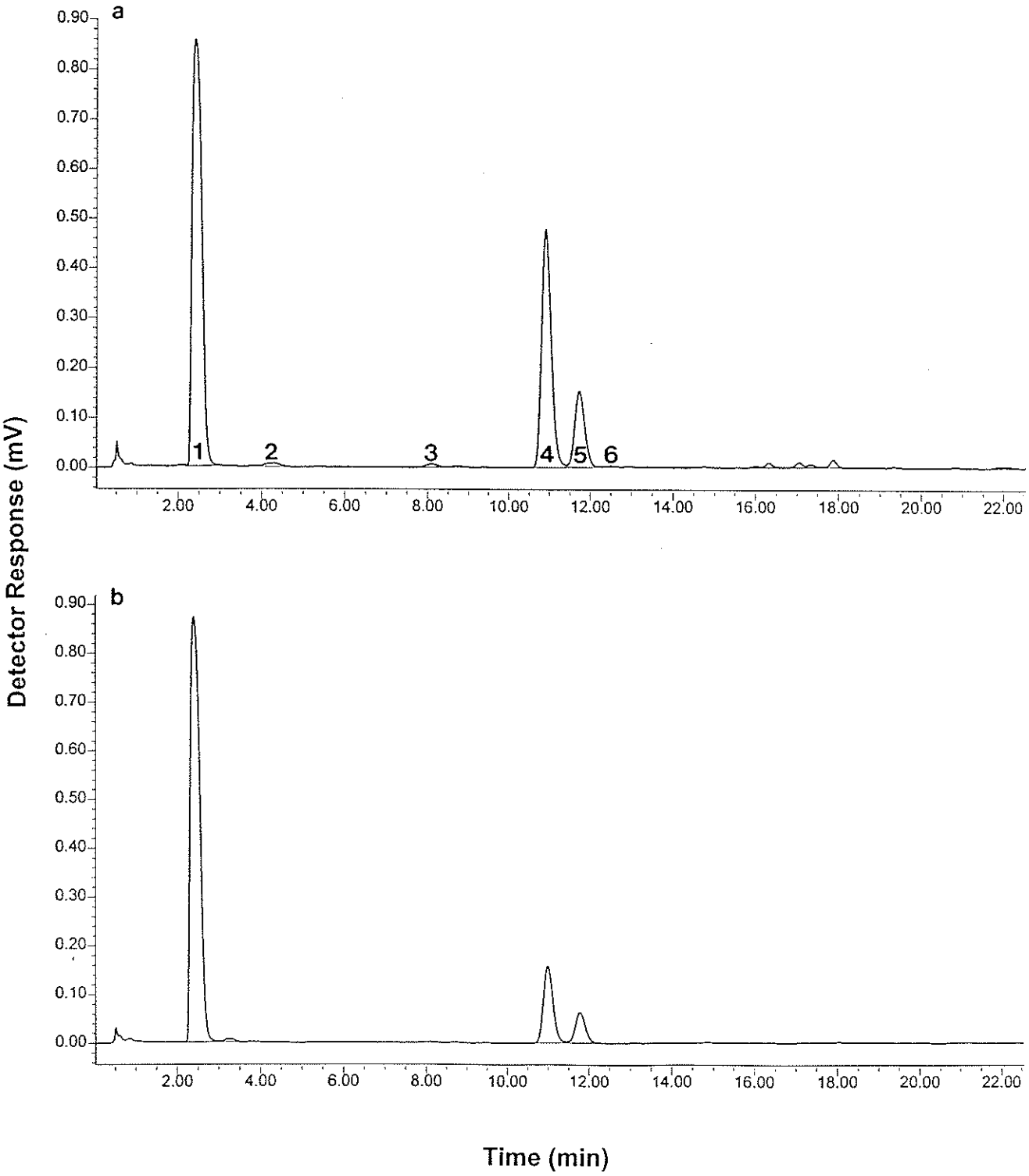
Generally, arabinoxylans from high amylose barley samples in both WE45 and WE95 contained the lowest levels of ferulic acid (5.3mg/g arabinoxylan on average), while those from normal varieties in WE45 and WE95 were richest in this compound (an average of 7.9 mg/g; Table 5.5). Significantly less total ferulic acid was observed in WE95 (Table

Table 5.5. Ferulic acid content in WE45 and WE95 fractions obtained from various barley samples.

BARLEY TYPE/VARIETY	WE45[1]			WE95		
	trans mg/g[2]	*cis* mg/g	**total mg/g**	*trans* mg/g	*cis* mg/g	**total mg/g**
Normal						
Falcon	4.49 ± .05	2.86 ± .24	**7.36** [c 3]	3.66 ± .22	2.13 ± .09	**5.79** [b]
CDC Dawn	6.85 ± .08	3.43 ± .24	**10.28** [a]	5.06 ± .08	3.07 ± .11	**8.13** [a]
High Amylose						
92-55-06-54	4.70 ± .07	2.14 ± .17	**6.84** [d]	2.66 ± .05	1.40 ± .10	**4.06** [d]
92-55-06-48	3.99 ± .00	1.95 ± .02	**5.93** [f]	3.01 ± .08	1.62 ± .16	**4.63** [c]
Waxy						
CDC Candle	4.15 ± .01	2.35 ± .02	**6.49** [e]	3.12 ± .00	2.58 ± .10	**5.70** [b]
SR93139	4.80 ± .03	2.24 ± .03	**7.04** [d]	2.06 ± .01	1.93 ± .02	**3.99** [d]
Zero Amylose Waxy						
SB94792	5.86 ± .05	3.12 ± .04	**8.97** [b]	3.45 ± .15	2.11 ± .01	**5.56** [b]
CDC Alamo	5.21 ± .26	3.45 ± .15	**8.66** [b]	3.17 ± .11	2.38 ± .11	**5.56** [b]
Average	5.01 ± .07	2.69 ± .11	**7.70** [A]	3.27 ± .09	2.15 ± .08	**5.43** [B]

[1] n ≥ 2 ± SD
[2] mg ferulic acid per g arabinoxylan in each fraction
[3] Means with different letters are significantly different ($p<0.05$); capital letters = differences among fractions, small letters = differences among varieties within each fraction.

Figure 5.4. High-performance liquid chromatographic (HPLC) profiles of phenolic acids extracted from WE45 (a), WE95 (b) of CDC Dawn. 1 = *p*-hydroxybenzoic acid (internal standard); 2 = caffeic acid; 3 = *p*-coumaric acid; 4 = *trans*-ferulic acid; 5 = *cis*-ferulic acid; 6 = sinapic acid


a
b
1
2
3
4
5
6
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
Detector Response (mV)
Time (min)

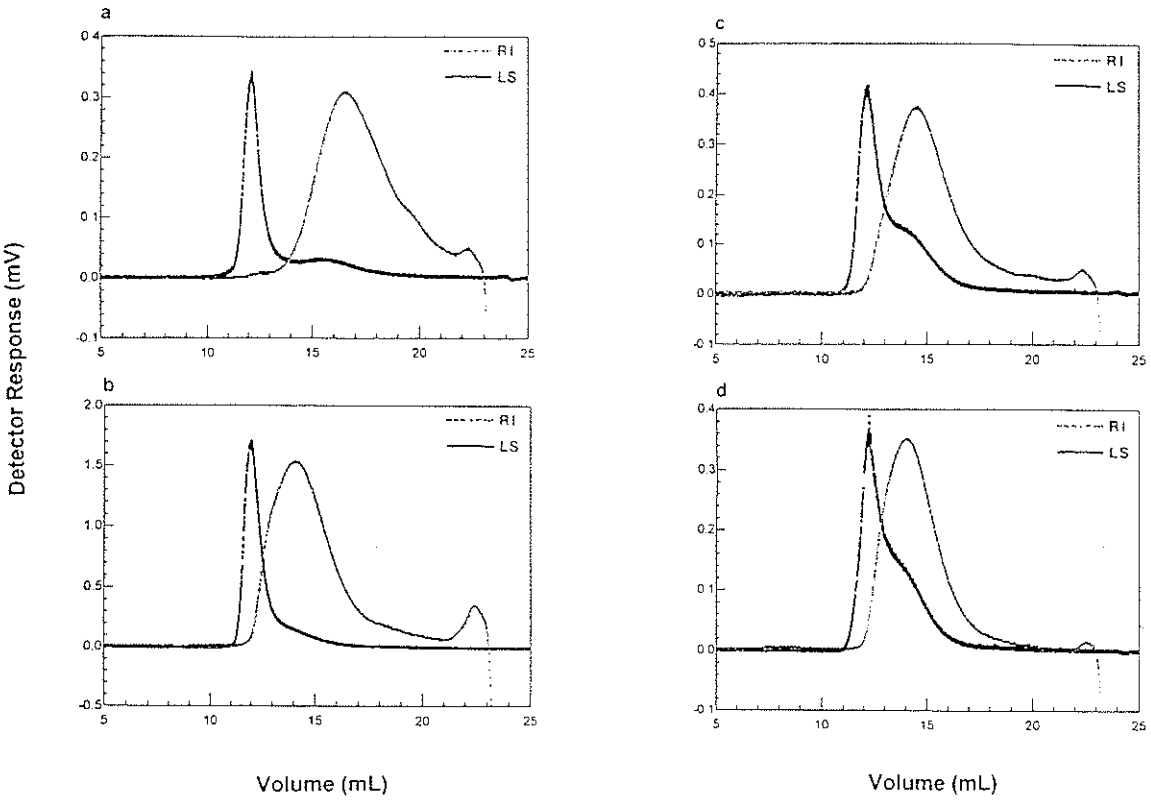
5.5). This finding implies that the lower solubility of arabinoxylans in WE95 is not associated with high ferulic acid content. Xylose to arabinose ratios in arabinoxylans were also not significantly different between WE45 and WE95. Other factors such as the substitution pattern of these arabinoxylans, or the ratio of *trans* to *cis* ferulic acid, which was found to be greater for WE45 (1.89) than WE95 (1.55), may be involved.

5.1.3. Molecular Characteristics of Water-Extractable Fractions

5.1.3.1. Molecular Weight. Weight average molecular weights (M_w), root mean square radii (R_g), polydispersity (M_w/M_n), and elution patterns of water-extractable fractions (WE45 and WE95) were measured using a HPSEC-MALLS-RI system to gain further insight into the molecular characteristics of these polysaccharides. Because it was determined from the previous analyses (protein, NMR, monosaccharide composition) that most of the isolated material in WE45 and WE95 fractions consisted of β-glucan, the molecular weight measurements and elution profiles were considered largely representative of the soluble β-glucan in the eight barley cultivars studied. However, the presence of arabinoxylans, particularly in WE45, might have contributed to the average molecular weight of the fractions.

Generally, broad and asymmetric peaks (RI signal) were observed for all water-soluble fractions (Figures 5.5.1, 5.5.2). However, differences in peak shape between the various WE45 fractions were clearly visible. Peaks representative of WE45 from Falcon, 92-55-06-54, and CDC Candle were broader, implying a wider distribution of molecular weights than those of the other WE45 fractions. The principal mode of separation of the

Figure 5.5.1. High-performance size exclusion chromatography of WE45 fractions with MALLS and RI detection; Falcon (a), CDC Dawn (b), 92-55-06-54 (c), 92-55-06-48 (d), CDC Candle (e), SR93139 (f), SB94792 (g), and CDC Alamo (h). Detector response indicated on y-axis is the response of the RI signal.


a
b
c
d
RI
LS
Detector Response (mV)
Volume (mL)
Volume (mL)

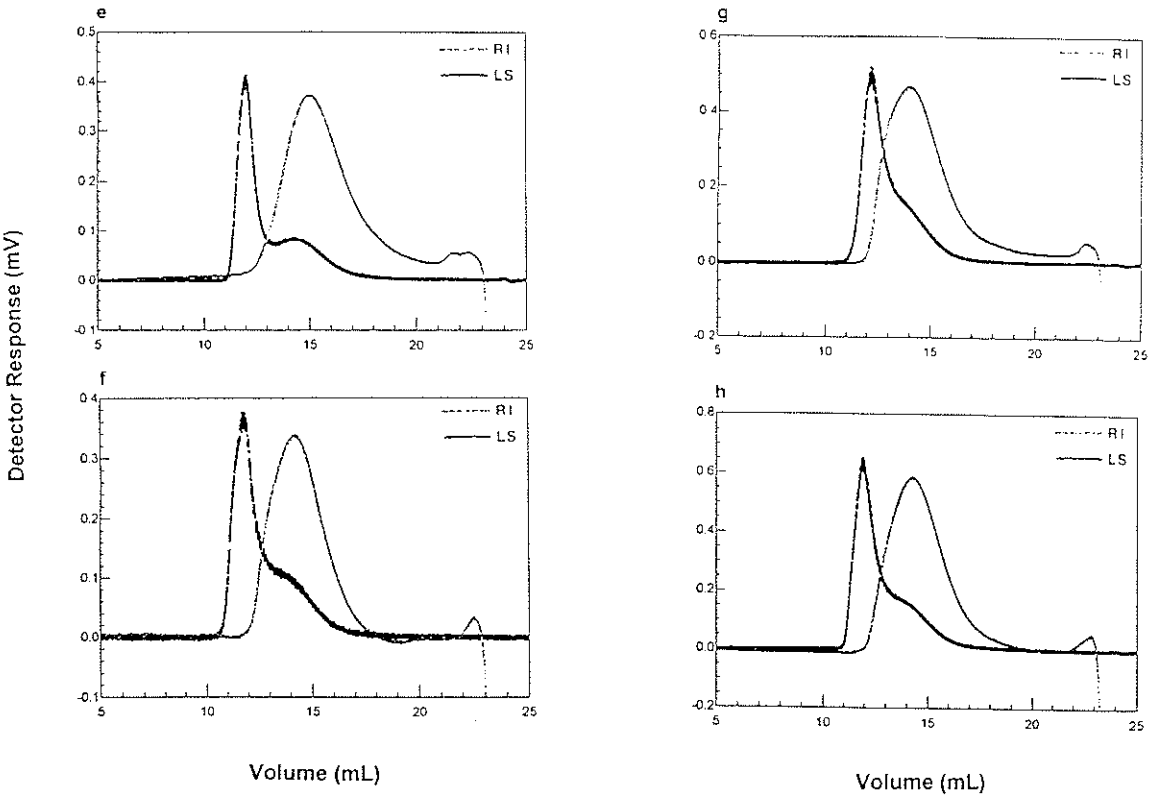
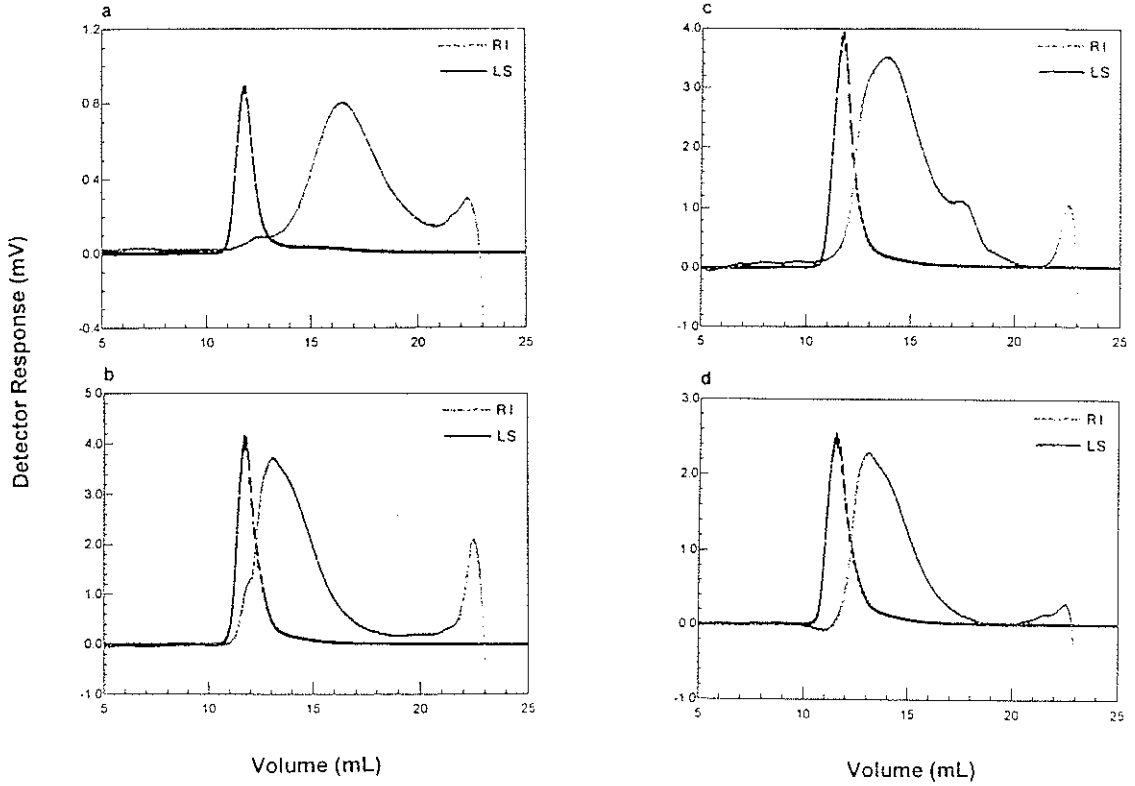
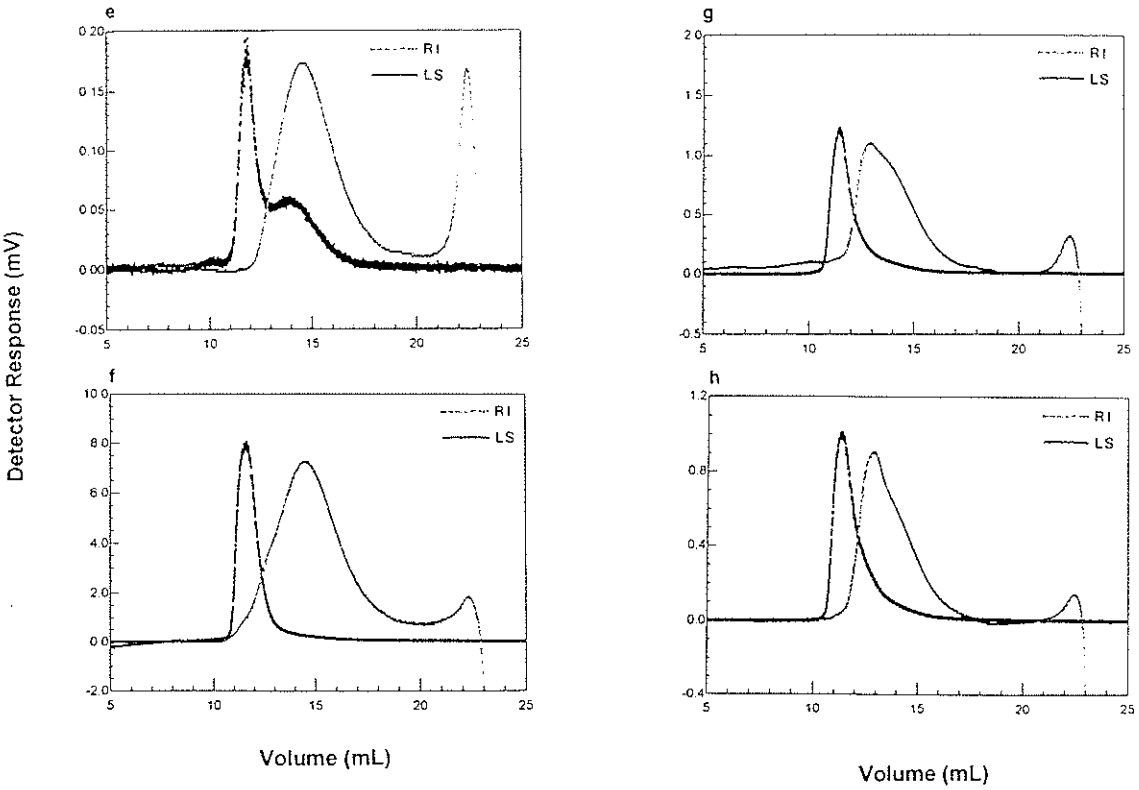

