Physicochemical and Structural Characteristics of Water-Extractable Arabinoxylan from Rye Lines Varying in Extract Viscosity and Its Relationship to End-Use Characteristics

A Thesis

Submitted to the College of Graduate Studies and Research

in Partial Fulfilment of the Requirements

for the Degree of Doctor of Philosophy

In the Department of Applied Microbiology and Food Science

University of Saskatchewan

Saskatoon, Saskatchewan

By

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Spring, 2001

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ABSTRACT

Five experimental rye (*Secale cereale* L.) lines ranging in extract viscosity from 5 to 95 cp were evaluated with respect to their physicochemical characteristics and those of their constituent water-extractable arabinoxylan.

Rye wholemeals contained significantly higher concentrations of total dietary fibre, soluble dietary fibre, total and water-extractable arabinoxylan, and β-glucan than did wheat or triticale. A significant positive correlation was observed between the extract viscosities of rye wholemeals and their soluble dietary fibre and water-extractable arabinoxylan contents. Gel filtration chromatography of rye water extracts revealed the presence of a high molecular weight fraction (HMWF), which was found in higher proportion in the ryes than in wheat or triticale. A significant positive correlation was observed between the proportion of the HMWF and the extract viscosity of rye wholemeal. Treatment of a water extract of rye with xylanase, followed by gel filtration chromatography, indicated that the HMWF consisted primarily of arabinoxylan. Successive treatment of a water extract of rye with α-amylase, lichenase, protease, and xylanase confirmed that the viscosity of the extract was primarily related to its content of arabinoxylan. Microscopic examination revealed that kernels of high extract viscosity rye had somewhat larger aleurone cells and thicker endosperm cell walls than did kernels of low extract viscosity rye.

Inclusion of rye, particularly high extract viscosity rye, in chick diets seriously impeded growth performance and feed efficiency. Part of the arabinoxylan survived breadmaking and exerted an effect in chicks, although substantially lower digesta viscosities were observed in chicks fed bread diets than those fed wholemeals.

Extract viscosities of rye flours were higher than those of corresponding wholemeals, indicating concentration of water-extractable arabinoxylan into flour during roller milling. Falling numbers of flours in the presence or absence of enzyme inhibitor correlated positively with their extract viscosities. Farinograms revealed the weakness of rye and triticale flours compared to wheat flour. Extract viscosity of rye flours and rye/wheat blends was positively correlated with dough stability, indicating higher strength for flours from higher extract viscosity ryes. Extract viscosity was negatively correlated with loaf volume of rye/wheat bread.

Water-extractable arabinoxylan (WEAX) was isolated from high, intermediate, and low extract viscosity rye. Structural analysis using H-NMR indicated that the WEAX from high extract viscosity rye was a less branched macromolecule having a lower degree of disubstituted Xylp residues and a higher degree of unsubstituted Xylp residues as compared to WEAX from intermediate and low extract viscosity ryes. Structural analysis using size exclusion HPLC/triple detection revealed that the WEAX from high extract viscosity rye had a higher molecular weight, a larger radius of gyration and a larger hydrodynamic radius, and a higher intrinsic viscosity compared to the WEAX from intermediate and low extract viscosity ryes.

ACKNOWLEDGEMENTS

I wish to express my sincere appreciation to Professor R. T. Tyler for his expertise, support, guidance and many helpful suggestions throughout the course of the project. His understanding and willingness to reach beyond academic boundaries is greatly appreciated. I greatly appreciate his criticism and editing of the thesis. I would like to express my appreciation and thanks to Professor G. L. Campbell for his support, help and valuable suggestions. His broad knowledge of rye and animal nutrition was of great help.

I would like to express my appreciation and thanks to the other members of my Advisory Committee: Professor G. J. Scoles, Dr. J. G. McLeod, and Dr. R. S. Bhatty for all their valuable suggestions, advice and time.

I would like to express my appreciation and thanks to the External Examiner Dr. M. J. Edney for his valuable suggestions, time and effort he spent on the thesis.

The technical assistance provided by Cindy Thomson, Lorna Mead, Doug Hassard, Donna Hay, Harv Braitenbach and Kuang Wu was greatly appreciated. The technical assistance provided by Brock Chatson for the H-NMR analysis was greatly appreciated.

Thanks to my dear husband Dr. E. M. Abdelaal for all his love, help, encouragement, support and many years of sacrifice and patience on helping me reach my goals. Lastly, I would like to dedicate this thesis to my dear mother, with all my love.

Financial support provided by the Agriculture Development Fund and the Value-Added Crops Strategic Research Program, Saskatchewan Agriculture and Food, Regina, SK is gratefully acknowledged.

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LIST OF ABBREVIATIONS

Araf	Arabinofuranosyl
AX	Arabinoxylan
A/X	Arabinose to xylose ratio
DDT	Dough development time
DP	Differential pressure
EV	Extract viscosity
F/G	Feed to gain ratio
FM	Fluorescence microscopy
GPC	Gel permeation chromatography
HMWF	High molecular weight fraction
HPGPC	High pressure gel permeation chromatography
HPLC	High pressure liquid chromatography
HRS	Hard red spring wheat
IV	Intrinsic viscosity
LS	Light scattering
MTI	Mixing tolerance index
MW	Molecular weight
R5	Low extract viscosity rye
R10	Low extract viscosity rye
R19	Intermediate extract viscosity rye

R30	Intermediate extract viscosity rye
R95	High extract viscosity rye
Rg	Radius of gyration
Rh	Hydrodynamic radius
RI	Refractive index
SDF	Soluble dietary fibre
SEM	Scanning electron microscopy
TAX	Total arabinoxylan
TDF	Total dietary fibre
WE	Water extract
WEAX	Water-extractable arabinoxylan
WEAG	Water-extractable arabinogalactan
WUAX	Water-unextractable arabinoxylan
Xylp	Xylopyranosyl

1. INTRODUCTION

Rye belongs to the genus *Secale*, which has many species. The most known species are *S. fragile* Bieberst and *S. cereale* L. The latter species is more extensively cultivated. Rye is not as old as wheat, but like wheat, it originated in southwestern Asia. During the first millennium B.C., rye cultivation extended to northern Europe. Later, rye gradually spread throughout most of Europe, and was eventually brought to North America and western South America by European settlers in the 16th and 17th centuries (Bushuk 1976).

In comparison to wheat, rye is of minor importance on the basis of world annual production. About 20.4 million tonnes of rye were produced in 1999, compared to 584 million tonnes of wheat and 134 million tonnes of barley (FAO 1999). The major rye producing region in 1999 was eastern Europe (Germany, 4.3 million tonnes; Poland, 4.0 million tonnes; Russian Federation, 3.8 million tonnes) which accounted for about 60% of world production (FAO 1999). Canada produces only 0.4 million tonnes annually, representing 2% of the total world production. Although a minor crop in western Canada in terms of acreage, rye is valued by producers for its agronomic characteristics.

Rye is recognized for its hardiness, i.e. its ability to grow on poor soils, adverse environmental conditions, and ravages by many pests. These properties contribute to the value of rye in sustainable agricultural systems. Certain diseases, especially those caused by fungi

such as powdery mildew and ergot, have long been considered the main disadvantages of rye. Fortunately, minimal infection by ergot may be achieved by the application of appropriate agronomic practices (Starzycki 1976).

The major limitation of rye production in Canada is market potential. Rye has historically found two major market outlets, as flour in bread and in animal feeds. Neither market has developed in North America to the same extent as in eastern Europe. In Europe, rye consumption is higher than in North America and extends beyond breads to other forms, including flaked breakfast cereal, rye porridge, baked rye berry pies, and snack foods such as crackers and rye crisps (Poutanen 1997). Another novel use is in the production of syrups, which are foremost a flavouring and colouring agent used to enhance the appeal of baked goods, cereals, confections, and beverages. Rye is also used in Canada for the production of potable alcohol and rye whisky, and occasionally as an adjunct in fuel alcohol manufacturing (Ingledew et al 1999).

Many of the attributes of rye grain, both positive and negative, have been related to cell wall constituents which contribute 5-15 percent of the grain (Voragen 1992). The principal polysaccharide constituents, of the cell wall in rye are arabinoxylan, β-glucan, and cellulose. Arabinoxylan (7-12%) is most abundant, followed by β-glucan (1-2%), and cellulose (1-2%) (Saastamoinen et al 1989). A primary distinction between rye and other cereals is the high concentration of total and water-extractable arabinoxylan in rye grain (Bach Knudsen et al 1994); arabinoxylan accounts for many of the functional characteristics of rye.

Various studies have demonstrated that viscosity in rye is attributable to water-extractable arabinoxylan (WEAX) (Bengtsson and Åman 1990; Boros et al 1993; Vinkx et

al 1993). In other studies, genetic variation in rye extract viscosity was more closely related to the molecular weight distribution of the WEAX (Girhammar and Nair 1992a; Scoles et al 1993) rather than to quantity. Viscosity may also be affected by the structure and configuration of the arabinoxylan (Bengtsson et al 1992a; Izydorczyk and Biliaderis 1992a), which constitutes a population of heterogenous molecules.

Arabinoxylan plays a key role in determining the processing characteristics of cereal grains (Kulp 1968; Fincher and Stone 1986; Meuser and Suckow 1986; McCleary et al 1986; Kühn and Grosch 1989; Bengtsson et al 1992a,b), where they are ascribed either a positive or negative role depending on the application. They affect milling behaviour, starch-gluten separation, dough handling and volume, crumb and crust characteristics of breads. In brewing and baking they limit the accessibility of starch granules to enzyme attack (Weipert 1997). The soluble and highly viscous arabinoxylan is recognized as the major antinutritive factor in rye grain in that they produce detrimental effects in monogastric animals, chicks in particular, by interfering with the digestion and absorption of all nutrients (GrootWassink et al 1989; Bedford and Classen 1992; McLeod et al 1996). While this is considered detrimental in animal feed where the economic considerations of rate-of-gain and feed conversion are paramount, they are considered beneficial in human diets, at least in western countries where overnutrition is a primary health concern.

It seems reasonable to expect that low and high extract viscosity ryes would have specific applications in food and feed, in that low extract viscosity rye would be particularly suitable for feed, whereas high extract viscosity rye would find application in food products. These prospects led to the development of breeding programs for ryes varying in extract

viscosity. The availability of such rye lines (Scoles et al 1993; McLeod et al 1996) also offers a novel means to explore the biochemistry of the constituents that govern rye functionality. Previous research of this sort has, for the most part, relied on adding extracted arabinoxylan fractions back to the system under study. To date, there exists only limited information concerning the behaviour during baking and feeding of rye lines possessing a wide range of extract viscosities. Genetic selection for low and high extract viscosity appears to decrease or increase the level of a high molecular weight arabinoxylan fraction (HMWF); however, more detailed structural differences may also exist (Bengtsson et al 1992a).

The primary purpose of this study was to identify and investigate biochemical and functional differences between low and high extract viscosity rye lines. The first objective was to establish the role of WEAX (differences in concentration, molecular weight, %HMWF) in determining the viscosities of the rye lines and to determine any morphological differences which existed among the lines. The second objective was to examine the end-use qualities of low and high extract viscosity ryes in breadmaking and as animal (poultry) feed. The third objective was to isolate and purify WEAX from high, intermediate and low extract viscosity ryes, and to examine the biochemical and structural properties of the isolated arabinoxylan using gel permeation chromatography, proton nuclear magnetic resonance and size exclusion high pressure liquid chromatography/triple detection. Achievement of these objectives should lead to a greater understanding of the behaviour of rye during processing and utilization and, ultimately, to the identification of new uses for rye or its WEAX in foods and feeds.

2. LITERATURE REVIEW

2.1. Grain Structure and Composition

2.1.1 Morphology

The rye grain is a small, one-seeded fruit or caryopsis, ranging from 6-8 mm in length and 2-3 mm in width (Simmonds and Campbell 1976). The ripe grain is free-threshing and normally greyish in colour, although a number of colour variants occur. As with wheat, the grain consists of the embryo attached via a scutellum to the endosperm and aleurone tissues (Figure 2.1). These are enclosed by the remnants of the nucellar epidermis, the testa (seed coat), and the pericarp (fruit coat). The latter layers surround the seed and adheres tightly to it. The grain possesses a crease which extends the full length of the ventral side.

Fluorescence microscopy has been used to study the microstructure of cereals including barley (Fulcher et al 1982), oat (Yiu 1986; Autio et al 1992), wheat and rye (Autio et al 1997). The last author studied structural differences between rye and wheat kernels. A bright fluorescence, indicative of intact cell walls, was obvious in the rye kernel and the cell walls were more evenly distributed throughout the kernel than in wheat, where the cell walls of the aleurone and inner endosperm were thicker than those of the outer endosperm. This would account for the high content of dietary fibre (arabinoxylan and β-glucan) in the rye grain.

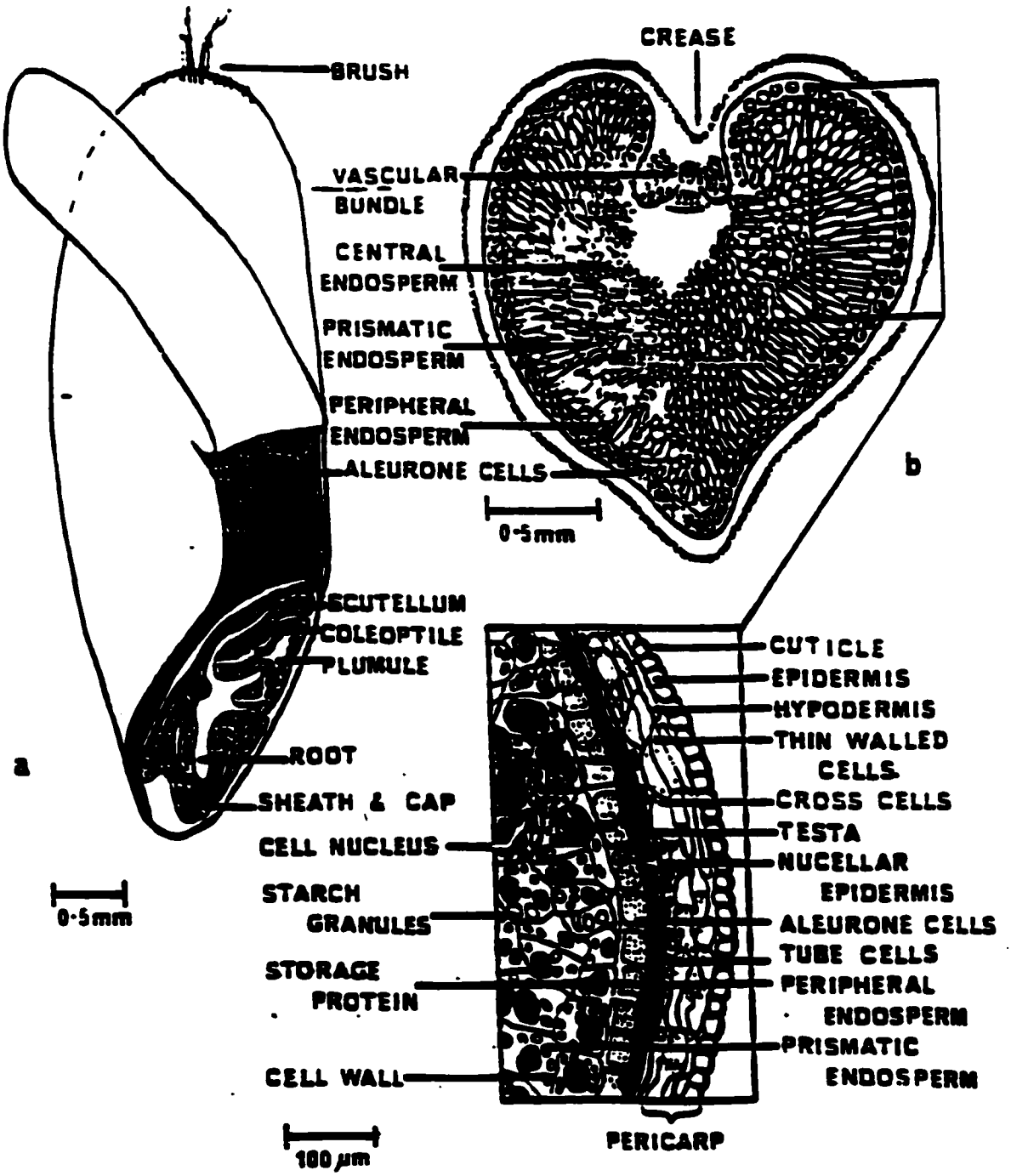


Figure 2.1 Diagram of a rye grain in longitudinal section (a) and in transverse mid-section (b) (Simmonds and Campbell 1976).

2.1.2 Nutrient Composition

The mature rye grain contains up to 80% total carbohydrates (starch, fibre, sugars), 15% protein, 2% fat, and 2% ash (Vinkx and Delcour 1996). The concentrations of the major nutrients in rye, wheat and triticale are presented in Table 2.1. In all cereals, starch was the main contributor to total carbohydrate, followed by dietary fibre. The starch content of the rye grain ranged from 55.5 to 64.5% (Pettersson and Åman 1987; Härkönen et al 1997), and did not differ greatly from that of wheat (62.4%) (Abdel-Aal et al 1995). Wheat starch granules have a bimodal size distribution, with about 3 to 4% (50 to 75% by weight) being lenticular (15 to 40 μm in length) and the remainder being small, spherical granules (1 to 10 μm in diameter). Rye starch granules also occur in two similarly distinctive populations. However, rye starch granules have a mean particle diameter greater than those of wheat and the size distribution covers a wider range (Berry et al 1971; Klassen and Hill 1971).

Like other cereal starches, rye starch granules show birefringence in polarized light and exhibit an A-type X-ray diffraction pattern. The birefringence endpoint temperature and gelatinization temperature range of rye starch granules were determined to be similar to those of wheat starch (Matz 1991). Berry et al (1971) and Klassen and Hill (1971) found that rye starch resembled wheat, durum and triticale starches in chemical composition, except for phosphorus content. The phosphorus content of rye starch (0.025%) was about half that of the other starches. The absolute density of rye starch (1.42 g/mL) was less than that of wheat starch (1.47 g/mL). The water binding capacity of rye starch (86.5%) was slightly higher than that of the wheat starch (85.0%). The α-amylase activity in rye starch was determined to be much higher than that in wheat, which is the major factor responsible for reducing the

Table 2.1 Nutrient Composition of the Rye Grain in Comparison with Wheat and Triticale (g/100 g db)

Constituent	Rye[1]	Wheat[2]	Triticale[3]
Starch	64.6	62.4	62.1
Crude protein (Nx6.25)	9.5	16.8	14.3
Total soluble sugars	6.6	3.3	5.5
Crude fat	2.4	2.1	2.2
Ash	1.9	1.7	2.0
Dietary fibre	16.5	12.5	16.8
Arabinoxylan	7.6	6.7[4]	7.6[4]
β-Glucan	2.3	0.6[5]	NF
Cellulose	2.6	2.5[6]	2.8[8]
Klason lignin	3.0	1.2[7]	3.9[8]

[1] Bengtsson and Åman (1990).
[2] Abdel-Aal et al (1995).
[3] Grela (1996).
[4] Saini and Henry (1989).
[5] Henry (1987).
[6] Lineback and Rasper (1988).
[7] Theander and Westerlund (1986).
[8] Jedel and Salmon (1994).
NF = not found.

amylograph viscosities of rye starch slurries (Klassen and Hill 1971).

The second major carbohydrate fraction in cereals is dietary fibre, the concentration of which in the rye kernel is about 17%. Although most of this fibre is insoluble, rye contains 3-4% soluble dietary fibre (Girhammar 1992). Rye fibre may be further categorized. For example, a rye sample (cv. Kungs II) containing 16.5% dietary fibre (dry basis) contained 2.3% of mixed linked (1→3), (1→4)-β-glucan, 7.6% of arabinoxylan, 2.6% of cellulose, and 3.0% of Klason lignin (Bengtsson and Åman 1990). In addition, rye also contains some arabinogalactan (Vinkx and Delcour 1996); the ratio of water-extractable arabinoxylan (WEAX) to arabinogalactan was reported to be 4.5 (Meuser et al 1986).

The protein content in the rye grain ranges from 9.5 to 14.7% (Pettersson and Åman 1987; Härkönen et al 1997) depending on cultivar, growing conditions, and climate. The higher values usually occurred in North America, where the level of nitrogen fertilizer applied was generally higher. Rye differs from wheat, barley, and most other cereals in having a comparatively high proportion of water- and salt-soluble proteins, both of which have a higher content of the limiting essential amino acid, lysine. As a result, the biological value of rye protein is considered to be superior to that of wheat and most other cereal proteins. However, its availability is reduced by other factors which restrict its true digestibility (Boros 1985).

The crude fat content of rye ranges between 1.5 and 2.5%, resembling the amount in other cereals such as wheat, barley and triticale (Simmonds and Campbell 1976; Härkönen et al 1997). The linolenic acid (C18:3) concentration (10.4%) tends to be higher in rye oil compared to wheat (5.7%) (Simmonds and Campbell 1976; Matz 1991).

Minor constituents of all cereals include minerals and vitamins. Ash is concentrated in the outer pericarp, aleurone layer, and endosperm cell walls of cereal kernels. The ash content of rye grain ranges from 1.9 to 2.1% (Härkönen et al 1997), which is similar to that of wheat (1.7%) (Abdel-Aal et al 1998) and triticale (2.0%) (Munck 1972). The mineral composition of rye ash is similar to that of other cereals (Matz 1991). Ash is particularly high in the aleurone layer of rye, due primarily to the presence of phytin granules or aleurone bodies, a mixture of potassium and magnesium salts of myoinositol hexaphosphate (Jacobsen et al 1971; Pomeranz 1973) surrounded by a protein-containing envelope (Lui and Altschul 1967). Like other cereals, rye is an important source of B-vitamins such as thiamine, nicotinic acid, riboflavin, pyridoxine, and pantothenic acid, as well as vitamin E. These components occur mainly in the embryo, scutellum, and aleurone layer (Simmonds and Campbell 1976).

In summary, rye does not differ substantially in composition from wheat and triticale in the quantitative sense; however, there are important differences with respect to how the constituents (i.e. protein, starch, arabinoxylan) behave during baking, brewing, etc.

2.1.3 Antinutrients in Rye

Numerous compounds have been implicated as having minor, potentially deleterious, effects on the utilization of the nutrients in rye grain by animals. These include alkylresorcinol phenolic compounds, trypsin inhibitors, and phytic acid (Rakowska 1994). Alkylresorcinol is a common name for alkyl and alkenyl derivatives of dihydroxybenzene. Toxicity of the isolated alkylresorcinol decreases with chain length and increases with the number of double bonds. However, low molecular weight homologues with alkyl chain lengths up to 13, which

are the most toxic, are absent from cereal grains. The chain length of alkylresorcinol in rye and wheat varies from 15 to 31. The total content of alkylresorcinol in rye grains varies from 0.9 to 2.0 mg/g, which is higher than that in wheat (0.2 to 0.6 mg/g) (Rakowska 1994). In rye grain, alkylresorcinols have been reported to not be harmful for poultry (Fernandez et al 1973) or swine (Honeyfield et al 1983), which is the basis for the current view that they are not a serious concern.

Trypsin inhibitors occur at a relatively high level in rye (4.0 U/g) compared to other cereals (wheat <1.0 U/g; triticale, 1.5-3.0 U/g) (Rakowska et al 1992). These authors reported that trypsin inhibitor isolated from rye or triticale had no effect on protein digestibility in rats and chickens when the dietary concentration was ≤1.4 U/g diet, but when added at higher levels (2.64 U/g diet), protein digestibility was depressed in rats by about 5.0%.

Phytic acid (myoinositol hexaphosphoric acid) and its salts are present in the aleurone cells of rye and other cereals (Kent-Jones and Amos 1967). The antinutritional effect of phytic acid is based on its ability to form highly insoluble and, therefore, indigestible salts of various cations (Fennema 1985). Phytic acid is largely blamed for complexing essential minerals and rendering them poorly available to monogastric animals (Deshpande and Damodaran 1990). It also interacts with proteins, resulting in reduced protein solubility and availability (Sathe and Salunkhe 1984). Phytic acid is also known to inhibit several enzymes such as pepsin (Camus and LaPorte 1976), α-amylase (Deshpande and Cheryan 1984), and trypsin (Singh and Krikorian 1982). The endogenous enzyme, phytase, is able to hydrolyse phytic acid (and its salts) to inositol and phosphoric acid. The phytase activity of rye has been reported to be

higher than that of wheat (Gontzea and Sutzescu 1968).

2.2 Non-starch Polysaccharides in Rye

Non-starch polysaccharides (NSP) are the main constituents of the cell walls in all parenchymatous and lignified tissues in cereals (Fincher and Stone 1986). These macromolecules impart a variety of properties based on the nature of the fibre e.g. viscosity of soluble fibre and rigidity of insoluble fibre. They are largely or completely indigestible by alimentary enzymes in monogastric animals (Fennema 1985). They also tend to remain insoluble during digestion, although the water-soluble fraction becomes extracted from plant structures. Rye contains 3-4% of soluble dietary fibre, which consists mainly of arabinoxylan (Åman et al 1997; Bengtsson and Åman 1990; Vinkx and Delcour 1996; Voragen et al 1992). This component was found to have a significant impact on the nutritive value of rye in human nutrition or as animal feed (Jones 1997).

2.3 Nutritional Benefits of Rye in Human Diets

Several factors in rye grain, and ultimately baked rye products, are conducive to a healthy diet. Soluble fibre is an important constituent in human diets. The beneficial effect of soluble fibre is attributed to its water-binding capacity and its consequent inhibitory effect on the absorption of saturated fatty acids and sodium (Åman et al 1997). Rye baked goods offer several other nutritional and health benefits over wheat bread because of its higher fibre, lysine, and mineral contents, and its lower caloric content (Seible 1975). Rye contains lignans, phytoestrogens, which have been attributed a role in preventing certain types of cancer

(Poutanen 1997). In a large-scale epidemiological study on elderly Finnish men, it was shown that the fibre complex in rye (in Finland, mainly consumed in food as wholemeal rye flour) may help the body to defend itself against heart attack and coronary death (Pietinen et al 1996). Recently, rye bran was shown to be more effective than oat or barley fibre in lowering the level of cholesterol in plasma and liver in hamsters, and was claimed to be a useful source of soluble fibre (Zhang et al 1993, 1994a,b; Rieckhoff et al 1999).

2.4 Antinutritional Effects of Rye in Animal Feeds

In spite of its usefulness in human nutrition, rye is considered to exhibit antinutritional effects in monogastric animals. While this is apparently contradictory to the situation in humans, actually it is not. The same factors which limit absorption in humans limit absorption and, therefore, growth in animals. In animal feeds, especially those for chicks, water-extractable arabinoxylan (WEAX) is recognized as the major factor responsible for depressed feed intake and impaired nutrient digestibility (Fernandez et al 1973; Lee and Campbell 1983; Fengler and Marquardt 1988a,b; Bedford et al 1991; Campbell and Bedford 1992; Marquardt et al 1994). Inclusion of 20% rye in a broiler chick diet decreased weight gain by 25% (Friesen et al 1991). Rybka et al (1993) determined that the level of total indigestible components (NSP plus indigestible protein) in rye averaged 197 mg/g, which is considered an intermediate value compared to barley (257 mg/g) and triticale and wheat (169 mg/g). The amount of indigestible protein associated with soluble and insoluble fibre that was not utilized by animals was found to be approximately 13-22% of the total protein in rye (Raczynska-Bojanowska and Rybka 1994).

