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Cereal arabinoxylans: advances in structure and physicochemical properties

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Arabinoxylans constitute a major fraction of cereal cell wall polysaccharides. They consist of a linear β-(1 → 4) linked xylan backbone to which α-L-arabinofuranose units are attached as side residues via α-(1 → 3) and/or α-(1 → 2) linkages. Several structural models have been put forward based on enzymic degradation studies and structure elucidation of oligosaccharides by NMR, methylation, and periodate oxidation techniques. These tentative models present different substitution patterns of arabinoses along the xylan chain. Cereal arabinoxylans exhibit a great deal of structural heterogeneity with respect to ratio of Ara*f*/Xyl*p*, substitution pattern of arabinoses, content of feruloyl groups and molecular size. The conformation and physicochemical properties (viscosity, gelation potential, intermolecular association) of arabinoxylans in aqueous solutions are dependent on the molecular features of these polysaccharides; specific structure–property relationships have been established in model and actual food systems. Wheat and rye arabinoxylans are important functional ingredients in baked products affecting the mechanical properties of dough, as well as the texture and other end-product quality characteristics.

INTRODUCTION

In 1927, non-starchy, gummy polysaccharides were isolated from bread wheat flours and shown to consist predominantly of the pentoses, arabinose and xylose (Hoffmann & Gortner, 1927; Freeman & Gortner, 1932). Similar polysaccharides were also found in durum wheat, rye, and barley, and were initially referred to as pentosans. However, since pentosans represent a heterogeneous group of polysaccharides which, in addition to pentose sugars, may also contain hexoses, hexuronic acids, and some proteins, the current nomenclature is more structure descriptive, identifying several polymeric components such as arabinoxylans or arabinogalactan peptides, depending on the molecular constitution of the polysaccharides. In recent years, wheat arabinoxylans in particular have stimulated much research interest since they have been proven to have significant influence on the water balance (Jelaca & Hlynka, 1971) and rheological properties of dough (Meuser & Suckow, 1986; Michniewicz *et al.*, 1991), retrogradation of starch (Gudmundsson *et al.*, 1991; Biliaderis & Izydorczyk, 1992), and bread quality (McCleary, 1986; Delcour *et al.*, 1991). Although arabinoxylans have been of interest to cereal chemists and technologists for many years, structural studies initiated in 1951 by Perlin were taken up only in the 1990s when a number of workers focused on the detailed structural characteristics of these polysaccharides.

This review surveys the most recent findings on cereal arabinoxylans, especially with regard to their location in grains, occurrence, structure, physicochemical and functional properties. An effort is also made to interrelate the molecular features and physicochemical properties of these biopolymers in solution and in food systems.

OCCURRENCE

Arabinoxylans have been identified in a variety of tissues of the main cereals of commerce: wheat, rye, barley, oat, rice, sorghum (Fincher & Stone, 1986), as well as in some other plants: pangola grass (Ford, 1989), bamboo shoots (Ishii, 1991) and rye grass (Hartley & Jones, 1976). Although these polysaccharides are minor components of entire cereal grains, they constitute an important part of plant cell walls. Thin walls that surround the cells in the starchy endosperm and the

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aleurone layer in most cereals consist predominantly of arabinoxylans (~60–70%); exceptions are endosperm cell walls of barley (~20%) and rice (~40%) (Fincher & Stone, 1986). Non-endospermic tissues of wheat, particularly the pericarp and testa, also have very high arabinoxylan content (~64%) (Selvendran *et al.*, 1980). In contrast, the walls of beeswing are rich in acidic polymers, glucuronoarabinoxylans (~60%) (Dupont & Selvendran, 1987; Brillouet & Joseleau, 1987). Glucuronoarabinoxylans have also been found in the husk of sorghum grain (Woolard *et al.*, 1976) and barley (Fincher & Stone, 1986), rice bran and endosperm (Shibuya & Iwasaki, 1985), and coleoptile cell walls of *Zea mays* (Nishitani & Nevins, 1988). Examination of the effects of genotypic and environmental differences on arabinoxylan levels in several cereals (Henry, 1986) pointed to the importance of these two factors; however, more systematic studies are necessary to establish more exact effects of these factors on the level and structural characteristics of arabinoxylans.

A large portion of cereal arabinoxylans cannot be extracted from the cell wall material with water. Differences in the water extractability of these polysaccharides might be related to differences in substitution patterns and in the extent of physical entanglement, covalent ester bonding between carboxyl groups of uronic acids and the hydroxyl groups of arabinoxylans, as well as the formation of diferulic acid bridges between adjacent arabinoxylan chains (Mares & Stone, 1973; Geissmann & Neukom, 1973*a*; Fincher & Stone, 1986; Gruppen *et al.*, 1992*a*).

Arabinoxylans, together with other polysaccharides, build up the cell walls of grain tissues and thus become part of the skeletal framework that maintains tissue integrity (Fincher & Stone, 1986). Because of their ability to absorb large amounts of water, they may reduce brittleness and provide some degree of elasticity and resistance of plant tissues to bending abuses. They could also be of some aid in allowing transport of dissolved metabolites and nutrients through the porous hydrated molecular network they establish around the cellulose crystallites. It has also been postulated that certain structural features of arabinoxylans permit some intermolecular alignment between polymer chains or non-covalent interactions of arabinoxylans with other polysaccharides (β-glucan, cellulose) and, therefore, formation of multicomponent gels in the complex matrix of the walls. In addition, the presence of ferulic acid residues on the arabinoxylan chains provides some potential for covalent polysaccharide–polysaccharide or polysaccharide–protein interactions (Fincher & Stone, 1986). Differences in the molecular features of arabinoxylans (degree of branching, spatial arrangement of arabinosyl substituents along the xylan backbone, or ferulic acid content) could alter the viscoelastic properties of the gels and hence the resilience, strength, and porosity of the wall matrix (MacGregor & Fincher, 1993). Another postulated function of arabinoxylans in cell walls of cereal grains is the inhibition of intercellular ice formation, ensuring winter survival of cereals (Kindel *et al.*, 1989); this effect could be attributed to the enhancement of viscosity and mechanical interference of the arabinoxylan gel network to the propagation of ice.

CHEMISTRY AND PHYSICOCHEMICAL PROPERTIES OF ARABINOXYLANS

Isolation, purification and fractionation of arabinoxylans

The water-soluble arabinoxylan in cereal tissues can be separated from the insoluble residues by means of aqueous extraction. The main task of all purification schemes is the separation of arabinoxylans from contaminating water-soluble proteins, α-D-glucans, β-D-glucans, and arabinogalactans.

Fincher and Stone (1974) reported that treating wheat flour with 80% ethanol prior to extraction with water, resulted in isolating water-soluble pentosans with only 2% protein. A relatively higher protein content in pentosan preparations (5.2%) was obtained in a study by Kim and D'Appolonia (1976), where crude papain was used in the purification procedure. A combination of heat treatment (95°C, 5 min) and adsorption of protein on a clay adsorbent (Vega clay) seems to be a more effective means of deproteinizing the water extracts of wheat flour containing cell wall polysaccharides (Crowe & Rasper, 1988; Izydorczyk *et al.*, 1991*a*). A recent study which utilized the latter method (Rattan *et al.*, 1995) reported arabinoxylan preparations with protein contents as low as 0.5% (w/w).

Small molecular weight α-D-glucans can be effectively hydrolysed with salivary α-amylase (Fincher & Stone, 1974; Izydorczyk *et al.*, 1991*a*), porcine α-amylase (Rattan *et al.*, 1995) or amyloglucosidase (Crowe & Rasper, 1988), although repeated treatments might be required to ensure complete hydrolysis. Any residual glucose found in the extracts after enzymic digestion seems to originate from β-D-glucans which can be selectively precipitated with 35–40% saturation of ammonium sulphate (Izydorczyk, 1993). This salt has also proven very efficient in separating arabinoxylans from arabinogalactan peptides present in water extracts of wheat flours. The latter can be removed by precipitation of arabinoxylan with saturated $(NH_4)_2SO_4$ or 80% ethanol (Fincher & Stone, 1974; Izydorczyk *et al.*, 1991*a*).

Various procedures have been reported for the isolation of water-unextractable cell wall material from cereal flours or brans. Most isolations involve the centrifugation of flour–water suspensions (Kim & D'Appolonia, 1976; Michniewicz *et al.*, 1990) or dough washings (D'Appolonia & McArthur, 1975) resulting in a sludge layer which is further purified with hydrolytic

enzymes (protease and amylase) to remove residual proteins and starch. Recently, Gruppen *et al.* (1989) reported a large-scale isolation of highly purified cell wall material from wheat endosperm based on dough-kneading in combination with wet-sieving. Alternative separation methods are based on wet-sieving and ultrasonication in aqueous ethanol (Mares & Stone, 1973) or removal of starch and intercellular proteins by organic solvents (Selvendran & Dupont, 1980).

The many solvents used for extraction of cell wall constituents include DMSO, urea, hydroxylamine–HCl, *N*-methylmorpholine *N*-oxide (Joseleau *et al.*, 1981), KOH (Dupont & Selvendran, 1987; Brillouet & Joseleau, 1987), NaOH (Michniewicz *et al.*, 1990), and $Ba(OH)_2$ (Gruppen *et al.*, 1991); the last solvent is preferred for its selectivity towards arabinoxylans. As reported by Gruppen *et al.* (1991), extraction with saturated $Ba(OH)_2$ (containing some $NaBH_4$) yielded a pure arabinoxylan fraction which represented approximately 80% of all arabinoxylan present in the water-unextractable cell wall material from wheat endosperm.