e
f
g
h
RI
LS
Detector Response (mV)
Volume (mL)
Volume (mL)

Figure 5.5.2. High-performance size exclusion chromatography of WE95 fractions with MALLS and RI detection; Falcon (a), CDC Dawn (b), 92-55-06-54 (c), 92-55-06-48 (d), CDC Candle (e), SR93139 (f), SB94792 (g), and CDC Alamo (h). Detector response indicated on y-axis is the response of the RI signal.


a
b
c
d
RI
LS
Detector Response (mV)
Volume (mL)
Volume (mL)


e
f
g
h
RI
LS
Detector Response (mV)
Volume (mL)
Volume (mL)

polymers on the column was size exclusion, meaning that the larger molecules elute sooner on the chromatogram, at a smaller elution volume. The most notable differences in elution volume among WE45 fractions was observed between that of Falcon and the other samples; this population (WE45 from Falcon) emerged later in the chromatogram, at an elution volume of 16.5 mL (from peak top), compared to approximately 14 mL for the other WE45 fractions.

Although some differences in elution volume were observed, they were generally small. The RI profile, however, is only a crude means of acquiring information on molecular weight, as two molecules with very different molecular weights may elute together if differences in their conformation allow them to have similar root mean square radii. Thus, the small differences in elution volumes observed in Figures 5.5.1 and 5.5.2 a to e are not necessarily indicative of small differences in molecular weight; light scattering is a more accurate means of molecular weight measurement and was therefore used to compare the average molecular weight of each fraction, while size exclusion chromatography was used for separation of the polymers in order to determine their distribution.

Despite the seemingly small differences in elution volume, an overall comparison of weight average molecular weights (M_w) between WE45 and WE95 indicated significant differences (Table 5.6); the average M_w in WE95, 4.29×10^6, was more than three times that of WE45 (1.29×10^6). These findings are contrary to those of Knuckles et al. (1997), who obtained higher molecular weights in barley β-glucans extracted with water at room temperature (23 °C) than in those extracted with water at 100 °C. However, this was likely

Table 5.6. Molecular weight (M_w), root mean square radius (R_g), and polydispersity (M_w/M_n) of WE45 and WE95 fractions.

BARLEY TYPE/VARIETY	WE45			WE95		
	M_w ($\times 10^{-6}$) [1] (g/mol)	R_g [1] (nm)	M_w/M_n [1]	M_w ($\times 10^{-6}$) [1] (g/mol)	R_g [1] (nm)	M_w/M_n [1]
Normal						
Falcon	0.22 [c 2]	58.25	4.56	1.50[c]	59.0	3.70
CDC Dawn	2.37 [a]	84.95	9.57	4.70[ab]	60.5	4.90
High Amylose						
92-55-06-54	2.45 [a]	113.95	10.30	6.30[a]	56.4	4.80
92-55-06-48	2.15 [a]	101.60	1.75	5.95[a]	59.7	4.50
Waxy						
CDC Candle	0.43 [c]	57.10	4.29	0.82[c]	66.0	4.70
SR93139	0.90 [b]	74.10	2.24	4.90[ab]	80.5	7.00
Zero Amylose Waxy						
SB94792	1.00 [b]	75.20	3.31	5.19[ab]	95.10	3.15
CDC Alamo	0.85 [b]	60.80	1.59	4.00[b]	96.45	2.01
Average	**1.292** [B]			**4.286**[A]		

[1] CV < 5.0 %; [2] Means with different letters are significantly different ($p < 0.05$); capital letters = differences among fractions, small letters = differences among varieties within each fraction.

a result of degradation of the polymers by β-glucanase during the extraction procedure at 100°C, as substantial β-glucanase activity was measured in these samples even after inactivation treatments (Knuckles et al., 1997). In the current study, higher molecular weights were observed for each barley variety in their WE95 fraction compared to WE45, and the magnitude of the differences varied significantly depending on the cultivar (Table 5.6). The differences in molecular weight between the two fractions seem to imply that the lower solubility of β-glucans in WE95 was somehow associated with their higher molecular weight and/or some other factor(s) influencing their molecular weight. Beer et al. (1997) did not find any evidence that the ease of extractability of β-glucans from oats was related to molecular weight, although in that study, sequential aqueous extractions were performed only at 90°C, and hence the effect of increasing extraction temperature had not been examined.

As was also the case in the findings of Knuckles and coworkers (1997), inter-varietal differences in molecular weight were observed ($p \leq 0.05$). No trend, however, between M_w and barley starch composition was apparent. Highest M_w among WE45 fractions were found in the two high amylose varieties (2.45×10^6 in 92-55-06-54, 2.15×10^6 in 92-55-06-48) and in normal barley CDC Dawn (2.37×10^6). The lowest M_w, on the other hand, occurred for material from CDC Candle and Falcon (0.43×10^6 and 0.22×10^6, respectively). The results were somewhat surprising, due to the fact that waxy sample SR93139 was previously measured as having the highest slurry viscosity of the eight cultivars, and therefore was expected to contain non-starch polysaccharides with the highest molecular weight; a similar phenomenon occurred in the other waxy variety, CDC Candle,

as its slurry viscosity was substantially higher than 92-55-06-54 and CDC Dawn (Figure 4.2) yet the molecular weight of the WE45 fraction was not. At an extraction temperature of 45 °C instead of 25 °C used in the slurry viscosity measurements, a greater proportion of higher molecular weight polymers may have become soluble in CDC Dawn, the high amylose varieties, and the zero amylose waxy types, relative to SR93139 and CDC Candle. Furthermore, the inconsistencies between slurry viscosities and the molecular weight measurements suggest that both the differences in content of soluble β-glucan and the differences in β-glucanase activity may have influenced slurry viscosity results, since these particular viscosity measurements were not adjusted for soluble β-glucan levels, and no attempts to inactivate β-glucanases were made.

All of the WE95 fractions were found to contain polymer populations of higher M_w relative to WE45; however, the magnitude of the differences in M_w among varieties was variable (Table 5.6). Hence, the relative ranking of M_w for the WE95 fractions changed slightly from WE45. High amylose variety 92-55-06-54 gave the highest M_w once again (6.30×10^6), followed by 92-55-06-48 (5.95×10^6). However, WE95 polymers from SB94792, SR93139, CDC Dawn, and CDC Alamo were also of high M_w. The extracted material from Falcon and CDC Candle had the lowest M_w of the WE95 fractions (Table 5.6).

The increases in M_w observed for the WE95 fractions compared to their WE45 counterparts were sometimes accompanied by a larger root mean square radius (R_g); in some cases, however, no increases in R_g were observed between WE45 and corresponding WE95 fractions, indicating (possibly) a more compact conformation of the latter. It is

possible also that the molecular structure of WE95 fractions predisposes them to greater intermolecular interactions resulting in a less extended conformation, despite the high molecular weight.

A somewhat higher polydispersity index was found in WE45 relative to WE95 , an indication that the polymers within each WE45 fraction generally had a wider range of molecular weights than those of WE95, and thus confirming the differences observed from the chromatograms (Table 5.6).

5.1.3.2. Linkage Composition. The ratios of β-(1→4) to β-(1→3) linkages in β-glucans in WE45 and WE95 fractions (one sample from each starch type) were calculated using the data obtained from ^{13}C-NMR (Figure 5.6), and are found in Table 5.7. Based on what has previously been reported, the resonance at ~103.9 ppm was assumed to originate from C-1 of 4-O-substituted glucose (Glc*p*) residues engaged in β-(1→3) linkages, while the doublet at 102.5 ppm from 3-O- and 4-O- substituted Glc*p* residues engaged in β-(1→4) linkages (Izydorczyk and MacGregor, 2000). Through integration of these two peaks, the ratio of β-(1→4) to β-(1→3) linkages was calculated. Thus, the ratio represents the amount of β-(1→4) linkages relative to β-(1→3) linkages, with a higher ratio possibly indicating the presence of longer blocks of contiguous β-(1→4)-linkages.

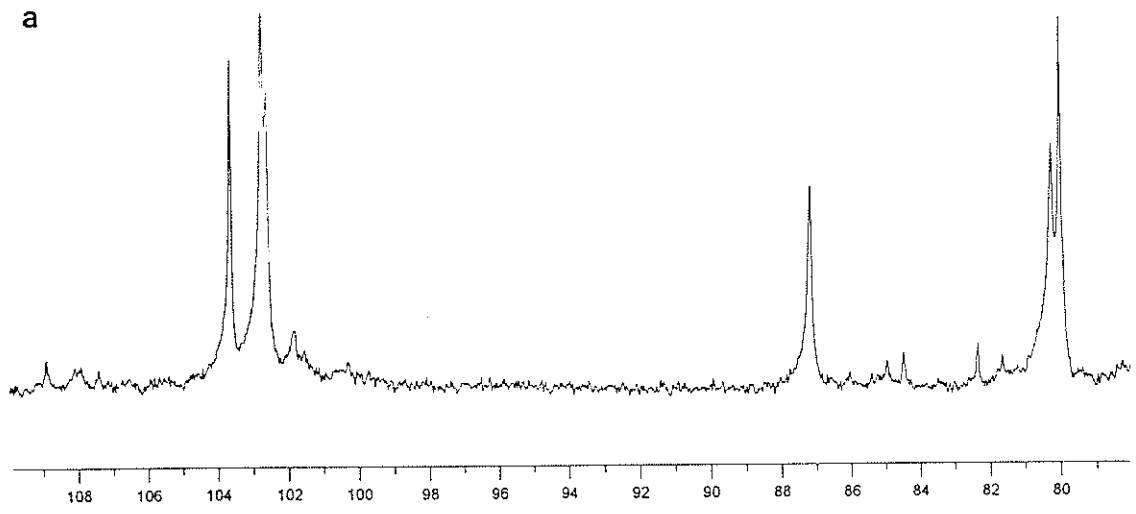
Of the four barleys examined, all had higher β-(1→4) to β-(1→3) linkage ratios in WE95 fractions compared to WE45; it is likely that the decreased solubility of WE95 fractions is at least partly a result of an increase in the length of the β-(1→4)-linked regions along the β-glucan chain (Figure 5.6). Ratios ranged from 1.86 to 2.23 in WE45 and 2.19 to 2.68 in WE95 (Table 5.7). The sample with the largest difference between its WE45

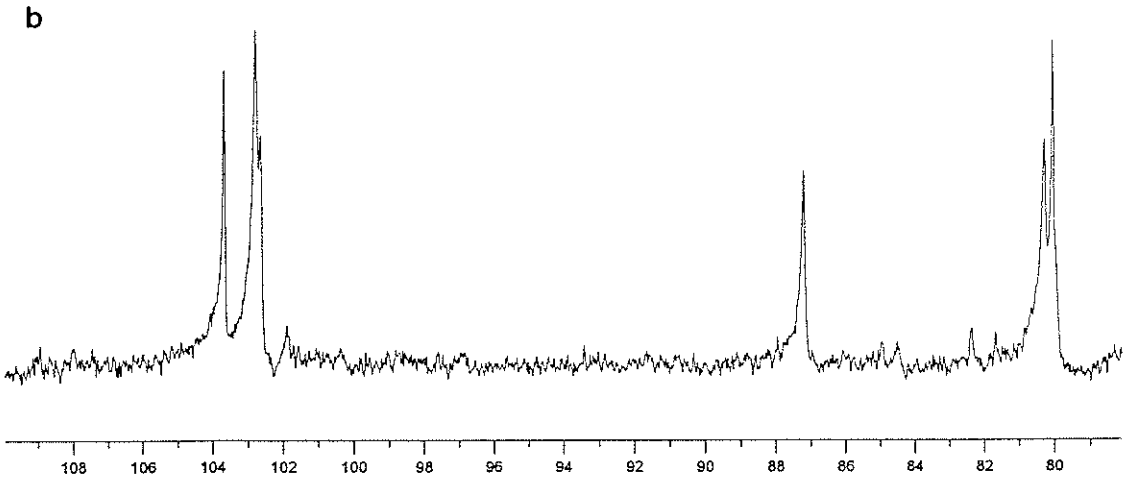
Table 5.7. Ratio of β-(1 → 4) to β-(1 → 3) linkages in water-extractable β-glucans.

BARLEY TYPE/VARIETY	β-(1 → 4) to β-(1 → 3) LINKAGE RATIO[1]	
	WE45	WE95
Normal		
Falcon	2.12	2.19
High Amylose		
92-55-06-48	1.86	2.68
Waxy		
SR93139	2.23	2.35
Zero Amylose Waxy		
SB94792	2.23	2.42

[1]obtained from ^{13}C-NMR data

Figure 5.6. Carbon-13 Nuclear Magnetic Resonance spectra of WE45 (a) and WE95 (b) fractions obtained from 92-55-06-48.


a
108
106
104
102
100
98
96
94
92
90
88
86
84
82
80


b
108
106
104
102
100
98
96
94
92
90
88
86
84
82
80

PPM

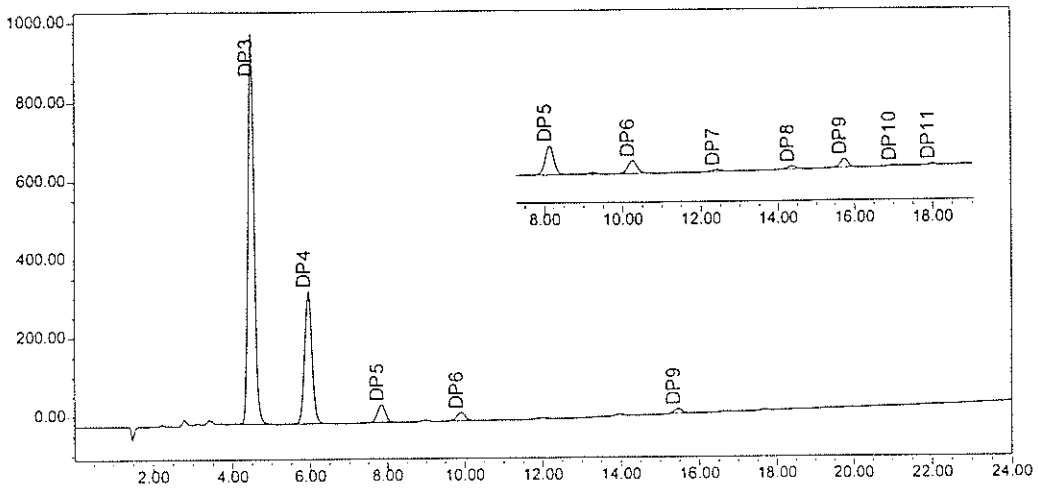
and WE95 fractions was high amylose variety 92-55-06-48, with a ratio of 1.86 in WE45 and 2.68 in WE95. A moderate increase was also evident in SB94792 (zero amylose waxy) and SR93139 (waxy), while Falcon (normal) showed only a slight increase (Table 5.7). It is conceivable that a higher ratio of β-(1→4) to β-(1→3) linkages might contribute to a more extended conformation of the β-glucan molecules, and/or might predispose them for intermolecular association via their cellulosic fragments.