Problems observed with the feeding of rye are associated with the content or the molecular weight of WEAX. Fengler and Marquardt (1988b) showed that the high viscosity of the gut contents of chicks fed rye was related to the concentration of WEAX. Bedford and Classen (1992) demonstrated that high viscosity in the gut was not correlated to total arabinoxylan content or to the content of WEAX, but only to the content of the highest molecular weight WEAX. Purified pentosan preparations (arabinoxylan + arabinogalactan) retained the majority of the antinutritional effects (Fengler and Marquardt 1988a,b). Also, removal of the growth-depressing effect was achieved by adding purified pentosanases to rye diets fed to chicks (Pettersson and Åman 1988; GrootWassink et al 1989). Fat retention was affected, with malabsorption of saturated and long-chain fatty acids being particularly severe, when rye was fed to chicks (Ward and Marquardt 1983).

2.4.1 Improvement of the Nutritive Value of Rye for Animal Feed

The problems associated with rye-based animal feeds can be overcome by several means. Campbell et al (1983) depolymerized the arabinoxylan using gamma irradiation, which increased the nutritive value of rye. Numerous studies have shown a beneficial response to enzyme supplementation of rye diets, with improvements noted in growth and feed conversion, as well as in digestibility (Campbell and Bedford 1992). GrootWassink et al (1989) demonstrated that the principal activity required was endo-xylanolytic; however, a purified xylanase was less effective than a crude preparation. Pawlik et al (1990) improved the nutritive value of rye by water-soaking and/or enzyme treatment, which reduced the viscosity of rye. This was attributed to endogenous or exogenous enzymes partially

hydrolyzing the viscous WEAX. When wheat was subjected to similar treatments, its nutritive value was also improved, but to a lesser extent. Synergism has been reported between endoxylanase and arabinosidase, xylosidase, or acetyl-xylan esterase (Seeta et al 1989; Tenkanen et al 1991) in model systems. However, the beneficial effect of xylanase could be affected by the structure of the arabinoxylan which varies among cereals, and the degree of substitution of the arabinofuranosyl (Araf) residues (Antoniou et al 1981; Henry 1985). Phenolic residues associated with arabinoxylan may also interfere with xylanase activity (Renard et al 1990).

2.5 Extract Viscosity of Rye Grain

Extract viscosity is the single most important functional characteristic associated with both the baking (Kühn and Grosch 1989; Weipert 1997; Autio et al 1998) and feeding quality of rye (Fengler and Marquardt 1988b; Campbell and Bedford 1992; Marquardt et al 1994). The predominant water-extractable non-starch polysaccharides in the endosperm of rye are WEAX and β-glucan, both of which have the ability to bind a large amount of water (McCleary et al 1986; Meuser and Suckow 1986). Water-unextractable arabinoxylan (WUAX) also binds water but without dissolving (Neukom 1976). In barley, there was a high correlation between the viscosity of an acidic extract and its soluble β-glucan content (Aastrup 1979; Bhatty et al 1987). The viscosity of rye, wheat and triticale extracts was attributed to their content of WEAX (Pettersson and Åman 1987; Bengtsson and Åman 1990; Boros et al 1993; Vinkx et al 1993; Härkönen et al 1995; Härkönen et al 1997; Girhammer 1992). Addition of Xylanase (from *Trichoderma reesei*) to either rye extracts or pure

arabinoxylan decreased viscosity significantly, confirming that the viscosity of rye extracts was attributable to arabinoxylan (Härkönen et al 1995).

2.5.1 Genotypic and Environmental Influences on Extract Viscosity

Both genetics and environment influence the extract viscosity of rye grain. Scoles et al (1993) identified genetic variability in extract viscosity among inbred rye lines; the genetic factor was affected by environment. Gan et al (1996, 1997) found substantial variability in extract viscosity among individual rye plants within an open-pollinated cultivar and among cultivars/lines. These authors found that a cultivar/line with higher mean extract viscosity exhibited larger variability in viscosity across environments and was more sensitive to environment than were those with low extract viscosity. Campbell et al (1989) reported similar differences in extract viscosity among locations for sixteen barley cultivars. Again, these differences were most apparent for high viscosity cultivars, whereas those exhibiting low viscosity were more uniform across locations. Absolute viscosity and β-glucan levels were affected by both environment and barley genotype, with climatic conditions that favoured rapid maturation conducive to elevated viscosity and β-glucan level (Aastrup 1979). Plaami et al (1989) found that environmental factors also affected total dietary fibre (TDF) content. These authors studied TDF in fifteen rye cultivars from Finland, and ten cultivars from Canada, Sweden, the USA, and the former USSR. High precipitation increased TDF content, whereas a high mean temperature during the growing period depressed TDF. A cold, wet season resulted in a high arabinoxylan content (Saastamoinen et al 1989). In contrast, Gan et al (1997) reported greater extract viscosity with grain growing in hot dry condition.

2.5.2 Relationship Between Kernel Characteristics and Extract Viscosity

Knowledge of the genetic correlations between extract viscosity (EV) and kernel characteristics would assist rye breeders in the selection of genotypes with low EV but high grain quality. Gan et al (1996) reported that EV was negatively correlated with kernel weight, positively correlated with falling number, and not correlated with test weight. The authors concluded that when seed numbers are too limited for viscosity analysis in early generations, kernel weight could be used as a non-destructive selection criterion, although it would not provide an equivalent opportunity to direct selection based on EV. Other seed characteristics such as colour, plumpness, and starch content did not appear to be related to EV or the level of high molecular weight arabinoxylan in rye grain (Scoles et al 1993).

2.6 Cell Walls of Cereal Grains

Cell walls of plant tissues perform a variety of diverse physiological functions (Fincher and Stone 1986). During development and maturity, the cell walls of grain tissues provide a skeletal framework and the intercellular cohesive forces maintain tissue integrity. During germination and development, they provide a conduit for the movement of water and low molecular weight solutes (sugars, amino acids, ions, and phytohormones) throughout the grain. In addition, they provide a protective barrier to insect and microbial penetration into the cellular contents. Germination begins with the endosperm and aleurone cell walls being selectively hydrolysed by enzymes arising from the overlying aleurone and scutellar epithelium, which initiates the process of mobilizing grain starch reserves (Fincher and Stone 1986).

The major constituents of cell walls are non-starch polysaccharides, which consist of arabinoxylan, β-glucan, pectin, cellulose, and lignin. They constitute an array of soluble and insoluble biopolymers assembled into a complex matrix. These components furnish the cell walls and the intracellular spaces in grain (Pomeranz 1985). While no specific models have been reported for rye specifically, or cereals in general, a general model has been developed which describes the association of polysaccharides and protein in primary sycamore cell walls (Albersheim et al 1973; Albersheim 1976). The cell wall is described as being similar to reinforced concrete, where cellulose fibrils correspond to reinforcing steel rods embedded in a matrix of pectin, hemicellulose, and protein (Keegstra et al 1973) (Figure 2.2). Xyloglucan is strongly attached to cellulose fibrils via hydrogen bonding; other hemicellulosic materials are covalently linked to xyloglucan, pectic polysaccharides and protein, forming a continuous network of cellulose fibrils. While no doubt overly simplistic, existing data does support this tentative structure (Lehninger 1978).

Although the structural and functional roles of plant cell walls are determined by the composition and organization of individual wall components (Hatfield et al 1999), these will differ among plant species and tissues. In the case of the rye kernel, the endosperm cell walls are largely arabinoxylan in composition. The total arabinoxylan content in rye grain ranges from 6.5% to 12.2% (Åman and Hesselman 1984; Henry 1987; Saini and Henry 1989; Saastamoinen et al 1989). Because of the importance of this non-starch polysaccharide in determining rye quality, its structure and characteristics will be discussed in detail.

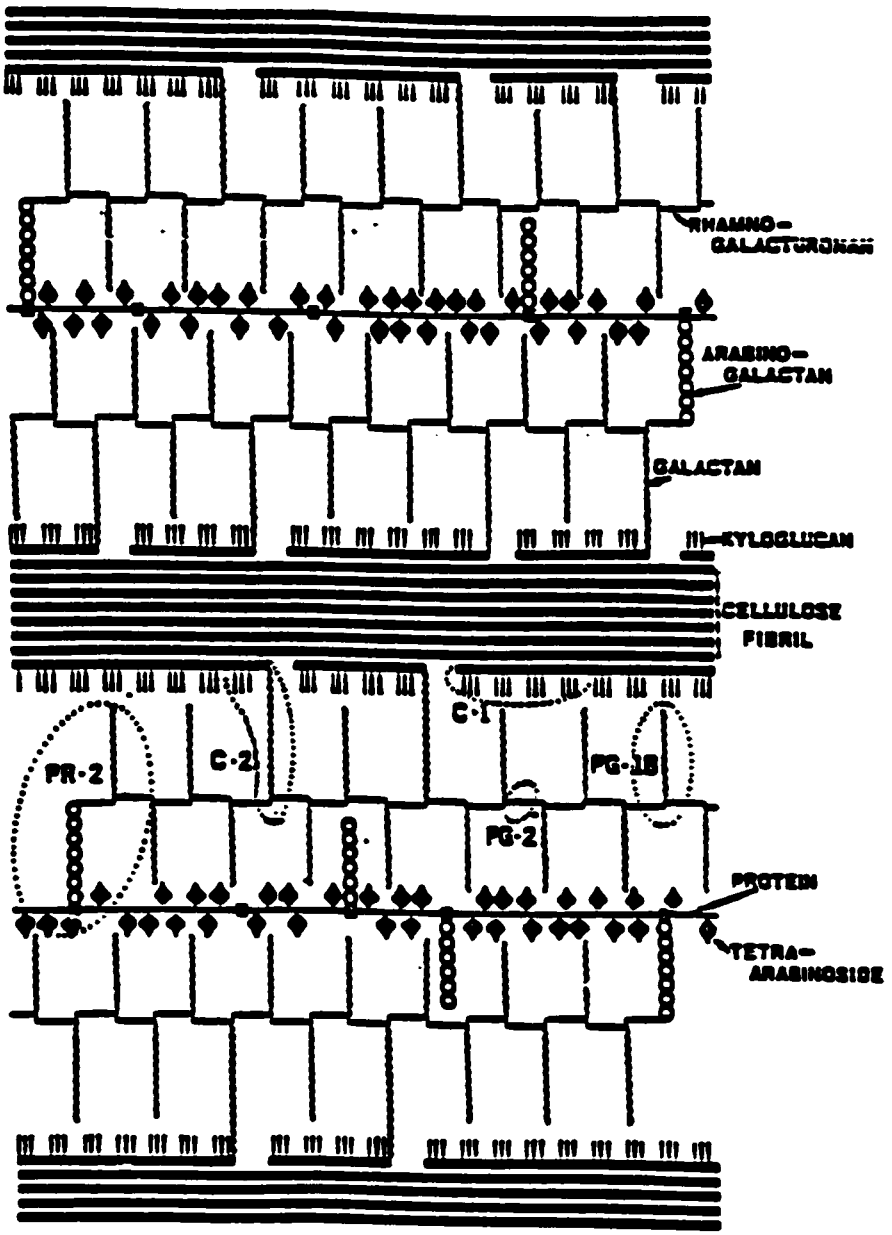


Figure 2.2 Tentative molecular structure for sycamore and other primary cell walls (Keegstra et al 1973). RP-2, PG-2, PG-1 are tri-, di- and monosaccharides resulted from the action of endopolygalacturonase on the galacturonosyl linkages of the main pectic chain. C-1, C-2 are neutral oligosaccharides and insoluble pectic fragments, respectively, that resulted from the action of endoglucanase on the xyloglucan.

2.6.1 Arabinoxylan

2.6.1.1 Occurrence, Content and Function in Cell Walls of Cereal Grains

Arabinoxylan occurs in various tissues of the main cereals of commerce, namely wheat, rye, barley, oat, rice, and sorghum (Fincher and Stone 1986). While this polysaccharide is minor component of cereal grains taken in their entirety, it constitutes a significant proportion of the cell wall (Fincher and Stone 1986). In rye cell walls, arabinoxylan is the major non-starch polysaccharide. The thin wall surrounding the cells in the starchy endosperm and in the aleurone layer in most cereals consists predominantly of arabinoxylan (60-70%), three exceptions being the endosperm cell wall of oat and barley (~20%) and rice (~40%) (Fincher and Stone 1986; Matz 1991).

Several physiological attributes of plants have been assigned to arabinoxylan because of its ability to absorb water. Arabinoxylan may reduce brittleness and provide some degree of elasticity to plant tissues. Arabinoxylan could also aid transport of dissolved metabolites and nutrients through the porous, hydrated molecular network established around the cellulose crystallites (Izydorczyk and Biliaderis 1995). It has been postulated that certain structural features of arabinoxylan permit either intermolecular alignment between polymer chains or non-covalent interaction with other polysaccharides (β-glucan, cellulose) and therefore, the formation of multicomponent gels in the complex cell wall matrix (MacGregor and Fincher 1993). Different molecular features of arabinoxylan (degree of branching, spatial arrangement of Araf substituents along the xylan backbone, ferulic acid content) may alter the viscoelastic properties of the gels and hence the resilience, strength, and porosity of the wall matrix (MacGregor and Fincher 1993). Another postulated function of arabinoxylan in cell

walls of cereal grains is the inhibition of intercellular ice formation, aiding winter survival of cereals (McNeil et al 1984); this effect was attributed to the enhancement of viscosity and the resultant mechanical interference of the arabinoxylan gel network to ice propagation. This would contribute to an important feature of rye for Saskatchewan - its winter hardiness.

A unique feature of arabinoxylan is the presence of ferulic acid covalently linked via an ester linkage to O-5 of the Araf residue (Geismann and Neukom 1973; Hoseney and Faubion 1981). Ferulic acid is capable of forming both ester and ether linkages, and therefore, may participate in cross-linking many cell wall macromolecules (Markwalder and Neukom 1976; Fry 1986; Hartly et al 1990; Gibeaut and Carpita 1992; Hatfield et al 1999). Some potential for covalent polysaccharide-polysaccharide or polysaccharide-protein interactions is also possible (Fincher and Stone 1986). Some studies (Markwalder and Neukom 1976; Fry 1986; Gibeaut and Carpita 1992) indicated that arabinoxylan in the cell wall is cross-linked by diferulic acid bridges. Photodimers (formed between two feruloyl residues without any loss of hydrogens) have been detected in Graminaceous cell walls (Hartley and Jones 1976; Ford and Hartley 1989; Hartely et al 1990). Cross-linking of cell wall components is expected to have a marked influence on numerous wall properties such as accessibility, extractability, plasticity, digestibility, and adherence (Hatfield et al 1999). These unique features of arabinoxylan contribute to its distinctive functional properties in the cereal grain during maturation and processing.

2.6.1.2 Water-Extractable and Unextractable Arabinoxylan

A major feature distinguishing arabinoxylan fractions is solubility. Arabinoxylan differs

in the ease by which they may be extracted using water. This has been attributed to differences in the arabinose substitution patterns on the xylan backbone (Izydorczyk and Biliaderis 1995). The extent of physical entanglement, covalent ester-bonding between carboxyl groups of uronic acids and the hydroxyl groups of arabinoxylan, as well as the formation of diferulic acid bridges between adjacent arabinoxylan chains (Geissmann and Neukom 1973; Mares and Stone 1973; Fincher and Stone 1986; Gruppen et al 1992) will affect solubility. In general, arabinoxylan from rye, wheat and barley was less branched than that from rice (Shibuya and Iwasaki 1985) or sorghum (Woolard et al 1976; Vietor 1992); as well, galactose and glucuronic acid substituents were evident in rice and sorghum..

The distribution of WEAX and WUAX differs among cereal grains and tissues. Most of the arabinoxylan in rye wholemeal is water-unextractable accounting for 70% of the total arabinoxylan (Delcour et al 1989; Saini and Henry 1989; Vinkx and Delcour 1996). The concentration of WEAX was higher in flour than in bran for both rye and wheat, and was similar among both fractions from triticale (Fengler and Marquardt 1988a). Compared to triticale and wheat, rye had the highest level of total arabinoxylan (7-12%) and WEAX (3-4%), being approximately five times of that in wheat and triticale (Hashimoto et al 1987; Saastamoinen et al 1989; Saini and Henry 1989). Whereas the bran fraction in rye constituted a much smaller proportion of the grain than in wheat, the levels of non-digestible soluble components for both species were very similar (Holas and Hampl 1973; Drews and Seibel 1976; Hashimoto et al 1987a; Delcour et al 1989). These authors concluded that the highest concentration of total arabinoxylan occurred in the bran, whereas the shorts contained the highest proportion of both WEAX and WUAX. This was confirmed by other studies (Fengler

and Marquardt 1988a; Delcour et al 1989; Härkönen et al 1997), where the level of WEAX in endosperm material from rye was much higher than that observed in the bran and shorts fractions indicating concentration of WEAX in the flour.

WEAX and WUAX have similar chemical compositions, but substitution differences are apparent (Hoseney 1984; Gruppen et al 1992). The arabinose to xylose (A/X) ratio ranged from 0.5-1.5 for WEAX from rye whereas for WUAX, the ratio ranged from 0.1-1.1 (Saini and Henry 1989; Vinkx et al 1995). Bengtsson and Åman (1990) reported a ratio of 0.48-0.55 for rye endosperm arabinoxylan (WEAX), which represented less substitution than for the bran counterparts (0.78) (Ebringerová et al 1994). Similarly, other studies (Izydorczyk et al 1991b; Cleemput et al 1993; Rattan et al 1994) showed that the A/X ratio in arabinoxylan from wheat endosperm, which was comparatively soluble, ranged from 0.5 to 0.7, but was generally lower than that found in wheat bran (1.02-1.07) (Brillouet and Joseleau 1987; Shiiba et al 1993). Others observed a similar A/X ratio for WEAX and WUAX in wheat (Medcalf and Gilles 1968; Meuser and Suckow 1986); however, the molecular weight of WUAX was higher. It was unresolved whether or not the higher molecular weight reflected cross-linking among xylan chains. Since genetic and/or climatic conditions affect both the content and solubility of arabinoxylan (Drews and Seibel 1976; Saastamoinen et al 1989), it may be possible to develop cereal grain with a particular configuration of arabinoxylan (WEAX and WUAX) through breeding.

2.6.1.3 Fine Structure of Arabinoxylan

The fine structure of arabinoxylan is a fundamental determinant of the properties of

this polysaccharide. Cereal arabinoxylan, like most polysaccharides, exhibites a high degree of heterogeneity. The underlying structure of arabinoxylan (Figure 2.3) is a linear backbone of(1→4)-β-D-xylopyranosyl (Xylp) residues, to which the α-L-Araf substituents are attached through O-2, O-3, or O-2,3 linkages (Fincher and Stone 1986; Poutanen et al 1991; Bengtsson et al 1992a; Vinkx and Delcour 1996).

Although arabinoxylans from various cereals and/or various plant tissues share basic structural features, they differ in the pattern of substitution on the xylan backbone. The A/X ratio, the relative proportions and sequence of the various linkages between these two sugars, and the presence of other substituents (Hoffman et al 1991; Bengtsson and Åman 1990; Shibuya et al 1983) also differ among sources.

2.6.1.3.1 Variation in Substitution on the Xylan Backbone

In addition to single unit substituents, a variety of di- and trimeric side chains have been identified as minor components of cereal grain arabinoxylan (Fincher and Stone 1986). The presence of O-2 monosubstituted Xylp residues has been verified in all cereal arabinoxylan. A near unity ratio of O-3 to O-2 monosubstituted Xylp residues would suggest almost equal distribution of the linkages in the polysaccharide. Single α-D-gluconopyranosyl residues and their 4-O-methylesters are common substituents (Fincher and Stone 1986), linked via O-2 of the xylosyl backbone residue. Glucuronoarabinoxylan occurs in the bran of rye and wheat and the hull of barley (Fincher and Stone 1986), as well as in endosperm and bran fractions of rice and sorghum (Woolard et al 1976; Shibuya and Iwasaki 1985). Also, acetyl groups are occasionally present in cereal arabinoxylan, particularly in

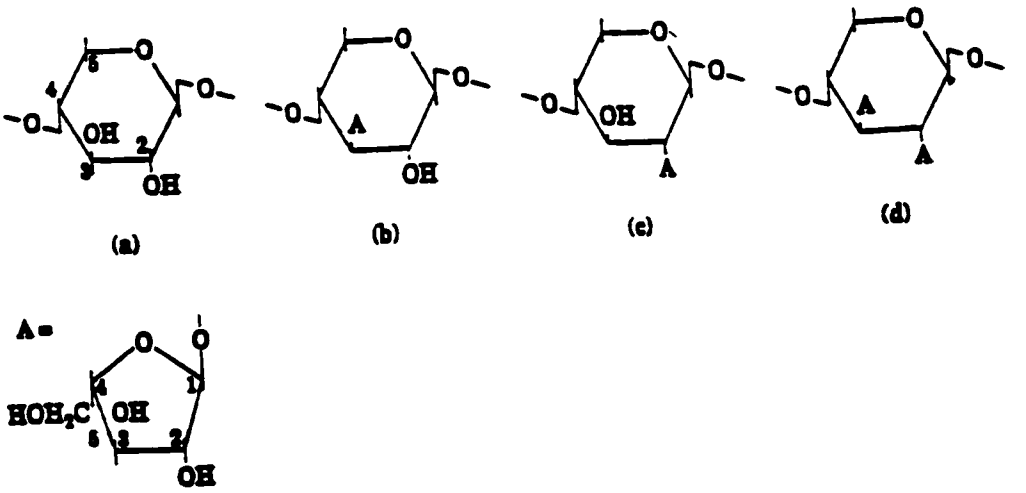


Figure 2.3 Structural elements of water-extractable arabinoxylan (WEAX) (Vinkx and Delcour 1996).

sorghum. Covalent bonds with lignin have been reported (Liyama et al 1994; Hatfield et al 1999).

The degree of arabinosyl substitution has a major impact on the structure and properties of arabinoxylan. Arabinosyl substitution stiffens the (1→4)-β-D-xylan backbone, resulting in a more extended conformation (Dea et al 1973). Anderwatha et al (1979) found higher degrees of substitution favoured water solubility. On the other hand, low arabinose substitution may permit hydrogen bonding between adjacent xylan chains, resulting in water-insolubility of the xylan. Arabinoxylan from rye, wheat and barley contains a comparatively high amount of unsubstituted Xylp residues and correspondingly low amounts of monosubstituted Xylp residues. Xylose residues may also be doubly-substituted; however, the proportion of doubly-substituted residues seems not to be related to the A/X ratio, and varies substantially among various arabinoxylan (Izydorczyk and Biliaderis 1995). The highest amount of doubly-substituted Xylp has been reported for wheat bran arabinoxylan (Izydorczyk and Biliaderis 1995).

2.6.1.3.2 Distribution of Arabinofuranosyl Residues on the Xylan Backbone

The distribution of Araf residues along the xylan backbone is probably of greater significance than the degree of substitution itself, since it affects the conformation and the capacity of arabinoxylan chains to interact with each other and/or with other polysaccharides (Andrewartha et al 1979). Certain arabinoxylan molecules possess structural features, segments of contiguously unsubstituted xylose residues, which might permit some associations over relatively short regions of the xylan backbone. The (1→4)-β-D-xylan in the

unsubstituted form could aggregate into insoluble complexes stabilized by intermolecular H-bonding (Andrewartha et al 1979). Aggregation of arabinoxylan molecules was found to be limited by steric hindrance imposed by the arabinosyl side-units which protrude from the xylan backbone. An unsubstituted (1→4)-β-xylan chain formed a 3-fold, left-handed helix, and in the solid state it appeared as an extended, twisted ribbon (Fincher and Stone 1986). This conformation is relatively flexible since it is supported by only one H-bond between two adjacent Xylp residues. When there was a low degree of arabinose substitution, non-covalent bonds (hydrogen bonds) between xylans and cellulose occured, resulting in cell wall stabilization (Liyama et al 1994). Addition of Araf residues stiffened the molecules by forcing the xylan backbone into an extended form.

2.6.1.3.3 Enzymatic Determination of Arabinoxylan Structure

Chain conformation and intermolecular associations have a direct bearing on the physical and functional properties of arabinoxylan. Based on enzymatic studies of rye arabinoxylan (using a semi-purified enzyme containing xylanase and arabinosidase activities prepared from crude xylanase from *Trichoderma reesei*), Bengtsson et al (1992a,b) proposed a model involving two distinct types of arabinoxylan polymers, or two types of regions within the arabinoxylan molecule (Figure 2.4a). The major polymer structure (arabinoxylan I) had a xylan chain substituted exclusively at O-3 of Xylp with Araf residues, whereas the minor polymer (arabinoxylan II) contained disubstituted O-2,3 Xylp residues. The successful separation of a minor fraction containing only un- and disubstituted Xylp residues (Vinkx et al 1993) favoured the hypothesis of Bengtsson et al (1992a) that two separate polymers occur

in rye arabinoxylan.

Wheat arabinoxylan seems to differ markedly from those of rye. Izydorczyk and Biliaderis (1994) used (1→4)-β-D-endoxylanase from *Trichoderma viride* to hydrolyze arabinoxylan fractions (F_{55} and F_{100}), obtained at 55 and 100% saturation, respectively, with ammonium sulphate. Different substitution patterns between the arabinoxylan chains were determined after hydrolysis using gel permeation chromatography, methylation, periodate oxidation, and proton nuclear magnetic resonance analysis. It was proposed that the less-substituted fraction F_{55} consisted of three structural domains (Figure 2.4b). The first region (I_{55}) constituted approximately 15% of fraction F_{55} and corresponded to the xylanase-resistant portion of F_{55}. This region contained high amounts of terminal Araf residues, most of which were linked to xylose by double substitution at O-2,3. The second region (II_{55}) (Figure 2.4b) constituted approximately 40% of F_{55} and contained a relatively high amount of terminal Araf residues at O-3 only. The third region (III_{55}) (Figure 2.4b) was less dense and contained sequences of contiguous (six or more) unsubstituted Xylp residues separating highly substituted regions. These unsubstituted regions were the most accessible to xylanase attack. In contrast, fraction F_{100} appeared to be made up mainly (75%) of a substituted region (I_{100}) (Figure 2.4b). This region contained terminal Araf residues linked to Xylp residues at O-2,3 and at O-2. Furthermore, in this region, four contiguous substituted Xylp residues are likely to occur as evidenced by periodate oxidation analysis. The only other region in F_{100} is region III_{100}, which like region III of F_{55}, was highly susceptible to enzymatic hydrolysis, yielding small oligosaccharides (degree of polymerization < 6).