In view of the fact that arabinoxylan preparations consist of several populations of arabinoxylan molecules which vary in structural characteristics, several fractionation techniques have been employed in an attempt to obtain more homogeneous fractions and thus explore structure–property relationships for these polymers. One of these techniques involving chromatography of pentosans complexed with sodium borate on diethyl aminoethyl (DEAE) cellulose column is based on the adsorption of polysaccharides to the cellulose matrix rather than on an ion exchange principle (Lineback *et al.*, 1977). The eluted fractions, obtained with a stepwise gradient of increasing borate or NaOH concentration, contain, however, a mixture of arabinoxylan and arabinogalactan peptide polymers which indicates a non-specific binding of pentosans to cellulose.

More recently, Dupont & Selvendran (1987) and Gruppen *et al.* (1992*a*) used anion-exchange chromatography for fractionation of alkali-extractable arabinoxylans from beeswing bran and endosperm of wheat. Binding of a portion of the material to the DEAE column suggested an acidic character of some arabinoxylan fractions. However, the mechanism of binding of arabinoxylans to the DEAE column remains somewhat unclear since none (Gruppen *et al.*, 1992*a*) or only traces (Dupont & Selvendran, 1987) of uronic acids were detected in some arabinoxylan fractions. In both studies, it was observed that the DEAE-bound arabinoxylans had a higher overall Ara/Xyl ratio than the unbound polymers. Dupont & Selvendran (1987) reported also that the higher content of phenolics in the DEAE-bound arabinoxylans might have influenced their elution pattern. In contrast to alkali-extractable arabinoxylans, water-extractable ones cannot be fractionated by anion-exchange chromatography (Gruppen *et al.*, 1992*a*).

Using fractional precipitation by stepwise addition of ammonium sulphate, several fractions of discrete chemical structures were obtained from water-soluble wheat endosperm arabinoxylans by Izydorczyk & Biliaderis (1992*a*). With increasing concentration of this salt, there was an increase in the ratio of Ara/Xyl and in the relative amount of doubly substituted xylose residues; simultaneously, ferulic acid content and the molecular size (as estimated by intrinsic viscosity measurements) of the isolated polysaccharide fractions decreased. Compared to the fractionation of proteins, selective precipitation with $(NH_4)_2SO_4$ has received very little attention with regard to fractionation of carbohydrate polymers. Although the exact mechanism of salting out neutral polysaccharides, such as arabinoxylans, remains obscure, studies by Izydorczyk & Biliaderis (1992*a*) indicated that fractional precipitation with this agent might be effected by intermolecular alignment and interaction of the unbranched portions between arabinoxylan chains. However, the relevance of feruloyl groups in the arabinoxylan molecules as well as that of the molecular size and conformation of the polymer chains cannot be excluded.

Fractionation of arabinoxylans by means of a graded ethanol precipitation technique also yields arabinoxylan families varying in their branching patterns (Hoffmann *et al.*, 1991*b*; Vietor *et al.*, 1992; Gruppen *et al.*, 1992*a*). However, while Vietor *et al.* (1992) and Gruppen *et al.* (1992*a*) reported, for alkali-extractable arabinoxylans from wheat and barley, an increase in Ara/Xyl ratio for fractions precipitating at higher alcohol concentrations, Hoffmann and co-workers (1991*b*) did not observe such relations. In contrast to the results published by Izydorczyk & Biliaderis (1992*a*), studies conducted by Hoffmann *et al.* (1991*b*) and Gruppen *et al.* (1992*a*) indicated the highly branched arabinoxylan fractions to be of higher molecular weight (as estimated by size exclusion chromatography and light scattering measurements) than their less branched counterparts. In addition, Vietor *et al.* (1992) reported no significant differences in molecular weight distributions (HPSEC) for fractions with varying Ara/Xyl ratio. These inconsistencies might reflect structural differences among arabinoxylans from different botanical origin but they might also be due to differences in the techniques and solvent conditions employed during measurement of the molecular weight; i.e. depending on solvent quality, chain aggregation events may influence the estimates of molecular weight for these polymers.

The polydisperse nature of arabinoxylans also allows their fractionation by means of molecular sieve chromatography. Although fractions with a broad range of molecular sizes (estimated by limiting viscosity measurements) were obtained using this approach, no substantial structural differences between the samples were revealed (Izydorczyk & Biliaderis, 1992*b*).

Structural analysis of arabinoxylans

Recent advances in the elucidation of the fine structure of cereal arabinoxylans have been possible via developments in appropriate analytical methods. In particular, nuclear magnetic resonance (NMR) has proven invaluable in studying the molecular structures of arabinoxylan polymers and oligomers. ^{13}C-NMR spectroscopy, a non-destructive probe of molecular structure, has become a method of choice for structure elucidation of native arabinoxylans (Fig. 1) (Ebringerova *et al.*, 1990; Bengtsson & Aman, 1990; Hoffmann *et al.*, 1991*b*; Izydorczyk & Biliaderis, 1992*a*, 1992*b*; Vinkx *et al.*, 1993). Most of the resonances in the ^{13}C-NMR spectra of arabinoxylans (Fig. 1) can be fully resolved (except those in the region of 73.6–74.7) and the assignments of the signals are well documented (Table 1). ^{13}C-NMR spectroscopy allows for fast determination of the nature, configuration, and relative content of the monosaccharide residues constituting the arabinoxylans as well as the type and amount of specific linkages; it offers, however, no information about the residue sequence in the chain. The availability of highly purified endo-β-D-xylanases from *Aspergillus* and *Trichoderma* species has allowed some researchers (Hoffmann *et al.*, 1991*a*, 1992*a*; Gruppen *et al.*, 1992*b*, 1993; Vietor, 1992; Bengtsson *et al.*, 1992; Izydorczyk, 1993) to examine the structure of cereal arabinoxylans in their finest detail. Hydrolysis of arabinoxylans with these enzymes provides a family of oligosaccharides containing various substituted and unsubstituted fragments. Following separation with chromatographic methods, ^{1}H-NMR spectroscopy (Fig. 2) combined with monosaccharide and methylation analyses, and molecular mass estimation (FAB–MS) have proven an excellent approach for assigning unambiguous structures for oligosaccharides containing up to 14 residues (Hoffmann *et al.*, 1992*a*; Gruppen *et al.*, 1992*b*). Because of the characteristic positions of the H-1

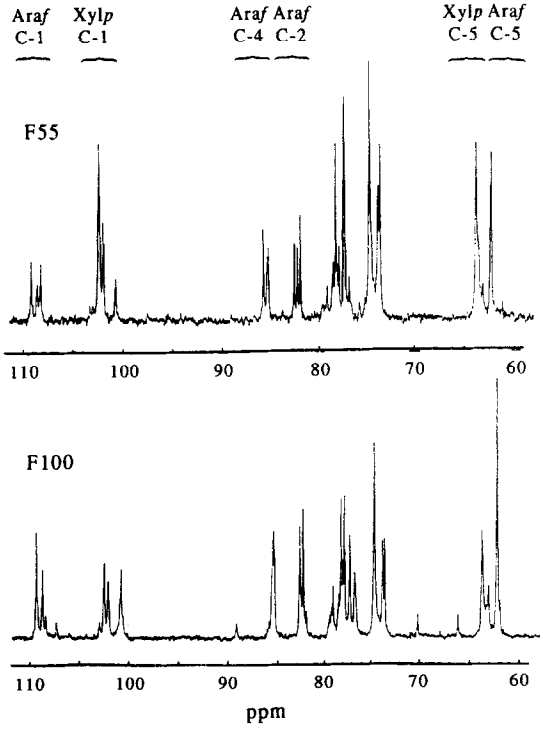

Fig. 1. ^{13}C-NMR spectra of two structurally distinct wheat arabinoxylan fractions obtained by fractional precipitation of water-extractable wheat endosperm arabinoxylan with 55% and 100% saturation of $(NH_4)_2SO_4$. The chemical shifts were assigned relative to 1,4-dioxane. Adapted from Izydorczyk (1993).

Table 1. ^{13}C-NMR chemical shift data of arabinoxylans[a]

	Chemical shifts (ppm)[c]				
Residue[b]	C-1	C-2	C-3	C-4	C-5
β-D-Xyl*p*	102.48	73.56	74.60	77.28	63.85
β-D-Xyl*p* (adj)	102.09				63.85
Element A					
β-D-Xyl*p*	100.79				63.21
α-L-Ara*f* (1 → 2)	109.46	82.30 82.41[d]		85.08	62.18
α-L-Ara*f* (1 → 3)	108.84	81.97		85.17 85.31[d]	62.18
Element B					
β-D-Xyl*p*	102.48	73.77	78.11	74.60	63.59
α-L-Ara*f* (1 → 3)	108.44	81.69	78.11	85.58	62.31

[a] Adapted from Hoffmann *et al.* (1991*ab*).
[b] β-D-Xyl*p* = →4)-β-D-Xyl*p* (1 →;β-D-Xyl*p* (adj) = →4)-β-D-Xyl*p* (1 →4)-β-D-Xyl*p*(1→adjoining element A and element B at the non-reducing end.
Element A = →4)[α-L-Ara*f*-(1 → 2)][α-L-Ara*f*-(1 → 3)]-β-D-Xyl*p*(1 →
Element B = →4)-α-L-Ara*f*-(1 → 3)]-β-D-Xyl*p*(1 →
[c] Relative to internal 1,4-dioxane (δ 67.40).
[d] Tentative assignment based on the assumption that small chemical shift differences occur when two elements A are linked together.