5.1.3.3. Analysis of Released Oligosaccharides Following Lichenase Digestion. Beta-glucans from WE45 and WE95 fractions were degraded mainly to tri- and tetra-saccharides as a result of lichenase digestion (Figure 5.7; Table 5.8), similar to the findings of Izydorczyk et al. (1998a) and Wood et al. (1994). Therefore, as has been previously established, the tri- and tetra-saccharides were considered as having originated from 3-O-β-D-cellobiosyl-D-glucose, and 3-O-β-D-cellotriosyl-D-glucose, respectively. Although peaks were identified up to DP 13, later-eluting peaks were also detected, suggestive of longer cellulosic regions on the β-glucan chain (Figure 5.7); this was also in accordance with previous studies.

Between WE45 and WE95 β-glucans, significant differences were seen in the proportions of DP 3 + DP 4 and DP ≥9 oligomers (Table 5.8). Lichenase digests of β-glucans extracted at 45°C contained from 90.15 to 91.48% DP 3 + DP 4, in contrast to 82.49 to 91.83% DP 3 + DP 4 for β-glucans extracted at 95°C. Moreover, the average percentage of DP 3 + DP 4 oligomers in WE45 (91.04%) was significantly greater ($p \leq 0.05$) than that of WE95 (87.17%). The lesser amount of DP 3 + DP 4 in WE95 fractions corresponded with increased levels of DP ≥9 oligomers in comparison to WE45 fractions.

Figure 5.7. High-performance anion exchange chromatography with pulsed amperometric detection of water-soluble oligosaccharides released during lichenase digestion of WE45 (a) and WE95 (b) from SB94792. Inset shows elution pattern of oligosaccharides with higher DP.

a


1000.00
800.00
600.00
400.00
200.00
0.00
DP3
DP4
DP5
DP6
DP9
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
DP5
DP6
DP7
DP8
DP9
DP10
DP11
8.00
10.00
12.00
14.00
16.00
18.00

b

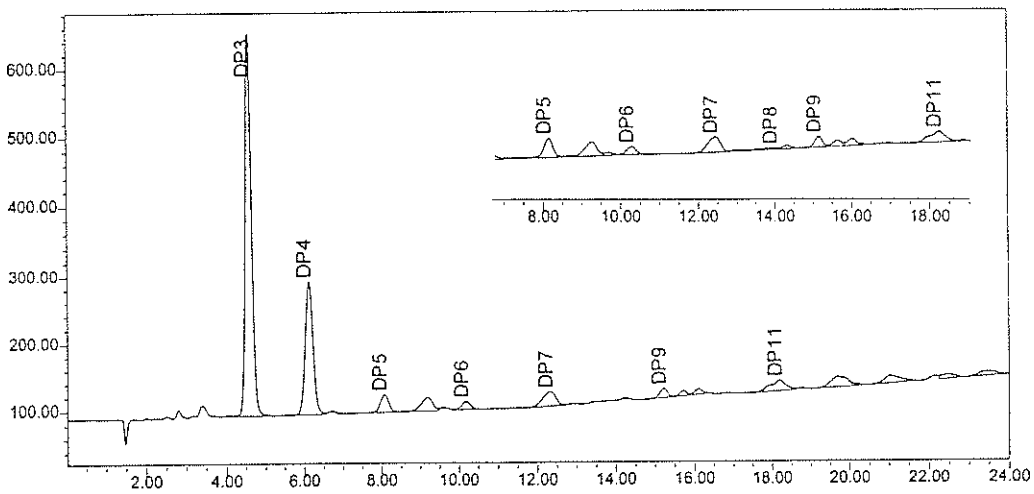

600.00
500.00
400.00
300.00
200.00
100.00
DP3
DP4
DP5
DP6
DP7
DP9
DP11
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
DP5
DP6
DP7
DP8
DP9
DP11
8.00
10.00
12.00
14.00
16.00
18.00

Table 5.8. Composition of oligosaccharides (mol %) released by lichenase from β-glucans in water-extractable fractions.

DP	Normal		High Amylose		Waxy		Zero Amylose Waxy	
	Falcon	CDC Dawn	92-55-06-54	92-55-06-48	CDC Candle	SR93139	SB94792	CDC Alamo
WE45								
3	60.87	59.38	62.88	63.45	64.19	64.15	64.30	64.33
4	29.28	31.01	28.23	27.60	27.26	27.11	27.26	27.11
5	4.27	3.98	3.99	3.97	3.99	4.02	4.12	4.12
6	2.07	2.10	2.04	1.96	1.95	2.02	2.02	2.00
7	0.46	0.48	0.38	0.35	0.40	0.42	0.37	0.39
8	0.49	0.57	0.51	0.52	0.47	0.46	0.44	0.47
9	1.29	1.63	1.39	1.35	1.27	1.30	1.15	1.14
>9	0.29	0.89	0.60	0.82	0.50	0.55	0.46	0.46
3 + 4	**90.15** $^{ab\ 1}$	**90.39** ^{ab}	**91.11** ^{a}	**91.04** ^{a}	**91.45** ^{a}	**91.25** ^{a}	**91.48** ^{a}	**91.44** ^{a}
3 : 4	**2.08** ^{D}	**1.91** ^{F}	**2.23** ^{C}	**2.30** ^{B}	**2.36** ^{A}	**2.37** ^{A}	**2.36** ^{A}	**2.38** ^{A}
WE95								
3	63.06	59.68	60.38	61.71	59.42	61.93	60.75	57.69
4	28.77	29.65	26.18	26.26	25.31	25.90	25.95	24.81
5	4.05	3.81	3.74	3.78	3.59	3.81	3.74	3.55
6	1.97	1.99	1.81	1.78	1.95	1.95	1.81	1.57
7	0.44	0.79	1.06	1.02	1.50	1.02	3.09	2.50
8	0.35	0.46	0.51	0.44	0.35	0.36	0.43	0.54
9	0.95	0.25	1.50	1.38	0.62	1.02	1.69	1.24
>9	0.43	3.38	4.83	3.65	7.39	3.98	2.55	8.13
3 + 4	**91.83** ^{a}	**89.33** ^{ab}	**86.56** ^{cd}	**87.96** ^{bc}	**84.73** ^{de}	**87.83** ^{bc}	**86.70** ^{cd}	**82.49** ^{e}
3 : 4	**2.19** ^{C}	**2.02** ^{E}	**2.31** ^{B}	**2.35** ^{AB}	**2.35** ^{AB}	**2.39** ^{A}	**2.34** ^{AB}	**2.33** ^{AB}

[1]Means with different letters are significantly different ($p < 0.05$); small letters = differences in DP 3 + DP 4 among samples; capital letters = differences in ratios of DP 3 to DP 4 among samples.

No significant difference in the average percentage of DP 5-8 oligomers between WE45 and WE95 fractions was observed. These results therefore confirm the ^{13}C-NMR data by verifying that the length of the cellulosic regions is greater in WE95 fractions, and further show an increase in β-(1→4)-linked regions of DP ≥9.

The overall difference in the average ratio of DP 3 to DP 4 oligosaccharides between WE45 and WE95 fractions (2.25 in WE45; 2.29 in WE95) was small, and not statistically significant at $p \leq 0.05$. Interestingly, however, ratios were significantly higher in the WE95 fractions of normal and high amylose varieties relative to their WE45 counterparts, while in WE95 from waxy samples, essentially no differences, or even slight decreases from WE45, were observed. Higher ratios of tri- to tetrasaccharides have previously corresponded with decreased solubility or extractability (Izydorczyk et al., 1998a), and it has been suggested 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 to lower β-glucan solubility (Tvaroska et al., 1983; Izydorczyk et al., 1998a). As a consequence, the increases in the ratio of tri- to tetrasaccharides between WE45 and WE95 fractions for normal and high amylose barleys may, along with the increase in cellulosic regions of DP ≥9, contribute to conformational differences translating to decreased solubility of the WE95 fractions.

The oligosaccharide profiles of WE45 β-glucans following lichenase digestion differed depending on barley starch composition and/or barley variety (Table 5.8). Beta-glucan digests from normal barleys consisted of fewer DP 3+4 oligosaccharides (90.15 to 90.39%) in comparison to samples with anomalous starch characteristics (91.04 to 91.48%;

$p \leq 0.05$); this was due to lesser amounts of cellotriosyl rather than cellotetraosyl regions, as the percentage of DP 3 oligomers in normal samples was lower than in waxy and high amylose, while levels of DP 4 were slightly higher. Among the six high amylose and waxy samples, amounts of DP 3+4 were lowest in high amylose (91.04 to 91.11%), intermediate in waxy (91.25 to 91.45%), and highest in zero amylose samples (91.44 to 91.48%). The lower levels of DP 3+4 in WE45 from normal varieties corresponded with greater amounts of DP 5-8, as well as greater amounts of DP ≥9 for CDC Dawn. CDC Dawn differed from Falcon mainly in the percentage of oligomers with DP ≥9 (Table 5.6). Decreased amounts of DP 3 in β-glucans from normal varieties produced significantly lower ratios of DP 3 to DP 4 (1.91 to 2.08) relative to the other barleys. Ratios were highest in waxy and zero amylose waxy samples (2.36 to 2.38) and slightly lower in high amylose (2.23 to 2.30). A recent study conducted by Jiang and Vasanthan (2000) analyzed lichenase-treated water-soluble β-glucans (extracted at RT) using matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) from several varieties of hull-less barley, three of which were examined in this study (CDC Dawn, CDC Candle, and CDC Alamo). Despite some discrepancies in the values because of the different methods employed, the trends they observed were similar; β-glucans from waxy types were found to have the highest DP 3 to DP 4 ratios (2.61), followed by high amylose (2.39) and normal (2.38) barley β-glucans . This compares with average ratios of 2.37 in waxy (including zero amylose waxy), 2.26 in high amylose, and 2.00 in normal samples, obtained in the present study.

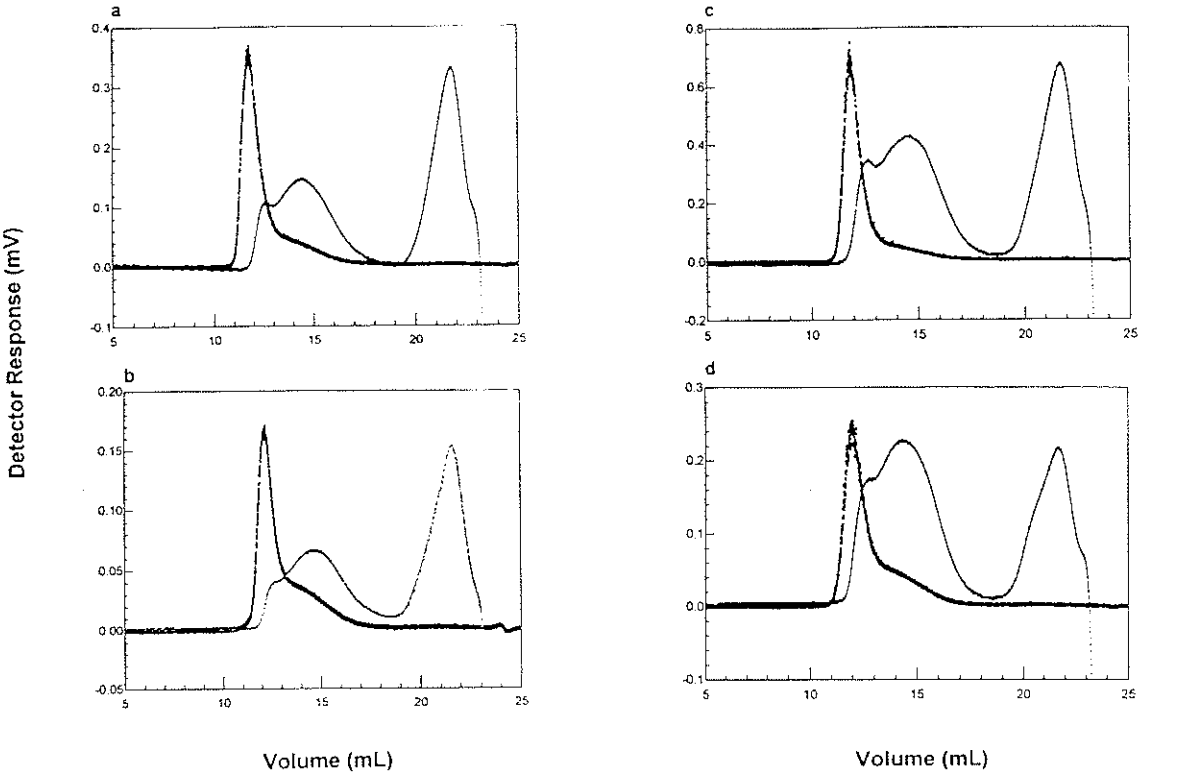
Focusing on the β-glucans with reduced solubility (WE95), differences between varieties in the relative proportions of released oligosaccharides were again detected.

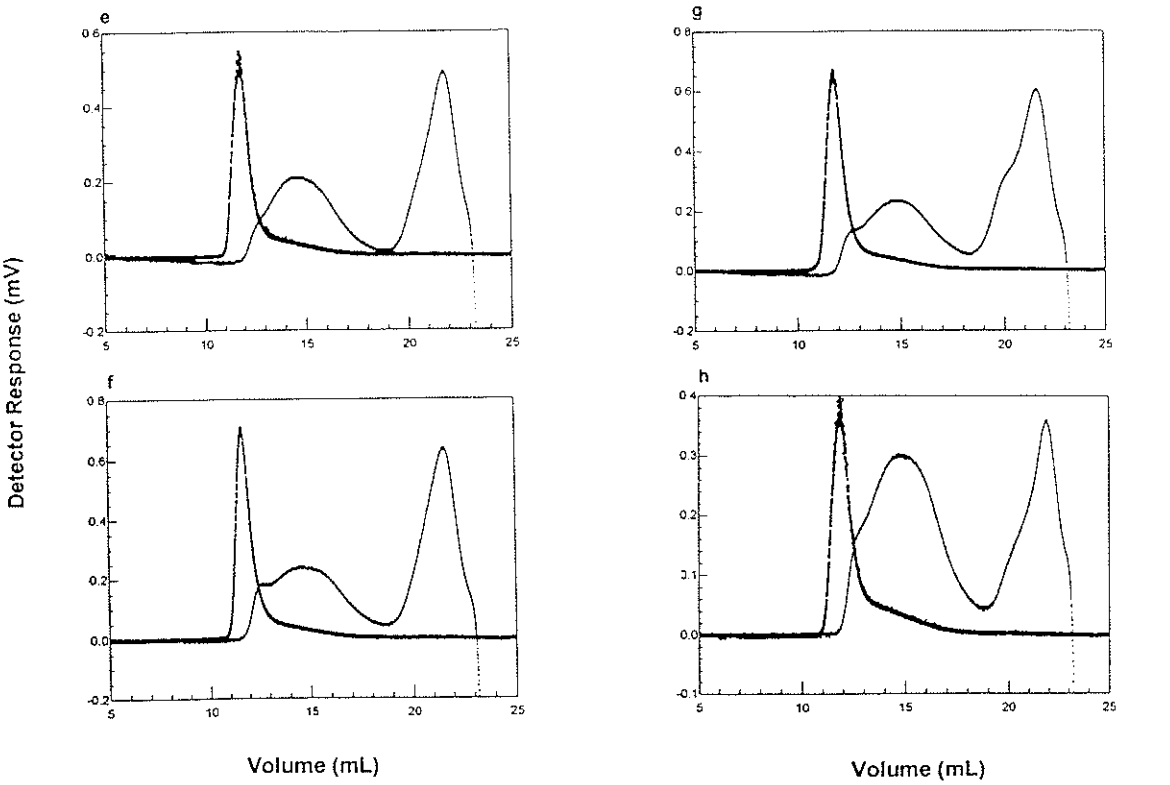
Opposite to the WE45 fractions, WE95 material from normal varieties consisted of significantly higher levels of DP 3+4 oligomers (89.33 to 91.83) than that isolated from high amylose and waxy lines (82.49 to 87.83). The smaller percentages of DP 3 + DP 4 in β-glucans from high amylose and waxy samples were generally associated with increased cellulosic regions of DP ≥9. The highest content of DP ≥9 fragments occurred in β-glucans from zero amylose waxy sample CDC Alamo (9.37%), and from waxy variety CDC Candle (8.01%). Similar to WE45 β-glucans from normal barleys, WE95 β-glucans from normal barleys had significantly lower DP 3 to DP 4 ratios.

5.1.4. Molecular Characteristics of $Ba(OH)_2$-extractable Arabinoxylans

5.1.4.1. Molecular Weight. The size exclusion chromatography profiles of $Ba(OH)_2$ fractions differed considerably from those of fractions that had been extracted with water (Figure 5.8). Two major polymer populations were detected in the $Ba(OH)_2$ fractions, as indicated by the presence of two peaks; the first one, eluting in the higher molecular weight region (11 to 19 mL elution volume) appearing asymmetric and broad, and indicating more than one population of polymers. The later eluting population was found to contain material absorbing UV light (Appendix I); it was deduced that this second population was likely protein, since it was known that fractions contained about 30% protein (Table 5.2). A small UV detector response occurred in the high molecular weight region of most of these fractions, which may point to some covalent interaction between protein and arabinoxylans; another possibility is that these proteins are not linked to the polysaccharides, but are rather of very high molecular weight due to aggregation and/or denaturation. However, it seemed

Figure 5.8. High-performance size exclusion chromatography of $Ba(OH)_2$ fractions with MALLS and RI detection; Falcon (a), CDC Dawn (b), 92-55-06-54 (c), 92-55-06-48 (d), CDC Candle (e), SR93139 (f), SB94792 (g), and CDC Alamo (h).


a
b
c
d
Detector Response (mV)
Volume (mL)
Volume (mL)
RI
LS


e
f
g
h
Detector Response (mV)
Volume (mL)
Volume (mL)

likely that the majority of the protein present in the $Ba(OH)_2$-extracted material was not covalently bound to arabinoxylans, since it eluted as a separate peak on the chromatograms.