The above structural model for wheat WEAX differs in some features from that

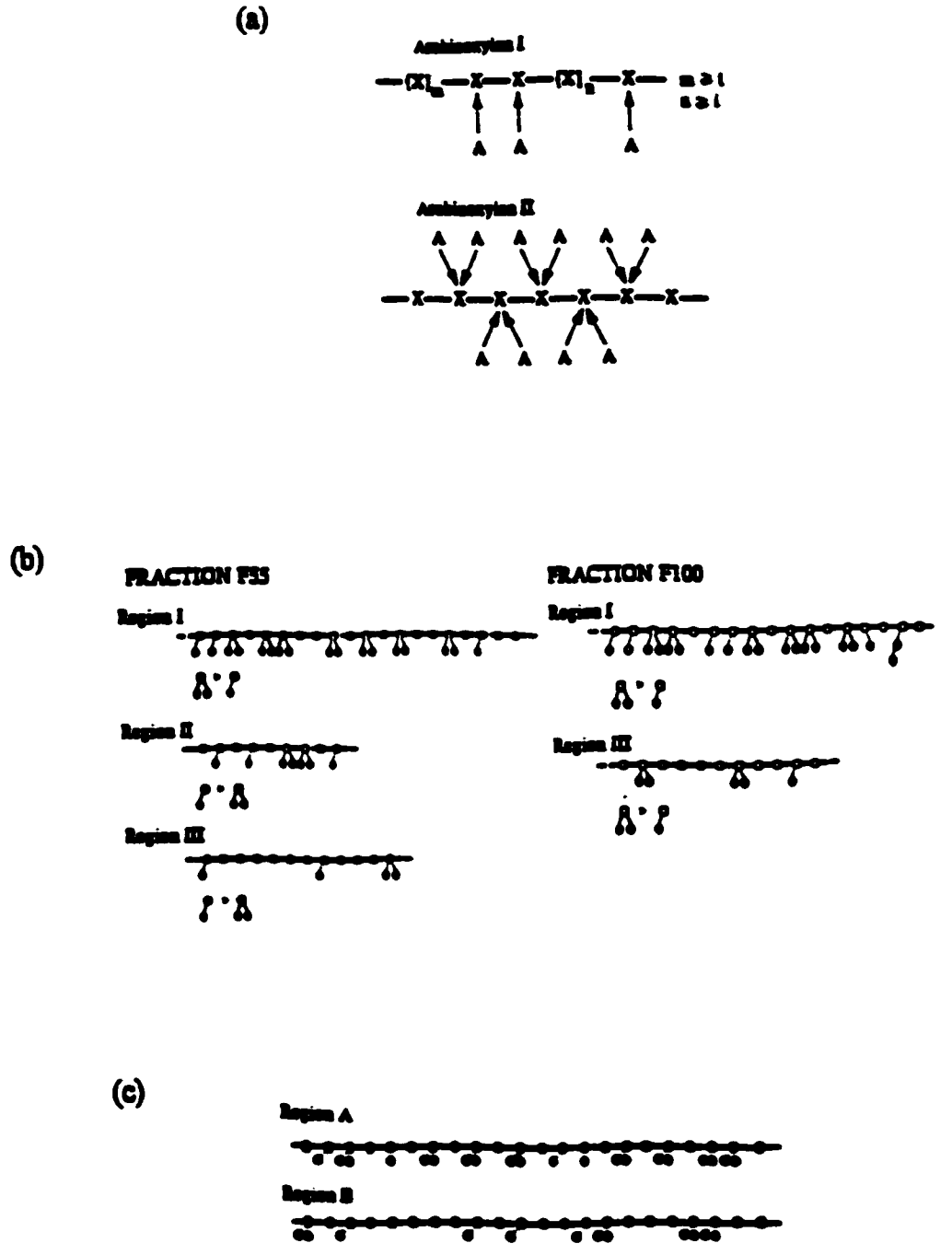


Figure 2.4 Tentative models for cereal arabinoxylan. (a) Water-extractable rye endosperm arabinoxylan (Bengetsson et al 1992a). (b) Water-extractable wheat endosperm arabinoxylan; two structurally distinct fractions obtained by stepwise precipitation of the native polymer with 55% and 100% saturation of ammonium sulfate (Izydorczyk 1993). (c) Alkali-extractable wheat endosperm arabinoxylan (Gruppen et al 1993)

proposed by Gruppen et al (1993) for alkali-extractable wheat arabinoxylan, which was based on enzymatic studies with two highly purified endoxylanases from *Aspergillus awamori*. They suggested the occurrence of two regions (A and B) having variable substitution patterns (Figure 2.4c). The highly branched region A was presumed to have a rather constant structure composed mostly of repeating tetrameric units of substituted and doubly arabinofuranosylated xylose residues. This region was also enriched in O-2 monosubstituted Xylp residues. The less dense region B, which alternates with region A, also included sequences of at least seven contiguous unsubstituted Xylp residues. Variation in the proportions of regions A and B and in the composition of the less densely branched region B could result in differences in A/X ratio among alkali-extractable arabinoxylan (Gruppen et al 1993).

Despite the similarities in A/X ratio and glycosidic linkage composition between alkali-extractable and the water-extractable arabinoxylan, the former was degraded during incubation with xylanase faster and to a greater extent. This could be explained by the presence of less contiguous and more isolated substituted xylose residues in the alkali-extractable arabinoxylan (Gruppen et al 1993).

2.6.1.4 Control of Arabinoxylan Synthesis

Variation in the structure of arabinoxylan is suggestive of the existence of control mechanisms during synthesis. Although arabinoxylan is a secondary gene product and, as such, is not under direct genetic control, some structural features support the existence of control elements (Izydorczyk and Biliaderis 1995). Hoffman et al (1992) and Gruppen et al (1993) observed that in the highly branched regions of wheat arabinoxylan, a disubstituted

Xylp residue was never preceded by a O-3 monosubstituted unit, and that a O-3 monosubstituted Xylp residue was rarely present adjacent to a O-2,3 disubstituted residues. During synthesis of cereal arabinoxylan, the transfer of L-Araf to the growing xylan chain and/or elongation of the backbone is most likely sterically hindered by the substitution pattern of the preceding residues.

2.6.1.5. Molecular Weight of Arabinoxylan

It is self evident that large molecules in general contribute more to viscosity than do small molecules. The molecular weight (MW) of cereal arabinoxylan is an important determinant of their physical properties in aqueous environments, including viscosity, water holding capacity and foam stabilization (Izydorczyk and Biliaderis 1992a).

The MWs reported for cereal arabinoxylan varies considerably depending on the method of their determination. Girhammar and Nair (1992b), utilizing high performance gel permeation chromatography (HPGPC), estimated the MW of rye arabinoxylan to range from 519,000 to 770,000, compared to 219,000-255,000 for wheat arabinoxylan. The WEAX of rye had an average MW of 275,000 (Girhammer and Nair 1992b), as determined by ultracentrifugation. Using size exclusion chromatography, Meuser et al (1986) found that the average MW for rye WEAX was 824,000 in wholemeal and 1,170,000 in bran. The MW of WUAX in the same range as that observed for WEAX (Vinkx et al 1995), indicating that size alone did not account for solubility differences. Ebringerova et al (1994) estimated the molecular parameters of the WEAX-protein complex from rye bran using HPGPC and gel chromatography coupled with viscometry and light-scattering. The high molecular weight

fraction had a MW $>10^6$. When this fraction was treated with pronase, the MW shifted to $10^4<MW>10^6$, indicating that the high-molecular weight fraction probably consisted of fragments of the native cell wall matrix, with arabinoxylan linked to a protein core.

While structurally similar, the WEAX of wheat is considered to be smaller than those of rye. The MW of wheat WEAX obtained by sedimentation ranged from 65,000-66,000 (Anderwartha et al 1979; Girhammar et al 1986). These values were much lower than those obtained by GPC; 70,000-1,000,000 (Fincher and Stone 1974); 217,000 (Girhammar et al 1986); 800,000-5,000,000 (Fincher and Stone 1986). Gruppen et al (1992) reported an average MW of 850,000 for alkali-extractable wheat arabinoxylan, as estimated by laser light scattering analysis. Extremely high molecular weight for barley endosperm arabinoxylan, up to 5,000,000 as estimated by GPC (MacGregor and Fincher 1993) emphasize the difficulty caused by molecular asymmetry using this method. In general, the MW of rye arabinoxylan is considered to be higher than that of wheat arabinoxylan and could range between 200,000 and 1,000,000 depending on the cultivar of rye and the method of MW determination (Izydorczyk and Biliaderis 1995).

Although extra arabinose residues will stiffen the arabinoxylan molecule, the length of the xylan backbone is a major determinant of the molecular size of arabinoxylan. Moreover, the flexibility of less-substituted arabinoxylan might permit intermolecular alignment over short sequences of contiguously 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 (Izydorczyk and Biliaderis 1995). 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) (Izydorczyk and Biliaderis 1995).

2.6.1.6 Intrinsic Viscosity of Arabinoxylan

The physicochemical characteristics of arabinoxylan and its physiological and technological functions depend, in part, on their intrinsic viscosity [η] (Girhammar and Nair 1992b). Whereas specific viscosity is the ratio of absolute viscosity of a fluid to that of a standard fluid, usually water, both at the same temperature, [η] is the ratio between specific viscosity and concentration. It has units of inverse density. The [η] of WEAX from rye was reported by Girhammar and Nair (1992b) to be 5.9 dL/g. This value is considered high compared to the [η] of other gums such as carrageenan (5.54 dL/g; Marrs 1986), guar gum (2.3-6.8 dL/g) and pectin (with low degree of methylation, 3.8 dL/g) (Izydorczyk et al 1991a), dextran (0.21 dL/g), beet arabinan (0.19 dL/g) and gum arabic (0.12-0.25 dL/g) (Fincher and Stone 1986).

Wheat arabinoxylan exhibited a lower [η] than that of rye arabinoxylan. Medcalf et al (1968) determined that the [η] for arabinoxylan from hard red spring wheat flours ranged from 2.5-3.1 dL/g. The [η] of arabinoxylan from Canadian wheat flours ranged from 2.8 to 5.5 dL/g, depending on cultivar (Izydorczyk et al 1991b; Rattan et al 1994). Andrewartha et al (1979) reported that partial removal of arabinosyl side-branches (by enzymic means) decreased asymmetry, and consequently, the limiting viscosity of arabinoxylan. Fractional precipitation (using ammonium sulphate) of wheat endosperm arabinoxylan isolated from several cultivars indicated that structural parameters and the limiting viscosities of the

fractions were related (Izydorczyk and Biliaderis 1992a,1993).

2.6.1.6.1 Factors Affecting the Viscosity of Arabinoxylan in Solution

The intrinsic viscosity of arabinoxylan is affected by its structure. For example, polymers with high [η] values had low A/X ratios, a high feruloyl residue content, and a low content of doubly-substituted Xylp. Furthermore, the relative amount of singly-substituted Xylp at O-2 vs. O-3 increased with decreasing [η], and short Araf side chains were more common in fractions having low [η]. Izydorczyk and Biliaderis (1995) reported that the high [η] of arabinoxylan is not solely determined by its asymmetrical conformation. Girhammar and Nair (1992b) found that temperature, pH, and salt concentration all affected the viscosity of solubilized rye arabinoxylan.

Apparent viscosity is the resistance of a substance to deformation under a particular set of conditions. The apparent viscosity of aqueous solutions of arabinoxylan was found to be affected by shear rate (Izydorczyk and Biliaderis 1992b) due to the disruption of the inter-molecular associations between polymers. At low shear, arabinoxylan solutions behaved like a Newtonian fluid; however, with increasing shear rate, they exhibited substantial shear thinning, typical of pseudoplastic materials. The magnitude of shear thinning depended on the molecular size of the arabinoxylan, as indicated by a lower non-Newtonian index (n) of the power law model ($\eta = k\gamma^{n-1}$) (Izydorczyk and Biliaderis 1992b).

A strong dependence of the viscosity of arabinoxylan solutions having various structural features on the concentration of these polysaccharides was reported by Izydorczyk and Biliaderis (1992a,b). In dilute solutions, the 'zero' shear rate specific viscosity $(\eta_{sp})_0$

increased linearly (slope≈1.0) with increasing arabinoxylan concentration. Above a critical concentration, however, an abrupt increase in the concentration dependence of $(\eta_{sp})_0$ was observed. The critical concentration (c*) at which this occurred depended on the hydrodynamic volume. Different values for c* among arabinoxylan fractions most likely reflect differences in the chain length and fine structural characteristics among these polymers (Izydorczyk and Biliaderis 1995). Thus, the behaviour of arabinoxylan in solution is determined by the overall asymmetrical conformation and the degree of polymerization, as well as the specific arrangement of arabinose residues along the xylan backbone.

2.6.1.7 Functional Properties of Arabinoxylan

In spite of being a relatively minor constituent in wheat and rye flours, arabinoxylan has a major impact on bread quality. The ability to bind large quantities of water (Bushuk 1966) and the ability to form viscous solutions or gels via cross-linking (oxidative gelation) (Geissmann and Neukom 1973) are significant attributes that can have important implications during mixing, dough development, and bread quality (loaf volume, crumb texture, and staling characteristics) (Amado and Neukom 1985; Meuser and Suckow 1986).

2.6.1.7.1 Oxidative Gelation of Arabinoxylan

An important property of arabinoxylan is its susceptibility to oxidative gelation. It has been suggested that oxidative gelation of arabinoxylan might change the rheological properties of a dough and influence bread volume (Hoseney 1984; Meuser and Suckow 1986). Oxidative gelation occurs when free-radical-generating agents (e.g. hydrogen

peroxide/peroxidase, ammonium persulphate, ferric chloride, linoleic acid / lipoxygenase) induce inter-chain covalent linkages through oxidative coupling of ferulic acid residues to the arabinoxylan (Figure 2.5) (Neukom and Markwalder 1978; Vinkx and Delcour 1996), resulting in the arabinoxylan chains forming three-dimensional networks (gels or viscous solutions). The influences of structure and molecular weight on the gelation capacity of arabinoxylan have been studied (Izydorczyk and Biliaderis 1992a,b). Only arabinoxylan fractions having a high ferulic acid content, high molecular weight, and a relatively unsubstituted xylan backbone structure were capable of extensive cross-linking to yield well-developed gel networks.

Ferulic acid associated with arabinoxylan is considered responsible for oxidative gelation (Neukom and Markwalder 1978; Izydorczyk et al 1990; Vinkx et al 1991; Vinkx and Delcour 1996; Hatfield et al 1999). Ferulic acid has three potential reactive sites that could participate in cross-linking of arabinoxylan chains, two on the aromatic ring and one at the double bond. Moore et al (1990) demonstrated that the aromatic ring of ferulic acid rather than the double bond (Hoseney and Faubion 1981) served as a cross-linking centre for arabinoxylan polymers. Since the presence of feruloyl groups is essential for cross-linking, their relative amounts and distribution along the chain backbone will influence the gelling potential of arabinoxylan (Izydorczyk et al 1991b; Izydorczyk and Biliaderis 1992a,b; Rattan et al 1994). It is also likely that the higher gelling potential of less-substituted arabinoxylan is due to the greater flexibility of the chain backbone, which promotes the establishment of cross-links by facilitating the initial contact between feruloyl groups of neighbouring arabinoxylan chains (Izydorczyk and Biliaderis 1992a,b). The hydration capacity of

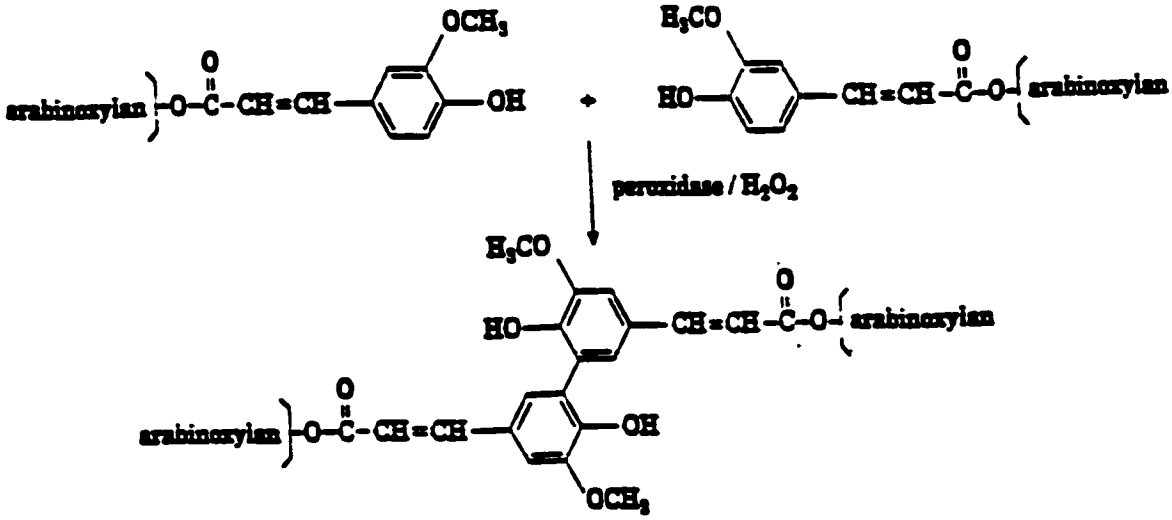


Figure 2.5 Cross-linking of arabinoxylan by formation of diferulic acid during oxidative gelation (Vinkx and Delcour 1996).

arabinoxylan is greatly increased upon oxidative gelation (Izydorczyk et al 1990); cross-linked arabinoxylan chains hold up to 100 x their weight of water.

A role for protein has been proposed in oxidative gelation. Neukom et al (1962) observed that a pure, protein-free arabinoxylan would not gel, indicating that glycoprotein was involved in the reaction. Later, Geissmann and Neukom (1973) concluded that oxidative gelation occurred as a result of oxidative phenolic coupling of ferulic acid residues, causing cross-linking of the polysaccharide molecules. In the presence of proteins, ferulic acid reacted with the N-terminal of an amino group, or with tyrosine residues (Neukom and Markwalder 1978). Vinkx et al (1991) found that the pentosan in a water extract from rye was necessary for oxidative gelation and that the protein moiety was of much less importance with respect to both the viscous properties of the extracts and the increase in viscosity upon gelation.

2.6.1.7.2 Arabinoxylan and Breadmaking

The water absorption, viscosity enhancing, and gelling properties of arabinoxylan have stimulated considerable interest in its effect upon the rheological behaviour of dough, loaf volume, and crumb texture. Interpretation of experimental results is not always straight forward, as the method of isolation, degree of purity, composition, and MW of arabinoxylan or pentosan preparations, level of supplementation, and the baking system employed all affect the impact of arabinoxylan on breadmaking (Hoseney 1984; Lineback and Rasper 1988).

When added to wheat flour, arabinoxylan clearly competes with other dough constituents for water. Significant increases in farinograph water absorption and dough development time were evident when pentosans or purified arabinoxylan were added to wheat

flour dough (Michniewicz et al 1991; Vanhamel et al 1993; Biliaderis et al 1995). The amount and MW of arabinoxylan are important determinants of the extent of these effects (Biliaderis et al 1995). When wheat flour was treated with a highly purified xylanase (McCleary 1986), it yielded sticky doughs and breads of low loaf volume and soggy texture. Alternatively, addition of crude (Delcour et al 1991; Michniewicz et al 1992) or purified (Biliaderis et al 1995) arabinoxylan to wheat flour enhanced loaf volume. The beneficial effect of arabinoxylan on loaf volume is not a linear relationship with respect to concentration; low levels are beneficial, but higher than optimal concentrations cause excessive viscosity in the dough and may even decrease the volume of the final baked product (Delcour et al 1991; Biliaderis et al 1995).

Another functional property attributed to arabinoxylan is its ability to retain gas in the dough (Hoseney 1984) and protect protein foams against thermal disruption (Izydorczyk et al 1992a). It is thought that the high viscosity of arabinoxylan add to the strength and elasticity of gluten-starch films surrounding gas bubbles in dough and thus slows the rate of CO_2 diffusion from dough during baking. The fineness and homogeneity of crumb texture was found to be directly related to the extent to which gas cells combine and collapse during heating (Izydorczyk and Biliaderis 1995).

2.6.1.7.3 Arabinoxylan and Bread Staling

Whereas arabinoxylan has no effect on starch gelatinization (Kim and D'Appolonia 1977; Gudmundsson et al 1991), it plays an important role in starch retrogradation and staling events in baked products. Biliaderis et al (1995) followed bread staling by measuring crumbs

firmness (large deformation mechanical tests) over a 7-day storage period. The crumb of breads fortified with arabinoxylan were consistently less firm than those of controls, indicating a positive effect of the arabinoxylan on the texture of bread crumbs which was related to higher moisture content. The additional water acted as a stabilizer of the gluten-starch composite matrix, thus lowering rigidity. The antifirming action was again dependent on both the amount and the molecular size of the added arabinoxylan.

Arabinoxylan may contribute to intermolecular associations required to establish a gel network of amylopectin, as demonstrated by Biliaderis and Izydorczyk (1992) through rheological studies on arabinoxylan-containing waxy maize starch gels. Contrary to an expected decrease in gel firming rate, the chain ordering of amylopectin (as assessed by calorimetry and X-ray diffraction) was enhanced in the presence of arabinoxylan. This effect was attributed to an acceleration of chain ordering due to an increase in the effective concentration of amylopectin.

2.6.1.8 Isolation and Fractionation of Arabinoxylan

Determination of the structural and functional properties of arabinoxylan, as well as industrial application, necessitates their isolation. Many approaches for extraction and purification of arabinoxylan have been employed. Since arabinoxylan is not easily solubilized, a major concern is maintenance of the structural integrity of arabinoxylan during extraction and purification.

2.6.1.8.1 Extraction Conditions and Arabinoxylan Stability

The maintenance of arabinoxylan structure during isolation and purification is critical to maintaining its physical and functional properties. Most isolation procedures include heat treatment (Cleemput et al 1993; Vinkx and Delcour 1996; Loosveld et al 1997; Delcour et al 1999) of the wholemeal in order to inactivate the endogenous enzymes. In some studies, arabinoxylan was isolated after refluxing the wholemeal with 80% alcohol (Fengler and Marquardt 1988a; Girhammar and Nair 1992a). In other studies, the wholemeal was heated at 130°C (Vinkx et al 1993; Vinkx et al 1995; Delcour et al 1999). Fengler and Marquardt (1988a) observed that the highest yield of arabinoxylan and the highest relative purity were obtained from autoclaved rye flour as compared to untreated flour, air-classified flour, or ethanol-boiled flour. Viscosity was more stable at a higher pH as opposed to a lower pH, and it was much less stable at a neutral pH in the raw state as compared to autoclaved rye flour, due to degradation by endogenous enzyme. These results indicate that arabinoxylan should be extracted from heat-treated rye and stored at a high pH. Extraction at a high pH was problematic because starch is soluble under these conditions (Fengler and Marquardt 1988a).

2.6.1.8.2 Isolation of Arabinoxylan Using Solubility Differences

Differences in solubility among arabinoxylan fractions permits isolation without appreciable losses. Saini and Henry (1989) extracted WEAX and WUAX from rye, wheat, and triticale using cold water, hot water, ammonium oxalate/ethylene diaminetetraacetic acid, 5% NaOH, or 15% NaOH. The total arabinoxylan content of all fractions averaged 12.2% for rye, 7.4% for triticale, and 6.6% for wheat. About half of the arabinoxylan was extracted

in the 5% NaOH-soluble fraction, with the residue fraction containing a much higher proportion of arabinose. In wheat, hot water was required to extract significant arabinoxylan, unlike rye and triticale where cold water was sufficient. The content of the cold water extractable fraction was higher in rye than in triticale. Beyond 5% NaOH there was little further solubilization of arabinoxylan, but again the highest levels were recorded for rye grain. The extractability of most arabinoxylan fractions was higher in rye than in wheat or triticale, except for the hot water and oxalate soluble fractions. The A/X ratios were less than 1.0 for most fractions, except for the residue fraction where arabinose was predominant. The residue fraction may be composed of an arabinoxylan in which the xylose chain is highly substituted with arabinose. The average ratios in all soluble and insoluble fractions were similar, but slightly higher for triticale than for wheat and rye. The wholemeals were subjected to hydrolysis and the total arabinoxylan determined, with the average level of arabinoxylan being significantly higher in rye than in wheat or triticale. This was consistent with a previous report (Henry 1985) where rye was reported to have a higher arabinoxylan content than did wheat or triticale, and the triticale arabinoxylan was more branched than that of rye or wheat.

Gruppen et al (1991) separated the water-unextractable cell wall constituents of wheat flour using various solvents. The extracts were compared in terms of yield, sugar composition, amino acid composition, and molecular weight distribution. Any of 1.0 M sodium hydroxide, 1.0 M hydroxylamine hydrochloride, saturated barium hydroxide or 4-methylmorpholine-N-oxide extracted most of the water-unextractable fraction, whereas 0.05 M sodium carbonate, 8.0 M urea or dimethylsulfoxide extracted only a small portion. Pure arabinoxylan (a limited amount) was extracted with sodium carbonate, whereas barium

hydroxide extracted a pure fraction representing 80% of the arabinoxylan present in the water-unextractable fraction, as well as a second fraction containing a mixture of arabinoxylan and glucan. The various extracts were very similar with respect to their protein content, amino acid composition, and MW (850,000).

A pilot-scale procedure for isolation of WEAX from rye was reported by Delcour et al (1999). The procedure involved extraction with water followed by incubation with α-amylase and subsequent precipitation using ethanol (1:4 v/v). A complete removal of protein material from the isolated arabinoxylan was achieved by clay treatment.

2.6.1.8.3 Isolation of Arabinoxylan by Differential Precipitation

Vinkx et al (1993) used various concentrations of ammonium sulphate, $(NH_4)_2SO_4$, to fractionate a rye water extract into three distinct arabinoxylan fractions differing markedly in both MW distribution and fine structure. A major fraction precipitated between 25 and 50% saturation and had an A/X ratio of 0.5 with virtually all branching at the O-3 position on the xylan backbone (as shown by H-NMR). This fraction had a lower MW than the other fractions, contained ferulic acid (0.04-0.07%), and exhibited oxidative gelation. A second (small) fraction that did not gel had an A/X ratio of 1.4 and precipitated between 75 and 100% $(NH_4)_2SO_4$ saturation. In this fraction, all branched Xylp residues were substituted at both O-2 and O-3 with Araf residues. A third fraction which precipitated between 50 and 75% $(NH_4)_2SO_4$ saturation was not a mixture of the other two arabinoxylan fractions.

Izydorczyk and Biliaderis (1993) fractionated arabinoxylan from ten wheat varieties using graded $(NH_4)_2SO_4$ and obtained three fractions, F_{65}, F_{75} and F_{100}; the number refer to

the saturation level of $(NH_4)_2SO_4$. Arabinoxylans from the various wheat cultivars were very heterogeneous, differing in their A/X ratio, ferulic acid content, and MW. The highest yield was obtained for F_{65} (53.3-85.9% of the total material recovered), which also had the highest MW and viscosity. The F_{75} fraction accounted for 10.2-35.7%, and F_{100} for 3.9-11.0%, of the total material recovered. The highest ferulic acid content and the lowest A/X ratio were obtained for fraction F_{65}, which also had the highest number of Xylp residues carrying only a single Araf side chain residue (at C-2 or C-3) and the lowest substitution at both C-2 and C-3. Unsubstituted Xylp residues also occurred more frequently in this fraction. The F_{75} and F_{100} fractions exhibited lower MWs and had lower levels of singly-substituted Xylp residues and higher levels of doubly-branched Xylp residues. Short Araf side chains were more commonly encountered in the low MW arabinoxylan fractions (F_{75} and F_{100}).

2.6.1.8.4 Fractionation Methods for Arabinoxylan

Other methods for arabinoxylan isolation not employing different solvents have been developed. Åman and Bengtsson (1991) fractionated the WEAX from rye grains using a sequential oxidation/reduction procedure which completely oxidized the arabinose and unbranched xylose residues in the intact polysaccharide. Quantitative analysis of the products revealed the presence of glycerol xylosides with one, two, or three xylose residues in the molar ratio of 1.00:0.86:0.02. Gel permeation chromatography was used to separate seven fractions, of which 1 and 2 were identified as mixtures of low MW components. Sugar analysis of fractions 3-7 showed that all contained xylose and glycerol residues.