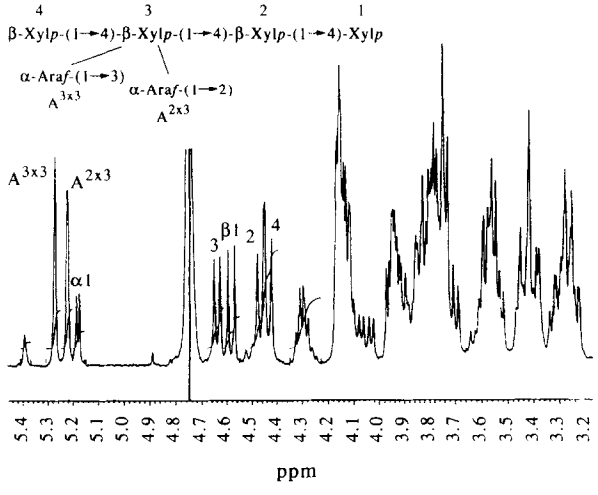

Fig. 2. ^{1}H-NMR spectrum of an arabinoxylan derived hexasaccharide. Adapted from Izydorczyk (1993).

resonances in relation to the branching patterns in arabinoxylans, the ^{1}H-NMR data of oligomer fragments can be used in identifying specific structural domains present in the polymeric arabinoxylans.

A complementary method of studying the arrangement of branching in arabinoxylans is the Smith degradation technique. Treatment of arabinoxylans with iodic acid salts–periodates promotes oxidation of L-arabinose and D-xylose units which do not carry any substituents (Ewald & Perlin, 1959; Aspinall, 1970). Selective removal of oxidized residues, followed by isolation and separation of the unoxidized portions of the molecules (i.e. glycerolxylosides with one, two, three or more xylopyranosyl residues), not only allows for the distinction among different modes of substitution patterns in arabinoxylans but also gives information about the length and amount of contiguously substituted xylopyranosyl residues in the chain. The only drawback of the Smith degradation is that the key step in the series of reactions, the oxidation of arabinoxylans with periodate, is often incomplete, due to formation of stable hemiacetal rings which prevent further oxidation of the molecules; this may lead to overestimation of unsubstituted xylose residues (Painter & Larsen, 1970; Ishak & Painter, 1971; Aman & Bengtsson, 1991). The problem, however, is relatively easily solved by repeated oxidation–reduction treatments, which ensure full oxidation of arabinoxylans (Ishak & Painter, 1971).

Methylation analysis of arabinoxylans for glycosidic linkage composition does not pose any more difficulties than those associated with other polysaccharides (undermethylation, incomplete hydrolysis, incomplete reduction and acetylation during formation of alditol acetates, etc.). The effective carbon response factors (Sweet *et al.*, 1975) are used for calculation of the molar quantities of permethylated alditols determined by gas liquid chromatography. Because 1,3,4,5-tetra-*O*-acetyl-(1-deuterio)-2-*O*-methyl-xylitol (originating from C(*O*)-3 singly substituted Xyl*p*) and 1,2,4,5-tetra-*O*-acetyl-(1-deuterio)-3-*O*-methyl-xylitol (originating from C(*O*)-2 singly substituted Xyl*p*) have very similar retention times, they cannot be resolved under most chromatographic conditions (Brillouet & Joseleau, 1987; Ebringerova *et al.*, 1990). However, their detection and quantitation can be achieved from the mass spectra by integration of signals of fragment ions characteristic of these two derivatives: m/e 118 (specific for 2-*O*-methyl-xylitol) and m/e 129 (specific for 3-*O*-methyl-xylitol) (Shibuya & Iwasaki, 1985; Vietor *et al.*, 1992; Gruppen *et al*, 1992*a*; Izydorczyk & Biliaderis, 1993).

Fine structure of cereal arabinoxylans

Cereal arabinoxylans consist of a chain backbone of (1 → 4)-linked β-D-xylopyranosyl residues to which α-L-arabinofuranose units are linked as side branches. The manner of attachment of arabinose units to the xylan backbone has been a matter of continuous research. The linkages of Ara*f* to C(O)-3 and to C(O)-2,3 of xylose residues have long been reported (Perlin, 1951; Goldschmid & Perlin, 1963; Westerlund *et al.*, 1990; Hoffmann *et al.*, 1991*a*). More recently, the presence of another linkage type, namely Ara*f* linked to C(O)-2 of Xyl*p* residues, has been verified for arabinoxylans from beeswing bran of wheat kernel (Brillouet & Joseleau, 1987), wheat endosperm (Gruppen *et al.*, 1992*a*; Izydorczyk & Biliaderis, 1993), barley endosperm (Victor *et al.*, 1992), corn cob heteroxylan (Ebringerova *et al.*, 1992), and rice endosperm and bran (Shibuya & Iwasaki, 1985).

Although most arabinofuranosyl residues in arabinoxylans are found as monomeric substituents, a small proportion of oligomeric side-chains, consisting of two or more arabinosyl residues linked via 1 → 2, 1 → 3, and 1 → 5 linkages, has been reported for some arabinoxylans (Table 2). Terminal galactosyl, glucosyl, and xylosyl residues can be present but are usually quantitatively minor and might originate from contaminant polysaccharides. Glucuronopyranosyl (and its 4-methyl ether) residues were found to constitute up to 4% by weight of the arabinoxylans from barley husk (MacGregor & Fincher, 1993). Recently, a water-soluble arabinoxylan–protein complex has been isolated from rye bran (Ebringerova *et al.*, 1994). A highly branched arabinoxylan was shown to be associated with a serine and glycine-rich protein moiety.

One of the unique features of arabinoxylans is the presence of ferulic acid covalently linked via an ester linkage to C(*O*)-5 of the arabinose residue (Smith & Hartley, 1983). Although it was once accepted that ferulic acid was esterified specifically to those arabinoses which were linked to C(*O*)-2 of xylose residues (Smith & Hartley, 1983), later studies with improved techniques have shown that arabinoses carrying feruloyl groups are linked to C(*O*)-3 of xylose units (Mueller-Harvey & Hartley, 1986). Ferulic acid is usually reported in wheat arabinoxylans in its *trans*-isomeric form; however, there are reports of up to 30–40% of *cis*-ferulic acid in wheat bran cell walls (McCallum & Walker, 1991). This might be explained by the fact that during a later stage of grain development, the bracts are more transparent towards UV light, and hence the amount of *cis*-isomers increases. Ferulic acid is capable of forming both ester and ether linkages and, therefore, it may participate in cross-linking reactions of cell wall macromolecules, thus making the graminaceous matter less susceptible to digestion. Photodimers (formed between two feruloyl residues without any loss of hydrogens) and diferulic acid (formed between two residues with a loss of two hydrogens) have been detected in plants (Ford & Hartley, 1989; Hartley & Jones, 1976).

Although arabinoxylans from various cereals and/or various plant tissues share the same basic chemical

Table 2. Structural features of arabinoxylans from cereal grains

Tissue	Extraction	Ara/Xyl	Linkage on Xyl*p*			Other substituents	Reference
			Type[a]	Amount (mol %)	3, 4/2, 4[b]		
Wheat							
Endosperm	Water	0.60	4	60		—	Perlin (1951)
			3, 4	20	nd		
			2, 3, 4	20			
Endosperm	Water	0.50	4	68.8		Short arabinose	Hoffmann *et al.* (1991*b*)
			3, 4	19.0	nd	side-chains	
			2, 3, 4	12.2			
Endosperm	Water	0.50	4	54.7		Short arabinose	Izydorczyk (1993)
			2, 4&3, 4	30.8	(23.4)	side-chains	
			2, 3, 4	14.5			
Endosperm	Alkali	0.56	4	62.1		Short arabinose	Gruppen *et al.* (1992)
			2, 4&3, 4	20.4	(8.2)	side-chains	
			2, 3, 4	17.5			
Bran	Water	0.57	4	37.5		Short arabinose	Shiiba *et al.* (1993)
			2, 4&3, 4	22.0	nd	side-chains	
			2, 3, 4	40.5			
	Water	1.07	4	20.8		Short arabinose	Shiiba *et al.* (1993)
			2, 4&3, 4	39.1	nd	side-chains	
			2, 3, 4	40.1		GluA	
Beeswing bran	Alkali	1.02	4	21.7		Short arabinose	Brillouet & Joseleau (1987)
			2, 4&3, 4	37.1	nd	side-chains	
			2, 3, 4	41.2		GluA	
Barley							
Endosperm	Alkali	0.72	4	56.6		—	Vietor (1992)
			2, 4&3, 4	24.5	(1.5)		
			2, 3, 4	18.9			
Rye							
Endosperm	Water	0.48	4	50.0		—	Bengtsson & Aman (1990)
			3, 4	47.2	nd		
			2, 3, 4	2.8			
	Water	0.55	4	42.3			Bengtsson & Aman (1990)
			3, 4	52.1	nd	—	
			2, 3, 4	5.6			
Bran	Alkali	0.78	4	41.0		Short arabinose	Ebringerova *et al.* (1990)
			2, 4&3, 4	33.0	(5.2)	side-chains	
			2, 3, 4	26.0			
Rice							
Endosperm	Alkali	0.80	4	16.4		Gal, Glc, GluA	Shibuya *et al.* (1983)
			2, 4&3, 4	78.1	(8.0)		
			2, 3, 4	5.5			
Bran	Alkali	0.93	4	20.8		Gal, GluA	Shibuya & Iwasaki (1985)
			2, 4&3, 4	63.7	(4.5)		
			2, 3, 4	15.5			
Sorghum							
Husk	Alkali	0.83	4	38.5		Short arabinose	Woolard *et al.* (1976)
			3, 4	46.1	nd	side-chains	
			2, 3, 4	15.4		GluA	
Endosperm	Alkali	0.87	nd	nd	nd	GluA	Vietor (1992)

[a] Numbers indicate positions at which Xyl*p* residues are substituted.
[b] Ratio of C(*O*)-3 to C(*O*)-2 monosubstituted Xyl*p* residues.
nd, not determined.

structure, they differ in the manner of substitution of the xylan backbone. The main differences are found in the ratio of arabinose to xylose, in the relative proportions and sequence of the various linkages between these two sugars, and in the presence of other substituents (Table 2). The ratio of Ara/Xyl in arabinoxylans from wheat endosperm may vary from 0.50 to 0.71 (Izydorczyk *et al.*, 1991*a*; Cleemput *et al.*, 1993; Rattan *et al.*, 1995) but it is usually lower than that found in bran (1.02–1.07) (Brillouet & Joseleau, 1987; Shiiba *et al.*, 1993). Similarly, rye endosperm arabinoxylans are less substituted (0.48–0.55)

(Bengtsson & Aman, 1990) than their bran counterparts (0.78) (Ebringerova *et al.*, 1990). In general, arabinoxylans from rice (Shibuya & Iwasaki, 1985) and sorghum (Woolard *et al.*, 1976; Vietor, 1992) seem to consist of more highly branched xylan backbones than those from wheat, rye, and barley, and they may contain galactose and glucuronic acid substituents, in addition to the pentose sugars.