Weight average molecular weights (M_w) of arabinoxylans extracted with saturated $Ba(OH)_2$ ranged from 0.64×10^6 to 2.22×10^6 (Table 5.9). The values reported for wheat arabinoxylans using gel filtration chromatography have varied from 0.02×10^6 to 5.0×10^6; however, there are difficulties in accurately measuring the molecular weight of asymmetrical molecules, such as arabinoxylans, by this method (MacGregor and Fincher, 1993; Izydorczyk et al, 1998b). The molecular weight reported by Gruppen et al. (1991) for wheat arabinoxylans extracted with $Ba(OH)_2$ and measured using laser light scattering was 0.85×10^6, falling within the range of those reported here.

Although significant differences in the M_w of arabinoxylan fractions were detected among varieties, there did not seem to be a correlation between M_w and barley starch type. However, varietal differences in M_w were found (Table 5.9). The highest molecular weights were obtained for fractions from SB94792 (2.22×10^6) and SR93139 (1.81×10^6). Fractions from Falcon and CDC Candle (1.22×10^6 and 1.21×10^6, respectively) also exhibited relatively high M_w values. Material from the high amylose samples, 92-55-06-54 (1.08×10^6) and 92-55-06-48 (0.90×10^6), and CDC Dawn (0.82×10^6) followed. Arabinoxylans from CDC Alamo were characterized as having the lowest M_w (0.64×10^6) of all $Ba(OH)_2$ fractions.

As expected, R_g generally increased with increasing M_w, with values between 57.95 and 91.45 nm (Table 5.9). Nevertheless, the order of R_g from greatest to least differed slightly from that of M_w, a possible indication that polymer aggregation may have occurred

Table 5.9. Molecular weight (M_w), polydispersity (M_w/M_n), and root mean square radius (R_g) of $Ba(OH)_2$ fractions[1].

BARLEY TYPE/VARIETY	M_w ($\times 10^{-6}$)[2] (g/mol)	R_g (nm)	M_w/M_n
Normal			
Falcon	1.22 [b 3]	83.85	2.3
CDC Dawn	0.82 [bc]	67.00	4.1
High Amylose			
92-55-06-54	1.08 [bc]	64.70	3.5
92-55-06-48	0.90 [bc]	83.70	2.8
Waxy			
CDC Candle	1.21 [b]	86.20	2.8
SR93139	1.81 [a]	91.45	4.0
Zero Amylose Waxy			
SB94792	2.22 [a]	88.95	4.9
CDC Alamo	0.64 [c]	57.95	3.0

[1] Mw of polymers within elution volume 11 – 19 mL as shown in Figure 5.8.
[2] CV < 5.0
[3] Means with different letters (column) are significantly different ($p < 0.05$).

in some samples, perhaps due to structural/conformational differences. Izydorczyk et al. (1998b) demonstrated that $Ba(OH)_2$-extractable arabinoxylans from malting barley possessed structural features that would allow for intermolecular association, which may have led to overestimation of molecular weights using size exclusion chromatography (Izydorczyk et al., 1998b). It is possible then, that such a phenomenon may have taken place to some extent in this investigation.

Polydispersity indices spanned a range of 2.3 to 4.9 (Table 5.9). Since values were greater than 1.0 in all $Ba(OH)_2$ fractions, these arabinoxylans would be considered polydisperse; wide ranges of molecular weights were represented in each individual fraction, as was also evident from their RI profiles.

5.1.4.2. Xylose Substitution Patterns (^{1}H-NMR Spectroscopy). The distribution of Ara*f* substituents along the xylan backbone, in addition to the ratio of xylose to arabinose, has been demonstrated to strongly influence the macromolecular characteristics of arabinoxylans. Detailed structural studies on wheat arabinoxylans have shown that those with a high intrinsic viscosity were characterized by a high xylose to arabinose ratio, as well as a low content of doubly substituted Xyl*p* residues on the backbone (Izydorczyk and Billiaderis, 1993). Xylose to arabinose ratios (Xyl*p*/Ara*f*) of the $Ba(OH)_2$ fractions had already been obtained in the current study from the monosaccharide composition, revealing that indeed these arabinoxylans possessed high xylose to arabinose ratios (1.70) relative to those in the other four fractions, WE45, WE95, $Ba(OH)_2/H_2O$, and NaOH (1.29 to 1.68). Information about substitution patterns of arabinoxylans were obtained from ^{1}H-NMR spectroscopy (Table 5.10) and the data used in conjunction with Xyl*p*/Ara*f* ratios. The

percentage of un-substituted (U-Xyl*p*), mono-substituted at the 2 (2-Xyl*p*) and 3 position (3-Xyl*p*), and di-substituted (2,3-Xyl*p*) xylose residues were calculated as described in Appendix II. Differences among varieties were revealed in the degree and manner of substitution of the xylan backbone (Table 5.10). Some general trends were noted when grouping fractions according to the barley type. The percentage of un-substituted xylose residues (U-Xyl*p*) was highest in normal and high amylose (57.3 to 62.4%), intermediate in waxy (56.4 to 56.5%), and lowest in zero amylose waxy varieties (51.01 to 53.07%). Consequently, the proportion of mono-substituted (2-Xyl*p*, 3-Xyl*p*) and di-substituted (2,3-Xyl*p*) xylose residues was greatest, on average, in fractions from zero amylose and waxy samples.

5.1.5. Sub-fractionation of Arabinoxylans

5.1.5.1. Yield. Because of the heterogeneity of arabinoxylans within the $Ba(OH)_2$-extractable material, the fractions were further separated by precipitation of the polymers at increasing levels of ammonium sulphate ($(NH_4)_2SO_4$) saturation (30 %, 40 %, 50 %, 65%, and 100 %). This procedure was carried out on $Ba(OH)_2$ fractions from four barley varieties, one from each starch type: Falcon (normal), 92-55-06-48 (high amylose), SR93139 (waxy), and SB94792 (zero amylose waxy). Since the sub-fractions obtained at 30 % salt saturation were extremely small (<10% yield) and found to consist primarily of protein (results not shown), they were not subsequently analyzed. Thus, four $Ba(OH)_2$ sub-fractions from each of four hull-less barley genotypes were assessed.

The percent yield of the four sub-fractions ($Ba(OH)_2$-40, $Ba(OH)_2$-50, $Ba(OH)_2$-65

Table 5.10. Substitution patterns[1] in arabinoxylans from $Ba(OH)_2$ fractions.

BARLEY TYPE/VARIETY	U-Xyl*p*[2]	2-Xyl*p*	3-Xyl*p*	2,3-Xyl*p*
Normal				
Falcon	57.31	11.14	13.63	17.92
CDC Dawn	62.39	6.21	12.04	19.36
High Amylose				
92-55-06-54	61.04	7.33	13.77	17.86
92-55-06-48	61.84	9.99	14.25	13.92
Waxy				
CDC Candle	56.42	8.92	16.05	18.61
SR93139	56.54	9.58	15.99	17.89
Zero Amylose Waxy				
SB94792	53.07	13.96	14.96	18.01
CDC Alamo	51.01	8.74	15.73	24.51

[1] obtained from ^{1}H-NMR and GC data; see Appendix II

[2] U-Xyl*p*: →4(Xyl*p*)1→ ; 2-Xyl*p*: →2,4(Xyl*p*)1→ ; 3-Xyl*p*: →3,4(Xyl*p*)1→ ; 2,3- Xyl*p*: →2,3,4(Xyl*p*)1→

and $Ba(OH)_2$-100) followed similar trends in each barley variety (Table 5.11). Yields were substantially lower for the $Ba(OH)_2$-40 material (9.19 % on average) compared to the other sub-fractions (19.54%, 22.48%, and 16.07% for $Ba(OH)_2$-50, -65, and -100, respectively); most of the arabinoxylans were precipitated at $(NH_4)_2SO_4$ saturation levels of 50% or greater. However, the difference in yield between $Ba(OH)_2$-40 and $Ba(OH)_2$-100 from 92-55-06-48 (8.40% and 9.86%, respectively) was not as great as those found in the other genotypes (Table 5.11). The $Ba(OH)_2$-extractable arabinoxylans from this variety gave the lowest yields of each of the $Ba(OH)_2$-40 and $Ba(OH)_2$-100 sub-fractions, which corresponded with notably higher yields of $Ba(OH)_2$-50 (24.44%) compared to $Ba(OH)_2$-50 from the other samples (17.28% to 18.32%). Moreover, the yield of $Ba(OH)_2$-50 from 92-55-06-48 was the highest of the sub-fractions from this variety. Among the other three barleys, yields were greatest for $Ba(OH)_2$-65 sub-fractions (21.28% to 24.88%)

Unfortunately, a portion of $Ba(OH)_2$-extracted material was not recovered in the fractionation procedure. The total yield of the four sub-fractions (63.44 to 71.44 %), when added to the yield of $Ba(OH)_2$-30 (~10 %), totaled 75 to 80 % instead of the theoretical 100%. The percent recovery was therefore lower than expected; it appears that a portion may have remained in solution even after precipitation at 100% salt saturation.

5.1.5.2. Protein Content. Protein was detected in all $Ba(OH)_2$ sub-fractions (Table 5.12), with the amounts differing, depending on both the sub-fraction and the barley variety. Levels were generally highest in the $Ba(OH)_2$-40 fractions, followed by $Ba(OH)_2$-100. Significantly less protein was measured in $Ba(OH)_2$-50 and $Ba(OH)_2$-65 sub-fractions (Table 5.12). These results point to the possibility of interactions between protein and

Table 5.11. Yield[1] (%) of $Ba(OH)_2$ sub-fractions obtained through ammonium sulphate precipitation.

BARLEY TYPE/VARIETY	FRACTION			
	$Ba(OH)_2$-40	$Ba(OH)_2$-50	$Ba(OH)_2$-65	$Ba(OH)_2$-100
	%	%	%	%
Normal				
Falcon	9.20	17.28	24.88	20.08
High Amylose				
92-55-06-48	8.40	24.44	22.44	9.68
Waxy				
SR93139	9.20	18.12	21.28	14.84
Zero Amylose Waxy				
SB94792	9.96	18.32	21.32	19.68

[1] w/w, based on $Ba(OH)_2$ material used for sub-fractionation.

arabinoxylans, occurring to a greater extent in sub-fractions $Ba(OH)_2$-40 and $Ba(OH)_2$-100 than in $Ba(OH)_2$-50 and $Ba(OH)_2$-65. The two sub-fractions with the least amount of protein, and hence, the highest purity, occurred in the $Ba(OH)_2$-50 and $Ba(OH)_2$-65 sub-fractions of high amylose barley, 92-55-06-48 (7.40 and 9.78 %, respectively). Moreover, within each sub-fraction of 92-55-06-48 except $Ba(OH)_2$-100, protein content was substantially lower than that of their counterparts. This was anticipated, as the protein content of the un-fractionated $Ba(OH)_2$ material in 92-55-06-48 (22.92 %) was also lower than that measured in the other corresponding fractions (27.08 to 33.62%).

5.1.5.3. Monosaccharide Composition. Similar to the $Ba(OH)_2$ fractions, the majority of the carbohydrates in the sub-fractions consisted of arabinose and xylose (>97.3%; Table 5.13). However, the sugars present in smaller amounts, particularly glucose and galactose, were concentrated in certain sub-fractions; glucose was most prominent within $Ba(OH)_2$-40 (0.82 to 2.42%) and $Ba(OH)_2$-50 (0.97 to 1.58%), while galactose was found primarily in $Ba(OH)_2$-100 (0.95 to 1.24%). $Ba(OH)_2$-65 sub-fractions contained little to no glucose, galactose, or mannose, and were therefore purest in arabinoxylan (Table 5.13).

Monosaccharide analysis confirmed the success of step-wise ammonium sulphate precipitation in the separation of the $Ba(OH)_2$-extractable polysaccharides. The four sub-populations of arabinoxylans differed in levels of arabinose and xylose, and consequently, in xylose to arabinose ratios (Table 5.13). Sub-fractions $Ba(OH)_2$-50, $Ba(OH)_2$-65, and $Ba(OH)_2$-100 experienced a marked decrease in Xyl/Ara ratios with each increase $(NH_4)_2SO_4$ concentration. Izydorczyk et al. (1998b) also observed the same trend; arabinoxylans obtained at higher saturation levels of $(NH_4)_2SO_4$ were more highly

Table 5.12. Protein content[1] (%w/w) in $Ba(OH)_2$ sub-fractions obtained through ammonium sulphate precipitation.

BARLEY TYPE/VARIETY	$Ba(OH)_2$-40	$Ba(OH)_2$-50	$Ba(OH)_2$-65	$Ba(OH)_2$-100
Normal				
Falcon	43.20 ± 0.51	23.58 ± 0.36	16.43 ± 0.20	35.27 ± 0.56
High Amylose				
92-55-06-48	25.93 ± 6.98	7.40 ± 0.10	9.78 ± 0.13	27.03 ± 0.18
Waxy				
SR93139	47.06 ± 0.44	19.15 ± 0.25	20.50 ± 0.20	26.26 ± 3.20
Zero Amylose Waxy				
SB94792	52.75 ± 0.05	23.90 ± 0.3	20.33 ± 0.13	33.65 ± 0.45

[1] n = 2 ± SD

Table 5.13. Monosaccharide composition of $Ba(OH)_2$ sub-fractions obtained by precipitation with ammonium sulphate.

Fraction/Type/Variety	% MOL OF CARBOHYDRATE MATERIAL[1]					
	Ara	Xyl	Glc	Gal	Man	Xyl*p* /Ara*f*
$Ba(OH)_2$-40						
Normal						
Falcon	32.91[b]	64.68[b]	2.42	tr[2]	nd	1.97[d]
High Amylose						
92-55-06-48	22.74[c]	76.44[a]	0.82	tr	nd	3.36[a]
Waxy						
SR93139	38.86[a]	59.28[c]	1.86	tr	tr	1.53[g]
Zero Amylose Waxy						
SB94792	31.88[b]	65.38[b]	2.15	tr	0.61	2.67[b]
Average	**31.47[C]**	**66.44[B]**				
$Ba(OH)_2$-50						
Normal						
Falcon	26.71[ab]	71.67[b]	1.58	tr	nd	2.68[b]
High Amylose						
92-55-06-48	26.99[a]	72.05[ab]	0.97	nd	nd	2.67[b]
Waxy						
SR93139	26.40[b]	72.52[a]	1.09	nd	nd	2.75[b]
Zero Amylose Waxy						
SB94792	26.86[ab]	71.90[ab]	1.23	tr	nd	2.05[c]
Average	**26.75[D]**	**72.03[A]**				
$Ba(OH)_2$-65						
Normal						
Falcon	38.02[c]	61.41[a]	0.55	tr	nd	1.62[e]
High Amylose						
92-55-06-48	40.92[a]	59.04[b]	tr	tr	nd	1.44[g]
Waxy						
SR93139	39.29[b]	60.70[a]	tr	tr	nd	1.55[ef]
Zero Amylose Waxy						
SB94792	40.11[ab]	59.57[b]	tr	tr	tr	1.49[fg]
Average	**39.58[B]**	**60.18[C]**				
$Ba(OH)_2$-100						
Normal						
Falcon	49.19[c]	49.54[a]	tr	0.95	tr	1.02[h]
High Amylose						
92-55-06-48	50.37[a]	48.58[b]	tr	0.99	tr	0.98[h]
Waxy						
SR93139	49.23[c]	49.69[a]	tr	1.01	nd	1.03[h]
Zero Amylose Waxy						
SB94792	49.58[b]	49.17[ab]	tr	1.24	tr	1.01[h]
Average	**49.59[A]**	**49.24[D]**				

[1] CV<5.0; [2] tr = trace (< 0.5 %); [3] nd = not detected; [4] means with different letters (column) are significantly different ($p<0.05$); capital letters = differences among fractions, small letters = differences among varieties within each fraction.

substituted, indicated by lower Xyl*p*/Ara*f* ratios, and hence, were more soluble. In the case of $Ba(OH)_2$-40 samples, ratios were more variable, and resulted in an average (2.28) lower than that of $Ba(OH)_2$-50 (2.69), but higher than those of the other two sub-fractions (1.01 to 1.53). Generally, sub-populations of arabinoxylans with higher degrees of substitution were isolated at higher salt concentrations.