Ciacco and D'Appolonia (1982) extracted water soluble pentosans from five wheat

flours (hard red spring, hard red winter, durum, western white, and soft red winter) and fractionated the extract using diethylaminoethyl cellulose (DEAE) chromatography into five fractions. The most abundant polysaccharides were arabinoxylan isolated as fractions 1 and 2. Ferulic acid was associated with all fractions. However, in most cases fraction 2 contained the highest ferulic acid level and had higher gelling capacities than fraction 1. The [η] measurements revealed higher values for the arabinoxylan fractions than for the remaining fractions.

2.7 The Milling of Rye

2.7.1 Rye Milling

Prior to incorporation into most products, rye grain is milled. One of the most critical functions in the technology of milling is the process of tempering or conditioning. The objectives of the tempering process are to reduce the hardness of endosperm so that it may readily be ground into flour and to toughen the bran to resist powdering. The objective of the milling process is to separate the bran and germ from the endosperm and subsequently reduce the endosperm to fine flour or meal having a particle size distribution appropriate for use in breadmaking (Bass 1988; Matz 1991; Sarkar 1993). Rye and wheat milling processes are similar.

2.7.1.1 Factors Affecting the Milling of Rye

One major consideration in rye tempering is the high content of arabinoxylan, which makes rye flour extremely hygroscopic. This tends to agglomerate the flour, which interferes

with sifting. For this reason, rye is milled at a lower moisture content than wheat. The rye kernel also does not have an intact hyaline layer external to the aleurone layer, thus water penetration into the kernel is rapid. Because of this, rye requires a shorter tempering time than does wheat (Matz 1991).

An important factor in rye milling is the presence of ergot sclerotia, which must be removed prior to milling. Another important factor is sprouting, which is not always visually detectable at harmful levels. Rye intended for milling is routinely tested for non-visual sprouting by the Falling Number test (Rozsa 1976). Rye grain has a strong tendency to sprout even under comparatively dry conditions such as in August/September in western Canada. During sprouting, the activity of α-amylase increases to a point where the flour can not be used for breadmaking (Rozsa 1976). A primary quality characteristic of rye is its α-amylase level because of the important contribution of starch rather than protein to rye breadmaking quality (Weipert 1997). The colour of rye grain is also considered an indication of quality; a light brown colour is preferred, because blue and green grains colour the flour grey (Rozsa 1976).

The 1000-kernel weight of rye is lower than that of wheat (Weipert 1997), which also affects milling. Some changes in the milling operations and the choice of milling rolls and sieves are required in order to compensate for the differences in kernel characteristics (bran thickness, the adherence of the endosperm to the bran, the greater friction of the rye kernel surface) between rye and wheat (Rozsa 1976).

Because rye kernel characteristics (size, protein) differ from one region to another, rye milling requirements differ accordingly. In North America, the protein content of rye tends

to be higher and the kernel is smaller and more vitreous than Western Europe, which grinds the rather low-protein, nonvitreous and large size kernel into fine flour with strict quality requirements. Eastern European rye mills grind all types of rye into a coarse flour, according to local quality specifications (Rozsa 1976).

2.7.2 Rye Flour

The most important fraction from rye milling is the flour. The average flour yield based on uncleaned grain is about 83%, which is higher than that generally obtained for wheat. This yield (extraction rate) varies widely from one mill to another. The two basic grades of rye flour produced in the U.S.A are dark and white (light) (Matz 1991). White flour represents about 80% of the rye flour produced. Mills attempt to meet the special requirements of customers by blending various proportions of these two flours. A blend of all of the flour streams coming off of a rye mill yields a straight grade flour which is sometimes called medium rye flour (Matz 1991). There are no standard rye flour grades in Canada; mills usually establish their own classifications according to clients' needs (Rozsa 1976). By-products of rye milling are used for animal feed and in formulated pet foods. Unlike wheat flour, chemical additives are seldom applied to rye flour, with the exception of chlorine, which may be added to increase brightness.

Extraction curves of mineral matter showed that at any stage of milling, rye is inferior to wheat. At the same extraction rate, rye flour will have a higher mineral content (Weipert 1997) and a very high 5-alkyl resorcinol content compared to wheat flour (Weipert and El-Baya 1977). The high content of mineral and phenolic constituents at any given extraction

rate is due more to the poor separation of rye endosperm from bran, rather than to the smaller rye kernel containing more mineral and bran and less endosperm. For economic reasons, most rye flour is milled to an extraction rate of 80% (mineral content of 1.0%; Weipert 1997).

2.7.2.1 Utilization of Rye Flour and Meal

There are many potential food and non-food applications where rye flour and/or its components can be used because of their unique functional properties. The flour is principally used in the production of bread, crackers, snack foods and in the preparation of flour mixes (Rozsa 1976). Sometimes rye flour is used as a filler or binder in sausage products. Other realized and potential applications for rye flour include as a brewing adjunct, in pet food, fish food, in the production of a gum having good foam stabilization properties, and in the production of soluble proteins which could be good yeast foods or foam builders. Rye meals are utilized in specialty products such as pumpernickel, whole grain breads, crackers, snack foods, mammi and rye crisps (Poutanen 1997).

2.8 Breadmaking with Rye

2.8.1 Comparison Between Rye and Wheat Dough

The inherent functional properties of rye flour cause the performance of rye flour in dough to differ from that of wheat. Rye proteins are not able to form as extensive a gluten network as wheat proteins, due in part, to the high arabinoxylan content of rye flour (Drews and Seibel 1976). The continuous phase of wheat dough is gluten, whereas that of wholemeal rye dough is primarily a protein-starch matrix. In wheat dough, the gluten is responsible for

its gas retaining ability, whereas in rye dough gas retention is attributed to the viscosity generated by WEAX (Meuser and Suckow 1986; He and Hoseney 1991). According to Holas et al (1992), rye protein forms neither the dough structure nor is the main water-absorbing material. The water-binding capacity of rye dough is largely dependent on WEAX and WUAX (Girhammer and Nair 1992a), whereas in wheat the water binding capacity depends mainly on gluten.

Most breads prepared from wheat or rye are made from flour rather than from whole grain meal, an important exception being traditional rye bread. Differences in the microstructure of wheat and rye doughs were observed using fluorescence microscopy (Autio et al 1997). The most significant difference was the particle size distribution. In wheat dough the particle size is 10 μm in diameter or less, in rye dough particles varied from under 10 μm to 1 mm in diameter, due to the presence of bran, aleurone, and endosperm particles. These would decrease in size during the baking process, especially if cell wall degrading enzymes were naturally present or had been added. Autio et al (1996) concluded that rye dough quality depended on the particle size distribution of the flour, the surface area of intact cell walls, and the water content.

2.8.2 Role of Arabinoxylan in Rye Dough

Arabinoxylan has important functional properties because of their water binding capacity and viscosity enhancing properties, which influence both dough and breadmaking characteristics (Meuser and Suckow 1986; Kühn and Grosch 1989; Delcour et al 1991; Maat et al 1992). In rye dough, arabinoxylan functions much as gluten does in wheat dough by

slowing the rate of CO_2 diffusion from the cell. Unfortunately, arabinoxylan does not have the rheological properties of gluten and thus the texture of rye flour dough is poorer than that of wheat flour dough (Hoseney 1984).

WEAX and WUAX have different functional roles in rye dough. WEAX form a sticky gel that keeps the dough together (Pomeranz 1985; Holas et al 1992), and also can affect the rate of starch degradation (Gudmundsson et al 1991). WUAX have a high water binding capacity and a capacity to swell (Kulp 1968; Kim and D'Appolonia 1977; Pomeranz 1985; Meuser and Suckow 1986), and were found to have important deleterious influences on the breadmaking properties of rye flour, such as dark grey colour, dull surface, and bitter taste (Meuser and Suckow 1985; Meuser and Suckow 1986; Kühn and Grosch 1989; Weipert 1993).

Considerable controversy exists concerning the impact of added arabinoxylan on wheat bread quality. Some authors have concluded that they have a negative impact (Roels et al 1993), others have reported no effect (D'Appolonia et al 1970), while still others have reported impressive positive effects (Michniewics et al 1992). These apparently conflicting results have been attributed to differences in the material from which arabinoxylan was obtained, the isolation techniques, impurities in the various isolates, or differences in breadmaking procedure (Courtin and Delcour 1998).

2.8.3 Rye Breads

In traditional rye bread, whole grain is used as raw material; most rye breads use rye flour mixed with wheat flour to aid dough formation and gas retention. The ratio of rye flour

to wheat flour varies from 10% rye /90% wheat to 90% rye/10% wheat (Drews and Seibel 1976; Poutanen 1997). Rye bread produced in the U.S.A usually contains 50% or more of wheat flour, unlike European rye bread, which is often made from 100% rye flour (Hoseney 1984). The traditional method of preparing rye bread uses the 'sour dough' formula. This procedure has the advantage that slightly damaged (sprouted) rye with increased α-amylase activity is usable, and the method produces a flavourful bread with a long shelf life (Rychlik and Grosch 1995).

The quality of rye bread is different from that of wheat bread. If baking volume (loaf volume of 100 g of flour) is considered the primary quality characteristic, rye is clearly inferior to wheat. It is recognized that this is a somewhat artificial criterion since consumers of rye bread would not necessarily choose rye on this basis. Whereas wheat flour yields bread volumes of 650 mL/100g and higher, sound rye flour yields volumes of only 280-300 mL/100g. The most important quality characteristics of rye bread are the physical properties of its crumb, which should be soft, resilient, and elastic. Crumb that is firm and dry, or wet, slack and inelastic, makes the bread unstable. Blending of rye flours of varying quality, blending of rye flour with wheat flour, and other remedies are used in baking (Weipert 1997). The latter include the addition of either mono- and diglycerides, carboxymethylcellulose, or guar gum, which yield rye breads with optimal loaf volume and a very good crumb structure (Mettler and Seibel 1995).

3. MATERIALS AND METHODS

3.1 Materials

Five rye (*Secale cereale* L.) lines exhibiting a range of extract viscosities were used in this study (Table 3.1). Low (R5) and high (R95) extract viscosity spring ryes were grown at Saskatoon, SK in 1997, and low (R10) and intermediate (R19, R30) extract viscosity fall ryes were grown at Swift Current, SK in 1996-97. Commercial samples of rye (cv. Prima), hard red spring wheat (*Triticum aestivum* L. cv. CDC Teal) and triticale (*Triticosecale* Witt. cv. Banjo) were obtained locally.

3.2 Preparation of Wholemeals

Grains were ground using a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO) to pass a 0.5 mm screen prior to analysis. Moisture content were determined in the wholemeals according to the AACC (1995) Method 44-15A.

3.3 Total and Soluble Dietary Fibre in Rye Wholemeals

Total and soluble dietary fibre contents of rye wholemeals were determined according to the AOAC (1999) procedure.

Table 3.1 Extract Viscosities and Growth Habits of Experimental Rye Lines and Commercial Cultivars From Rye, Triticale and Wheat Grains

Line / Cultivar	Designation	Extract Viscosity[1] (cp)	Growth Habit
Rye HVS-97	R95[2]	95.0 ± 1.9[3]	Spring
Rye 8591-SD	R30	30.0 ± 1.1	Fall
Rye 8591-SD	R19	19.0 ± 0.9	Fall
Rye Saratove-5	R10	9.9 ± 0.2	Fall
Rye LVS-97	R5	4.9 ± 0.2	Spring
Rye (Prima)	Rye	12.5 ± 0.3.3	Fall
Triticale (Banjo)	Triticale	2.3 ± 0.1	Spring
Wheat, CDC (Teal)	Wheat	2.3 ± 0.1	Spring

[1] 1:5 (w/v) wholemeal to water ratio.
[2] each experimental line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[3] mean ± standard deviation (n = 3).

3.4 Sugar Analysis

Monosaccharides were determined in rye wholemeals, water extracts, freeze-dried water extracts and purified arabinoxylan preparations after hydrolysis with sulphuric acid and derivatization to their alditol acetates according to Blakeney et al (1983). The derivatized sugars were quantified on a Hewlett-Packard Model 5880A gas liquid chromatograph (GLC) equipped with a fused silica DB-23 capillary column of 0.25 mm i.d x 30 m and 0.25 μm film thickness (J&W Scientific, Folsom, CA) and a flame ionization detector. The injector, column and detector were maintained at 275, 230, and 300°C, respectively. A standard mixture of arabinose, xylose, mannose, galactose and glucose (Sigma Chemical Co., St. Louis, MO) was prepared and used for calibration and β-D allose was used as the internal standard. Total and soluble arabinoxylan contents were estimated by summation of the arabinose and xylose contents (x 0.88). All analyses were performed in duplicate.

3.5 β-glucan

β-glucan contents of the wholemeals, freeze-dried water extracts, and purified arabinoxylan preparations were determined using the enzymatic method of McCleary and Glennie-Holmes (1985).

3.6 Microscopy of High and Low Extract Viscosity Rye Kernels

The microstructure of low (R5) and high (R95) extract viscosity rye kernels was observed by scanning electron microscopy (SEM) and by fluorescence microscopy (FM). For SEM, cross sections were obtained from the central portion of the seed using a sharp blade,

mounted on aluminium studs with wax and sputter-coated with gold (S 1508 - Sputter Coater, Edwards, High Vacuum, West Sussex, U.K.). Samples were viewed with an SEM (Phillips SEM 505, Eindhoven, The Netherlands) at 30 kV. Representative images were recorded (Polaroid film, type 665, Polaroid Corp., Cambridge, MA).

For FM, cross sections of approximately 5 mm thickness were obtained from the central portion of the seed using a sharp blade, fixed in 5% (v/v) glutaraldehyde in 0.1 M phosphate buffer (pH 7), and dehydrated with an aqueous ethanol series (70, 80, 95%) for 2 h at each concentration. The dehydrated sections were embedded in JB-4 Embedding medium (Canemco, St. Laurent, PQ). Sections (5 μm in thickness) were cut using a microtome (Sorvall JB-4, Du Pont Co., Biomedical Division, Newtown, CT), stained with 0.01% (w/v) Calcofluor (White M2R New) (Sigma) for 2 min, washed with deionized water for 1 min, and then air-dried. The sections were examined under UV light using a Nikon Microphot-FXA microscope (Nikon Co., Tokyo, Japan). Representative images were recorded (Kodak film, type P1600, Eastman Kodak Co., Rochester, NY).

3.7 Viscosity Measurements

Rye, wheat and triticale wholemeals, flours, and breads were extracted using water at a solids to water ratio of 1:5 (w/v) for 60 min. The mixtures were centrifuged (3000 x g, 10 min), and the viscosities of the supernatants were measured as described by Scoles et al (1993) using a Brookfield Cone-Plate Viscometer (Model LVTDCP-11, Brookfield Engineering Laboratories Inc., Stoughton, MA) equipped with spindle CP-40 and maintained at 25°C.

The viscosity of chick digesta after centrifugation (3000 x g, 10 min) and of 1% aqueous solutions of freeze-dried water extracts and purified arabinoxylan preparations from high (R95), intermediate (R30) and low (R5) extract viscosity rye wholemeals were determined as described above.

3.8 Effect of Dry Heat Treatment of Rye Wholemeal on Extract Viscosity

The effectiveness of the dry heat treatment (130°C, 90 min) of the wholemeal in inhibiting the activity of endogenous enzymes was monitored by measuring the viscosity of a water extract (1:10 meal to water ratio, w/v) of R95 wholemeal over time (30, 60, 90, 120 min).

3.9 Molecular Weight Distributions

Rye, wheat and triticale wholemeals were heated at 130°C for 90 min to inactivate endogenous enzymes (Delcour et al 1999), and then extracted with water at a meal : water ratio of 1:10 (w/v) for 90 min at room temperature (approximately 25°C). Purified arabinoxylan was solubilized in water at 4°C at an arabinoxylan:water ratio of 0.1:100 (w/v). The water extracts and arabinoxylan solutions were filtered through 0.45 μm disposable filters (Millipore-HA, Millipore Co., Bedford, MA); 0.5 mL of the filtrate was loaded on a gel filtration column (Econo Column 100 x 1.5 cm, BIO-RAD, Mississauga, ON) containing Sephacryl S-500 (Pharmacia, Uppsala, Sweeden). The sugars were eluted with 0.3% NaCl at a flow rate of 12 mL/h. Fractions of 4 mL were collected and an aliquot of each fraction was analyzed for total sugar content by the phenol-sulphuric acid method (Dubois et al 1956).

The column was calibrated with standard dextran from *Leuconostoc mesenteroides* strain 13-512 (average molecular weights of approximately $10x10^3$, $20x10^3$, $126x10^3$, $300x10^3$, $575x10^3$ and $4x10^6$) (Sigma). Sugar content was calculated as glucose for dextran standards, and as pentose for rye extracts. The content of a high molecular weight fraction in each extract was expressed as a percentage of the total carbohydrate applied to the column.

3.10 Enzyme Treatment of Water Extracts from Ryes

3.10.1 Rye Extract Viscosity as Affected by α-Amylase, Protease, Lichenase and Xylanase Treatment

High extract viscosity rye (R95) wholemeal was heated and extracted with water as described in section 3.9. Three aliquots of 1 mL of the extract were incubated with each enzyme at the optimum pH and temperature for each enzyme. The concentrations of the enzymes and the incubation times were applied according to Bhatty et al (1991), except that xylanase incubation time was 2, 4, 6, 8, 10 and 60 min. The viscosities of the extracts incubated with the other enzymes were determined after 10, 20, 30, 40, 50 and 60 min.

3.10.2 Xylanase Treatment of the High Molecular Weight Fraction

Low extract viscosity rye (R5) wholemeal was heated and extracted with water as described in section 3.9 The extract was dialysed against deionized water overnight using membrane tubing with a MW cut-off of 6000-8000 (Spectra Medical Industries Inc., Los Angeles, CA) to eliminate low molecular weight components. A 30 µL aliquot of the xylanase preparation used in section 3.10.1, was added to 1.0 mL of the dialysed extract and incubated

for 15 min at 40°C. The extract was filtered through a 0.45 μm disposable filter (described in section 3.9), and 0.5 mL of the filtered extract was subjected to gel filtration column as described in section 3.9. Samples of the untreated extract, before and after dialysis, were also filtered and subjected to gel permeation chromatography.

3.11 Milling Tests

The optimal tempering moisture level for the milling of rye grain was determined using 100 g sample of the commercial rye tempered to various moisture levels (10.5, 12.5, or 14.0%) for 8 h. Samples were milled in a Quadrumat Jr. flour mill (C.W. Brabender Instruments, Inc., South Hackensack, NJ) equipped with a 60 mesh (US) reel sifter. The tempering level of 12.5% moisture resulted in the highest flour extraction rate, without inducing a significant depression in loaf volume, as observed during preliminary baking experiments, was chosen for use in the milling of the experimental rye lines. Wheat and triticale were milled at 15.0% and 14.0% moisture, respectively. Additional sifting (60 mesh) was applied to the bran fraction, such that an extraction rate of approximately 77% was achieved for all of the experimental rye lines.

3.12 Rheological Properties of Rye, Wheat and Triticale Flours

3.12.1 Viscosity

Viscosities of water extracts of the flours were determine as described in section 3.7.

3.12.2 Farinography

Water absorption of flours and dough stability were determined using a farinograph

(C.W. Brabender Instruments, Inc., South Hackensack, NJ) according to AACC (1995) Method 54-21 (50 g flour, 14% moisture basis). Values obtained from the farinograph curves were as follows: dough development time, the time interval (min) between the first addition of water to the point of maximum dough consistency; arrival time, the elapsed time (min) between the commencement of mixing and the point where the top of the curve first intersected the 500 Brabender Unit (BU) line; stability, the difference between departure time (leaving the 500 BU line) and arrival time; mixing tolerance index, the difference in BU from the top of the curve at the peak to the top of the curve measured 5 min after the peak.

3.12.3 Falling Number

Falling Numbers for all rye flours in the presence and absence of enzyme inhibitor (0.2% mercuric acetate) were determined according to AACC (1995) Method 56-81B.

3.13 Breadmaking with Rye, Wheat, and Triticale Flours

AACC (1990) method 10-10A was used to determine the maximum replacement level of rye flour in rye/wheat blends that did not have a dramatic effect on the baked loaf volume using the low (R10) and high (R95) extract viscosity ryes at several levels (0-70% rye flour in 10% increments). The maximum replacement level was determined on the basis of loaf volume and general loaf acceptability. Subsequently, all rye flours were used at the replacement level identified in the first experiment (30% rye/70% wheat) in order to determine the effect of rye extract viscosity on loaf characteristics. Bread was also prepared from a 30% triticale/70% wheat blend. Rapeseed displacement (National Manufacturing

Company, Division of TMCO, Inc., Lincoln, NE) was used to measure the loaf volume of the various breads.

3.14 Feeding Experiment

3.14.1 Breadmaking with Wheat and Rye Whole Grain Flours

Breads were prepared from low (R5) and high (R95) extract viscosity rye and wheat whole grains using the lean formula (whole grain flour + yeast + water + salt) procedure described in AACC (1990) Method 10-10. Breads were dried at 40°C in a forced-air oven and ground (Laboratory Mill size 811, Christy Norris Engineers Ltd., Chelmsford, U.K.). The ground breads were incorporated into chick diets as described in section 3.14.2.

3.14.2 Chick Feeding Trial

Low (R5) and high (R95) extract viscosity rye and wheat wholemeals (prepared in section 3.2) and breads (prepared in section 3.14.1) were presented to male broiler chicks housed in a thermostatically-controlled battery brooder (Animal Care Unit, Western College of Veterinary Medicine, University of Saskatchewan, Saskatoon, SK). The chicks were given a commercial chick starter for 1 d prior to distribution to treatment groups. Fourteen chicks were housed per pen, with 5 pens per dietary treatment. The experimental period was 14 d, terminating when the chicks were 15 d old. The chicks were weighed at the start and at the end of the experimental period and weighed amounts of feed (as meals) were provided as required. The compositions of the experimental diets (percent) were as follows: ground grain or ground bread (rye or wheat), 60.0; soy meal, 33.0; tallow, 4.0; limestone, 1.16; dicalcium

phosphate, 1.27; salt, 0.25; D,L methionine, 0.20; choline chloride, 0.083; vitamin/trace mineral premix to meet requirements (NRC 1994). Feeding of the chicks was staggered (45 min intervals) to allow digesta collection at a constant time after the initial feeding. Pooled digesta (collected from the duodenum/jejunum) from three chicks constituted a single replicate, with five replicates per dietary treatment. The digesta samples were placed on ice and taken to the laboratory immediately following collection, centrifuged (3,000 x g, 10 min), and their viscosities determined before and after xylanase (used in section 3.10.1) treatment.

3.15 Isolation of Water-Extractable Arabinoxylan (WEAX)

The procedure used for the preparation of water extracts (WE) and purified arabinoxylan (AX) is presented schematically in Figure 3.1. Wholemeals from high (R95), intermediate (R30), and low (R5) extract viscosity ryes were heated (130°C, 90 min) and then extracted with water at a meal to water ratio of 1:10 (w/v) for 90 min. The extracts were incubated with α-amylase (1.2 mL, 2900 U/mL) (Sigma) at 90°C for 30 min to hydrolyze contaminating starch. The high temperature would also denature protein. The mixture was cooled (25°C) and centrifuged (3000 x g, 15 min), the supernatant was collected, and then incubated with amyloglucosidase (0.3 mL, 200 U/mL) (Sigma) at 60°C for 12 h. The mixture was cooled (25°C), and recentrifuged (3,000 x g, 20 min). The supernatant was incubated with lichenase (β-glucan kit, Biocon, Lexington, KY) for 2 h at 40°C before dialysis (48 h, 4°C) using membrane tubing (molecular weight cut-off of 6000-8000; Spectra Medical Industries, Inc., Los Angeles, CA) to eliminate low molecular weight components. The dialyzed supernatant was treated with a clay suspension (Montmorillonite, Sigma) to remove

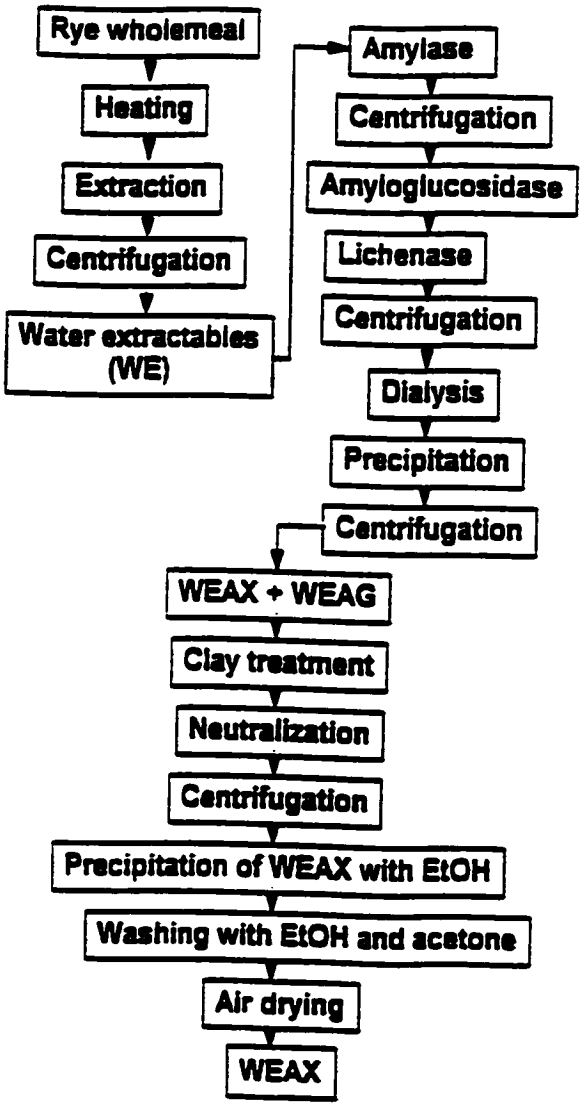


Figure 3.1 Procedure for isolation of water-extractable arabinoxylan. WEAX = water extractable arabinoxylan, WEAG = water extractable arabinogalactan.

protein (15:1 clay to protein ratio, w/w). The pH of the supernatant was adjusted to 3.0 before stirring for 30 min (room temperature, approximately 25°C). The suspension was neutralized (pH 7.0) and centrifuged (10,000 x g, 30 min) to remove the clay-protein complex. Ethanol (95%) was added (to a final ethanol concentration of 80%), the solution was stirred for 30 min, left overnight at 4°C, and centrifuged (10,000 x g, 30 min). The sediment (predominantly WEAX) was re-solubilized in water (300 mL), and WEAX was separated from water-extractable arabinogalactan (WEAG) by ethanol precipitation (final ethanol concentration of 65%). The precipitated WEAX was recovered by centrifugation.

3.16 Composition of Freeze-Dried Water Extracts and Isolated WEAX

Freeze-dried water extract (WE) and purified water-extractable arabinoxylan (WEAX) were analyzed for moisture (vacuum oven, 60°C, 7 h), ash (AACC (1995) Method 08-30), β-glucan (enzymatic method of McCleary and Glennie-Holmes 1985) and starch (Chiang and Johnson 1977). Protein was determined in the WE using the Kjeldahl procedure according to AACC (1995) Method 46-11A. For purified WEAX, protein was determined according to the method of Lowry et al (1951) using bovine serum albumin as a reference protein. Monosaccharide compositions were measured as described in section 3.4.