Table 2 summarizes the findings on the glycosidic linkage composition in cereal arabinoxylans. Marked by a relatively low degree of branching, arabinoxylans from wheat, rye, and barley contain a rather high amount of unsubstituted Xyl*p* residues and a relatively low amount of monosubstituted Xyl*p* residues, compared to the more highly branched arabinoxylans from rice and sorghum. The proportion of doubly substituted residues seems not to be related to the arabinose to xylose ratio and varies substantially among various arabinoxylans. The highest amounts of doubly substituted Xyl*p* has been reported for wheat bran arabinoxylans. The presence of C(*O*)-2 monosubstituted xylose residues has been verified in all cereal arabinoxylans except those of rye endosperm. This type of xylose substitution appears to be a structural feature characteristic especially of barley arabinoxylans; a close to one ratio of C(*O*)-3 to C(*O*)-2 monosubstituted Xyl*p* residues would suggest almost equal distribution of both linkages in the polysaccharide.

Cereal arabinoxylans, like most polysaccharides, exhibit a high degree of endogenous micro-heterogeneity. It is, therefore, not possible to assign a single structure to arabinoxylans. In order to obtain a better insight into the structural characteristics of individual, relatively homogeneous arabinoxylan populations, several investigators (Vietor *et al.*, 1992; Gruppen *et al.*, 1992*a*; Izydorczyk & Biliaderis, 1992*a*) have recently fractionated arabinoxylans using ethanol and ammonium sulphate graded precipitation techniques. As indicated in Table 3, an increase in concentration of alcohol or the ammonium salt resulted in arabinoxylan fractions with continuously increasing Ara/Xyl ratios. This higher degree of branching was also accompanied by variations in the relative proportions of un-, mono-, and disubstituted Xyl*p* residues. Highly substituted arabinoxylan fractions contained less unsubstituted Xyl*p* and were enriched in C(*O*)-2,3 disubstituted residues. In contrast to water- and alkali-extractable wheat arabinoxylans which showed a decreasing ratio of C(*O*)-3/C(*O*)-2 monosubstituted Xyl*p* residues, the relative amount of C(*O*)-3 monosubstituted Xyl*p* residues in barley arabinoxylans was found to be relatively independent of the Ara/Xyl ratio (Vietor, 1992).

The distribution of arabinosyl substituents along the xylan backbone is probably of greater importance than the degree of substitution itself, since it affects the conformation (Andrewartha *et al.*, 1979) and the capacity of arabinoxylans to interact with each other and/or with other polysaccharides. Chain conformation and intermolecular associations have a direct bearing on certain physical and functional properties of these macromolecules.

Table 3. Linkage composition for xylose residues in arabinoxylan fractions

Arabinoxylan fraction	Ara/Xyl	Mode of linkage on Xyl*p* (mol %)				
		4[a]	2, 4	3, 4		2, 3, 4
Alkali-extractable barley endosperm[1] (Vietor, 1992)						
20 EtOH[b]	0.43	71[c]	4	15	(3.7)[d]	9
30	0.44	65	6	14	(2.3)	15
60	0.71	55	9	14	(1.5)	22
70	1.07	33	15	18	(1.2)	33
Alkali-extractable wheat endosperm (Gruppen *et al.*, 1992*a*)						
20 EtOH[b]	0.36	51.2[e]	16.3[f]		(26.8)[d]	4.8
30	0.46	46.6	14.1		(18.5)	8.3
40	0.55	42.1	11.4		(8.5)	10.8
50	0.68	34.8	8.6		(3.9)	14.9
60	0.80	27.8	7.3		(1.8)	17.6
Water-extractable wheat endosperm (Izydorczyk, 1993)						
55 $(NH_4)_2SO_4$[g]	0.50	36.9[e]	20.8[f]		(23.4)[d]	9.8
60	0.67	37.3	10.4		(9.9)	12.8
70	0.80	30.2	11.4		(8.5)	12.9
80	0.88	25.0	10.5		(6.0)	14.0
100	0.91	20.0	10.3		(4.1)	16.5

[a] Numbers indicate positions at which Xyl*p* residues are substituted.
[b] Numbers indicate ethanol concentration at which the fractions were obtained.
[c] In mol % of total Xyl*p* residues.
[d] Numbers in parentheses refer to ratio of C(*O*)-3 to C(*O*)-2 monosubstituted Xyl*p*.
[e] In mol % of total sugar residues present.
[f] Sum of 2,4- and 3,4-xyloses.
[g] Numbers indicate saturation level of $(NH_4)_2SO_4$ at which the fractions were obtained.

According to the early work by Perlin and co-workers (Ewald & Perlin, 1959; Goldschmid & Perlin, 1963), wheat endosperm arabinoxylans consist mostly of highly branched regions where singly C(*O*)-3 or doubly C(*O*)-2,3 substituted xylose residues are separated by single unsubstituted xylose residues. At lengths of approximately 20–25 xylose units, relatively smooth domains of at least two to five (and possibly more) unsubstituted xylosyl residues may be present.

More recently, the distribution of arabinosyl residues in water-soluble arabinoxylans from wheat endosperm was examined by Izydorczyk & Biliaderis (1994). Two structurally distinct arabinoxylan fractions, obtained at 55 and 100% saturation with ammonium sulphate, were hydrolysed with a (1 → 4)-β-D-endoxylanase from *Trichoderma viride*. Based on the differences in the relative distribution of oligosaccharide fragments (on a Bio Gel P-2 column) and in their structural characteristics (as revealed by methylation, periodate oxidation analyses, and ^{1}H-NMR), different

substitution patterns between these arabinoxylans were unravelled. It was proposed that the less substituted fraction F55 is built up of three structural domains (Fig. 3a). Region I_{55}, corresponding to the indigestible portion of F55, contains high amounts of terminal arabinoses, most of which are linked to xyloses doubly substituted at C(*O*)-2,3. Periodate oxidation (Smith degradation) demonstrated that xylose residues carrying arabinose substituents are present either isolated, in pairs, or even as three contiguously substituted residues; the first arrangement occurs most frequently, whereas the third arrangement the least. Although, no direct evidence was given in these studies, clusters of three contiguous disubstituted xylose residues are unlikely to be present in the native structure due to steric hindrance. However, blocks of two neighbouring disubstituted xylose residues have been reported (Hoffmann *et al.*, 1992*b*). Region I_{55} constitutes approximately 15% of fraction F55. Region II_{55}, yielding the larger oligomers upon hydrolysis, also contains a relatively high amount of terminal arabinoses, but the majority of them are linked to xylose residues at C(*O*)-3 only. Fragments corresponding to this region are much shorter than those in I_{55} and constitute approximately 40% of the F55 fraction. The highly substituted domains are