In terms of xylose to arabinose (Xyl*p*/Ara*f*) ratios, variation was generally low among samples within each of the sub-fractions except $Ba(OH)_2$-40 (Table 5.13). An extremely low level of substitution/ high Xyl*p*/Ara*f* (3.36) was obtained in $Ba(OH)_2$-40 of high amylose variety 92-65-06-48. Therefore, the significantly higher Xyl*p*/Ara*f* ratio of the un-fractionated $Ba(OH)_2$ arabinoxylans (Figure 5.3) from 92-55-06-48 (1.92 ; $p \leq 0.05$) was presumably due to the presence of this $Ba(OH)_2$-40 sub-population. The next highest ratio among $Ba(OH)_2$-40 arabinoxylans was found in those of zero amylose waxy sample SB94792 (2.05), followed by normal variety Falcon (1.97), and finally, waxy variety SR93139 (1.53). The ranking of Xyl*p*/Ara*f* ratios among the $Ba(OH)_2$-50 arabinoxylans was nearly the reverse although the ratios spanned a narrow range SR93139 (2.75) > Falcon (2.68) > SB94792 and 92-55-06-48 (2.67). Among $Ba(OH)_2$-65 arabinoxylans, high amylose variety 92-55-06-48 contained those with the lowest Xyl*p*/Ara*f* ratio (1.44), after Falcon (1.62), SR93139 (1.55) and SB94792 (1.49). Arabinoxylans of $Ba(OH)_2$-100 sub-fractions were the most homogenous with respect to their Xyl*p*/Ara*f* ratios, as the range was narrow (0.98 to 1.03). Nevertheless, the ranking of the ratios closely resembled that of the $Ba(OH)_2$-50 sub-fractions: SR93139(1.03) > Falcon (1.02) > SB94792 (1.01) > 92-55-06-48 (0.98).

5.1.5.4. Molecular Weight. The size exclusion chromatography profiles of each $Ba(OH)_2$ sub-fraction, as with the un-fractionated material, showed two polymer populations (Figure 5.9). Again, a large UV detector response was obtained at approximately 22 mL elution volume, indicating the presence of protein. Although the protein content of these later eluting populations was not quantified, evidence that they consisted largely of protein was given by the fact that their RI profiles corresponded with the trend in protein content; the later eluting, lower molecular weight peaks, had a considerably lower RI response in the two sub-fractions with the lowest protein levels, $Ba(OH)_2$-50 and -65 of high amylose sample 92-55-06-48 (7.40% and 9.78%, respectively). Similarly, in comparing the earlier eluting peaks between the various sub-fractions, those of $Ba(OH)_2$-40 populations were found to exert a lower RI response relative to $Ba(OH)_2$-50, $Ba(OH)_2$-65, and $Ba(OH)_2$-100. This was particularly true of $Ba(OH)_2$-40 of Falcon, SR93139, and SB94792; these samples also contained the highest protein levels (43.20 to 52.75%). All populations within the elution volume range of 11 to 19 mL were still broad; however, differences in polydispersity values were observed; fractions precipitated at increasing salt saturation had increasingly higher M_w/M_n (Table 5.14). The data therefore indicated that the ammonium sulphate precipitation technique was, in fact, successful in isolating populations of varying weight average molecular weight, polydispersity, and root mean square radii.

Figure 5.9. High-performance size exclusion chromatography of $Ba(OH)_2$-40 (a), $Ba(OH)_2$-50 (b), $Ba(OH)_2$-65 (c), and $Ba(OH)_2$-100 (d) sub-fractions from 92-55-06-48 with MALLS and RI (top), and MALLS and UV(bottom) detection.

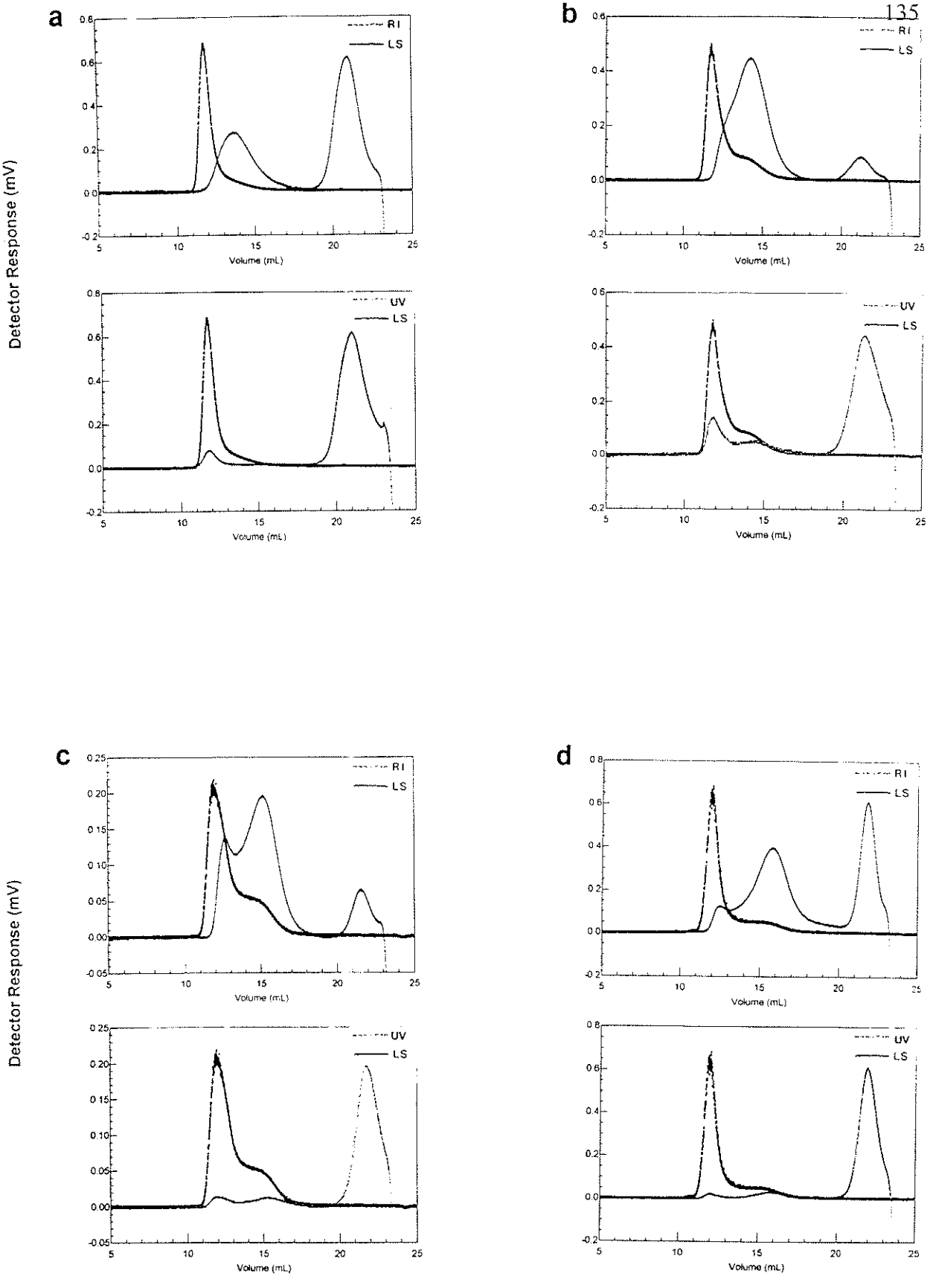

a
b
c
d
RI
LS
UV
Detector Response (mV)
Volume (mL)

Table 5.14. Molecular weight (M_w), polydispersity (M_w/M_n), and root mean square radius (R_g) of $Ba(OH)_2$ sub-fractions obtained by ammonium sulphate precipitation[1].

BARLEY TYPE/VARIETY	M_w ($\times 10^{-6}$)[2] (g/mol)	M_w/M_n[2] (nm)	R_g[2]
$Ba(OH)_2$-40			
Normal			
Falcon	4.77	1.6	91.90
High Amylose			
92-55-06-48	4.98	1.9	117.20
Waxy			
SR93139	3.84	2.4	82.30
Zero Amylose Waxy			
SB94792	4.37	2.6	74.80
Average	**4.49**[A 3]	**2.3**	**97.69**
$Ba(OH)_2$-50			
Normal			
Falcon	1.94	4.8	95.05
High Amylose			
92-55-06-48	0.90	1.9	89.15
Waxy			
SR93139	2.37	3.6	88.40
Zero Amylose Waxy			
SB94792	1.95	2.8	89.75
Average	**1.79**[B]	**3.3**	**90.59**
$Ba(OH)_2$-65			
Normal			
Falcon	1.09	2.9	82.85
High Amylose			
92-55-06-48	0.70	2.1	79.25
Waxy			
SR93139	1.06	4.6	80.60
Zero Amylose Waxy			
SB94792	0.52	6.9	70.00
Average	**0.84**[B]	**4.2**	**78.17**
$Ba(OH)_2$-100			
Normal			
Falcon	0.81	4.2	78.35
High Amylose			
92-55-06-48	0.90	3.8	71.95
Waxy			
SR93139	0.75	6.3	75.25
Zero Amylose Waxy			
SB94792	0.75	4.8	76.50
Average	**0.80**[B]	**4.8**	**75.51**

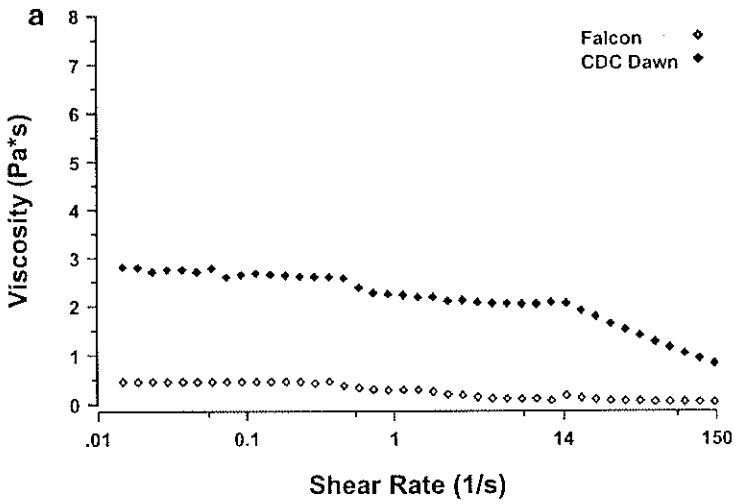
[1] Mw of polymers within elution volume 11 – 19 mL; [2] CV < 5.0;
[3] Means with different letters (column) are significantly different ($p < 0.05$).

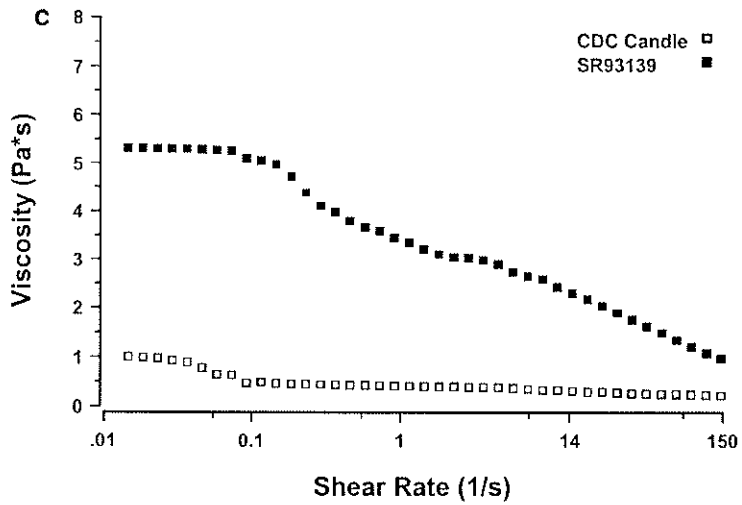
5.1.6. Viscoelastic Behaviour of Water-Soluble β-glucans

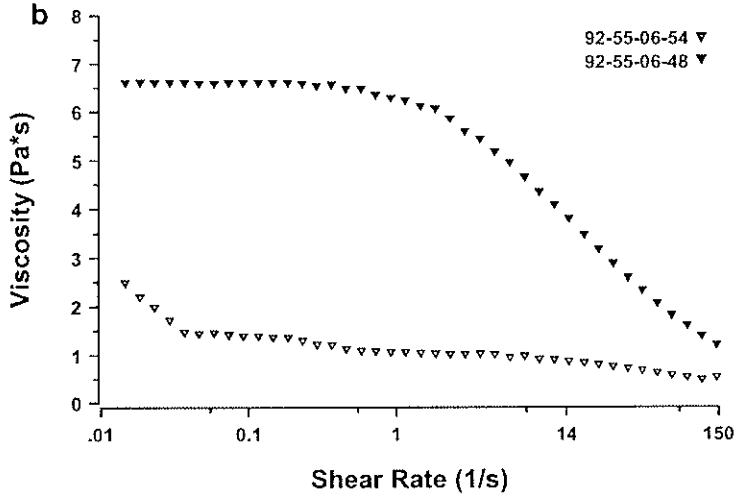
5.1.6.1. Steady Shear Flow Curves. The apparent viscosities of WE45 and WE95 solutions were measured over a shear rate range of 0.01/s to 150/s (Fig. 5.10.1, 5.10.2). All fractions exhibited flow behaviour typical of polysaccharide solutions, in that they showed evidence of shear thinning with increasing shear rates. However, samples differed drastically in the magnitude of their apparent viscosities (particularly at lower shear rates), and subsequently, the degree of shear thinning varied; fractions with higher viscosities in the lower shear rate region generally showed more extensive shear thinning than those with lower viscosities in the same region of the spectra.

In almost all instances, WE95 fractions had apparent viscosities higher than their WE45 counterparts, which follows the trend observed for M_w between the two fractions (i.e. WE95 fractions had significantly higher M_w than WE45; $p \leq 0.05$). As is shown in Figures 5.10.1 and 5.10.2, the apparent viscosities differed dramatically depending on the barley variety. In WE45, maximum apparent viscosities, occurring at the lower shear rates, ranged from 0.3 Pa·s (Falcon), to 6.6 Pa·s (92-55-06-48). WE95 fractions had maximum viscosities of 0.3 Pa·s (Falcon) to 46 Pa·s (92-55-06-48). Thus, among both WE45 and WE95 fractions, those from Falcon and 92-55-06-48 exhibited the lowest and highest apparent viscosities, respectively, although the spread of the viscosities, like M_w values, was greater among WE95 samples. Of the WE45 fractions, SR93139 ranked second in maximum apparent viscosity behind 92-55-06-48, followed by CDC Alamo and SB94792, CDC Dawn, 92-55-06-54, CDC Candle, and Falcon. The ranking of the WE95 fractions changed slightly due to the higher viscosity of the material from the two zero amylose

Figure 5.10.1. Steady Shear Flow Curves of 2% solutions of WE45 at 15 °C; normal (a), high amylose (b), waxy (c), zero amylose waxy (d).


a
Falcon
CDC Dawn
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
.01
0.1
1
14
150


c
CDC Candle
SR93139
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
.01
0.1
1
14
150


b
92-55-06-54
92-55-06-48
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
.01
0.1
1
14
150

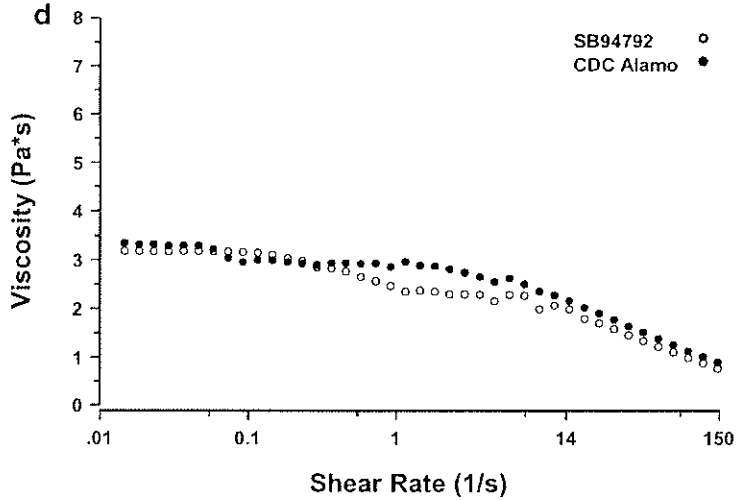
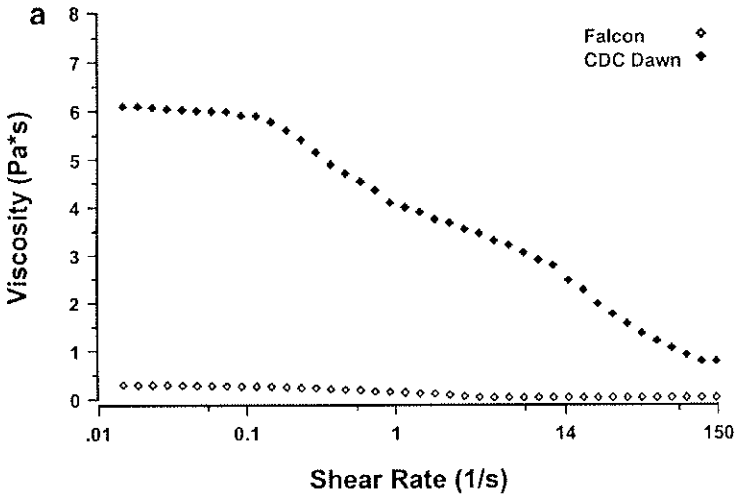
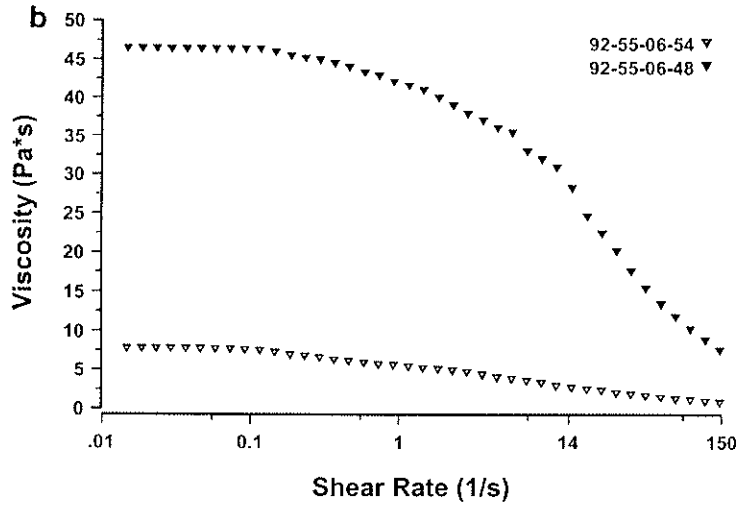
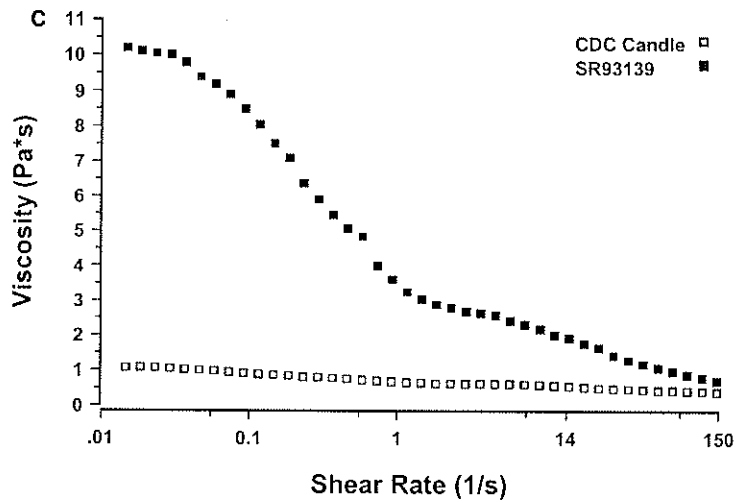
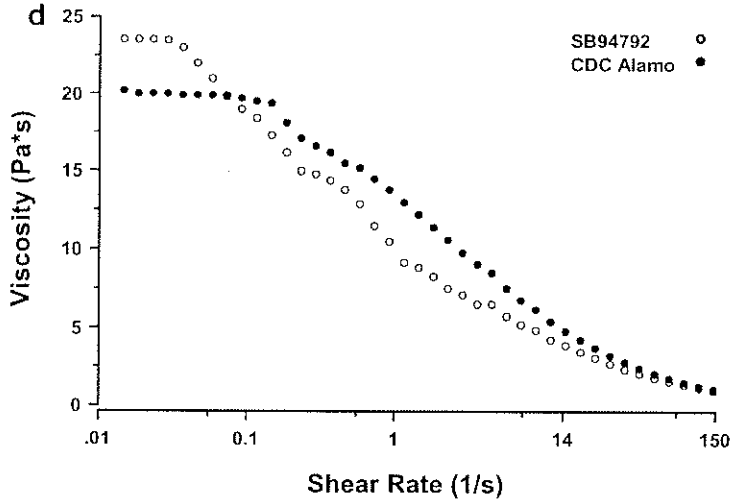