Sucrose and fructose were determined in the freeze-dried water extract using the oximes method. Sugars were extracted with 80% methanol, followed by purification and derivatization with STOX reagent (oxime internal standard, Pierce Co., Rockford, IL) to form the oxime derivatives prior to silylation with hexamethyldisilazane (Sigma). The derivatized sugars were analysed using the GLC described in section 3.4 but equipped with a fused silica

DB-5 capillary column (0.2 mm i.d. x 10 m, 0.25 μm film thickness) (J&W Scientific, Folsom, CA). The initial temperature (180°C) was held for 6 min, and then increased to 320°C at 15°C/min. The injector and detector temperatures were both 300°C. A standard containing fructose, sucrose, glucose and raffinose was used for calibration.

3.17 Structural Analysis of WEAX

3.17.1 Nuclear Magnetic Resonance (H-NMR)

Pure WEAXs from high (R95), intermediate (R30) and low (R5) extract viscosity ryes were dissolved in D_2O (99.9%) with stirring (120 min, 4°C) followed by lyophilization. This step was repeated to remove extraneous H-signals from the spectrum. The deuterium-exchanged dry material was finally dissolved in D_2O (2.0 mg/mL) for H-NMR spectrum determination. H-NMR spectra were recorded on a 500 MHz instrument (Bruker Analytic Gmbh, Rheinstetten/Karlsruhe, Germany) at 67°C. The pulse repetition time was 0.01 sec with the number of scans varying from 100 to 500. Chemical shifts were referenced to an acetone internal standard (δ 2.2 ppm). The Plant Biotechnology Institute, National research Council, Saskatoon, SK provided the equipment and technical assistance required for the determination of H-NMR spectra.

3.17.2 Size Exclusion High Pressure Liquid Chromatography with Triple Detection

Pure WEAXs from high (R95), intermediate (R30), and low (R5) extract viscosity ryes were analyzed using a size exclusion high pressure liquid chromatograph equipped with a Mix Bed column (VisoGel GMPWXL, Viscotek Co, Houston, TX) and a triple detector

system (Viscotek Model T60). The triple detector system consisted of refractive index (RI), laser light scattering photometer (LS) and viscometer (differential pressure, DP) detector, connected in series. The column and the triple detector system were maintained at ambient temperature (approximately 25°C). The LS angle was 90° and the laser wavelength was 670 nm. Samples were dissolved in 0.05M phosphate buffer (pH 7.0) at a concentration of 0.15%. The injection volume was 78 μL and the samples were eluted with 0.1 M $NaNO_3$, at a flow rate of 0.5 mL/min. On-line detection of the radius of gyration ($\sqrt{r^2g}$) and the hydrodynamic radius (Rh) were obtained using the LS photometer. A Mark-Houwink plot was produced by plotting the logarithm of intrinsic viscosity versus the logarithm of molecular weight.

4. RESULTS

4.1 Extract Viscosity, Total and Soluble Dietary Fibre, Total and Water-Extractable Arabinoxylan, and β-Glucan

Water extracts (1:5, w/v) of rye wholemeal were higher in viscosity than those of wheat or triticale (Table 4.1). The viscosities of the rye extracts ranged from 5-95 cp, compared to 2 cp for the triticale and wheat extracts. All rye wholemeals (including that of the commercial cultivar) were higher in total dietary fibre (TDF), soluble dietary fibre (SDF), total arabinoxylan (TAX), water-extractable arabinoxylan (WEAX), and β-glucan than were wheat or triticale wholemeals. Wheat and triticale contained similar levels of TDF, SDF, and β-glucan. Wheat was higher in TAX and WEAX than was triticale. Among ryes, extract viscosity was weakly correlated with TDF ($r = 0.55$, $p < 0.05$) and TAX ($r = 0.57$, $p < 0.05$) content, and strongly correlated with SDF ($r = 0.90$, $p < 0.05$) and WEAX ($r = 0.89$, $p < 0.05$) content. No relationship was evident between extract viscosity and β-glucan content of rye. When WEAX was calculated as a percentage of TAX, the differences among ryes were more obvious. The WEAX content of triticale, in absolute terms and as a proportion of TAX, was much lower than that of the ryes or wheat.

Arabinose and xylose were determined in freeze-dried water extracts of rye, wheat, and triticale wholemeals as a means of determining the arabinoxylan concentrations in the extracts (Table 4.2). Rye extracts were higher in arabinoxylan than those of wheat or triticale.

Table 4.1 **Extract Viscosity, Total Dietary Fibre, Soluble Dietary Fibre, Total Arabinoxylan, Water-Extractable Arabinoxylan, and β-Glucan Concentrations (% of Dry Matter) of Wholemeals from Experimental Ryes and from Commercial Cultivars of Rye, Triticale, and Wheat**

	Extract Viscosity[1] (cp)	TDF[2] (%)	SDF[3] (%)	TAX[4] (%)	WEAX[5] (%)	WEAX (% of TAX)	β-Glucan (%)
Experimental Ryes[6]							
R95	95.0 ± 1.9[7]	17.0 ± 0.1	5.2 ± 0.0	7.3 ± 0.0	2.5 ± 0.02	33.7	1.9 ± 0.01
R30	30.0 ± 1.1	16.2 ± 0.3	4.0 ± 0.0	6.8 ± 0.1	2.0 ± 0.02	30.1	2.5 ± 0.01
R19	19.0 ± 0.9	16.8 ± 0.5	4.6 ± 0.1	6.7 ± 0.2	2.0 ± 0.01	30.0	1.9 ± 0.06
R10	9.9 ± 0.2	14.7 ± 0.2	4.0 ± 0.0	5.6 ± 0.0	1.3 ± 0.01	23.4	2.0 ± 0.01
R5	4.9 ± 0.2	16.5 ± 0.2	3.8 ± 0.2	6.7 ± 0.0	1.5 ± 0.01	22.1	1.8 ± 0.08
Commercial Cultivars							
Rye	12.5 ± 0.3	15.2 ± 0.1	4.1 ± 0.2	7.0 ± 0.2	1.8 ± 0.01	25.2	2.1 ± 0.08
Triticale	2.3 ± 0.1	12.3 ± 0.1	1.2 ± 0.1	4.1 ± 0.1	0.5 ± 0.02	12.7	0.6 ± 0.00
Wheat	2.3 ± 0.1	11.6 ± 0.2	1.4 ± 0.1	4.9 ± 0.1	1.0 ± 0.02	20.3	0.6 ± 0.01

[1] wholemeal to water ratio of 1:5 (w/v).
[2] total dietary fibre.
[3] soluble dietary fibre.
[4] total arabinoxylan.
[5] water-extractable arabinoxylan.
[6] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[7] mean ± standard deviation (n = 3).

Table 4.2 **Arabinoxylan Contents (% of Dry Matter) and Arabinose/Xylose Ratios of Freeze-Dried Water Extracts of Wholemeals of Experimental Ryes and of Commercial Cultivars of Rye, Triticale, and Wheat**

	Arabinose (%)	Xylose (%)	AX[1] (%)	A/X[2]
Experimental Ryes[3]				
R95	6.6 ± 0.04[4]	10.6 ± 0.04	15.1 ± 0.01	0.62 ± 0.01
R30	5.8 ± 0.01	9.0 ± 0.03	13.0 ± 0.03	0.64 ± 0.01
R19	5.5 ± 0.03	7.6 ± 0.01	11.5 ± 0.08	0.73 ± 0.01
R10	3.5 ± 0.01	5.7 ± 0.01	8.1 ± 0.01	0.62 ± 0.01
R5	3.7 ± 0.01	5.7 ± 0.03	8.3 ± 0.03	0.66 ± 0.01
Commercial Cultivars				
Rye	4.4 ± 0.03	7.4 ± 0.04	10.5 ± 0.01	0.61 ± 0.02
Triticale	2.3 ± 0.00	2.3 ± 0.01	4.0 ± 0.01	0.99 ± 0.01
Wheat	3.5 ± 0.01	4.6 ± 0.02	7.1 ± 0.03	0.77 ± 0.03

[1] arabinoxylan = (arabinose + xylose) x 0.88.
[2] arabinose to xylose ratio.
[3] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[4] mean ± standard deviation (n = 3).

A significant positive correlation ($r = 0.98$, $p < 0.05$) existed between the extract viscosities of the rye wholemeals (Table 4.1) and the arabinoxylan contents of their freeze-dried water extracts (Table 4.2). The A/X ratios among the ryes were similar indicating that the extent of arabinoxylan branching was similar among ryes. The A/X ratio of the triticale extract was much higher than those of the ryes or wheat, and that of wheat was somewhat higher than those of the ryes.

4.2 Microstructure of Low and High Extract Viscosity Rye Grain

The aleurone consisted of a single layer of cells in both the low (R5) and the high (R95) extract viscosity ryes (Figure 4.1), although individual aleurone cells appeared slightly larger in R95. Fluorescence microscopy confirmed the SEM results and showed that the cell walls of the starchy endosperm of R95 were thicker than those of R5 (Figure 4.2).

4.3 Effect of Heat Treatment of Rye Wholemeal on Extract Viscosity

The effect of heat treatment (130°C, 90 min) of rye wholemeal prior to extraction on extract viscosity was determined by extracting heated and unheated wholemeals with water for 30, 60, 90 and 120 min (Figure 4.3). Extract viscosity was greater after 60 min than after 30 min for both the heated and unheated wholemeals. Viscosity declined progressively with an increase in extraction time beyond 60 min for unheated wholemeal. Extracts of heated wholemeal exhibited similar viscosities at extraction times of 60, 90 and 120 min. The results confirmed the necessity of using heated wholemeal and an extraction time of 60 min or longer in the preparation of water extracts from rye, as reported by others (Vinkx and Delcour 1996;

Figure 4.1 Scanning electron micrographs of representative cross sections from low (R5; A) and high (R95; B) extract viscosity rye kernels.

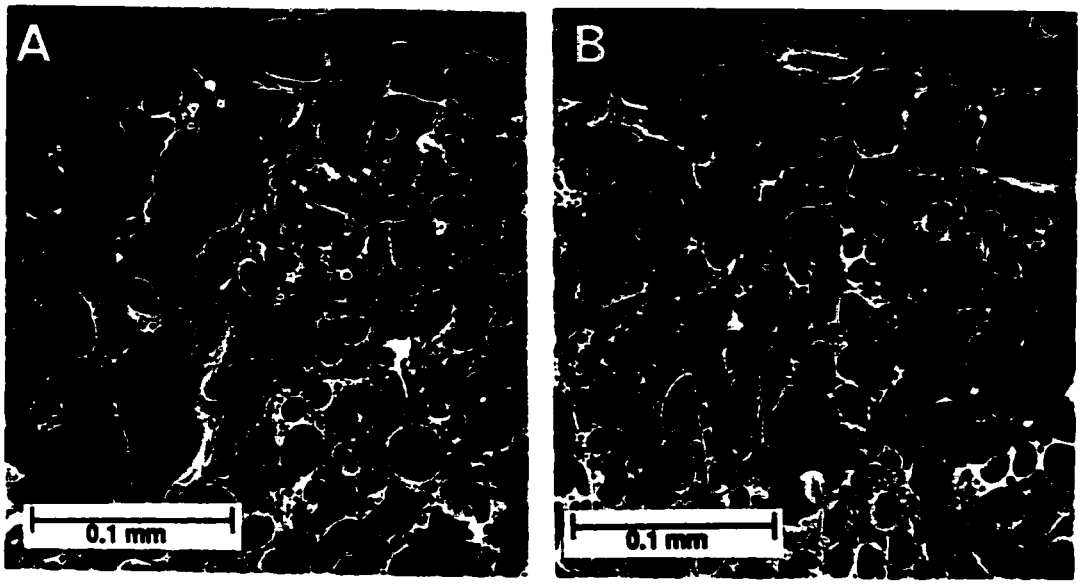
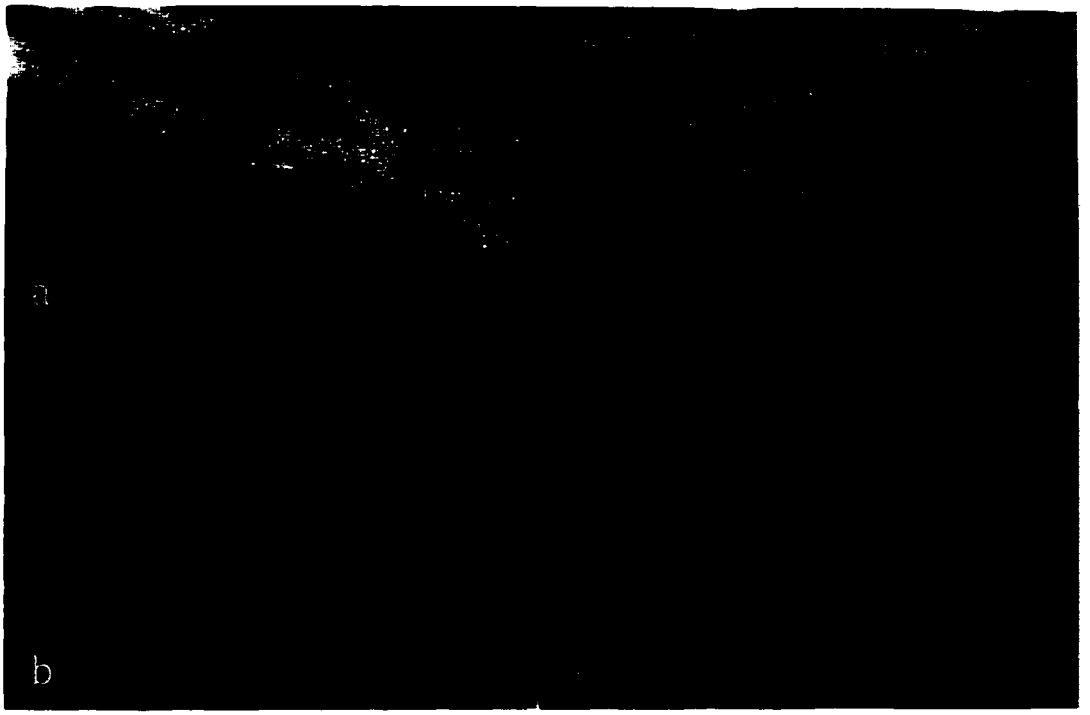

A
0.1 mm
B
0.1 mm

Figure 4.2 Fluorescence micrographs of representative cross sections from low (R5; left) and high (R95; right) extract viscosity rye kernels showing aleurone layers (top) and endosperm cell walls (bottom). Magnification was 400X.


a
b

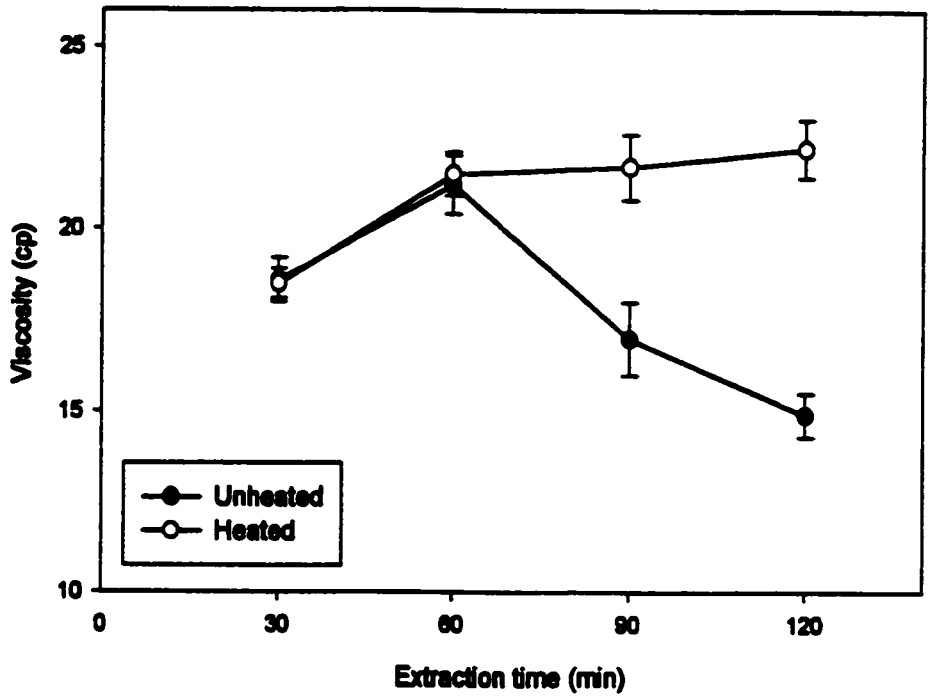


Figure 4.3 Effect of extraction time on the viscosity of water extracts from heated and unheated high extract viscosity rye (R95) wholemeal at a wholemeal to water ratio of 1:10 (w/v).

Delcour et al 1999).

4.4 Gel Filtration Chromatography

Water extracts from rye wholemeals were fractionated on Sephacryl S-500 (Figure 4.4). A high molecular weight fraction (HMWF) eluted between 80 and 140 mL, corresponding to a weighted average molecular weight (MW) of 500,000. A low molecular weight fraction (weighted average MW of 12,000) eluted between 140 and 200 mL. Molecular weight estimations were based on dextran standards (Figure 4.5). The proportion of the total carbohydrate loaded on the column represented by the high molecular weight fraction (%HMWF) varied among the rye lines (20-39%), as shown in Table 4.3. The %HMWF in water extracts was strongly correlated ($r = 0.84$, $p < 0.05$) with the water extract viscosity of the wholemeal. Wheat and triticale water extracts were also fractionated on Sephacryl S-500 (Figure 4.6); the %HMWF in both extracts was low compared to values for the rye extracts, with the wheat extract containing a higher proportion (16%) than did the triticale extract (6%) (Table 4.3). The viscosities of the rye water extracts at a ratio of 1:10 (w/v) wholemeal to water was considerably lower (ranging from 4-18 cp), compared to their corresponding values at a ratio of 1:5 (w/v) wholemeal to water (ranging from 5-95 cp; Table 4.1). Wheat and triticale water extracts at a ratio of 1:10 (w/v) did not differ significantly from those at a ratio of 1:5 (w/v) wholemeal to water (average 2 cp).

4.5 Enzyme Treatment of Water Extracts From Rye

Treatment of the water extract from high extract viscosity rye (R95) wholemeal with

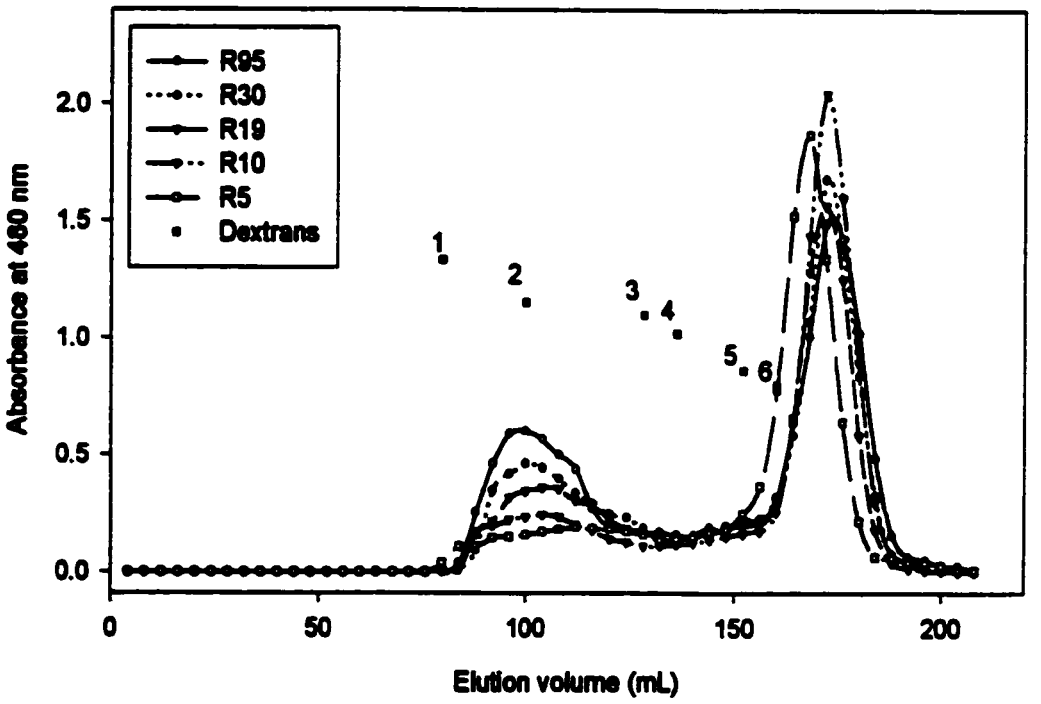


Figure 4.4 Molecular weight distributions of water extracts (1:10 w/v) of wholemeals from experimental ryes determined by gel filtration chromatography using Sephacryl S-500. Each rye line is designated as R (rye) followed by its wholemeal extract viscosity (in cp). Dextran standard MWs (approximate): 1=$4x10^6$, 2=$575x10^3$, 3=$300x10^3$, 4=$126x10^3$, 5=$20x10^3$, 6=$10x10^3$.

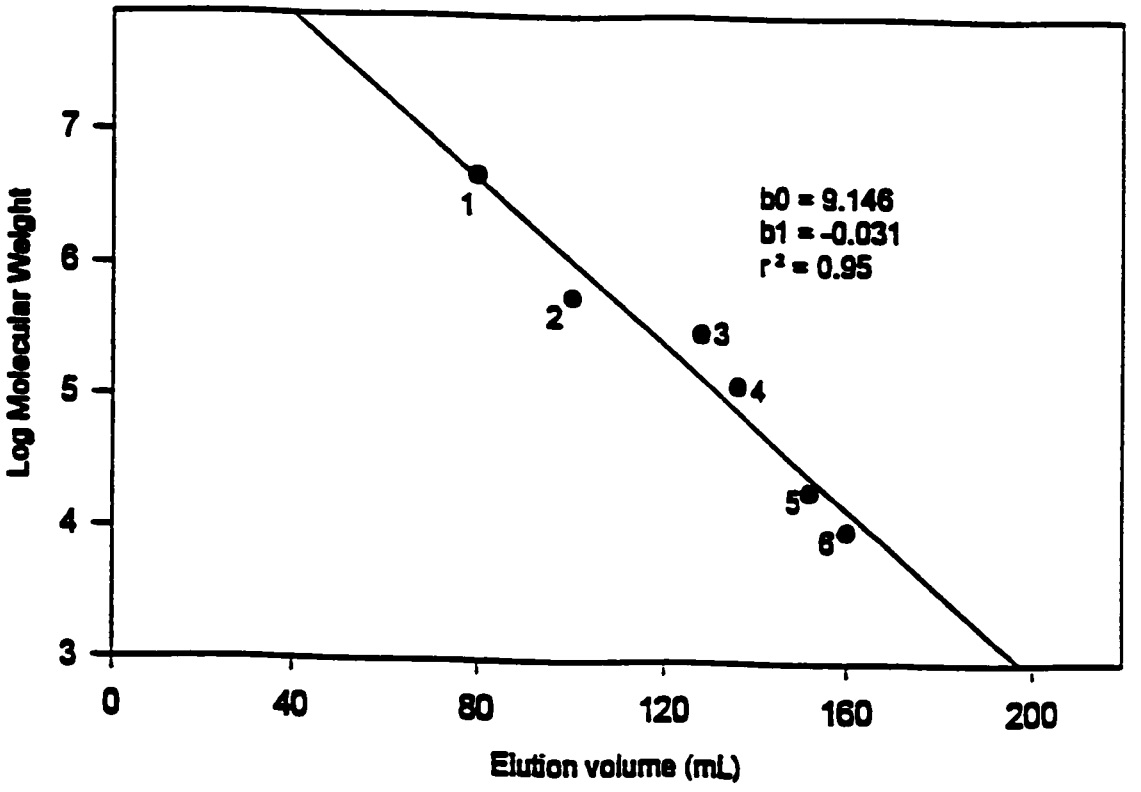


Figure 4.5 Gel filtration (Sephacryl S-500) calibration curve prepared using dextran standards. MWs (approximate): 1=$4x10^6$, 2=$575x10^3$, 3=$300x10^3$, 4=$126x10^3$, 5=$20x10^3$, 6=$10x10^3$.

Table 4.3 **Comparison of Total Carbohydrate Content, Percentage of a High Molecular Weight Fraction (HMWF) and Extract Viscosity of Water Extracts of Wholemeals from Experimental Ryes and from Commercial Cultivars of Rye, Triticale, and Wheat**

	Total Carbohydrate[1] (mg/mL)	HMWF[2] (%)	Extract Viscosity[3] (cp)
Experimental Ryes[4]			
R95	6.0 ± 0.1	38.7 ± 0.5	18.3 ± 0.2[5]
R30	4.8 ± 0.3	34.2 ± 0.4	7.9 ± 0.2
R19	5.2 ± 0.1	32.0 ± 1.3	6.6 ± 0.2
R10	4.8 ± 0.2	20.9 ± 0.4	5.1 ± 0.1
R5	5.3 ± 0.1	20.1 ± 0.5	3.8 ± 0.2
Commercial Cultivars			
Rye	5.8 ± 0.1	27.5 ± 0.6	4.6 ± 0.1
Triticale	3.7 ± 0.1	6.1 ± 0.0	2.3 ± 0.0
Wheat	2.2 ± 0.2	16.4 ± 0.0	1.9 ± 0.0

[1] total carbohydrate loaded on the column.
[2] the proportion of the total carbohydrate loaded on the column represented by the high molecular weight fraction.
[3] viscosity of water extract (1:10 w/v).
[4] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[5] mean ± standard deviation (n = 3).

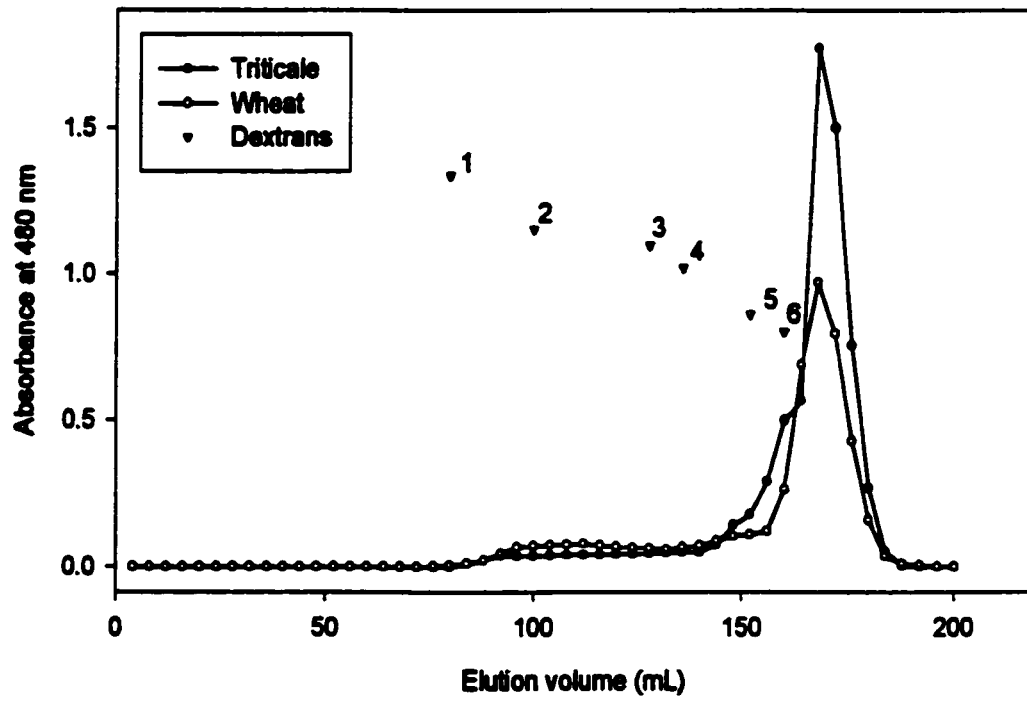


Figure 4.6 Molecular weight distributions of water extracts (1:10 w/v) of triticale and wheat wholemeals as determined by gel filtration chromatography using Sephacryl S-500. Dextran standard MWs (approximate): 1=$4x10^6$, 2=$575x10^3$, 3=$300x10^3$, 4=$126x10^3$, 5=$20x10^3$, 6=$10x10^3$.