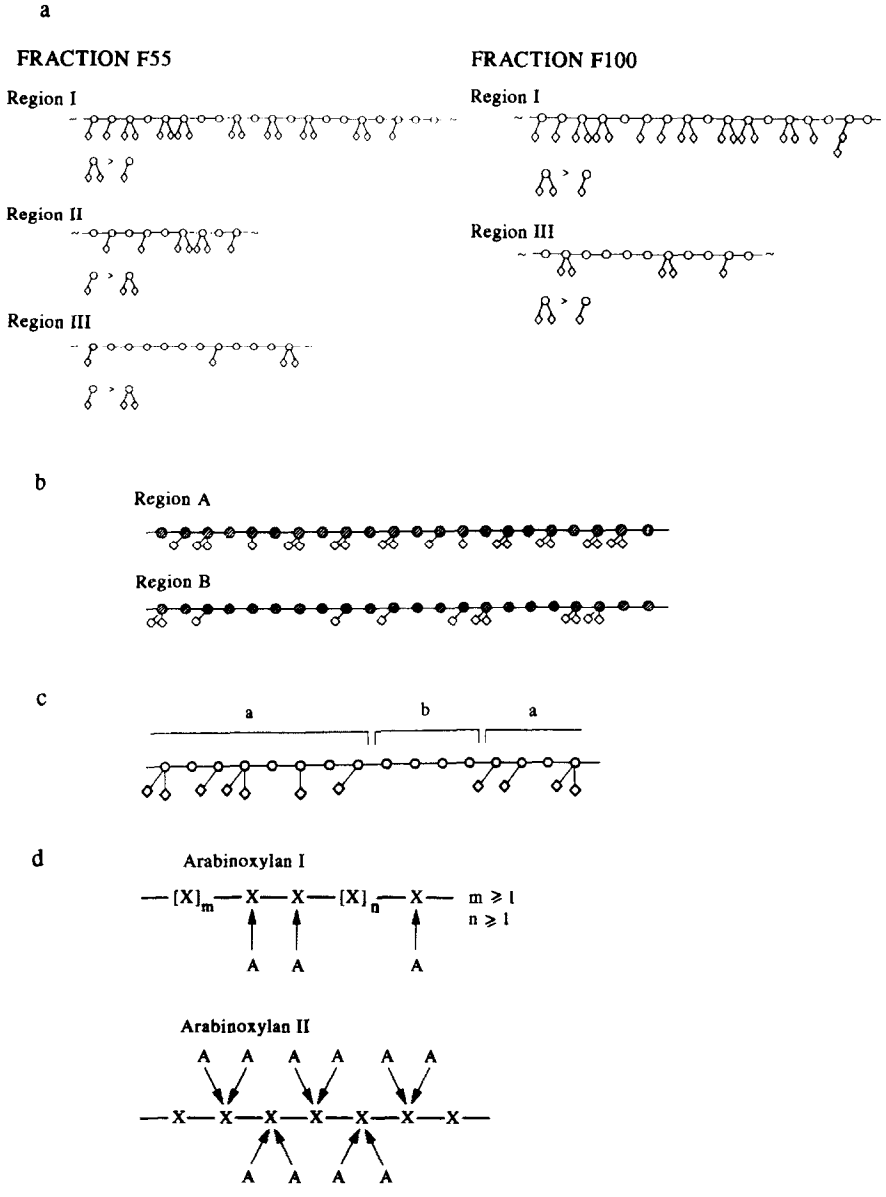

Fig. 3. Tentative models for cereal arabinoxylans. (a) Water-extractable wheat endosperm arabinoxylan; two structurally distinct fractions obtained by stepwise precipitation of the native polymer with 55% and 100% saturation of $(NH_4)_2SO_4$. Adapted from Izydorczyk (1993). (b) Alkali-extractable wheat endosperm arabinoxylan. Adapted from Gruppen *et al.* (1993). (c) Alkali-extractable barley endosperm arabinoxylan. Adapted from Vietor (1992). (d) Water-extractable rye endosperm arabinoxylan. Adapted from Bengtsson *et al.* (1992).

separated by less dense regions (III_{55}) containing sequences of contiguous (at least up to six but possibly more) unsubstituted xylose residues; these regions are the most accessible to xylanase attack. In contrast, fraction F100 appears to be made up mainly (75%) of the most highly substituted region I_{100} (Fig. 3a). This region, in addition to high amounts of terminal arabinoses linked to xylose residues at C(*O*)-2,3 is also enriched in C(*O*)-2 monosubstituted Xyl*p* residues, and short arabinose side-chains. Furthermore, in this region, four contiguously substituted xylose residues are likely to occur as evidenced by periodate oxidation analysis. The only other region in F100 is III_{100} (18%) which, like III_{55}, is highly susceptible to enzymic hydrolysis yielding small oligosaccharides (degree of polymerization (DP) < 6). However, among the enzyme-resistant oligomers of III_{100}, the diarabinoxylxylotetraose with one internal doubly substituted xylose residue predominates over the arabinosylxylotetraose segment with one singly substituted xylose; the reverse is true regarding the distribution of these fragments in the III_{55} digests. It was, therefore, suggested that the water-soluble arabinoxylans of wheat endosperm are composed of a range of polymeric structures varying between the two extreme models of F55 and F100. Variations in structure of arabinoxylans are likely to be due to differences in the ratio of region I to region II to region III, as well as to the differences in the organization of these structural domains (especially I and III).

The above structural model for water-extractable arabinoxylans differs in some features from the one proposed by Gruppen *et al.* (1993) for alkali-extractable arabinoxylans. On the basis of enzymic studies with two highly purified endoxylanases from *Aspergillus awamori*, Gruppen's group suggested the occurrence of two regions (A and B) with variable substitution patterns in alkali-extractable wheat arabinoxylans (Fig. 3b). The highly branched region A is presumed to have a rather constant structure composed mostly of repeating tetrameric units of unsubstituted and doubly arabinofuranosylated xylose residues. This region is also enriched in C(*O*)-2 monosubstituted xylose residues. The less dense region B, which alternates with region A, includes also sequences of at least seven contiguously unsubstituted xylose residues. Thus, the differences in Ara/Xyl ratio among alkali-extractable arabinoxylans were ascribed to variations in the proportion of regions A/regions B and to the composition of the less densely branched region B (Gruppen *et al.*, 1993). Instead, structural differences among water-extractable polymers were attributed to variations in composition of both highly and less branched, regions I and III (Izydorczyk & Biliaderis, 1994). Moreover, the alkali-extractable polymers have been reported to contain less contiguous and more isolated substituted xylose residues than water-extractable arabinoxylans (Gruppen *et al.*, 1993). This may explain why the former, containing a similar Ara/Xyl ratio and glycosidic linkage composition, are degraded faster and to a greater extent than the latter.

The structure of arabinoxylans from barley endosperm appears to be somewhat more regular than that from wheat. A recent study on the water-insoluble barley arabinoxylans, hydrolysed with a purified endoxylanase from *Aspergillus awamori*, indicated two distinct substitution patterns for these polysaccharides (Vietor, 1992, Fig. 3c). In a major region, singly or doubly substituted xyloses are clustered together and separated by single unsubstituted residues. Blocks of this type are interlinked by short unbranched regions of unsubstituted Xyl*p* residues (at least four). Because of negligible differences in the type and relative abundance of fragments with DP $\leqslant$ 10 in the enzyme digests from various barley arabinoxylans, the differences in Ara/Xyl ratio for these polysaccharides were ascribed exclusively to the variations in the relative proportions of the unbranched regions (Vietor, 1992).

Rye arabinoxylans seem to differ markedly from those of wheat and barley. Based also on enzymic studies, Bengtsson *et al.* (1992) proposed a distinct model involving two types of arabinoxylan polymers or two types of regions in the arabinoxylan molecules (Fig. 3d). The major polymer structure (arabinoxylan I) has a xylan chain substituted exclusively at C(*O*)-3 of Xyl*p* with arabinosyl groups, whereas the minor polymer (arabinoxylan II) contains disubstituted C(*O*)-2,3 Xyl*p* residues. The successful separation of a minor fraction containing only un- and disubstituted xylose residues (Vinkx *et al.*, 1993) favours the hypothesis of Bengtsson and co-workers that two separate polymers exist in rye arabinoxylans; however, the monosubstituted polymer has not yet been isolated to endorse such a view.

Despite the observed high degree of structural heterogeneity among cereal arabinoxylans, all recent studies point to a non-random distribution of arabinosyl residues along the xylan backbone. Gruppen *et al.* (1993) and Vietor (1992) found that the frequency of two contiguously substituted xylosyl residues and of unsubstituted blocks was too high to be predicted in arabinoxylans with a statistically random distribution of arabinosyl groups. Although arabinoxylans are secondary gene products and as such not under strict genetic control, some of their fine structural features indicate that their biosynthesis, at least to some extent, might be controlled. The findings of Gruppen *et al.* (1993) and Hoffmann *et al.* (1992*b*) that in the highly branched regions of wheat arabinoxylans the unsubstituted xylose residue is never preceded by a C(*O*)-3 monosubstituted unit or that elements containing C(*O*)-3 monosubstituted Xyl*p* are rarely present next to C(*O*)-2,3 disubstituted ones suggest that during synthesis of cereal arabinoxylans the transfer of L-Ara*f* to the growing xylain chain and/or elongation of the backbone is sterically hindered by the substitution pattern of the preceding residues.

Physicochemical properties of arabinoxylans

Molecular weights reported for cereal arabinoxylans vary depending on the method of their estimation. For water-extractable wheat arabinoxylans, molecular weight values obtained by sedimentation range from 65 000 to 66 000 (Andrewartha *et al.*, 1979; Girhammar *et al.*, 1986). These values are much lower than those obtained by gel filtration: 800 000–5 000 000 (Fincher & Stone, 1986), 70 000–1 000 000 (Fincher & Stone, 1974), 217 000 (Girhammar *et al.*, 1986). Extremely high molecular weight values for barley endosperm arabinoxylans of up to 5 000 000 (MacGregor & Fincher, 1993), estimated by gel filtration chromatography, emphasize the difficulties in accurately measuring the molecular weight of asymmetrical molecules by this method. For alkali-extractable wheat arabinoxylans, Gruppen *et al.* (1992*a*) reported a weight-average molecular weight of 850 000, as estimated by laser light scattering analysis. The water-extractable arabinoxylans of rye endosperm are considered to be larger than those of wheat. Recently, Girhammar and Nair (1992), utilizing high performance gel permeation chromatography, estimated the molecular weight of rye arabinoxylans to range between 519 000 and 770 000 compared to the range of 219 000–255 000 for wheat. Depending on solvent quality, chain aggregation may be partially responsible for such a wide variation in the estimates of molecular weight of these polymers.

The elution profiles of wheat endosperm arabinoxylans indicate a very broad distribution of molecular size. The high ratio of weight-average molecular weight to number-average molecular weight (M_w/M_n) reported for alkali-extractable wheat arabinoxylans (1.3–2.5) (Gruppen *et al.*, 1992*a*), water-extractable wheat (4.1) and rye arabinoxylans (8.5) (Girhammar & Nair, 1992) strongly points to their inherent polydispersity.

The fine structural features of arabinoxylans are of fundamental importance in understanding the properties of these polysaccharides. An unsubstituted (1 → 4)-β-xylan chain forms a 3-fold, left-handed helix, and in the solid state it appears as an extended, twisted ribbon (Fincher & Stone, 1986). This conformation is relatively flexible since it is supported only by one H-bond between two adjacent xylosyl residues. The (1 → 4)-β-xylan in the unsubstituted form aggregates into insoluble complexes, stabilized by intermolecular H-bonding (Andrewartha *et al.*, 1979). Aggregation of arabinoxylan molecules is limited by steric hindrance imposed by the arabinosyl side-units which protrude from the xylan backbone. Nevertheless, certain arabinoxylan molecules possess structural features (segments of contiguously unsubstituted xylose residues) which might permit some intermolecular alignment and interchain associations over relatively short regions of the xylan backbone.