d
SB94792
CDC Alamo
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
.01
0.1
1
14
150

Figure 5.10.2. Steady Shear Flow Curves of 2% solutions of WE95 at 15°C; normal (a), high amylose (b), waxy (c), zero amylose waxy (d).


a
Falcon
CDC Dawn
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
.01
0.1
1
14
150


b
92-55-06-54
92-55-06-48
Viscosity (Pa*s)
Shear Rate (1/s)
0
5
10
15
20
25
30
35
40
45
50
.01
0.1
1
14
150


c
CDC Candle
SR93139
Viscosity (Pa*s)
Shear Rate (1/s)
0
1
2
3
4
5
6
7
8
9
10
11
.01
0.1
1
14
150


d
SB94792
CDC Alamo
Viscosity (Pa*s)
Shear Rate (1/s)
0
5
10
15
20
25
.01
0.1
1
14
150

varieties, resulting in the following rank order: 92-55-06-48 > SB94792 > CDC Alamo > SR93139 > CDC Dawn > 92-55-06-54 > CDC Candle > Falcon. Viscosity measurements were performed at least in duplicate, and the coefficients of variation (CV) were less than 5.0, indicating a high degree of precision.

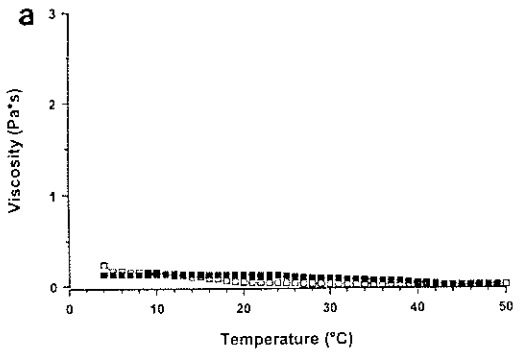
Even for WE45 and WE95 polymers from barley varieties with similar starch composition, large variations in viscoelastic behaviour were observed, particularly between fractions from normal, high amylose, and waxy barley types. Those from zero amylose varieties, on the other hand, had similar viscoelastic properties. The differences observed can only partly be accounted for by M_w.

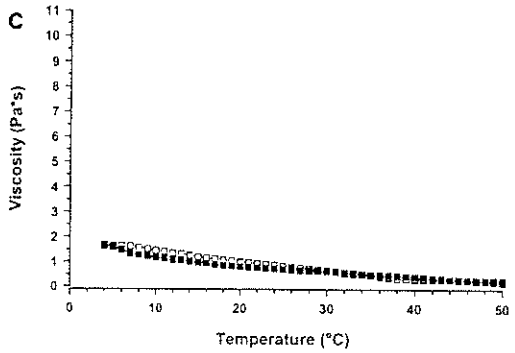
Among the barley cultivars studied here, fractions of NSP from high amylose sample 92-55-06-48 seem to have the greatest potential for use as a functional ingredient in foods, from the point of view of soluble β-glucan flow behaviour. In addition to M_w, factors such as polydispersity, solubility, and molecular structure may also influence the viscoelastic properties of these polymers, and should therefore be taken into consideration when determining the barley cultivar and the processes chosen in the manufacture of functional foods using barley non-starch polysaccharides.

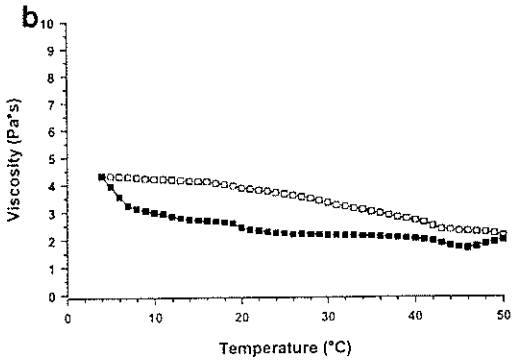
5.1.6.2. Temperature Effects. To see how the water-extractable fractions, WE45 and WE95, behaved when subjected to changes in temperature, 2% solutions were heated and cooled at a constant shear rate (Fig. 5.11.1, 5.11.2). All WE45 and WE95 fractions showed a decrease in viscosity with increasing temperature, and a subsequent increase in viscosity when cooled. Not all fractions resumed their original viscosity at the end of the heating/cooling cycle , however, and in almost all cases, the increase in viscosity during

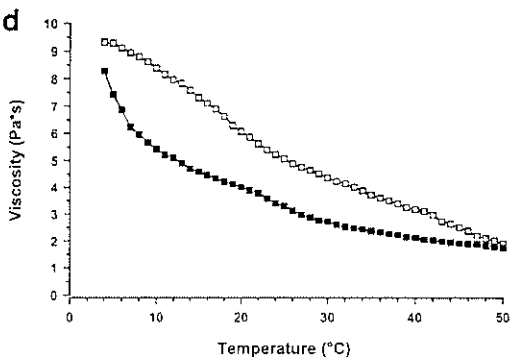
cooling occurred at a slower rate than the viscosity decrease during heating; this was true particularly in the early stages of cooling. It is likely that all samples would have eventually reached their original viscosity with a longer cooling time, since the polymers may have simply not had enough time to reform the molecular entanglements present initially. Nevertheless, it is interesting to note that polymers in WE45 fractions generally were able to reach or at least approach their original viscosity by the end of the experiment, whereas WE95 polymers, with the exception of those from CDC Candle, were not as successful. Perhaps this is a result of structural differences, such as increased length of cellulosic regions in β-glucans from WE95 fractions, or simply that WE95 fractions were more viscous, and having a greater degree of molecular entanglements originally, more time was required to reform them. Despite the differences observed, all water-extractable fractions from the barley varieties examined tended to retain their viscosity. This is in agreement with the work of Böhm and Kulicke (1999), who found that repeated heating (80°C) and cooling (RT) of concentrated barley β-glucan solutions (6% w/v) did not result in any detectable changes in viscoelastic behaviour. Therefore, based on their response to heating and cooling, these water-soluble polymers all have potential in food applications, although their nutritional and functional impacts may vary and require further investigation.

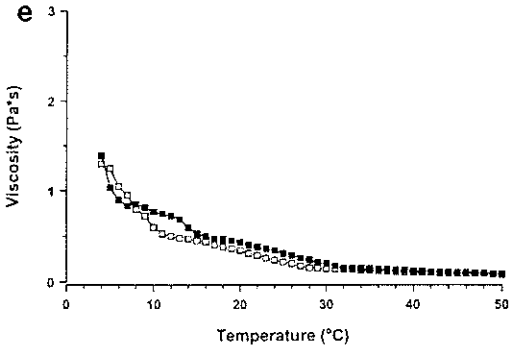
Figure 5.11.1. Effect of heating/cooling on viscosity of WE45 fractions at a constant shear rate (2.31/s); Falcon (a), CDC Dawn (b), 92-55-06-54 (c), 92-55-06-48 (d), CDC Candle (e), SR93139 (f), SB94792 (g), and CDC Alamo (h). Open squares = heating; closed squares = cooling.

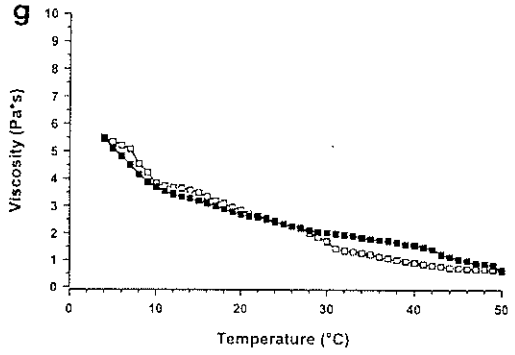

a
Viscosity (Pa*s)
Temperature (°C)

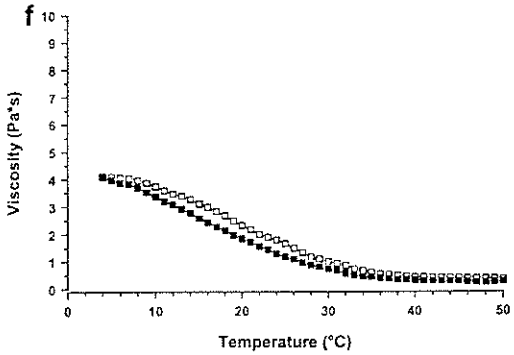

c
Viscosity (Pa*s)
Temperature (°C)


b
Viscosity (Pa*s)
Temperature (°C)


d
Viscosity (Pa*s)
Temperature (°C)


e
Viscosity (Pa*s)
Temperature (°C)


g
Viscosity (Pa*s)
Temperature (°C)


f
Viscosity (Pa*s)
Temperature (°C)

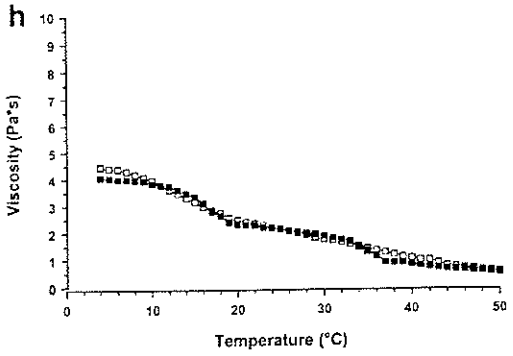
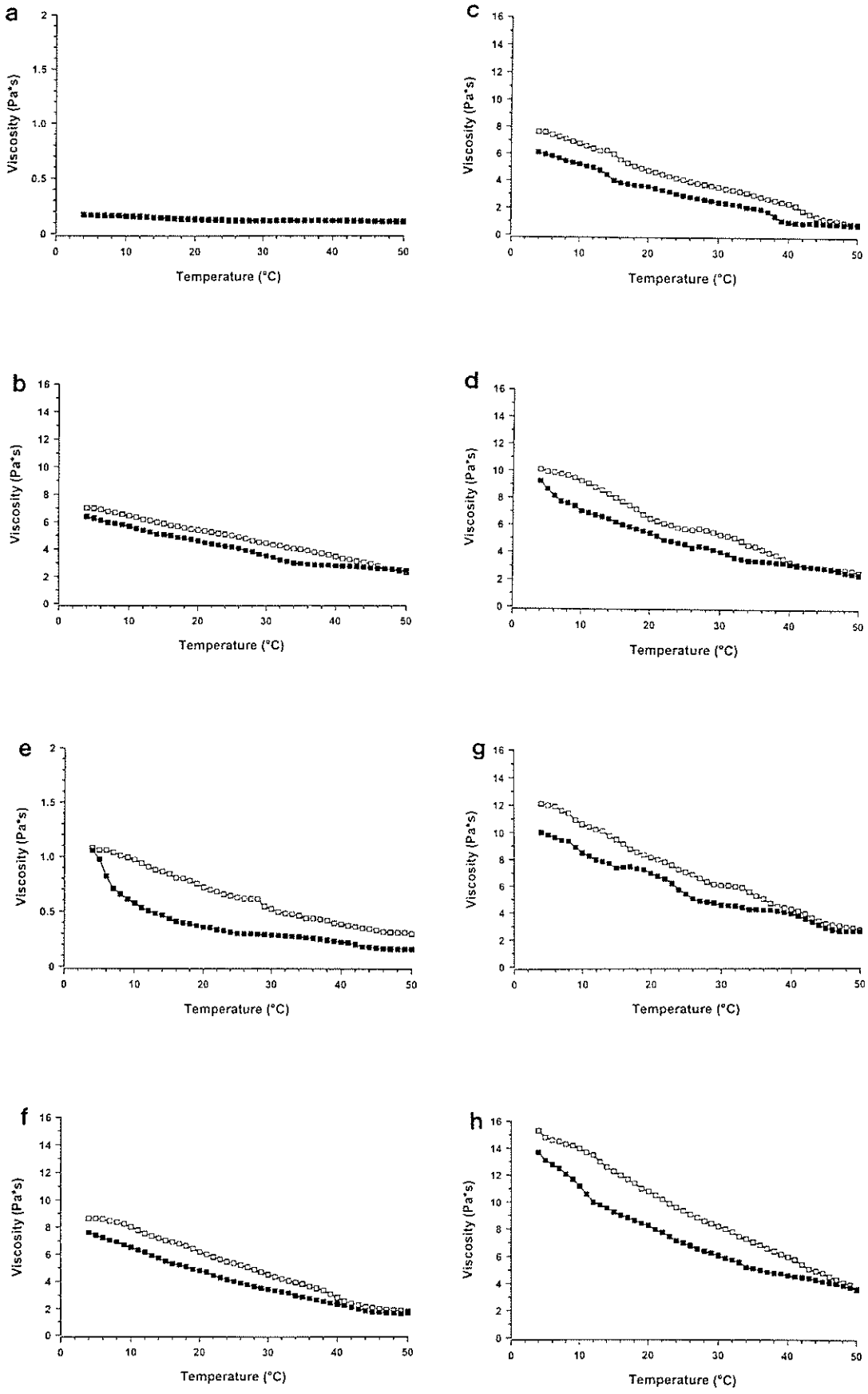

h
Viscosity (Pa*s)
Temperature (°C)

Figure 5.11.2. Effect of heating/cooling on viscosity of WE95 fractions at a constant shear rate (2.31/s); Falcon (a), CDC Dawn (b), 92-55-06-54 (c), 92-55-06-48 (d), CDC Candle (e), SR93139 (f), SB94792 (g), and CDC Alamo (h). Open squares = heating; closed squares = cooling.


a
Viscosity (Pa*s)
Temperature (°C)
c
Viscosity (Pa*s)
Temperature (°C)
b
Viscosity (Pa*s)
Temperature (°C)
d
Viscosity (Pa*s)
Temperature (°C)
e
Viscosity (Pa*s)
Temperature (°C)
g
Viscosity (Pa*s)
Temperature (°C)
f
Viscosity (Pa*s)
Temperature (°C)
h
Viscosity (Pa*s)
Temperature (°C)

5.2. SUMMARY

For the purpose of their detailed characterization, β-glucans and arabinoxylans were sequentially extracted from eight cultivars of hull-less barley, resulting in five fractions: WE45, WE95, $Ba(OH)_2$, $Ba(OH)_2/H_2O$, and NaOH. Differences in yield and composition between the five fractions and cultivars indicated differences in solubility and structure of the polymers both within the barley grain and between similar fractions from different barley varieties. However, in all fractions, β-glucans and/or arabinoxylans were the major carbohydrates. The water-extractable material (WE45 and WE95), contained mainly β-glucans, while the $Ba(OH)_2$-extractable material was made up almost entirely of arabinoxylans ($Ba(OH)_2/H_2O$, and NaOH material contained a heterogenous mixture of β-glucans and arabinoxylans). Therefore due to the less heterogenous mixture of the two polysaccharides in WE45, WE95, and $Ba(OH)_2$, these three fractions were chosen for further study.