α-amylase or lichenase did not significantly reduce its viscosity (Figure 4.7). A slight reduction in extract viscosity resulted from the addition of protease. A very marked reduction in the viscosity of the extract resulted from treatment with xylanase.

Dialysis of the water extract from low extract viscosity rye (R5) wholemeal removed most of the low molecular weight components (Figure 4.8). Subsequent incubation of the dialyzed rye extract with xylanase markedly reduced the % HMWF; a concomitant increase in the low molecular weight peak was observed.

4.6 Milling Test to Determine the Optimal Tempering Moisture for Rye

A preliminary milling experiment was conducted using the commercial rye cultivar to establish the optimal tempering moisture for the experimental ryes, which were available in limited quantities. Tempering to 10.5% moisture resulted in a flour yield of 80.3% (Table 4.4). At 12.5% and 14.0% moisture, extraction rates were reduced to 72.3% and 67.1%, respectively. A tempering moisture of 12.5% was chosen for the milling of the experimental ryes, as this provided an acceptable flour yield and an acceptable loaf volume, as determined from a preliminary baking trial with flour from the commercial rye (results not shown). In the milling of the experimental ryes, additional flour (≤60 mesh) was sifted from the bran and combined with the flour fraction, resulting in extraction rates of 77-78% for all flours.

4.7 Physical Properties of Flours and Doughs

4.7.1 Extract Viscosities of Milling Fractions, Flours, and Wholemeals

The viscosities of water extracts of flours, shorts, and bran fractions from the

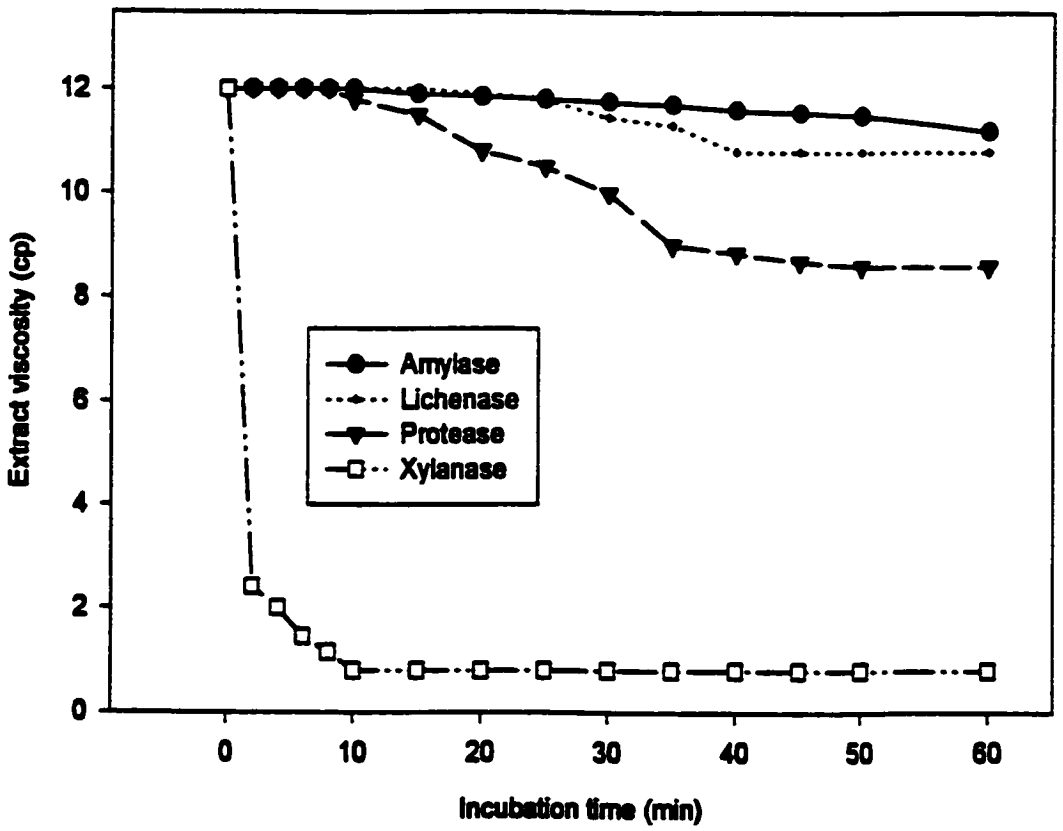


Figure 4.7 Effect of amylase, lichenase, protease and xylanase treatment on the viscosity of a water extract from high extract viscosity rye (R95) wholemeal. The ratio of wholemeal to water was 1:10 (w/v).

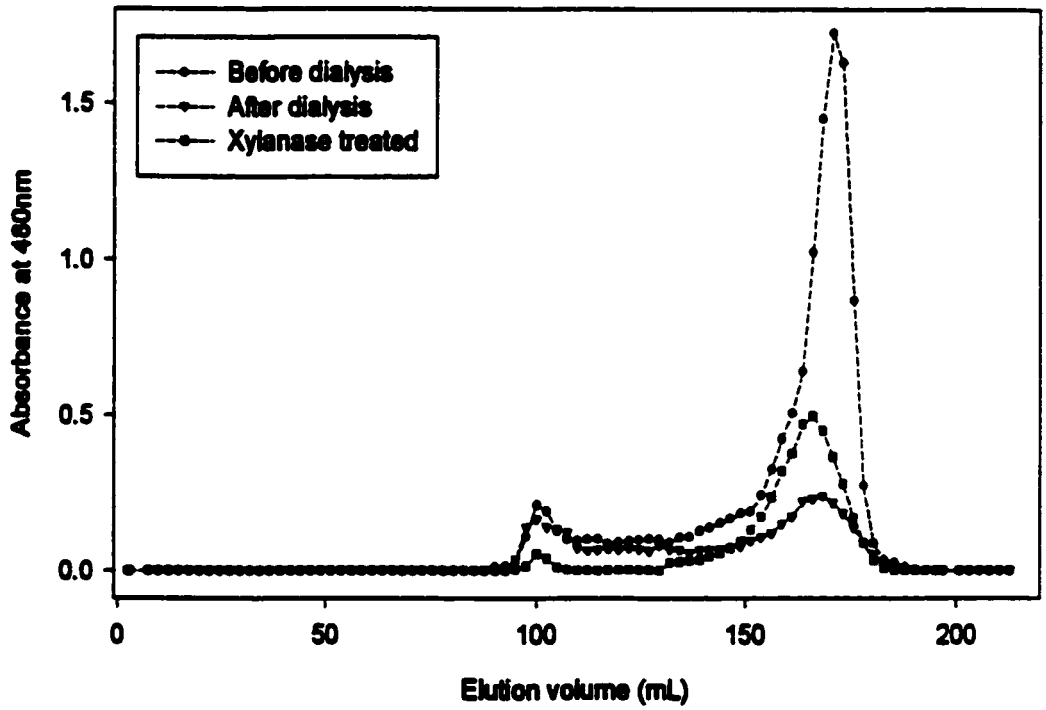


Figure 4.8 Molecular weight distribution of a water extract from low extract viscosity rye (R5) at a wholemeal to water ratio of 1:10 (w/v) before dialysis, after dialysis, and after dialysis and xylanase treatment.

Table 4.4 Yields and Extract Viscosities of Milling Fractions from a Commercial Rye Cultivar (Prima)[1] Generated at Various Tempering Moistures

Milling Fraction	Tempering Moisture (%)	Particle Size (μm)	Yield (%)	Viscosity[2] (cp)
Flour	10.5	< 250	80.3 ± 1.9[3]	28.8 ± 0.4
	12.5		72.3 ± 1.1	26.3 ± 0.1
	14.0		67.1 ± 0.9	25.6 ± 0.9
LSD[4]			3.1	2.7
Shorts	10.5	250-425	7.1 ± 0.2	8.4 ± 0.1
	12.5		7.8 ± 0.1	9.2 ± 0.4
	14.0		7.1 ± 0.2	16.9 ± 0.2
LSD			0.5	2.1
Bran	10.5	> 425	12.6 ± 0.5	6.2 ± 0.1
	12.5		20.4 ± 0.2	5.6 ± 0.1
	14.0		25.8 ± 0.6	6.4 ± 0.3
LSD			1.1	0.4

[1] extract viscosity of wholemeal was 12.5 cp.
[2] extract viscosities determined at a 1:5 (w/v) sample to water ratio.
[3] mean ± standard deviation (n = 3).
[4] least significant difference at $p < 0.05$.

commercial rye sample were determined as an indication of the distribution of WEAX among the fractions (Table 4.4). Tempering moisture had little effect on the extract viscosity of any particular fraction, with the exception of the relatively high extract viscosity exhibited by the shorts fraction generated at 14.0% moisture. The extract viscosities of the flour fractions were 3-4 times those of corresponding shorts fractions, and 4-5 times those of corresponding bran fractions, with the exception of the shorts fraction generated at 14.0% moisture which had an extract viscosity approximately two-thirds that of the corresponding flour.

Extract viscosities of flours from rye, wheat, and triticale reflected the extract viscosities of the wholemeals (Figure 4.9). The viscosities of the rye flour extracts ranged from 9-199 cp, compared to 5-95 cp for the wholemeals. A highly significant positive correlation ($r = 0.99$, $p < 0.05$) was observed between the extract viscosities of the rye wholemeals and those of their corresponding flours. Wheat and triticale flours exhibited very low extract viscosities (2-3 cp) compared to the rye flours.

4.7.2 Farinograph Characteristics of Flours From Rye, Wheat, and Triticale, and Rye/Wheat and Rye/Triticale Blends

Farinograms (Figure 4.10, Table 4.5) showed rye and triticale flours to be very weak in comparison to wheat flour. Compared to wheat flour, rye flours exhibited similar water absorptions, much shorter arrival and dough development times, much higher mixing tolerance indices and much lower stabilities. Compared to the rye flours, triticale flour exhibited a low water absorption, very short arrival and dough development times, a similar mixing tolerance index and a higher stability. Extract viscosity of rye flour was negatively

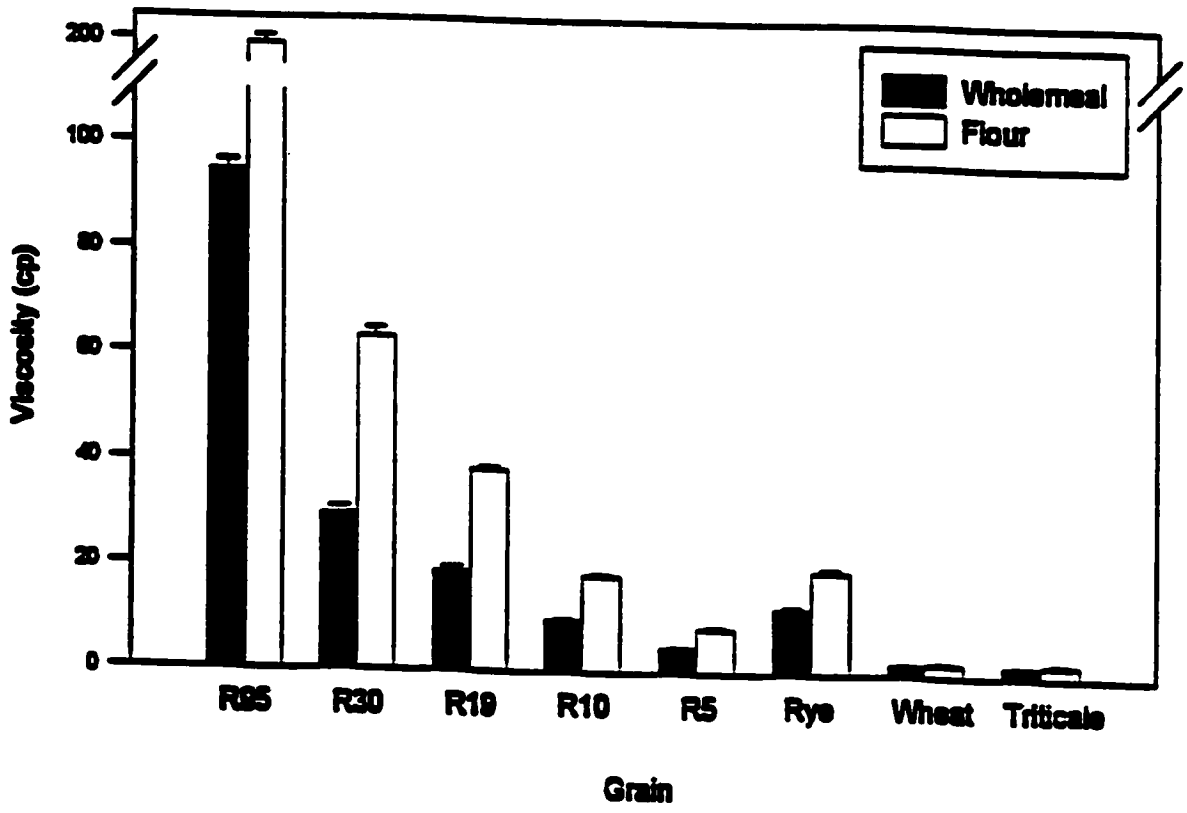


Figure 4.9 Extract viscosities of wholemeals and flours from experimental ryes, and from commercial cultivars of rye, wheat, and triticale. Each experimental rye line is designated as R (rye) followed by the extract viscosity of its wholemeal (in cp).

Figure 4.10 Farinograms of flours from commercial cultivars of wheat, rye, and triticale and from experimental rye lines, individually and in blends with wheat flour at a ratio of 30% rye or triticale:70% wheat (w/w). Each experimental rye line is designated as R (rye) followed by the extract viscosity of its wholemeal (in cp).

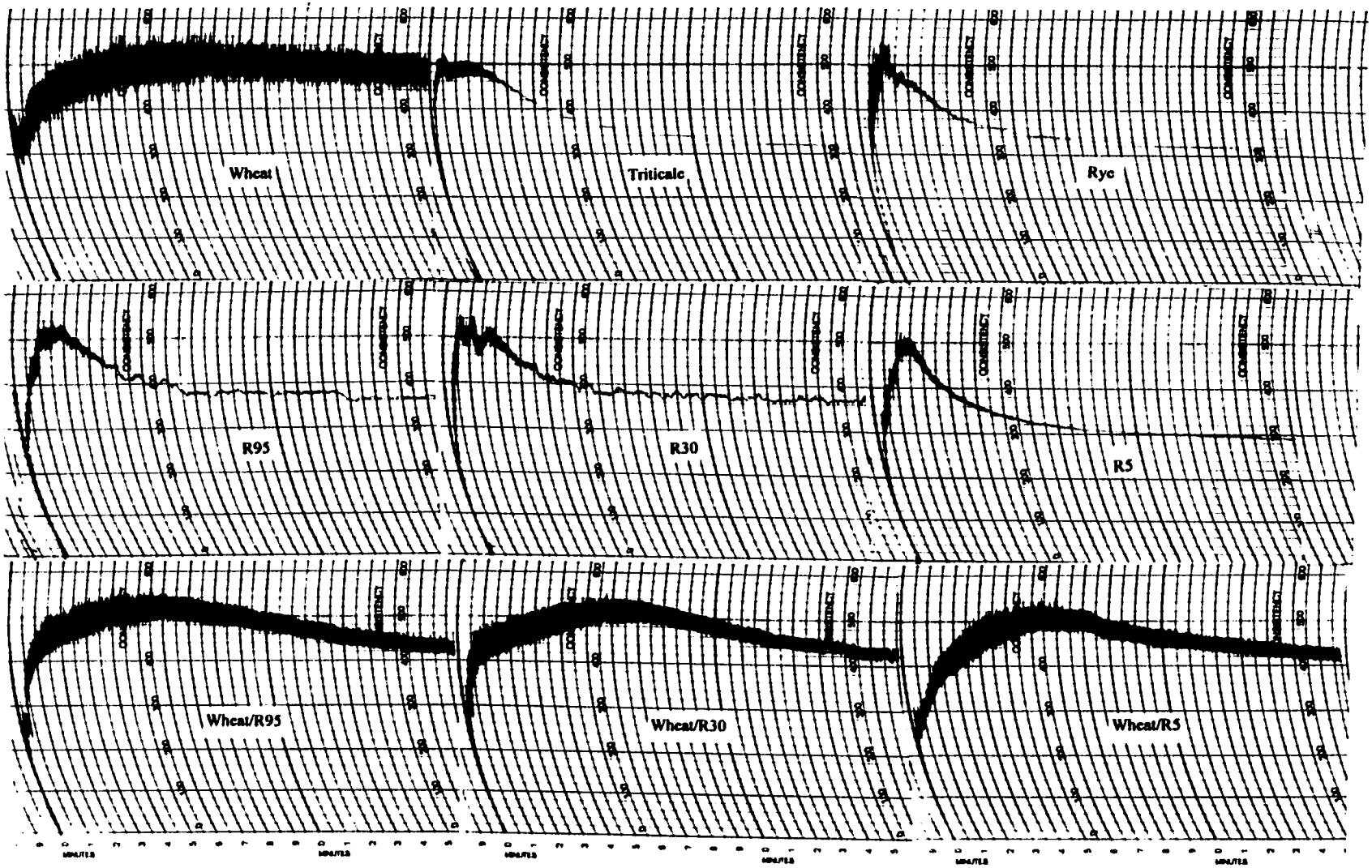

Wheat
Triticale
Rye
R95
R30
R5
Wheat/R95
Wheat/R30
Wheat/R5

Table 4.5 Farinograph Characteristics of Experimental Rye Flours, and Flours from Commercial Cultivars of Rye, Wheat, and Triticale Individually and in Blends with Wheat Flour

	Water Absorption (%)	Arrival Time (s)	DDT[1] (s)	MTI[2] (BU)	Stability[3] (s)
Experimental Ryes[4]					
R95	73.3 ± 0.1[5]	55.0 ± 2.5	92.5 ± 3.5	125.0 ± 7.1	97.5 ± 4.0
R30	68.1 ± 0.2	30.0 ± 0.1	52.5 ± 2.5	122.5 ± 3.5	117.5 ± 3.5
R19	72.8 ± 0.4	45.0 ± 0.1	87.5 ± 3.5	165.0 ± 7.1	60.0 ± 0.1
R10	66.3 ± 0.4	42.5 ± 1.5	63.5 ± 2.9	157.5 ± 3.5	47.5 ± 0.5
R5	72.3 ± 0.4	67.5 ± 3.0	80.0 ± 3.4	172.5 ± 3.5	47.5 ± 1.5
Rye	66.1 ± 0.2	57.5 ± 2.5	87.5 ± 3.5	127.5 ± 3.5	62.5 ± 2.5
LSD[6]	0.8	9.5	11.3	10.1	13.8
Rye/Wheat Blends (30:70, w/w)					
R95	70.3 ± 1.1	157.5 ± 6.6	322.5 ± 10.6	20.0 ± 0.1	540.0 ± 22.4
R30	69.3 ± 0.4	127.5 ± 5.6	345.0 ± 15.2	47.5 ± 1.5	465.0 ± 21.2
R19	70.0 ± 0.1	180.0 ± 0.6	332.5 ± 3.5	37.5 ± 1.5	382.5 ± 10.6
R10	67.8 ± 0.4	157.5 ± 6.6	315.0 ± 21.2	22.5 ± 1.0	472.5 ± 10.6
R5	67.3 ± 0.4	175.0 ± 7.1	322.5 ± 10.6	52.5 ± 2.5	322.5 ± 10.6
Rye	66.3 ± 1.1	127.5 ± 5.6	315.0 ± 0.1	20.0 ± 0.1	450.0 ± 20.4
LSD	2.3	13.4	23.9	6.8	51.1
Wheat	66.7 ± 0.4	115.0 ± 5.1	315.0 ± 14.2	5.0 ± 0.0	1155.0 ± 21.2
Triticale	59.8 ± 0.4	15.0 ± 0.1	37.5 ± 1.5	137.5 ± 3.5	130.0 ± 6.1
Triticale/Wheat	64.3 ± 0.4	112.5 ± 5.3	285.0 ± 12.2	22.5 ± 1.0	585.0 ± 21.2

[1] dough development time.
[2] mixing tolerance index (in Brabender Units).
[3] dough stability (in seconds).
[4] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[5] mean ± standard deviation (n = 3).
[6] least significant difference at $p < 0.05$.

correlated ($r = -0.65$, $p < 0.05$) with mixing tolerance index and positively correlated ($r = 0.64$, $p < 0.05$) with dough stability. No relationship was observed between extract viscosity and either water absorption, arrival time, or dough development time.

The farinograph characteristics of rye and triticale flours were much improved when blended with wheat flour (Figure 4.10). A significant positive correlation ($r = 0.85$, $p < 0.05$) existed between the extract viscosities of the rye/wheat blends and their farinograph water absorptions (Figure 4.11, Table 4.5). The arrival times of the blends were considerably longer than those of wheat flour and the individual rye flours (Table 4.5). Dough development times of the blends were similar to those of wheat. Mixing tolerance indices of the rye/wheat blends were intermediate to those of wheat and rye. The triticale/wheat blend tended to follow the trends exhibited by the rye/wheat blends with respect to its farinograph characteristics.

4.7.3 Falling Number

Significant differences in falling number were detected among the rye flours when measured with or without enzyme inhibitor (Table 4.6). Falling number was directly related to the extract viscosity of rye flour in the presence ($r = 0.73$, $p < 0.05$) or absence ($r = 0.65$, $p < 0.05$) of the inhibitor. In the presence of the enzyme inhibitor, falling number increased markedly for all samples with the exception of wheat, and most dramatically for the commercial rye and triticale samples.

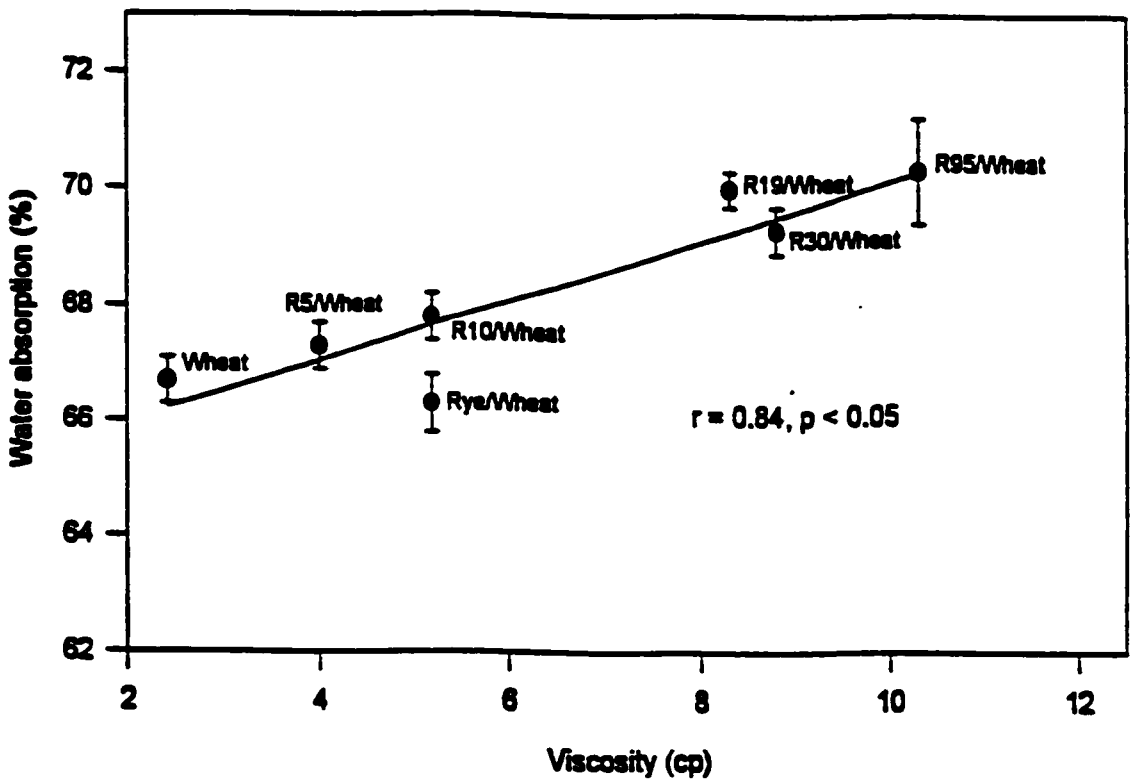


Figure 4.11 Relationship between the extract viscosities of rye/wheat blends (30%:70%, w/w) and their farinograph water absorptions. Each experimental rye line is designated as R (rye) followed by the extract viscosity of its wholemeal (in cp).

Table 4.6 **Falling Numbers of Flours from Experimental Rye Lines and of Flour from Commercial Cultivars of Rye, Triticale, and Wheat, With and Without the Addition of an Enzyme Inhibitor[1]**

Flour	Falling Number (s)
Without enzyme inhibitor	
R95[2]	295.5 ± 6.4[3]
R30	287.5 ± 3.5
R19	274.0 ± 4.2
R10	263.0 ± 6.4
R5	141.5 ± 2.1
Rye	82.5 ± 3.5
Triticale	112.0 ± 1.4
Wheat	422.0 ± 11.3
LSD[4]	10.3
With enzyme inhibitor	
R95	402.0 ± 3.0
R30	377.0 ± 5.5
R19	387.0 ± 4.5
R10	313.0 ± 2.0
R5	297.0 ± 2.5
Rye	314.0 ± 2.5
Triticale	330.1 ± 11.3
Wheat	450.0 ± 6.0
LSD	15.7

[1] mercuric acetate (0.2%, w/v).
[2] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[3] mean ± standard deviation (n = 3).
[4] least significant difference at $p < 0.05$.

4.8 Baking Quality

4.8.1 Determination of the Appropriate Level of Rye Flour in Rye/Wheat Blends

To determine the maximum level of rye flour that could be incorporated into a rye/wheat blend without causing a marked decline in loaf quality, flours from two rye lines (R95 and R10) representing extremes in flour extract viscosity (199 and 20 cp, respectively, Figure 4.9) were substituted for wheat flour at levels of up to 70% rye flour (in 10% increments). Despite the differences in extract viscosity of the rye flours, loaf volume declined in similar fashion as the level of each rye flour increased (Figures 4.12 and 4.13), although R95 had a somewhat greater depressing effect on loaf volume than did R10. Beyond 30% rye flour in the blend, loaf volume and other bread characteristics (including crust smoothness and crumb stickiness and density) became progressively less satisfactory as the proportion of the rye flour in the blend increased. Accordingly, 30:70 rye/wheat blends were used in subsequent baking studies with the experimental rye lines.