Addition of arabinosyl substituents appears also to stiffen the molecules by maintaining the xylan backbone more extended. The asymmetry of arabinoxylans was confirmed by a very high value (140) of the axial ratio (the length of the molecule in relation to its width) for wheat endosperm arabinoxylans (Andrewartha *et al.*, 1979) and that of the Simha shape factor (507) for rye arabinoxylans (Girhammar & Nair, 1992). Also, the exponent 'a' (from the Mark–Hauwink equation) equal to 0.98, reported for rye arabinoxylans is characteristic of a polymer chain with restricted flexibility (Anger *et al.*, 1986). In contrast, for corn cob heteroxylans with an unusually low degree of substitution (Ara/Xyl 0.07), the 'a' value was estimated to be 0.50 (Ebringerova *et al.*, 1992), a value characteristic of molecules in unperturbed coil-shaped structures. It is presumed, therefore, that in aqueous media, arabinoxylans adopt an extended wormlike conformation.

As a result of their rather stiff conformation, arabinoxylans exhibit very high viscosity in aqueous solutions, compared to the intrinsic viscosity of other polysaccharides such as dextran (0.21 dl/g), beet arabinan (0.19 dl/g), or gum arabic (0.12–0.25 dl/g) (Fincher & Stone, 1986). The intrinsic viscosity values reported by Medcalf *et al.* (1968) for arabinoxylans from hard red spring wheat flours ranged between 2.5 and 3.1 dl/g. D'Appolonia and MacArthur (1975) reported values between 0.8 and 3.1 dl/g for arabinoxylans from conventional height and semidwarf hard red spring flours. The intrinsic viscosity of arabinoxylans from Canadian flours may vary from 2.75 to 5.48 dl/g depending on the wheat cultivar (Izydorczyk *et al.*, 1991*b*; Rattan *et al.*, 1995). Andrewartha *et al.* (1979) reported that partial removal of arabinosyl side-branches (by enzymic means) decreased the asymmetry and, consequently, the limiting viscosity of arabinoxylans. Fractional precipitation (using ammonium sulphate) of wheat endosperm arabinoxylans isolated from several cultivars revealed some interesting relationships among structural parameters and limiting viscosity of the fractions (Izydorczyk & Biliaderis, 1992*a*, 1993). Polymers with high [η] values had high Xyl/Ara ratios, a high feruloyl residue content and low content of doubly substituted Xyl*p* (Fig. 4). Furthermore, the relative amount of singly substituted Xyl*p* at C(*O*)-2 vs C(*O*)-3 increased with decreasing [η], and short Ara*f* side chains were more common in fractions of low limiting viscosity. Ebringerova and Hromadkova (1992) also reported higher [η] values 9265 cm^3/g in DMSO) for the low (Ara/Xyl 0.14) and irregularly substituted water-insoluble rye bran arabinoxylans than for their more highly substituted (Ara/Xyl 0.78) water-soluble counterparts (169 cm^3/g in DMSO). These results might seem unexpected since usually, increased arabinose substitution is associated with increased asymmetry of arabinoxylan molecules and thus with higher

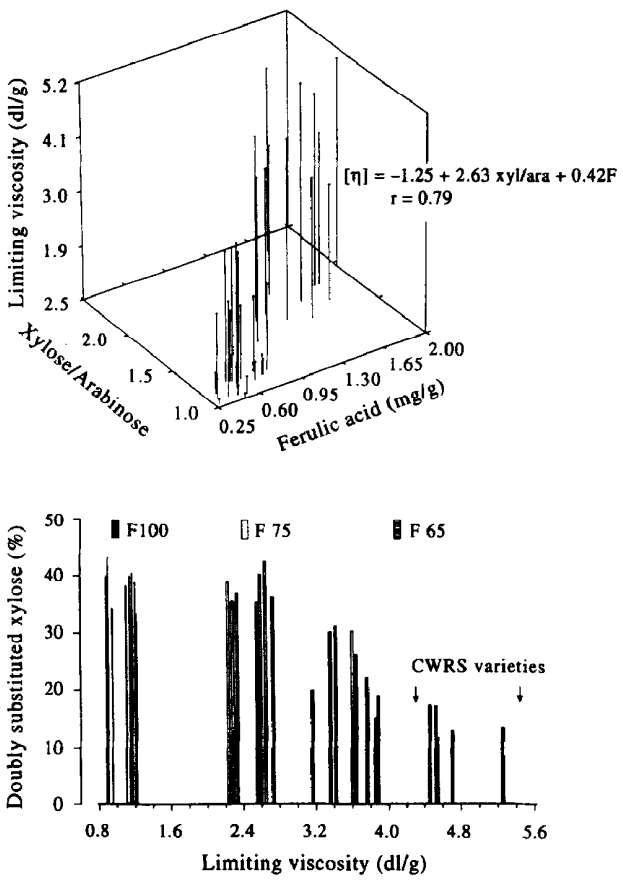

Fig. 4. Relationships between structural features and limiting viscosity of wheat endosperm arabinoxylan fractions; F65, F75, F100 denote fractions obtained at 65, 75, and 100% saturation with $(NH_4)_2SO_4$. Adapted from Izydorczyk and Biliaderis (1993).

hydrodynamic volume. However, the high intrinsic viscosity of arabinoxylans is not solely governed by their asymmetrical conformation. Although extra arabinose residues will stiffen the molecules, the length of the chain backbone is another determinant of the size of these polysaccharides. Moreover, the flexibility of less substituted arabinoxylans might permit intermolecular alignment over short sequences of continuously unsubstituted xylose residues, which would lead to formation of H-bond stabilized macrostructures; this in turn would lead to overestimation of the molecular weight of these polymers by some techniques. It is likely that only a certain portion of the entire arabinoxylan population might form aggregate structures; also, since the molecules will interact only over short sequences, such aggregates might be labile and prone to dissociation under certain conditions (concentration, solvent quality, shear). The behaviour of arabinoxylans in solution, would, therefore, be influenced not only by the overall asymmetrical conformation or the DP but also by the specific arrangement of arabinose residues along the xylan backbone.

The solution behaviour of arabinoxylans with various structural features indicates a strong dependence of viscosity on the concentration of these polysaccharides (Izydorczyk & Biliaderis, 1992*a*, *b*). In dilute solutions, the 'zero' shear rate specific viscosity $(\eta_{sp})_0$ increased linearly (slope $\approx$ 1.0) with increasing arabinoxylan concentration. Above certain concentrations, however, an abrupt increase in the concentration dependence of $(\eta_{sp})_0$ is observed (slope $\approx$ 2), which corresponds to the onset of coil overlap among the polymer chains, and the critical concentration (c^*) at which it occurs depends on the hydrodynamic volume (Fig. 5). The differences in the values of c^* among the arabinoxylan fractions (Fig. 5, inset) most likely reflect differences in the chain length and fine structural characteristics among these polymers. In a more detailed study using arabinoxylan subfractions, obtained by molecular sieve chromatography, two critical concentrations, c^* and

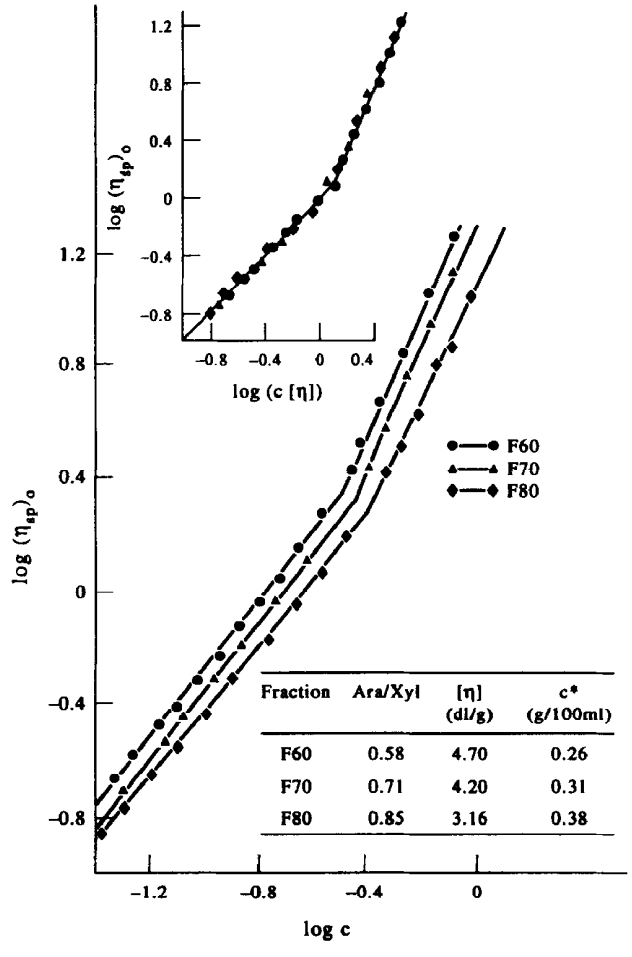

Fig. 5. Concentration dependence of 'zero shear' rate specific viscosity $(\eta_{sp})_0$ for aqueous solutions of water-extractable wheat arabinoxylan fractions; fractions obtained by stepwise precipitation of the native arabinoxylan with $(NH_4)_2SO_4$ (numbers indicate saturation level of $(NH_4)_2SO_4$ at which the fractions were obtained). Inset: 'zero shear' rate specific viscosity as a function of the coil overlap parameter ($c[\eta]$). Adapted from Izydorczyk and Biliaderis (1992*a*).

c^{**}, were identified (Izydorczyk & Biliaderis, 1992*b*). These critical polymer concentrations delimit three distinct regimes of the $(\eta_{sp})_0$ dependence on concentration: the dilute, semidulute, and concentrated regions, having slopes 1.1, 2.0–2.6, and 3.7–3.9, respectively. The existence of these three domains provides additional evidence for a rigid, rod-like conformation of arabinoxylans in solution as has been shown for polysaccharides with cellulosic backbone (Cuvelier & Launay, 1986).