Upon analysis of the water-extractable β-glucans, variation in molecular structure between those β-glucans extracted at 45°C and those extracted at 95°C was observed. Higher M_w, higher β-(1→4) to β-(1→3) linkage ratios measured by ^{13}C-NMR, as well as greater amounts of cellulosic regions of DP greater than 9 and greater amounts of cellotriosyl units measured after digestion with lichenase, were found in polymers from WE95 fractions compared to WE45. This also translated to higher apparent viscosity in the WE95 fractions. Therefore, water extraction at high temperature may prove to be advantageous in the isolation of β-glucans for nutritional and/or functional purposes; more research is needed to establish whether these β-glucans of lesser solubility, once isolated

and incorporated into food, exhibit significantly different physiological behaviour compared to those extracted at lower temperatures. Extracting at a temperature of 45°C may prove to be more cost effective from a production standpoint, especially if the physiological and nutritional benefits of a higher extraction temperature are not substantial.

Intervarietal differences were observed through ^{13}C-NMR and analysis of oligosaccharides following lichenase digestion; however, most evident were the differences in weight average molecular weight (HPSEC-MALLS) between WE45 and WE95 fractions from different cultivars. Results indicated that varieties such as high amylose and waxy, having non-starch polysaccharides of higher molecular weight than those in normal barley genotypes, may have greater potential in food and nutritional applications.

Similar to β-glucans, arabinoxylans from the various fractions and cultivars varied in solubility and structure. A portion of arabinoxylans was solubilized in water at 45 and 95°C, and extracted along with the β-glucans; the amount of arabinoxylan extracted was significantly higher in high amylose varieties ($p>0.05$). Arabinoxylans in WE45 and WE95 had a higher degree of branching compared to their alkali-soluble counterparts, as indicated by their lower Xyl*p*/Ara*f* ratios. Among the $Ba(OH)_2$ fractions, t he substitution pattern of the xylan backbone correlated with some small differences in M_w, R_g, and M_w/M_n. When the $Ba(OH)_2$ arabinoxylans were sub-fractionated with ammonium sulphate, separation based on Xyl*p*/Ara*f*, M_w, M_w/M_n, and R_g was achieved; however, polydispersity indices remained high, pointing to the extreme heterogeneity of these polysaccharides. ^{1}H-NMR analysis of the sub-fractions would indicate whether separation was based on the substitution patterns. To date, little is known of the physiological effect of arabinoxylan

consumption, and studies examining the viscoelastic behaviour of isolated arabinoxylans, together with those looking at their cholesterolaemic and/or glycaemic effects, would be helpful in determining whether these polymers in barley deserve greater attention.

6. GENERAL CONCLUSIONS

The present work has shown that non-starch polysaccharides from hull-less barley may vary dramatically in content, solubility and molecular characteristics depending on the cultivar. The use of enzymes and/or sonication during aqueous extraction, and hydrothermal pretreatment of the grain, were found to increase the pool of water-extractable NSP (β-glucans/arabinoxylans) and prevent their hydrolysis, respectively. Such treatments may therefore be useful in enhancing the desired properties of barley for food use. However, in the quest to achieve the maximum health benefits of barley (through its incorporation into food products), initial selection of cultivars with high content, solubility, and viscosity of β-glucans and arabinoxylans is critical, and hence necessitates the determination of which barley cultivars best meet these criteria. In this particular study, molecular features such as the length of cellulosic regions in β-glucans, Xyl*p*/Ara*f* in arabinoxylans, weight average molecular weight of water- and alkali-extractable fractions ($BaOH_2$), were found to vary significantly depending on the barley genotype. The impact of such molecular differences on their physiological properties is not certain, but the varying viscoelastic properties of the water-soluble fractions indicate that it may be possible for two barley varieties containing similar amounts of soluble NSP to have different nutritional characteristics.

Future research efforts should therefore focus on increased understanding of the relationships between the structure of β-glucans and arabinoxylans, shown here to vary depending on the cultivar, and their physiological or functional properties. This would include studies examining the effects of consumption of β-glucans and arabinoxylans varying in source (cultivar) and molecular features, on glycaemic index and/or serum

cholesterol, as well as the effects of processing on these relationships as the polymers exist in cereal based products and/or functional foods. In addition to the use of enzymic, physical, and/or hydrothermal treatment, further work to optimize efficiency and cost-effectiveness, such as the extraction from, or addition of, milling byproducts rather than the whole grain, may also render the manufacture of concentrates and foods rich in barley β-glucan/arabinoxylan more feasible. All such efforts would assist in the expanding role of barley as a food grain, thereby increasing its value as a crop while contributing to the improved health of our population.

7. LITERATURE CITED

Aastrup, S. 1979. The relationship between the viscosity of an acid flour extract of barley and its β-glucan content. Carlsberg Research Communication. 44:289-304.

Åman, P., and C.W. Newman. 1986. Chemical composition of some different types of barley grown in Montana, U.S.A. J. Cereal Sci. 4:133-141.

Andersson, A.A.M., Elfverson, C., Andersson, R., Regner, S., and P. Åman. 1999. Chemical and physical characteristics of different barley samples. J. Sci. Food Agric. 79: 979-986.

Antoniou, T, Marquardt, R.R., and P.E. Cansfield. 1981. Isolation, partial characterization, and antinutritional activity of a factor (Pentosans) in rye grain Feeding experiments with chicks. J. Agric. Food Chem. 39:1240-1247.

Bamforth, C.W., Moore, J., Proudlove, M.O., Bartholome, B., and G. Williamson. 1996. The dissolution of β-glucans. New dimensions. Abstract. J. Inst. Brew. 102:313.

Bartolomé, B., Faulds, C.B., and G. Williamson. 1997. Enzymic release of ferulic acid from barley spent grain. J. Cereal Science. 25:285-288.

Beer, M.U., Wood, P.J., and J. Weisz. 1997. Molecular weight distribution and (1-3)(1-4)-β-D-glucan content of consecutive extracts of various oat and barley cultivars. Cereal Chem. 74:476-480.

Bhatty, R.S. 1986. Physicochemical and functional (bread making) properties of hull-less barley fractions. Cereal Chem. 63:31-35.

Bhatty, R.S. 1987. Relationship between acid extract viscosity and total soluble and insoluble beta-glucan contents of hulled and hull-less barley. Can. J. Plant Sci. Rev. Can. Phytotechnie. 67:997-1008.

Bhatty, R.S. 1995. Laboratory and pilot plant extraction and purification of β-glucans from hull-less barley and oat brans. J. Cereal Sci. 22:163-170.

Bhatty, R.S. 1997. Milling of regular and waxy starch hull-less barleys for the production of bran and flour. Cereal Chem. 74:693-699.

Bhatty, R.S. 1999. β-Glucan and flour yield of hull-less barley. Cereal Chem. 76:314-315.

Bhatty, R.S., Berdahl, J.D., and G.I. Christison. 1975. Chemical composition and digestible energy of (feed) barley. Can. J. Anim. Sci. 55:759-764.

Bhatty, R.S.; MacGregor, A.W.; B.G. Rossnagel, B.G. 1991. Total and acid-soluble β-glucan content of hull-less barley and its relationship to acid-extract viscosity. Cereal Chem. 68:221-227.

Biliaderis, C.G., Izydorczyk, M., and O. Rattan. 1995. Effect of arabinoxylans on bread-making quality of wheat flours. Food Chem. 53:165-171.

Böhm, N., and W.-M.. Kulicke. 1999. Rheological studies of barley (1-3),(1-4)-β-glucan in concentrated solution: investigation of the viscoelastic flow behaviour in the sol-state. Carbohyd. Res. 315:293-301.

Breitmaier, E., and W. Voelter. 1987. Carbon-13 NMR Spectroscopy - High-resolution methods and applications in Organic Chemistry and Biochemistry. 3rd ed. VCH Publishers, New York NY.

Burkus, Z., and F. Temelli. 1998. Effect of extraction conditions on yield, composition, and viscosity stability of barley β-glucan gum. Cereal Chem. 75:805-809.

Carriere, C.J., and G.E. Inglett. 1998. Solution viscoelastic properties of Oatrim-10 and cooked oat bran. Cereal Chem. 75:354.

Cawley, R.W. 1964. The role of wheat flour pentosans and other gums on gluten-starch loaves. J. Sci. Food Agric. 15:834-838.

Courtin, C.M., and J.A. Delcour. 1998. Physicochemical and bread-making properties of low molecular weight wheat-derived arabinoxylans. J. Agric. Food Chem. 46: 4066-4073.

Cummings, J.H., and H.N. Englyst. 1995. Gastrointestinal effects of food carbohydrate. Am. J. Clin. Nutr. 61:938S-945S.

D'Appolonia, B.L., Gilles, K.A., and D.G. Medcalf. 1970. Effect of water-soluble pentosans on gluten-starch loaves. Cereal Chem. 47:194-204.

Dervilly, G., Saulnier, L., Roger, P., and J. Thibault. 2000. Isolation of homogeneous fractions from wheat water-soluble arabinoxylans. Influence of the structure on their macromolecular characteristics. J. Agric. Food Chem. 48:270-278.

Doehlert, D.C., Zhang, D., and W.R. Moore. 1997. Influence of heat pretreatments of oat grain on the viscosity of flour slurries. J. Sci. Food Agric. 74:125-131.

Edney, M.J., Marchylo, B.A., and A.W. MacGregor. 1991. Structure of total barley β-glucan. J. Inst. Brew. 97:39-44.

Ellis, R.P., Swanston, J.S., Rubio, A., Perez-Vendrell, A.M., Romagosa, I., and J.L.

Englyst, H., Wiggins, H.S., and J.H. Cummings. 1982. Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates. Analyst. 107:307-318.

Freeman, M.E., and R.A. Gortner. 1932. The gums of the cereal grains. Food Chem. 9: 506-517.

Gan, Z., Ellis, P.R., and J.D. Schofield, J.D. 1995. Mini review: Gas cell stabilization and gas retention in wheat bread dough. J. Cereal Sci. 21: 215-230.

Gómez, C., Navarro, A., Manzanares, P., Horta, A., and J.V. Carbonell. 1997a. Physical and structural properties of barley (1-3),(1-4)-β-D-glucan. Part I.. Determination of molecular weight and macromolecular radius by light scattering. Carbohydr. Polym. 32:7-15.

Gómez, C., Navarro, A., Manzanares, P., Horta, A., and J.V. Carbonell. 1997b. Physical and structural properties of barley (1-3),(1-4)-β-D-glucan. Part II. Viscosity, chain stiffness and macromolecular dimensions. Carbohydr. Polym. 32:17-22.

Gómez, C., Navarro, A., Manzanares, P., Horta, A., and J.V. Carbonell. 1997c. Physical and structural properties of barley (1-3),(1-4)-β-D-glucan. Part III. Formation of aggregates analyzed through its viscoelastic and flow behaviour. Carbohydr. Polym. 32:141-148.

Gruppen, H., Hamer, R.J., and A.G.J. Voragen. 1991. Barium hydroxide as a tool to extract pure arabinoxylans from water-insoluble cell wall material of wheat flour. J. Cereal Sci. 13:275-290.

Gruppen, H., Kormelink, F.J.M., and A.G.J. Voragen. 1993. Water-unextractable cell wall material from wheat flour. 3. A structural model for arabinoxylans. J. Cereal Sci. 18:111.

Gudmundsson, M., and A.C. Eliasson, 1996. Some physical properties of barley starches from cultivars differing in amylose content. J. Cereal Sci. 16:95-105.

Hagerman, A.E. and R.L. Nicholson. 1982. High-performance liquid chromatographic determination of hydroxycinnamic acids in the maize mesocotyl Phenol metabolism, Zea mays, corn. J. Agric. Food Chem. 30:1098-1102.

Hartley, R.D. and E.C. Jones. 1976. Diferulic acid as a component of cell walls of *Lolium multiflorum*. Phytochem. 15:157-1160.

Hecker, K.D., Meier, M.L., Newman, R.K., and C.W. Newman. 1998. Barley β-glucan is effective as a hypocholesterolaemic ingredient in foods. J. Sci. Food Agric. 77:179-183.

Henry, R.J., and A.H.D. Brown. 1987. Variation in the carbohydrate composition of wild

barley (Hordeum spontaneum). Plant Breeding. 98:97-103.

Inglett, G.E., and S.B. Grisamore. 1991. Maltodextrin fat substitute lowers cholesterol. Food Technol. 45:104.

Ishii, T. 1991. Acetylation at O-2 of arabinofuranose residues in feruloylated arabinoxylan from bamboo shoot cell-walls. Phytochem. 30:2317-2320.

Izydorczyk, M.S., and C.G. Biliaderis. 1992. Influence of structure on the physicochemical properties of wheat arabinoxylan. Carbohydr. Polym. 17:237-247.

Izydorczyk, M.S., and C.G. Biliaderis. 1995. Cereal arabinoxylans: advances in structure and physicochemical properties. Carbohydr. Polym. 28:33-48.

Izydorczyk, M.S. and C.G. Biliaderis. 1996. Gradient ammonium sulphate fractionation of galactomannans. Food Hydrocoll. 10:295-300.

Izydorczyk, M.S., and C.G. Biliaderis. 2000. Pp. 361-383 in Novel Macromolecules in Food Systems. G. Doxastakis and V. Kiosseoglou (eds.). Elsevier Publ. Co., Amsterdam.

Izydorczyk, M.S., Biliaderis, C.G., and Bushuk, W. 1991. Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. Cereal Chem. 68:39-144.

Izydorczyk, M.S., and A.W. MacGregor. 2000. Evidence of intermolecular interactions of β-glucans and arabinoxylans. Carbohydr. Polym. 41:417-420.

Izydorczyk, M.S., Macri, L.J., and A.W. MacGregor. 1998a. Structure and physicochemical properties of barley non-starch polysaccharides - I. Water-extractable β-glucans and arabinoxylans. Carbohydr. Polym. 35:249-258.

Izydorczyk, M.S., Macri, L.J., and A.W. MacGregor. 1998b. Structure and physicochemical properties of barley non-starch polysaccharides - II. Alkali-extractable β-glucans and arabinoxylans. Carbohydr. Polym. 35:259-269.

Jadhav, S.J., Lutz, S.E., Ghorpade, V.M., and D.K. Salunkhe. 1998. Barley: Chemistry and value-added processing. Crit. Rev. Food Sci. 2:123-171.

Jelaca, S.L., and I. Hlynka. 1972. Effect of wheat-flour pentosans in dough, gluten, and bread. Cereal Chem. 49: 489-495.

Jenkins, D.J.A., Jenkins, A.L., Wolever, T.M.S., Rao, A.V., and L. Thompson. 1985. Fiber and starchy foods: Gut function and implications in disease. Am. J. Gastroenterol. 81:920-930.

Jenkins, D.J.A., Jenkins, A.L., Wolever, T.M.S., Vuksan, V., Venket Rao, A., Thompson, L. and R.G. Josse. 1995. Effect of reduced rate of carbohydrate absorption on carbohydrate and lipid metabolism. Eur. J. Clin. Nutr. 49:S68-S73.

Jiang, G. and T. Vasanthan. 2000. MALDI-MS and HPLC quantification of oligosaccharides of lichenase-hydrolyzed water-soluble beta-glucan from ten barley varieties. J. Agric. Food Chem. 48: 3305-3310.

Kahlon, T.S., and F.I. Chow. 1997. Hypocholesterolemic effects of oat, rice, and barley dietary fibers and fractions. Cereal Foods World. 42:86-92.

Kalra, S., and S. Jood. 2000. Effect of dietary barley β-glucan on cholesterol and lipoprotein fractions in rats. J. Cereal Sci. 31:141-145.

Karpinnen, S., Liukkonen, K., Aura A., Forssell, P., and K. Poutanen. 2000. In vitro fermentation of polysaccharides of rye, wheat and oat brans and inulin by human faecal bacteria. J. Sci. Food Agric. 80:1469-1476.

Klamczynski, A.P., and Z. Czuchajowska. 1999. Quality of flours from waxy and non-waxy barley for production of baked products. Cereal Chem. 76:530-535.

Knuckles, B.E., Chiu, M.M., and A.A. Betschart. 1992. Beta-glucan enriched fractions from laboratory-scale dry milling and sieving of barley and oats. Cereal Chem. 69:198-202.

Knuckles, B.E., and M-C.M. Chiu. 1999. Beta-glucanase activity and molecular weight of beta-glucans in barley after various treatments. Cereal Chem. 76:92-95.

Knuckles, B.E., Yokohama, W.H., and M-C.M. Chiu. 1997. Molecular characterization of barley β-glucans by size-exclusion chromatography with multiple-angle laser light scattering and other detectors. Cereal Chem. 74:599-604.

Lee, C.J., Horsley, R.D., Manthey, F.A., and P.B. Schwarz. 1997. Comparisons of β-glucan content of barley and oat. Cereal Chem. 74:571-575.

Lee, Y., Schwarz, P., and B. D'Appolonia. 1998. Functional properties of β-glucans from wheat starch and flour. Food Sci. Biotechnol. 7:51-55.

Lowry, O.L., Rosebrough, N.J., Farr, A.L., and R.J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Loosveld A., Grobet P.J., and J.A. Delcour. 1997. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. J. Agric. Food Chem. 45:1998-2002.

MacGregor, A.W., and G.B. Fincher. 1993. Carbohydrates of the barley grain. pp. 73-130 in Barley Chemistry and Technology. A.W. MacGregor and R.S. Bhatty (eds). American Association of Cereal Chemists; St Paul.

Megazyme Int. Ireland Ltd. Mixed-linkage beta-glucan assay procedure. Megazyme (Aust.) Pty. Ltd., 2/11 Ponderosa Parade, Warriewood (Sydney) Australia.