4.8.2 Baking Quality of 30:70 Rye/Wheat and Triticale/Wheat Blends

The extract viscosities of the rye/wheat blends were positively correlated ($r = 0.81$, $p < 0.05$) with, but substantially lower than, those of the corresponding rye flours (Table 4.7). In all cases, the loaf volume of the blend was lower than that obtained with the wheat flour control. Extract viscosity of experimental rye/wheat flour blends was negatively correlated ($r = -0.74$, $p < 0.05$) with loaf volume and specific volume ($r = -0.73$, $p < 0.05$), and positively correlated ($r = 0.73$, $p < 0.05$) with loaf weight. Loaf weights and specific volumes of breads prepared from the rye/wheat blends were slightly higher and substantially lower,

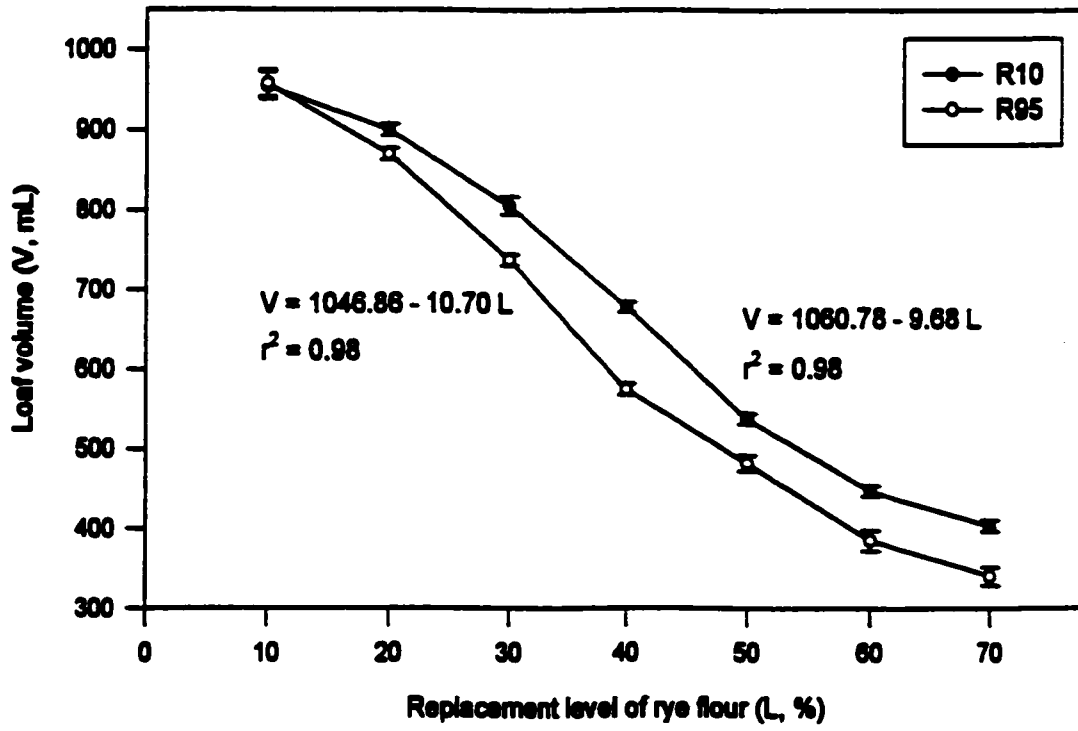


Figure 4.12 Effect of level of replacement of wheat flour by low (R10) and high (R95) extract viscosity rye flour on bread loaf volume.

Figure 4.13 Appearance of bread baked from wheat flour replaced with 10-70% low (R10; top) or high (R95, bottom) extract viscosity rye flour.


0
10
20
30
40
50
60
70
0
10
20
30
40
50
60
70

Table 4.7 Extract Viscosity and Baking Quality of Rye/Wheat and Triticale/Wheat Blends (30:70, w/w) Compared to that of the Wheat Flour Control

	Extract Viscosity (cp)	Loaf Volume (mL)	Loaf Weight (g)	Specific Volume (mL/g)
Blends[1]				
R95	10.3 ± 0.5[2]	793.3 ± 7.6	141.9 ± 1.2	5.6 ± 0.1
R30	8.8 ± 0.3	818.3 ± 7.6	141.9 ± 0.4	5.8 ± 0.0
R19	8.3 ± 0.1	841.7 ± 7.6	142.9 ± 0.9	5.9 ± 0.0
R10	5.2 ± 0.0	838.3 ± 2.9	140.1 ± 0.5	6.0 ± 0.1
R5	4.0 ± 0.0	815.0 ± 13.2	142.2 ± 0.6	5.7 ± 0.1
Rye	5.9 ± 0.0	896.7 ± 17.6	139.1 ± 0.6	6.4 ± 0.1
Triticale	2.3 ± 0.0	945.0 ± 13.2	139.7 ± 0.1	6.8 ± 0.1
Wheat	2.4 ± 0.1	971.7 ± 5.8	138.0 ± 0.4	7.0 ± 0.1
LSD[3]	0.7	17.7	1.2	0.1

[1] each experimental rye line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[2] mean ± standard deviation (n = 3).
[3] least significant difference at $p < 0.05$.

respectively, than corresponding values for the wheat flour control. The baking characteristics of the triticale/wheat blend were very similar to those of the wheat flour control.

4.9 Chick Feeding Experiment

The highest average body weight (398 g) was attained by chicks fed the wholemeal wheat diet, followed by those of chicks fed the low extract viscosity rye (R5) diet (335 g) and those fed the high extract viscosity rye (R95) diet (280 g) (Table 4.8). Feed conversion (F/G) followed the same trend, with significant differences between wheat and both ryes, but not between ryes.

Diet extract viscosity and digesta viscosity reflected the extract viscosities of the corresponding wholemeals and breads (Table 4.8). Diet extract viscosities were reduced somewhat when the rye and wheat wholemeals were included in the form of bread. Digesta viscosities were reduced by approximately 50, 80 and 75% for wheat, R5 and R95 respectively, when the wholemeals were incorporated as bread.

Treatment of digesta with xylanase resulted in marked decreases in viscosity. Corresponding viscosities of digesta from chicks fed the high and low extract viscosity rye wholemeal and wheat wholemeal diets, with and without xylanase treatment, were 8.1, 2.3 and 0.6 cp and 196.0, 82.3 and 5.9 cp, respectively.

Table 4.8 **Results Obtained from a Chick Feeding Experiment Using Diets Containing Wheat, Low (R5), or High (R95) Extract Viscosity Rye Wholemeals or Breads Made from Whole Grain Flours**

Treatment	Weight Gain (g)	Feed Conversion (F/G)	Extract Viscosity (cp)	Diet Extract Viscosity (cp)	Digesta Viscosity (cp)
Wholemeals					
Wheat	398.3 ± 14.0[1]	1.44 ± 0.05	2.4 ± 0.0	1.5 ± 0.0	5.9 ± 0.2
R5[2]	335.4 ± 10.0	1.61 ± 0.02	4.5 ± 0.0	3.4 ± 0.1	82.3 ± 3.2
R95	280.4 ± 19.8	1.71 ± 0.06	95.0 ± 1.1	9.3 ± 0.6	196.0 ± 5.0
LSD[3]	44.6	0.16	3.1	0.3	39.2
Breads					
Wheat	ND[4]	ND	2.1 ± 0.1	1.4 ± 0.1	2.9 ± 0.3
R5	ND	ND	3.7 ± 0.1	3.1 ± 0.1	13.2 ± 0.1
R95	ND	ND	28.2 ± 1.1	6.8 ± 0.1	57.6 ± 0.3
LSD			0.4	0.5	2.4

[1] mean ± standard deviation (n = 5).
[2] each experimental rye line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[3] least significant difference at $p < 0.05$.
[4] not determined.

4.10 Characterization of WEAX from High, Intermediate, and Low Extract Viscosity Ryes

4.10.1 Freeze-Dried Water Extracts

The chemical compositions of freeze-dried water extracts from three rye wholemeals were determined as an aid to the establishment of an appropriate procedure for the isolation of highly purified WEAX preparations (Table 4.9). A higher yield (17 g/100 g meal) of the freeze-dried water extract (WE) was obtained from R5 wholemeal than from R95 or R30 (15 g/100 g meal in each case). The viscosities of 1% aqueous solutions prepared from the WEs were determined to be 2.1, 2.5 and 4.8 cp for R5, R30 and R95, respectively. The levels of simple sugars were generally similar in hydrolyzates of the three extracts. Glucose accounted for a high percentage of the extracts, followed by mannose. Sucrose averaged 11.6% for the three extracts. The concentrations of arabinoxylan in the extracts reflected their viscosities; arabinoxylan accounted for 15, 13 and 8% of the extracts from R95, R30 and R5, respectively. All extracts contained significant concentrations of protein, with the extract from R5 exhibiting the highest amount (35%). Ash content was similar in the three extracts average (8.5%). Starch and β-glucan were present in small amounts in the three extracts.

4.10.2 Composition and MWs of Purified WEAX

The lowest yield of WEAX was obtained from the low extract viscosity (R5) rye wholemeal (0.7 g/100 g meal) (Table 4.10); the yields from R30 and R95 were similar (1.5 and 1.6 g/100g, respectively). Aqueous solutions (1% w/v) of the WEAX isolated from the three ryes differed markedly in viscosity; viscosities were approximately 32, 76 and 566 cp

Table. 4.9 **Yield, Viscosity and Composition[1] of Freeze-Dried Water Extracts from High (R95), Intermediate (R30), and Low (R5) Extract Viscosity Rye Wholemeals (% Dry Basis)**

	R95[2]	R30	R5
Yield (g/100 g meal)	15.2 ± 0.2[3]	15.0 ± 0.2	17.2 ± 0.3
Viscosity of 1% solution (cp)	4.8 ± 0.1	2.5 ± 0.0	2.1 ± 0.1
Glucose	26.1 ± 0.2	26.4 ± 0.2	27.0 ± 0.6
Sucrose	11.9 ± 0.2	12.5 ± 0.2	10.5 ± 0.2
Mannose	9.6 ± 0.0	10.1 ± 0.1	8.3 ± 0.1
Xylose	10.6 ± 0.0	9.0 ± 0.1	5.7 ± 0.1
Arabinose	6.6 ± 0.0	5.8 ± 0.1	3.7 ± 0.1
Galactose	1.8 ± 0.0	1.7 ± 0.1	2.0 ± 0.0
Fructose	1.0 ± 0.0	1.2 ± 0.0	1.6 ± 0.0
Arabinose/Xylose ratio	0.62	0.64	0.65
Arabinoxylan[4]	15.1 ± 0.1	13.0 ± 0.1	8.3 ± 0.1
Protein	25.7 ± 0.0	23.8 ± 0.1	34.5 ± 0.1
Ash	8.2 ± 0.0	8.7 ± 0.0	8.7 ± 0.0
Starch	3.4 ± 0.0	4.2 ± 0.0	5.6 ± 0.0
β-Glucan	3.2 ± 0.0	3.1 ± 0.0	2.7 ± 0.0

[1] concentrations of simple sugars were determined in hydrolyzed extracts.
[2] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[3] mean ± standard deviation (n = 3).
[4] (arabinose + xylose) x 0.88.

Table 4.10 Yield, Viscosity and Composition[1] of Water-Extractable Arabinoxylan Isolated from High (R95), Intermediate (R30), and Low (R5) Extract Viscosity Ryes (% Dry Basis)

	R95[2]	R30	R5
Yield (g/100 g meal)	1.6 ± 0.0[3]	1.5 ± 0.0	0.7 ± 0.0
Viscosity (cp) of 1% solution	566.0 ± 2.8	76.0 ± 0.2	32.4 ± 0.3
Xylose	62.8 ± 0.6	66.9 ± 0.4	63.2 ± 0.6
Arabinose	37.9 ± 0.2	36.7 ± 0.4	38.1 ± 0.2
Arabinoxylan[4]	88.6 ± 0.3	91.2 ± 0.4	89.2 ± 0.4
Protein	4.3 ± 0.1	3.8 ± 0.0	4.1 ± 0.1
Ash	1.7 ± 0.1	0.8 ± 0.0	1.1 ± 0.0
A/X[5] ratio	0.60	0.55	0.60
Approximate MW[6]	730,000 ± 650	476,000 ± 720	269,000 ± 520

[1] arabinose and xylose were determined in hydrolyzed isolates.
[2] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[3] mean ± standard deviation (n = 3).
[4] arabinoxylan = (arabinose + xylose) x 0.88.
[5] arabinose to xylose ratio.
[6] determined using gel filtration chromatography.

for WEAX from R5, R30 and R95, respectively. Analysis of the WEAX confirmed their high level of purity (approximately 90% AX) (Table 4.10). Arabinose and xylose were the only sugars present in significant amounts in hydrolyzates of the WEAX. The parities of the WEAX preparations confirmed the efficacy of the isolation procedure. The A/X ratios of the three WEAX were similar, with WEAX from R30 exhibiting an A/X ratio (0.55) which was slightly lower than the A/X ratios of WEAX from R5 or R95 (0.60 in both cases).

Purified WEAX from the three ryes exhibited single peaks when subjected to gel filtration, eluting between 80 and 150 mL (Figure 4.14). There were significant differences in weighted average molecular weight (MW) among the three WEAX, with WEAX from R5 exhibiting the lowest MW (269,000), WEAX from R30 an intermediate MW (476,000), and WEAX from R95 the highest MW (730,000) (Table 4.10).

4.11 Fine Structural Properties of Purified WEAX

4.11.1 Proton Nuclear Magnetic Resonance (H-NMR)

The H-NMR spectra of WEAX isolated from R95, R30 and R5 are presented in Figure 4.15. The absence of peaks between δ 4.70-4.80 ppm in the spectra of the WEAX indicated no contamination by β-glucan (Bengtsson et al 1992; Cleemput et al 1993). There was also no contamination with arabinogalactan as indicated by the absence of a peak at δ 5.26 ppm (Figure 4.15) (Loosveld et al 1997). More detailed representations of the L-arabinofuranosyl anomeric proton regions (δ 5.40-5.20 ppm) of the three WEAX are presented in Figure 4.16. In this region, the WEAX exhibited three major peaks. The first peak (δ 5.40 ppm) resulted from the anomeric protons of α-L-arabinofuranosyl (Araf) linked

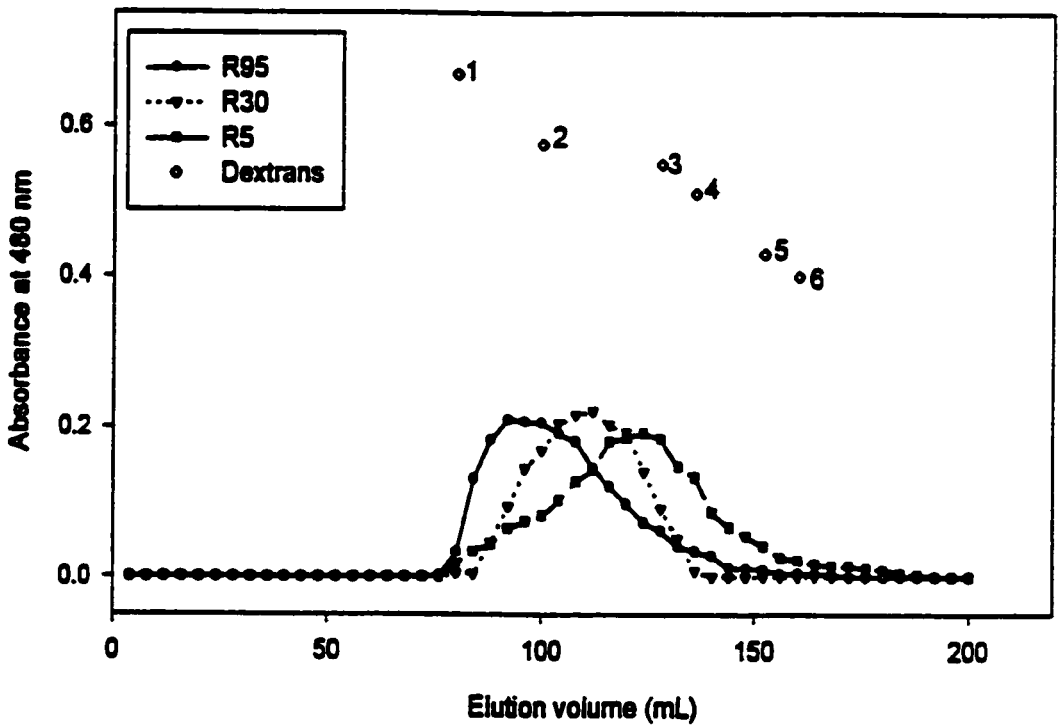
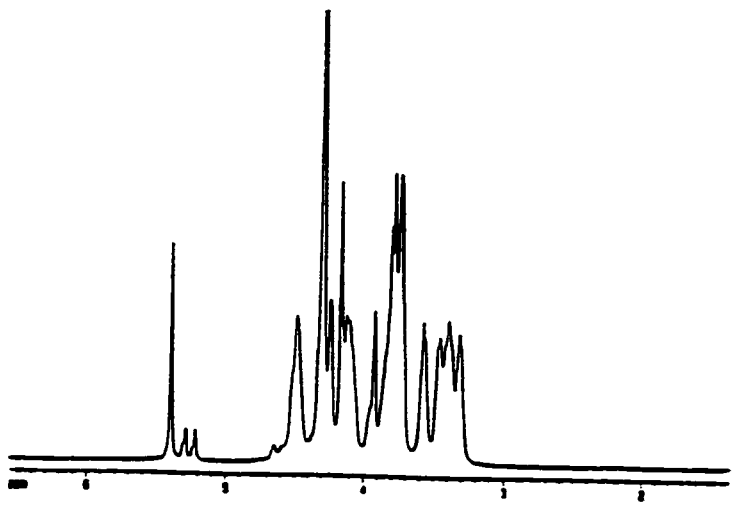
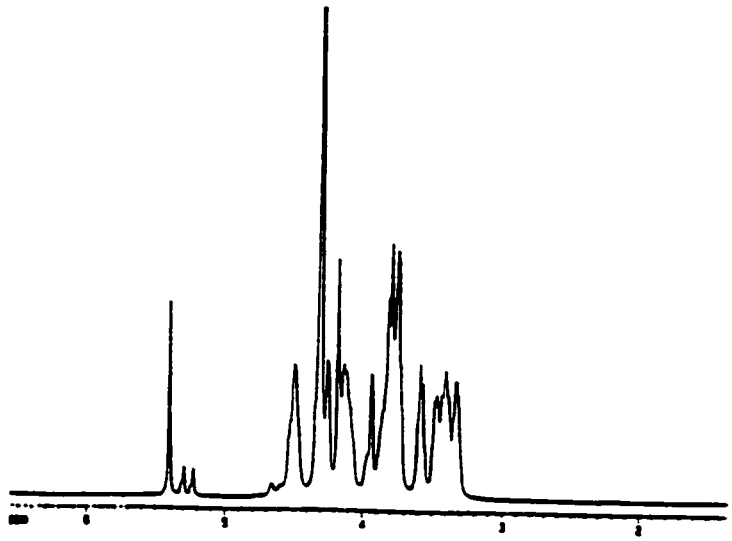
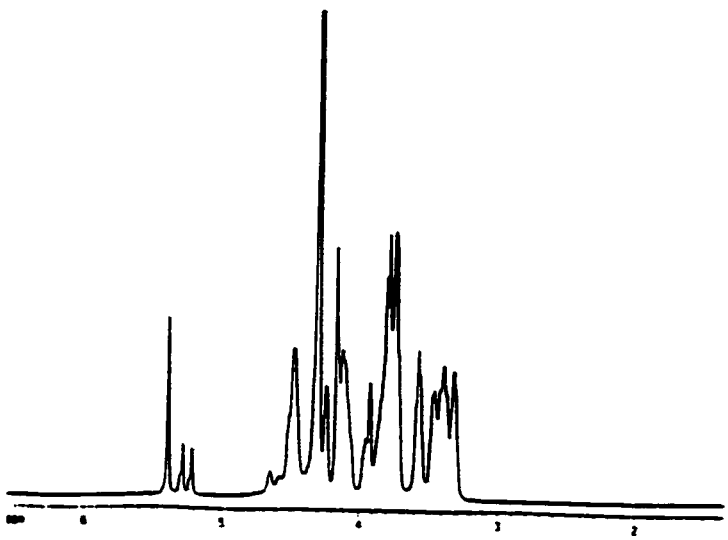


Figure 4.14 Molecular weight distributions of purified water-extractable arabinoxylan isolated from high (R95), intermediate (R30), and low (R5) extract viscosity rye wholemeals as determined by gel filtration chromatography using Sephacryl S-500. Rye lines are designated as R (rye) followed by the extract viscosity (in cp) of the wholemeal. Dextran standard MWs (approximate): 1=$4x10^6$, 2=$575x10^3$, 3=$300x10^3$, 4=$126x10^3$, 5=$20x10^3$, 6=$10x10^3$.

Figure 4.15 Proton nuclear magnetic resonance (H-NMR) spectra of purified WEAX from high (R95; top), intermediate (R30; middle), and low (R5; bottom) extract viscosity rye wholemeals.







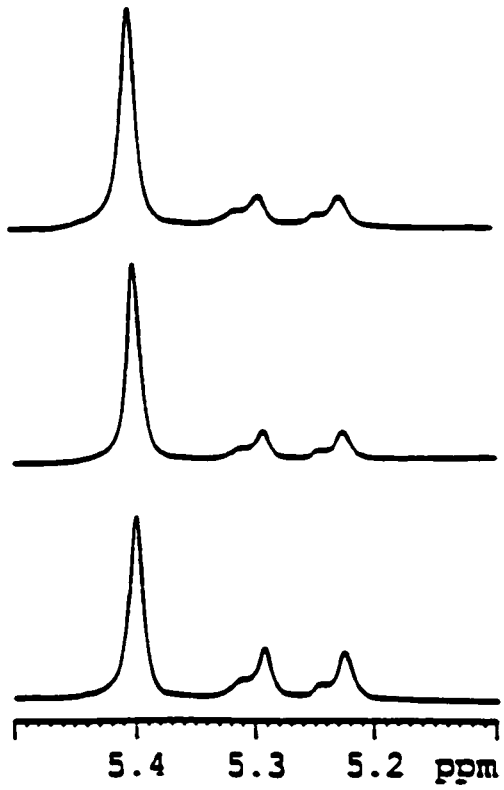


Figure 4.16 **Arabinose anomeric proton regions of the proton nuclear magnetic resonance (H-NMR) spectra of purified WEAX from high (R95; top), intermediate (R30; middle), and low (R5; bottom) extract viscosity rye wholemeals.**

to O-3 of xylopyranosyl (Xylp) residues of the WEAX (Bengtsson et al 1992; Hoffmann et al 1992; Cleemput et al 1993; Vinkx et al 1993; Roels et al 1999). The remaining two peaks (δ 5.30 and δ 5.23 ppm) represent the anomeric protons of Araf linked to O-2 and O-3 of the same Xylp residue (Bengtsson et al 1992; Hoffmann et al 1992; Cleemput et al 1993; Vinkx et al 1993; Roels et al 1999).

The proportions of un-, mono-, and disubstituted Xylp residues in the three WEAX were calculated by quantitative integration of the anomeric proton peaks of the individual Araf residues (Westerlund et al 1990; Cleemput et al 1993) on the assumption that all arabinose residues were terminally linked (as shown by the spectra). Water extractable arabinoxylan from R5 had a higher percentage of disubstituted Xylp residues (28%) compared to WEAX from R30 and R95 (18% and 15%, respectively) (Table 4.11). Water extractable arabinoxylan from R30 had a higher percentage of monosubstituted Xylp residues (59%) compared to WEAX from R5 and R95 (49 and 51%, respectively). The highest percentage of unsubstituted Xylp residues (34%) was obtained for WEAX from R95, compared to 23% and 24% for WEAX from R5 and R30, respectively.

4.11.2 Size Exclusion High Pressure Liquid Chromatography (HPLC) with Triple Detection

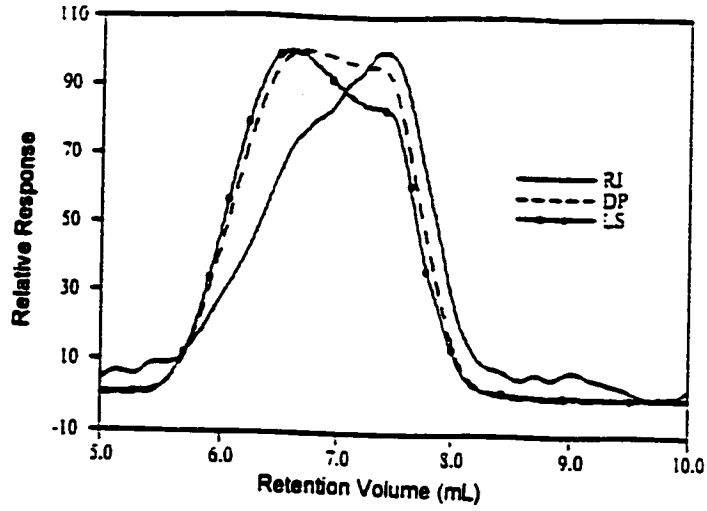
Structural differences among the WEAX from R95, R30, and R5 were further characterized using size exclusion HPLC with refractive index, light-scattering, and differential pressure detection. The outputs obtained from the three detectors for the three WEAX are presented in Figure 4.17.

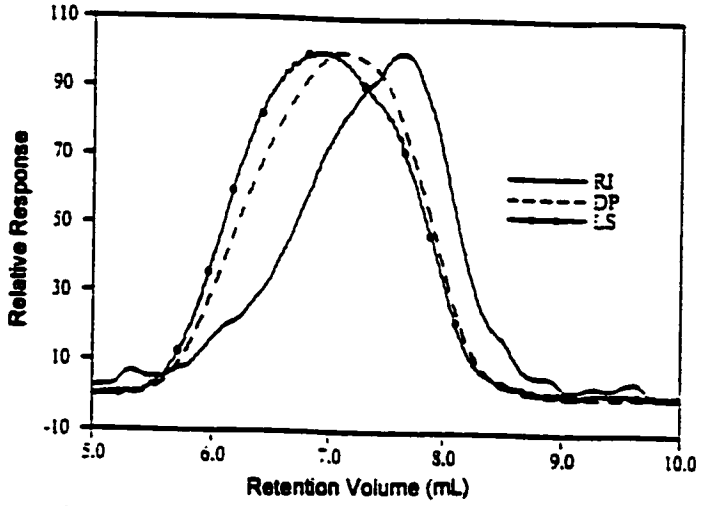
Table 4.11 Fine Structure of Purified Water-Extractable Arabinoxylan Isolated from High (R95), Intermediate (R30), or Low (R5) Extract Viscosity Rye Wholemeals, as Determined by H-NMR and High Pressure Size Exclusion Chromatography/Triple Detection

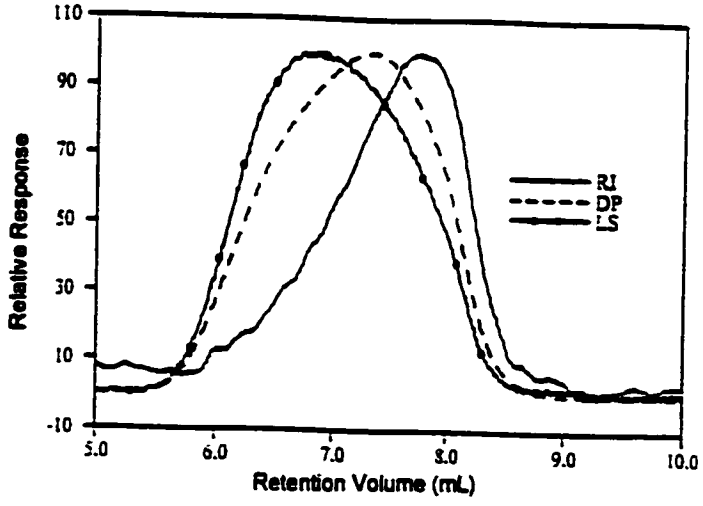
Parameter	R95[1]	R30	R5
Unsubstituted Xylp (%)	34	24	23
Monosubstituted Xylp (%)	51	59	49
Disubstituted Xylp (%)	15	18	28
$Xylp_{disub}/Xylp_{monosub}$[2]	0.29	0.31	0.57
MW[3]	494,950 ± 23971[4]	280,400 ± 18809	199,050 ± 5162
Rg (nm)[5]	54.5 ± 1.2	37.1 ± 1.2	29.1 ± 0.5
Rh (nm)[6]	41.9 ± 0.9	28.5 ± 1.0	22.4 ± 0.4
IV (dL/g)[7]	10.2 ± 0.2	6.2 ± 0.3	4.3 ± 0.2
'a' value[8]	0.57 ± 0.0	0.72 ± 0.0	0.67 ± 0.0

[1] each line is designated as R (rye) followed by its wholemeal extract viscosity (in cp).
[2] ratio of disubstituted xylose residues to monosubstituted xylose residues.
[3] weighted average molecular weight.
[4] mean ± standard deviation (n = 2).
[5] weighted average radius of gyration.
[6] weighted average hydrodynamic radius.
[7] intrinsic viscosity.
[8] slope of Mark-Houwink plot.