The apparent viscosity of aqueous solutions of arabinoxylans is largely dependent on the shear rate (Izydorczyk & Biliaderis, 1992*b*). At low shear, arabinoxylan solutions behave like a Newtonian fluid; however, with increasing shear rate, they exhibit substantial shear thinning, typical of pseudoplastic materials (Fig. 6). The beginning of the shear rate zone marked by τ (identifying the onset of shear thinning) progressively shifts toward the higher shear rates in order of decreasing molecular weight of the samples. Also, the much lower non-Newtonian index 'n' (of the power law model, $\eta = k\dot{\gamma}^{n-1}$) for F1 vs F5 indicates that the magnitude of shear thinning depends on the molecular size of arabinoxylan.

In the presence of free radical-generating agents (e.g. hydrogen peroxide/peroxidase, ammonium persulphate, ferric chloride, linoleic cid/lipoxygenase), arabinoxylans are capable of forming three-dimensional networks (gels or viscous solutions). This unique property of water extracts of wheat flour was first described by Durham (1925). Ferulic acid associated with arabinoxylans has been considered to be responsible for oxidative gelation. Numerous hypotheses concerning the mechanism of this reaction have been developed. Detection of diferulic acid

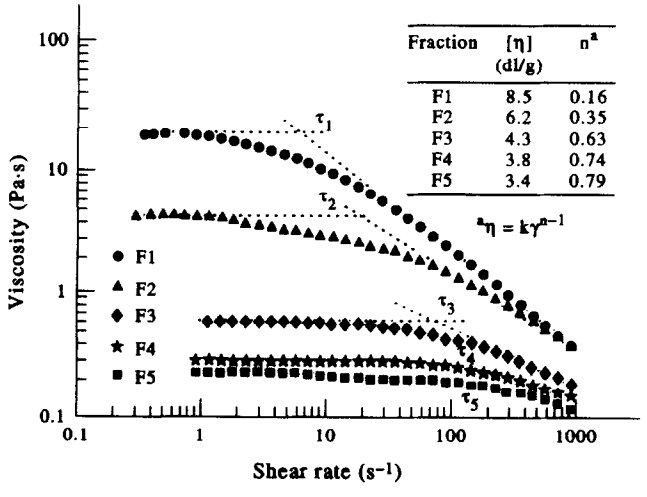

Fig. 6. Effect of shear rate on the apparent viscosity of aqueous solutions of water-extractable wheat endosperm arabinoxylan fractions (2.0% w/v) at 20°C; fractions of different molecular size were obtained by a molecular sieve chromatography. Adapted from Izydorczyk and Biliaderis (1992*b*).

in oxidized arabinoxylan systems indicates that cross-linking occurs through the coupling of two adjacent ferulic acid residues (Geissmann & Neukom, 1973*b*). In the presence of proteins, ferulic acid could be linked to the N-terminal of an amino group or to tyrosine residues (Neukom & Markwalder, 1978); however, such linkages have not yet been isolated. Ferulic acid has three potential reactive sites that could participate in cross-linking of arabinoxylans: two on the aromatic ring and one at the double bond. The most recent studies by Moore *et al.* (1990) clarified that the aromatic ring of ferulic acid and not the double bond (Hoseney & Faubion, 1981; Thibault & Garreau, 1987) serves as a cross-linking centre for the arabinoxylan polymers.

Small amplitude shear oscillatory measurements have proven very effective in following the development of three-dimensional networks in solutions of arabinoxylans undergoing oxidative gelation (Izydorczyk *et al.*, 1990). The mechanical spectrum of an arabinoxylan solution before the addition of the oxidant (H_2O_2/peroxidase) (Fig. 7, inset a) is typical of a viscous solution; the viscous component, loss modulus (G''), predominates over the storage modulus (G') in the range of frequencies tested. Following oxidative gelation (Fig. 7, inset b), the G' prevails over the G'', and the spectrum becomes typical of a solid-like material. The G'–time profile exhibits an initial rapid rise of the storage modulus followed by a plateau region (Fig. 7). This behaviour reflects an initial formation of covalent linkages between ferulic acid residues of adjacent arabinoxylan chains; once sufficient cross-links have formed, they impede further movement of chains and thus prevent further cross-linking (Izydorczyk *et al.*, 1990). It has also been established that the rate and extent of gel rigidity development is dependent on polymer and oxidant concentration (Izydorczyk *et al.*, 1990; Rattan *et al.*, 1995).

Recent studies (Izydorczyk & Biliaderis, 1992*a*, *b*) have clearly indicated the importance of structure and molecular weight on the gelation capacity of arabinoxylans. It was found that only arabinoxylan fractions having high ferulic acid content, high molecular weight, and a relatively unsubstituted xylan backbone structure were capable of extensive cross-linking, yielding well-developed gel networks (Table 4) (Izydorczyk & Biliaderis, 1992*a*, *b*). Since the presence of feruloyl groups is pivotal for cross-linking, their relative amounts and distribution along the chain backbone must influence the gelling potential of arabinoxylans. In several studies (Izydorczyk *et al.*, 1991*b*; Izydorczyk & Biliaderis, 1992*a*, *b*; Rattan *et al.*, 1995) significant correlations were shown between the rigidity of the cross-linked arabinoxylan gels, as manifested by G', and the intrinsic viscosity $[\eta]$ of the native polysaccharides. It is also likely that the higher gelling potential of less substituted arabinoxylans is due to the greater flexibility of the chain backbone which allows the establishment of

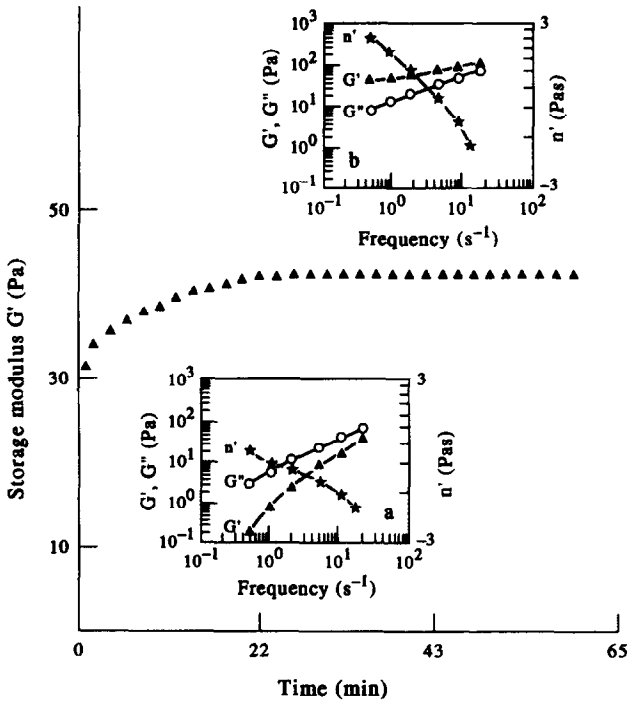

Fig. 7. Development of storage modulus, G', with time of a typical water-extractable wheat endosperm arabinoxylan (2.5%, w/v) treated with horseradish peroxidase (0.22 PU/ml) and H_2O_2 (3 ppm). Data were collected at a frequency of 1.0 s^{-1} and strain of 4% at 15°C. Insets represent the mechanical spectra of the arabinoxylan solutions before the addition of (a) the oxidant; and (b) after gel network development.

cross-links by facilitating the initial contact between feruloyl groups of neighbouring arabinoxylan chains. For native arabinoxylans isolated from several bread wheat flours, the G' of cross-linked hydrogels could be well described by a multiple linear regression model in which $[\eta]$ and the content of feruloyl groups are the two independent variables (Biliaderis *et al.*, 1995).

FUNCTIONAL PROPERTIES OF ARABINOXYLANS

Many conflicting studies have been reported on the functional role of pentosans, particularly as it relates to the breadmaking process (Hoseney, 1984; Lineback & Rasper, 1988). The diversity of experimental findings may be attributed to several factors: method of isolation, degree of purity, composition of pentosan preparations, level of supplementation, and the various baking systems employed by cereal chemists and technologists. However, with most evidence suggesting that the functional properties of pentosans are primarily due to the arabinoxylan component and with the development of improved isolation and purification procedures, recent studies have thrown more light on this subject.

When added to wheat flour, arabinoxylans clearly compete with other constituents of dough for water. Several studies (Michniewicz *et al.*, 1991; Vanhamel *et al.*, 1993; Biliaderis *et al.*, 1995) showed significant increases in the farinograph water absorption and dough development time when pentosans or purified arabinoxylans were included. The amount and molecular size of arabinoxylans are important determinants of the extent of these effects (Biliaderis *et al.*, 1995). The hydration capacity of arabinoxylans could be greatly increased upon oxidative gelation (Izydorczyk *et al.*, 1990). Cross-linked arabinoxylans were shown to hold up to 100 g of water per gram of polymer. Interestingly, the hydration properties of cross-linked arabinoxylans are not sensitive to electrolytes, contrary to most synthetic hydrogels and cross-linked sugar beet pectins (Izydorczyk *et al.*, 1990; Thibault & Garreau, 1987). The relative ranking of arabinoxylans to imbibe and hold water increases with the cross-linking density of the gel network (Izydorczyk & Biliaderis, 1992*b*) up to an optimum level; at very high degrees of cross-linking, however, swelling is impeded and the water holding capacity decreases.