McCleary, B.V., and I. Shameer. 1987. Assay of malt β-glucanase using azo barley glucan: an improved precipitant. J. Inst. Brew. 93:87-90.

McDonald, A.M.L., and Stark, J.R. 1988. A critical examination of procedures for the isolation of barley starch. J. Inst. Brew. 94:125-132.

Miller, S.S. and R.G. Fulcher. 1995. Oat endosperm cell walls. II. Hot-water solubilization and enzymatic digestion of the wall. Cereal Chem. 72:428-432..

Miller, S.S. Fulcher, R.G., Sen, A., and J.T. Arnason. 1995. Oat endosperm cell walls: I. Isolation, composition, and comparison with other tissues. Cereal Chem. 72:421-427.

Molina, C. 1997. The development of β-glucanase and degradation of β-glucan in barley grown in Scotland and Spain. J. Cereal Sci. 26:75-82.

Newman, R.K., Lewis, S.E., Newman, C.W., Boik, R.J., and Ramage, R.T. 1989. Hypocholesterolemic effect of barley foods on healthy men. Nutr. Rep. Int. 39:749-758.

Newman, R.K., and C.W. Newman. 1991. Barley as a food grain. Cereal Foods World. 36:800-805.

Nordkvist, E., Salomonsson, A.C. and P. Åman. 1984. Distribution of insoluble bound phenolic acids in barley grain. J. Sci. Food Agric. 35:657-661.

Oscarsson, M, Andersson, R., Salomonsson, A-C., and P. Åman. 1996. Chemical composition of barley samples focusing on dietary fibre components. J. Cereal Sci. 24:161-170.

Oscarsson, M., Parkkonen, T., Autio, K., and P. Åman. 1997. Comparison and microstructure of waxy, normal, and high amylose barley samples. J. Cereal Sci. 26:259-264.

Pence, J.W., Elder, A.H., and D.K. Mecham. 1950. Preparation of wheat flour pentosans for use in reconstituted doughs. Cereal Chem. 27: 60-66.

Perez-Vendrell, A.M., Brufau, J., Molina-Cano, J.L., Francesch, M., and J. Guasch. 1996. Effects of cultivar and environment on β-(1,3)-(1,4)-D-glucan content and acid extract

viscosity of Spanish barleys. J. Cereal Sci. 23:285-292.

Perlin, A.S. 1951. Structure of the soluble pentosans of wheat flours. Cereal Chem. 28: 382-393.

Pietinen, P., Rimm, E.B., Korhonen, P., Hartman, A.M., Willett, W.C., Albanes, D., and J. Virtamo. 1996. Intake of dietary fibre and risk of coronary heart disease in a cohort of Finnish men. Circulation 94:2720-2727.

Pomeranz, Y. 1988. Wheat. Chemistry and Technology, 3rd Ed. American Association of Cereal Chemists, Minnesota.

Ralph, J., Quideau, S., Grabber, J.H., and R.D. Hatfield. 1994. Identification and synthesis of new ferulic acid dehydrodimers present in grass cell walls. J. Chem. Soc. Perkin Trans. 1:3485-3498.

Roels, S.P., Collado, M., Loosveld, A.M., Grobet, P.J., and J.A. Delcour. 1999. Variation in the degree of D-xylose substitution in water-extractable European durum wheat (*Triticum durum* Desf.) semolina arabinoxylans. J. Agric. Food Chem. 47:1813-1816.

Rouau, X., and D. Moreau. 1993. Modification of some physicochemical properties of wheat flour pentosans by an enzyme complex recommended for baking. Cereal Chem. 70(6): 626-632.

Saulnier, L., Andersson, R., and P. Åman. 1997. A study of the polysaccharide components in gluten. J. Cereal Sci. 25:121-127.

Saulnier, L., Gevaudan, S., and J.K. Thibault. 1994. Extraction and partial characterization of β-glucan from the endosperms of two barley cultivars. J. Cereal Sci. 19:171-178.

Schoch, T.J. 1964. Iodimetric determination of amylose. In: Methods in Carbohydrate Chemistry. Vol 4. Whistler, R.L. ed. Academic Press, New York, pp 157-160.

Schooneveld-Bergmans, van Dijk, Y., Beldman, G., and A. Voragen. 1999a. Physicochemical characteristics of wheat bran glucuronoarabinoxylans. J. Cereal Sci., 29:49-61.

Schooneveld-Bergmans, Beldman, G., and A. Voragen. 1999b. Structural features of (glucurono)arabinoxylans extracted from wheat bran by barium hydroxide. J. Cereal Sci., 29:63-75.

Schooneveld-Bergmans, M., Hopman, A., Beldman, G., and A. Voragen. 1998. Extraction and partial characterization of feruloylated glucuronoarabinoxylans from wheat bran. Carb. Polym. 35:39-47.

Schneeman, B.O. 1998. Properties of non-digestible polysaccharides associated with health. Abstract. Plant Polysaccharide Symposium, University of California, Davis, CA.

Slavin, J.L. 1987. Dietary fiber: Classification, chemical analysis and food sources. J. Am. Diet. Assoc. 87:1164-1171.

South, J.B., and Morrison, W.R. 1990. Isolation and analysis of starch from single kernels of wheat and barley. J. Cereal Sci. 12:43-51.

Stone, B.A., and A.E Clarke. 1993. Chemistry and biology of (1→3)-β-glucans. La Trobe University Press, Bundoora.

Stuart, I.M., Loi, L., and G.B. Fincher. 1988. Varietal and environmental variation in (1→3,1→4)- β-glucan levels and (1→3,1→4)- β-glucanase potential in barley: Relationship to malting quality. J. Cereal Sci. 7:61-71.

Sulaiman, B.D., and Morrison, W.R. 1990. Proteins associated with the surface of wheat starch granules purified by centrifuging through caesium chloride. J. Cereal Sci. 12:53-61.

Temelli, F. 1997. Extraction and functional properties of barley β-glucan as affected by temperature and pH. J. Food Sci. 62:1194-1201.

Theander, O., Westerlund, E., and P. Åman. 1993. Structure and components of dietary fiber. Cereal Foods World 38(3): 135-141.

Tvaroska, I., Ogawa, K., Deslandes, Y., and R.H. Marchessault. 1983. Crystalline conformation and structure of lichenan and barley beta-glucan. Can. J. Chem. 61:1608-1616.

Vårum, K.M., Smidsrod, O., and Brant, D.A. 1992. Light scattering reveals micelle-like aggregation in the (1-3),(1-4)-beta-D-glucans from oat aleurone. Food Hydrocol. 5: 497-511.

Vukic Vranjes, M., and C. Wenk. 1995. The influence of extruded vs. untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. Animal Feed Sci. Technol. 54: 21-32.

Wang, L., Miller, R.A., and R.C. Hoseney. 1998. Effects of (1-3),(1-4)-β-D-glucans of wheat flour on bread making. Cereal Chem. 75:629-633.

Wang, L, Newman, R.K., Newman, C.W., and P.J. Hofer. 1992. Barley beta-glucans alter intestinal viscosity and reduce plasma cholesterol concentrations in chicks. J. Nutr. 122:2292-2297.

Wood, P.J. 1993. In Oat bran (ed. P.J. Wood), Amer. Assoc. Cereal Chem., St. Paul, MN, p. 83.

Wood, P.J., Anderson, J.W., Braaten, J.T., Cave, N.A., Scott, F.W., and C. Vachon. 1989. Physiological effects of beta-D-glucan rich fractions from oats. Cereal Foods World. 34:878-882.

Wood, P.J., Braaten, J.T., Scott, F.W, Riedel, K.D, Wolynetz, M.S., and M.W. Collins. 1994. Effect of dose and modification of viscous properties of oat gum on plasma glucose and insulin following an oral glucose load. Brit. J. Nutr. 72:731-743.

Wood, P.J., Weisz, J., and B.A. Blackwell. 1991. Molecular characterization of cereal β-D-glucans. Structural analysis of oat β-D-glucan and rapid structural evaluation of β-D-glucans from different sources by high-performance liquid chromatography of oligosaccharides released by lichenase. Cereal Chem. 68:31-39.

Wood, P.J., Weisz, J., and B.A. Blackwell. 1994. Structural studies of (1-3),(1-4)-β-D-glucans by ^{13}C-nuclear magnetic resonance spectroscopy and by rapid analysis of cellulose-like regions using high-performance anion-exchange chromatography of oligosaccharides released by lichenase. Cereal Chem. 71:301-307.

Wood, P.J., Weisz, J., Fedec, P., and V.B. Burrows. 1989. Large-scale preparation and properties of oat fractions enriched in (1→3)(1→4)-β-D-glucan. Cereal Chem. 66:97-103.

Wood, P.J., Weisz, J., and W. Mahn. 1991. Molecular characterization of cereal β-glucans. II. Size-exclusion chromatography for comparison of molecular weight. Cereal Chem. 68:530-536.

Woodward, J.R., and G.B. Fincher. 1982. Substrate specificities and kinetic properties of two (1-3),(1,4)-β-D-glucan endo-hydrolases from germinating barley (Hordeum vulgare). Carbohydr. Res. 106:11-122.

Woodward, J.R., Phillips, D.R., and G.B. Fincher. 1988. Water soluble (1→3) (1→4) -β-D-glucans from barley (Hordeum vulgare) endosperm: IV. Comparison of 40C and 65C soluble fractions. Carbohydr. Polym. 8:85-98.

Wu, Y.V., Stringfellow, A.C., and G.E. Inglett. 1994. Protein- and β-glucan-enriched fractions from high-protein, high β-glucan barleys by sieving and air classification. Cereal Chem. 71:220-223.

Xue, Q., Newman, R.K., Newman, C.W., and C.F. McGuire. 1991. Waxy gene effects on β-glucan, dietary fiber content and viscosity of barleys. Cereal Research Communications. 19:399-404.

Xue, Q., Wang, L., Newman, R.K., Newman, C.W., and H. Graham. 1997. Influence of the hull-less, waxy starch and short-awn genes on the composition of barleys. J. Cereal Sci. 26:251-257.

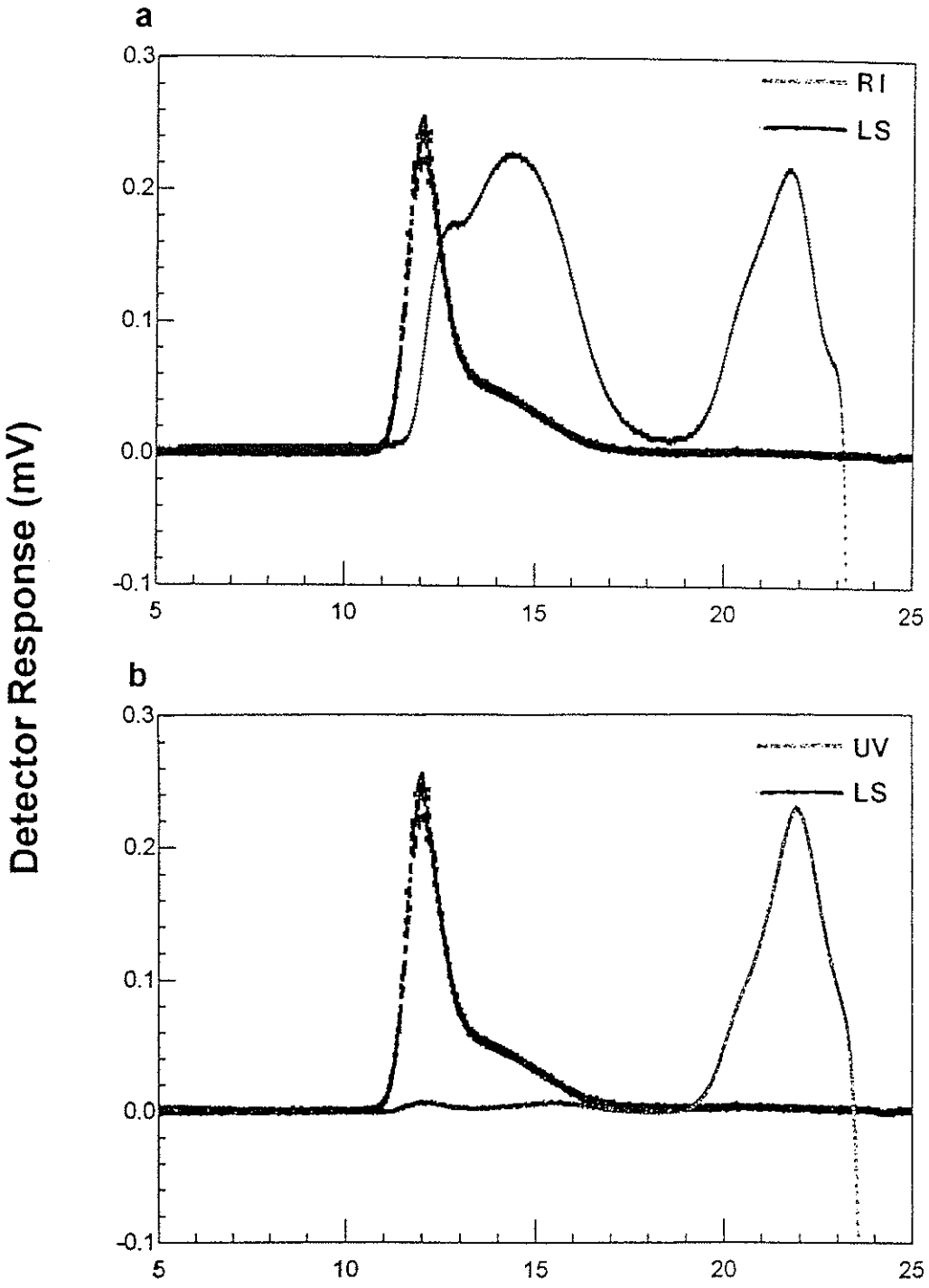
Yokohama, W.H., Hudson, C.A., Knuckles, B.E., Chiu, M-C.M, Sayre, R.N., Turnlund, J.R., and B.O. Schneeman. 1997. Effect of barley β-glucan in durum wheat pasta on human glycemic response. Cereal Chem. 74:293-296.

You, S., Fiedorowicz, M., and S. Lim. 1999. Molecular characterization of wheat amylopectins by multi-angle laser light scattering analysis. Cereal Chem. 76:116-121.

Zhang, D., Doehlert, D.C., and W.R. Moore. 1997. Factors affecting viscosity of slurries of oat groat flours. Cereal Chem. 74:722-726.

Zheng, G.H., Rossnagel, B.G., Tyler, R.T., and R.S. Bhatty. 2000. Distribution of β-glucan in the grain of hull-less barley. Cereal Chem. 77:140-144.

APPENDIX I. HIGH-PERFORMANCE SIZE EXCLUSION CHROMATOGRAPHY OF THE $Ba(OH)_2$ FRACTION FROM 92-55-06-48 WITH MALLS AND RI DETECTION (a) AND WITH MALLS AND UV DETECTION (b). DETECTOR RESPONSE INDICATED ON THE Y-AXIS IS THE RI AND UV RESPONSE, RESPECTIVELY.



APPENDIX II. CALCULATION OF THE DISTRIBUTION OF UN- (U-Xyl*p*), MONO- (2-Xyl*p*, 3-Xyl*p*) , AND DI-SUBSTITUTED (2,3-Xyl*p*) XYLOSE RESIDUES IN ARABINOXYLANS FROM $Ba(OH)_2$ FRACTIONS.

X = weight percentage of Xyl*p*
A = weight percentage of Ara*f*
X_0 = weight percentage of U-Xyl*p*
X_1 = weight percentage of 2-Xyl*p* and 3-Xyl*p*
X_2 = weight percentage of 2,3-Xyl*p*

$$X = X_0 + X_1 + X_2 \quad (1)$$

$$A = X_1 + 2X_2 \quad (2)$$

(1) From the ^{1}H-NMR spectra, integrate the peaks at δ 5.36 (X_1), and δ 5.26 and δ 5.19 ppm (X_2), and determine the ratio of X_1/X_2:

$$a = X_1/X_2 \text{ or } X_1 = aX_2$$

(2) From the GC analysis, calculate the A/X ratio:

$$A/X = b \text{ or } bX = A$$

It follows that

$$A = aX_2 + 2X_2$$
$$A = (a + 2)X_2 \quad (3)$$

Since $bX = A$,

$$bX = (a + 2)X_2 \quad (4)$$

Rearranging equation 4:

$$X_2/X = b/(a + 2)$$

Since $X_1 = aX_2$ or $X_1/a = X_2$ (1),

$$(X_1/a)/X = b/(a + 2)$$
$$X_1/X = ab(a + 2) \quad (5)$$

We know that

$$100\% = [X_0/X + (X_1/X) + (X_2/X)]*100\% \quad (6)$$

Therefore

$$(X_0/X)*100\% = 100\% - [X_1/X + X_2/X]*100\%$$

or

$$X_0/X = 1 - [ab(a + 2)] - b/(a + 2)$$

or

$$X_0/X = 1 - (a + 1)[b/(a + 2)]$$

Within the arabinose anomeric proton region of the ^{1}H-NMR spectra (δ 5.2 - 5.5 ppm), all $Ba(OH)_2$ fractions had three major peaks. The first peak at δ 5.36 ppm represents the anomeric protons (H1) of Ara*f* linked to the O-3 positions of Xyl*p* residues of arabinoxylans (Roels et al., 1999). The other two peaks (δ 5.26 and 5.19 ppm) represent the anomeric protons (H1) of Ara*f* linked to the O-2 and O-3 positions of the same Xyl*p* residue (Roels et al., 1999).