Addition of water-soluble pentosans (Delcour *et al.*, 1991; Michniewicz *et al.*, 1992) or purified arabinoxylans (Biliaderis *et al.*, 1995) to wheat flour has been shown to enhance the loaf volume of breads. Earlier studies by McClearly (1986) demonstrated the importance of arabinoxylans in the breadmaking process. Wheat flours treated with a highly purified xylanase yielded sticky doughs and breads of low loaf volume and soggy texture. The beneficial effect of arabinoxylans on the loaf volume is controlled, however, by the concentration of these polysaccharides in the dough system. Higher than the optimum concentration of arabinoxylans would cause viscosity built up in the dough and consequently hinder or even decrease the volume of the final baked products (Delcour *et al.*, 1991; Biliaderis *et al.*, 1995). The concentration of arabinoxylans which maximally improves the loaf volume is, in turn, dependent on the nature of the base flours and the molecular weight of arabinoxylan preparations (Fig. 8).

The role of arabinoxylans in starch retrogradation and staling events in baked products has also been the subject of several studies. Biliaderis *et al.* (1995), who followed the bread staling by measuring crumb firmness (large deformation mechanical tests), showed that over a 7 day storage period, crumbs of breads fortified with arabinoxylans were consistently less firm than those of controls (Fig. 9). The positive effect of arabinoxylans on the texture of bread crumbs was related to the increased moisture content of these samples; water acting as a plasticizer of the gluten–starch composite matrix lowers the rigidity of the products. The antifirming action of arabinoxylans was dependent on the amount and molecular size of the added polymers into the bread formulation. Rheological studies on arabinoxylan

Table 4. Rheological parameters of arabinoxylan networks[a]

Arabinoxylan fraction[b]	Ara/Xyl	Ferulic acid[c] (mg/g)	$[\eta]$ (dl/g)	Polymer concentration (%)	G'^c (Pa)	G''^c (Pa)	$\tan\delta^d$
F60	0.58	1.88 ± 0.03	4.70	0.25	0.94 ± 0.05	0.62 ± 0.04	0.66
				0.50	8.25 ± 0.40	1.18 ± 0.20	0.14
				0.75	25.00 ± 1.20	0.85 ± 0.05	0.03
				1.00	35.00 ± 3.00	1.18 ± 0.20	0.03
F70	0.71	0.88 ± 0.01	4.20	1.0	0.33 ± 0.03	0.60 ± 0.05	1.83
				2.0	2.13 ± 0.50	3.92 ± 0.50	1.84
				2.5	4.20 ± 0.50	7.70 ± 1.00	1.83
				3.0	12.00 ± 1.25	17.30 ± 2.05	1.44
F80	0.85	0.66 ± 0.05	3.16	1.0	0.05 ± 0.02	0.22 ± 0.02	4.68
				3.0	0.60 ± 0.05	2.92 ± 0.50	4.86
				4.0	3.04 ± 0.20	10.70 ± 0.90	3.30
				5.0	6.77 ± 1.05	19.60 ± 1.50	2.89
F95	0.88	0.70 ± 0.04	1.90	5.0	0.02 ± 0.01	0.20 ± 0.05	10.00

[a] Solutions of arabinoxylan fractions were treated with horseradish peroxidase (0.22 PU/ml) and H_2O_2 (1.5 ppm); the reported values are those obtained after 1 h reaction (adapted from Izydorczyk & Biliaderis, 1992*a*).
[b] Arabinoxylan fractions obtained upon fractional precipitation with $(NH_4)_2SO_4$; the numbers refer to saturation level of $(NH_4)_2SO_4$ at which the fractions were obtained.
[c] $n = 3 \pm$ s.d.
[d] $\tan\delta = G''/G'$.

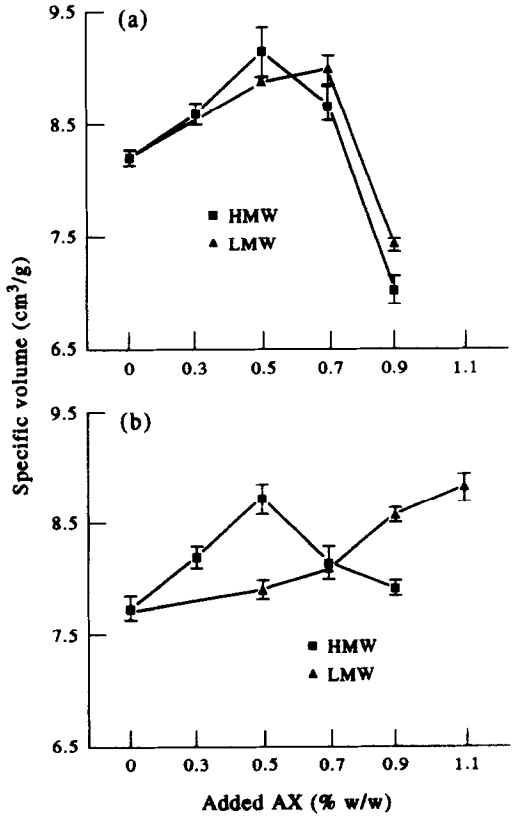

Fig. 8. Effect of added arabinoxylans (high and low molecular weight, HMW and LMW) on the specific volume of breads made from (a) Canada western red spring; and (b) Canada prairie spring wheat flours. Adapted from Biliaderis *et al.* (1995).

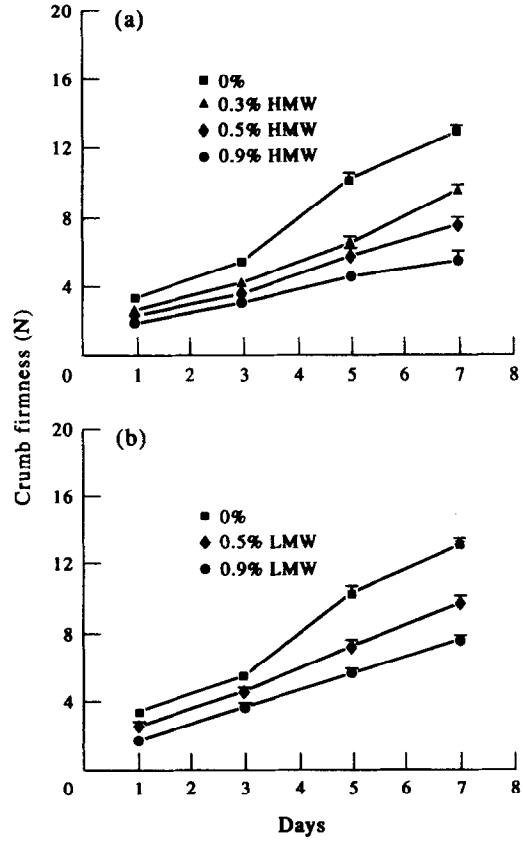

Fig. 9. Effect of added (a) high (HMW) and (b) low molecular weight (LMW) arabinoxylans (0–1.1%, w/w flour basis) on crumb firmness during storage of breads made from Canada western red spring wheat flours. Adapted from Biliaderis *et al.* (1995).

containing waxy maize starch gels (Biliaderis & Izydorczyk, 1992) indicated that these polymers interfered with the intermolecular associations required to establish a gel network structure of amylopectin. Contrary to this decreased gel firming rate, chain ordering of amylopectin (as assessed by calorimetry and X-ray diffraction) was enhanced in the presence of arabinoxylans. These effects may be attributed to acceleration of chain ordering due to an increase in the effective concentration of amylopectin. Breads supplemented with arabinoxylans also showed increased rates of starch retrogradation, as monitored by calorimetry (Biliaderis *et al.*, 1995), the enthalpy values of the staling endotherm of fortified bread crumbs were higher than those of control bread during storage at 7°C. In this case, the enhanced kinetics of structure development might be attributed to the higher moisture content of arabinoxylan-containing breads; earlier studies by Zeleznak & Hoseney (1986) showed that between 20 and 45% water content, retrogradation increases with increasing moisture. At first glance, the results of bread staling obtained by calorimetry seem to contradict those obtained with firmness tests. However, the two techniques measure different properties of the ageing baked products. Thus, while calorimetry is sensitive to the development of short and/or long range order of the amylopectin, crumb firmness measurements assess the changes in mechanical properties of the entire complex starch–gluten matrix. Although both processes occur concurrently during storage, they are influenced in different ways by the moisture content of the product.

Another possible functional property of arabinoxylans is associated with their ability to retain gas in the dough (Hoseney, 1984). Arabinoxylans have been shown to protect protein foams against thermal disruption (Izydorczyk *et al.*, 1992*a*). It is thought that the high viscosity of arabinoxylans will add to the strength and elasticity of gluten–starch films surrounding the gas bubbles and thus slow down the rate of CO_2 diffusion from dough during baking; fineness and homogeneity of crumb texture is directly related to the extent to which gas cells combine and collapse during heating. More studies are needed, however, to provide data supporting the above considerations.

The contribution of arabinoxylans to the malting and brewing qualities of barley grains has not yet been well elucidated. Although some evidence now exists that arabinoxylans together with β-glucans cause low yield extracts, high wort viscosities resulting in filtration problems, and formation of certain types of haze in beer, more research effort must be devoted in this area to better understand and control the effects of these polysaccharides on the end-use quality of barley (MacGregor & Fincher, 1993). Furthermore, treatment of wheat flours with hemicellulases improves the processing of low quality wheat flours into starch and gluten (Weegels *et al.*, 1991); i.e. enzymic hydrolysis of cell wall polysaccharides, including arabinoxylans, facilitates a more effective separation of starch granules and gluten.

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