Figure 4.17 Triple detector chromatograms of purified WEAX from high (R95; top), intermediate (R30; middle) or low (R5; bottom) extract viscosity rye wholemeal, as determined by high performance size exclusion chromatography/triple detection. RI = refractive index detector, DP = differential pressure detector, LS = light scattering detector.


110
90
70
50
30
10
-10
Relative Response
5.0
6.0
7.0
8.0
9.0
10.0
Retention Volume (mL)
RI
DP
LS


110
90
70
50
30
10
-10
Relative Response
5.0
6.0
7.0
8.0
9.0
10.0
Retention Volume (mL)
RI
DP
LS


110
90
70
50
30
10
-10
Relative Response
5.0
6.0
7.0
8.0
9.0
10.0
Retention Volume (mL)
RI
DP
LS

The MWs of the three WEAX, as determined by the light scattering detector, were approximately 495,000, 280,000, and 199,000 for R95, R30 and R5, respectively. Significant differences in the radius of gyration (Rg) and the hydrodynamic radius (Rh) among the three WEAX were observed (Table 4.11), with both Rg and Rh being proportional to extract viscosity.

The intrinsic viscosities of the WEAX from R95, R30 and R5 were 10.2, 6.2 and 4.3 dL/g respectively (Table 4.11). Mark-Houwink plots were obtained by plotting the intrinsic viscosity values of the WEAX against their MWs (Figure 4.18). The WEAX from R95 exhibited an 'a' value of 0.57, which was lower than the 'a' values obtained for WEAX from R30 (0.72) and R5 (0.67) (Table 4.11).

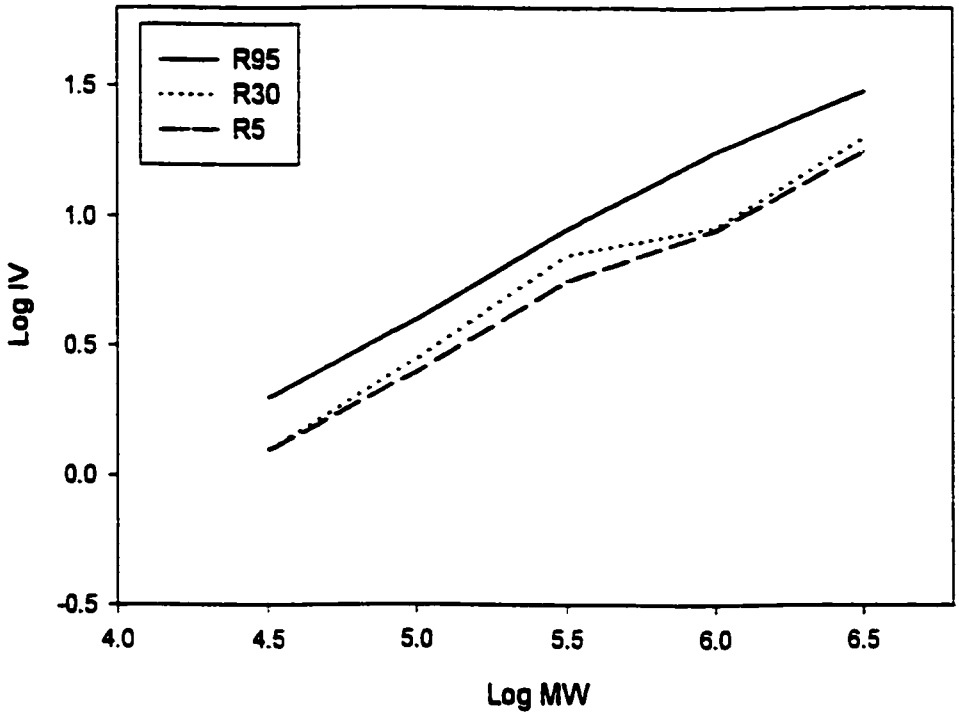


Figure 4.18 Mark-Houwink plots of purified WEAX from high (R95), intermediate (R30) or low (R5) extract viscosity rye, as determined by high performance size exclusion chromatography with triple/detection. IV = intrinsic viscosity, MW = molecular weight.

5. DISCUSSION

5.1 Rye Composition and Extract Viscosity

The significant positive correlation obtained between the extract viscosities of rye wholemeals and their WEAX contents (Section 4.1) supports the view that WEAX concentration is a primary determinant of extract viscosity in rye (Bengtsson and Åman 1990; Boros et al 1993; Vinkx et al 1993). The high extract viscosities of rye wholemeals compared to those of triticale and wheat were consistent with their comparatively high contents of SDF and WEAX (Table 4.1). The relatively high concentrations of SDF, TAX, WEAX and β-glucan detected in rye wholemeals (Table 4.1), compared to those in wheat and triticale, are in keeping with the results of others (Simmonds and Campbell 1976; Saini and Henry 1989; Voragen et al 1992). Åman et al (1997) reported that rye and wheat, respectively, contained 15.9% and 11.1% of TDF, 9.1% and 6.0% of TAX and 1.8% and 0.8% of β-glucan. No relationship was observed between extract viscosity and the A/X ratio in water extracts (Table 4.2). Despite exhibiting a relatively low extract viscosity, comparable to that of wheat, triticale WEAX exhibited the highest A/X ratio, i.e. the highest degree of branching. Saini and Henry (1989) also reported a higher degree of branching in triticale WEAX than in wheat WEAX.

The strong positive correlation observed between the extract viscosity of rye

wholemeal and the %HMWF in the extract (Section 4.4) suggested that differences among ryes in extract viscosity were also related to the MW of the WEAX. These results were confirmed later by the determination that WEAX isolated from ryes differing in extract viscosity indeed differed in MW (Section 4.11). Similar results have been reported by others (Girhammar and Nair 1992b; Scoles et al 1993; Vinkx et al 1993). The major contribution of the HMWF to rye extract viscosity was confirmed by observing the effect of various enzyme treatments on the viscosity and MW profile of wholemeal extracts. Treatment of the extract from R95 with either α-amylase or lichenase indicated that the contribution of starch or β-glucan to the viscosity of the extract was very small in comparison to that of WEAX (Figure 4.7). The slight reduction in extract viscosity resulting from the addition of protease may have been due to hydrolysis of peptide bridges between WEAX and other constituents. Similar results were obtained by Ebringerovà et al (1994), who observed a decrease in average MW of the WEAX-protein complex isolated from rye bran after incubation with protease. The marked reduction in the viscosity of the extract after treatment with xylanase confirmed that the viscosity of the extract from R95 was caused principally by WEAX. Similar results have been reported by others (GrootWassink et al 1989; Bedford et al 1991; Girhammar and Nair 1992b; Boros et al 1993; Vinkx et al 1993). Xylanase treatment (Figure 4.8) of the extract from R5 wholemeal indicated that most of the HMWF consisted of WEAX.

5.2 Rye Milling

Use of a tempering moisture of 10.5% (Table 4.4) did not appear to toughen the bran of rye sufficiently to result in a good separation of bran and flour, which resulted in a high

level of contamination of the flour fraction with bran as indicated by the high flour yield and the relatively low loaf volume obtained from this flour. The use of a tempering moisture of 12.5% apparently toughened the bran and consequently resulted in a better separation of bran and flour. The low flour yield obtained at a tempering moisture of 14.0% may have been due to the hygroscopic arabinoxylan causing the flour to agglomerate, which would interfere with sifting (Rozsa 1976). Härkönen et al (1997) also observed a low flour yield (50%) when rye was milled at 14.0% moisture.

5.3 Distribution of WEAX in Rye Milling Fractions

The distribution of WEAX in the roller-milled fractions (flour, shorts, bran) was determined by measuring the viscosities of water extracts of the fractions obtained at different tempering levels (Table 4.4). The results indicated that WEAX was concentrated in flour, due to its occurrence in the cell walls of the starchy endosperm (Fincher and Stone 1986). This interpretation is supported by the results of Härkönen et al (1997). The significant effect of a higher tempering moisture (14.0%) on the extract viscosity of the shorts fraction was presumably a reflection of the higher proportion of flour in this fraction, although this was not reflected in its yield.

Microscopic examination revealed that the R95 kernel had somewhat larger aleurone cells and thicker endosperm cell walls than did the R5 kernel (Section 4.2). It is tempting but probably unrealistic to correlate this observation with the higher extract viscosity and WEAX content of R95 (Table 4.1), given the rather small differences in WEAX content (in absolute terms) between R95 and R5.

5.4 Contribution of WEAX to the Physicochemical Properties of Rye Flours and Doughs

The extract viscosities of wholemeals and flours from ryes (Figure 4.9) reflected differences in the levels of WEAX in flour and wholemeal (Bengtsson et al 1992b). The low extract viscosities of wheat and triticale wholemeals and flours were consistent with their low WEAX contents and low %HMWFs. Viscosity is an important rheological property of dough that contributes to mixing resistance; the resistance of dough to mixing during successive stages of its development may be determined using the farinograph (Tipples et al 1982). Farinograms typical of weak flours were observed for all rye flours and triticale flour (Figure 4.10), in that they all absorbed water and formed dough rapidly in comparison to wheat flour. According to Tipples et al (1982), the shape of the farinograph curve provides a fair indication of the inherent strength of a flour. A weak flour gives a curve indicating a rapid build up in dough consistency to a sharply defined peak, followed by a rapid breakdown, which was the case with rye and triticale flours in the current study. In contrast, strong flours are usually associated with a much slower build up in consistency, a relatively longer time to achieve peak consistency, a less sharply defined maximum and a much more gradual drop in consistency following peak development, which was the case with wheat flour in the current study.

The high water binding capacity of WEAX (Grahammer and Nair 1992b) in the rye flours, as indicated by their high farinograph water absorptions (Table 4.5), resulted in sticky, weak doughs exhibiting short development times. The comparatively low WEAX content of triticale flour compared to wheat flour and the rye flours was reflected in its low water

absorption and very short dough development time. The positive correlation observed between the extract viscosities of the rye flours and stability (Table 4.5), and the negative correlation between extract viscosity and mixing tolerance index (Table 4.5), revealed the positive effect of extract viscosity (i.e WEAX content) of rye flour on dough strength.

Blending rye or triticale flours with wheat flour at a ratio of 30% rye or triticale:70% wheat resulted in similar farinograph properties for all blends despite their differences in extract viscosity (Section 4.7.2). Obviously, the gluten strength of the wheat flour component in the blend was sufficient to mask the effect of differences in extract viscosity on dough characteristics. However, the large increase in arrival time for all blends compared to either wheat or rye flour (Table 4.5) did indicate that the presence of rye flour in the system reduced its dough forming ability and increased the time required to form a consistent dough.

Falling number is an indirect measure of α-amylase activity, in which a lower number (i.e. lower viscosity) is generally assumed to reflect higher α-amylase activity (Matz 1991). Weipert (1997) stated that the arabinoxylan in rye may reduce the amount of starch damage incurred during milling and, therefore, may protect the starch from degradation by α-amylase to some extent. The extract viscosities of the rye flours were reflected in their falling numbers (Table 4.6), as ryes exhibiting higher extract viscosities exhibited higher falling numbers, both in the presence and absence of enzyme inhibitor. The relatively large increases in falling number observed for the low extract viscosity rye (R5) and the commercial rye sample with the inclusion of enzyme inhibitor reflected either higher α-amylase levels or xylanase levels, or both in these samples or higher levels of damaged starch as described by Weipert (1997), or both. The small increase in falling number observed for wheat flour indicated a low level

of α-amylase activity in the wheat sample.

5.5 WEAX and Rye Baking Quality

Blending either R10 or R95 flour with wheat flour (up to 70% rye flour) affected the resulting loaf volumes in similar fashion (Section 4.8.1). The reduction in loaf volume in each case (Figures 4.12 and 4.13) would be expected to result from both dilution of the wheat gluten by the rye flour and the increase in the level of WEAX in the blend. The somewhat lower reduction in loaf volume caused by R10 flour compared to R95 flour suggested a direct negative effect of WEAX content on loaf volume. However, considering the marked difference in extract viscosity between R10 flour (20 cp) and R95 flour (199 cp) (Figure 4.9), it was concluded that extract viscosity (i.e. WEAX content) would be a minor consideration in the selection of rye grain for its baking performance in rye/wheat blends.

Biliaderis et al. (1995) demonstrated that the impact of WEAX derived from wheat on loaf volume was determined by both the concentration and the molecular weight of WEAX in the dough system; a linear increase in loaf volume was associated with an increase in WEAX concentration up to an optimum concentration, beyond which loaf volume again decreased due to viscosity build-up in the dough. The optimum concentrations of WEAX were determined to be 0.7 and 0.5% (w/w, WEAX:flour) for low (135,000) and high (202,000) molecular weight WEAX, respectively. The differences among the experimental rye lines in WEAX content (Table 4.1) and molecular weight (Table 4.11) would explain the negative correlation obtained between extract viscosity and loaf volume (Table 4.7) of bread baked from their flours when blended with wheat flour. Tao and Pomeranz (1967) reported

that WEAXs in flours varied widely in their effects on breadmaking quality. Shogren et al (1987) reported that WEAX in hard wheat had a small negative effect on loaf volume and that TAX had an additional negative effect. Courtin and Delcour (1998) reported no correlation between extract viscosity of flour and loaf volume of wheat bread baked from flours fortified with up to 3% of wheat WEAX. Cawley (1964) and Rouau et al (1994), however, attributed the superior baking quality of bread made from reconstituted flour (gluten, starch and arabinoxylan) to high viscosity. These apparently conflicting results could be due to differences in the source, degree of purity, composition or MW of the arabinoxylan or pentosan preparation, the level of supplementation or the baking process employed (Hoseney 1984; Lineback and Rasper 1988).

5.6 WEAX and the Nutritional Value of Rye

The impact of WEAX on the nutritional value of rye was evident in the chick feeding experiment (Section 4.9), where the poorest body weight gains and feed conversions were associated with the high extract viscosity rye diet (Table 4.8). This confirms previous reports that high digesta viscosity in chicks fed diets containing rye seriously impeded growth and feed use efficiency (GrootWassink et al 1989; Friesen et al 1991; Bedford and Classen 1992; Campbell and Bedford 1992; McLeod et al 1996). These results are consistent with an earlier observation that viscosity reduction achieved with arabinoxylan depolymerization (xylanase supplementation) or water extraction improved the feeding value of rye grain (Campbell and Bedford 1992).

Partial hydrolysis of the arabinoxylan during the breadmaking process was evident,

given the lower extract viscosities of the bread diets compared to the wholemeal diets (Table 4.8), and the significantly lower digesta viscosities of chicks consuming bread compared to those fed wholemeal (Table 4.8). This would indicate a change (i.e. reduction) in the molecular weight of WEAX during breadmaking. Cleemput et al (1997) reported changes in the molecular weight of WEAX during fermentation, which were attributed, in part, to enzymatic hydrolysis. Yeh et al (1980) reported the loss of a high molecular weight WEAX fraction after mixing of hard red wheat dough. The results of the current study indicate that a portion of the WEAX in rye survived the breadmaking process and, hence, while detrimental in animal feeding, might have beneficial effects on human health when incorporated into bread or related products.

5.7 Isolation of WEAX from Three Experimental Rye Wholemeals

Prior to isolation of WEAX, the effect of dry heating of the wholemeal on extract viscosity was examined (Section 4.3). The higher viscosity of water extracts obtained from both heated and unheated wholemeals after 60 min of extraction compared to 30 min was apparently due to the extraction of more WEAX at the longer extraction time. The reduction in viscosity of the extract from unheated wholemeal after 90 min of extraction was attributed to the activity of endogenous enzymes, principally xylanase. Similar results were reported by Boros et al (1993). Pawlik et al (1990) reported a reduction in the viscosity of extracts from unheated rye wholemeal of ~15% over a 180 min incubation period (at 25°C). The stability of the viscosity of the extract from heated wholemeal at extraction times of 60 min and longer confirmed the effectiveness of the heat treatment in inactivating endogenous enzymes and the

completeness of WEAX extraction at 60 min, and indicated that the degree of starch dextrinization did not increase substantially over the heating period. That little starch was dextrinized by the heating process was confirmed by the insignificant reduction in extract viscosity which resulted from the incubation of a water extract from rye with α-amylase (Figure 4.7).

Knowledge of the chemical composition of the freeze-dried water extracts (Table 4.9) assisted in the development of a protocol for the isolation of WEAX from rye. The presence of significant levels of low molecular weight sugars in water extracts was in agreement with results reported by others (Henry and Saini 1989; Bengtsson and Åman 1990), and necessitated the use of a dialysis step to eliminate these naturally occurring low molecular weight monosaccharides and others generated during amylase and lichenase treatments. The significant amount of protein in the water extracts was also in agreement with the results of others (Fengler and Marquardt 1988a,b; Courtin and Delcour 1998; Delcour et al 1999) and required the use of an adsorptive clay treatment to eliminate this protein.

The procedure employed for the isolation of WEAX from rye was actually a combination of several existing procedures. The α-amylase/amyloglucosidase treatment was employed by Loosveld et al (1997). Delcour et al (1999) applied α-amylase treatment followed by clay treatment. Precipitation of WEAX with aqueous ethanol (65%, v/v, ethanol/extract), leaving arabinogalactan in the supernatant, was employed by Cleemput et al (1993).

Analysis of the WEAX isolated from R95, R30 and R5 wholemeals (Table 4.10) confirmed the high purity of the isolates. The residual protein in the WEAX may have resulted

from protein covalently attached to WEAX structure (Ebringerovà et al 1994). Despite the similarity in the degree of purification of the three WEAXs, there were significant differences in the viscosities of 1% aqueous solutions of the three WEAXs, suggesting differences in their molecular structures.

5.8 Extract Viscosity and Its Relationship to the Structure of WEAX

Information on the degree of branching was obtained using H-NMR (Section 4.11.1). The results (Figure 4.16 and Table 4.11) confirmed that the high viscosity of WEAX from R95 was due, in part, to its low degree of di- and monosubstituted Xylp residues and its high degree of unsubstituted Xylp residues, resulting in a less compact structure compared to WEAX from R30 and R5. According to Wyatt (1993), a high degree of disubstitution of a polymer will cause its hydrodynamic volume to be small and its density to be high, which would be expected to generate low viscosity in aqueous solution, which was the case for WEAX from R5 or R30. Courtin and Delcour (1998) similarly reported that high molecular weight WEAX isolated from wheat flour, which exhibited high viscosity, was low in disubstituted and high in mono- and unsubstituted Xylp residues, compared to a lower molecular weight WEAX purified from a wheat arabinoxylan concentrate. Izydorczyk and Biliaderis (1993) obtained (from wheat) a high molecular weight, high viscosity WEAX fraction exhibiting a low degree of disubstituted Xylp residues and a high degree of monosubstituted Xylp residues.

Despite differences in viscosity and in the degree of mono- and disubstitution, the purified WEAXs from R5 and R95 exhibited similar A/X ratios. This could be related to

differences in the level of a proposed arabinan (Ebringerovà et al 1994) or to the shorter chain length (i.e. low MW) of the more highly branched WEAX from R5. Similar A/X ratios were observed for low and high viscosity WEAX isolated from rye bran (Ebringerovà et al 1994), despite higher disubstitution in the low viscosity fraction. Izydorczyk and Biliaderis (1995) reported that the proportion of disubstituted residues was not related to the A/X ratio, and varied considerably among various WEAXs.

Bengtsson et al (1992a) proposed a model for rye WEAX (Figure 2.3) having two regions within the WEAX structure. The major region (AX I) had a xylan chain with arabinosyl substituted exclusively at O-3 of Xylp, whereas the minor region (AX II) contained disubstituted O-2,3 Xylp residues. In this respect, the structures of the WEAX in the current study may reflect different combinations of AXs I and II. The higher viscosity WEAX from R95 may have contained a higher proportion of the type I AX, whereas the lower viscosity WEAX (from R30 and R5) may have contained more of the disubstituted type II AX.

The MWs determined by light scattering (Section 4.11.2) were substantially lower (Figure 4.17 and Table 4.11) than corresponding values obtained by gel filtration chromatography (Figure 4.14 and Table 4.10). According to Izydorczyk and Biliaderis (1995), the flexibility of the less-substituted WEAX in high viscosity polymers would permit intermolecular alignment over sequences of continuously unsubstituted Xylp residues, thereby increasing their apparent MWs. The non-availability of standards similar in structure to the WEAX tested would lead to overestimation of MW by gel filtration, and would explain the lower MWs determined for the WEAX by light scattering. Gruppen et al (1992) reported a weighted average molecular weight of 850,000 for alkali-extractable wheat arabinoxylan, as

determined by light scattering. Meuser et al (1986), using gel filtration chromatography, reported weighted average MWs of 824,000 for WEAX from rye wholemeal, and 1,170,000 for WEAX from rye bran. Fincher and Stone (1986) reported a MW of 70,000-1,000,000 for WEAX from wheat as determined by gel filtration chromatography, which was much higher than that obtained by sedimentation (65,000-66,000) (Andrewartha et al 1979; Girhammar et al 1986). The extremely high molecular weights for barley endosperm arabinoxylan (up to 5,000,000) as determined by gel filtration chromatography emphasize the difficulties caused by molecular asymmetry in using this method of MW determination (MacGregor and Fincher 1993).

The differences observed among the three WEAXs in MW, Rg and Rh (Table 4.11) were consistent with their viscosity measurements. Rg is said to be influenced by the conformation of the macromolecule and dependent upon its internal mass distribution, whereas Rh reflects the end-to-end size of the molecule in solution (Wyatt 1993). The higher Rh value obtained for WEAX from R95 supports the interpretation that this polymer is able to occupy a larger space in aqueous solution as a consequence of being a less dense molecule, resulting in the ability to exert higher viscosity. Conversely, the smaller Rg and Rh of WEAX from R30 or R5, in addition to their lower MWs, confirmed their compactness and, hence, the lower viscosities of solutions of these polymers. The significant differences observed in the intrinsic viscosities (Table 4.11) of the three WEAXs provide further support for the conclusion that the relatively high viscosity of WEAX from R95 was related to its higher molecular weight, larger Rg and Rh and lower degree of branching.

Structural differences among the WEAXs were detected from the slopes of the Mark-

Houwink plots (Figure 4.18 and Table 4.11). A high slope ('a' value), as obtained for WEAX from R5 or R30, is considered characteristic of a polymer chain with restricted flexibility (Ebringerovà et al 1994; Izydorczyk and Biliaderis 1995). In contrast, a low 'a' value, as obtained for WEAX from R95, is characteristic of an unperturbed coiled configuration (Ebringerovà et al 1994; Izydorczyk and Biliaderis 1995). These results confirmed again that WEAX from R95 was a long chain, extended macromolecule with a relatively low degree of branching.

5.9 Structure of WEAX and Its Relationship to End-Use Characteristics

The structural characterization of the WEAXs isolated from the three rye wholemeals allows for some explanation of the behaviour of flours from these ryes during breadmaking. The molecular structure of WEAX from R95 is best described as an extended, long chain polymer with a large Rg and Rh and a high degree of unsubstituted Xylp residues. These structural features would facilitate hydrogen bonding, in the dough system, between gluten from wheat flour and the unsubstituted region of the WEAX. Hydrogen bonding would enhance the strength of the dough as indicated by the relatively high farinograph stability of the R95/wheat blend (Table 4.5). The relatively low loaf volume obtained with the R95/wheat blend suggests, however, that hydrogen bonding between arabinoxylan and gluten was disrupted during baking, which resulted in a loss of gas-retentive strength and, consequently, reduced loaf volume. More extensive dehydration of gluten by the higher concentration and higher molecular weight of arabinoxylan in R95 flour would also contribute to the inferior loaf volume obtained with the R95/wheat blend. Conversely, the lower concentration of WEAX

in flour from low (R5) or intermediate (R30) extract viscosity ryes along with its comparatively high degree of di- and monosubstitution of Xylp residues, as well as its lower molecular weight Rg and Rh, would hydrogen bond less effectively with gluten in dough (reducing dough strength) and would compete, to a lesser extent, with gluten for moisture (which would enhance loaf volume). Udy (1957) reported that gluten proteins in wheat interacted with water-soluble polysaccharides (pentosans) through hydrogen bonds. The degree of interaction was found to depend on the molecular size (degree of polymerization), branching and shape of the pentosans. These bonds appear to contribute to the net strength of the dough structure at pre-oven temperatures, such that physical dough tests such as farinography fail to reveal its inherent weakness.

6. SUMMARY AND CONCLUSIONS

The availability of rye lines exhibiting a wide range of extract viscosities (5-95 cp) afforded the opportunity to investigate the physical and chemical properties of these ryes. Accordingly, a series of experiments on the composition, physiochemical properties, breadmaking properties and nutritional value for chicks of these rye lines was undertaken. Studies included the isolation of WEAX from three lines representing low, intermediate and high extract viscosity, and the investigation of the structural characteristics of the WEAX.

Rye wholemeals contained significantly higher concentrations of TDF, SDF, TAX, WEAX, and β-glucan than did wheat or triticale. A significant positive correlation was obtained between the extract viscosities of rye wholemeals and their SDF and WEAX contents. Gel filtration chromatography of water extracts of rye revealed the presence of a high molecular weight fraction (HMWF), which was found in higher proportion in the ryes than in wheat or triticale. A significant positive correlation was also observed between the proportion of the HMWF and extract viscosity of rye wholemeal. Treatment of a water extract of rye with xylanase, followed by gel filtration chromatography, indicated that the HMWF consisted primarily of arabinoxylan. Successive treatment of a water extract of rye with α-amylase, lichenase, protease and xylanase confirmed that the viscosity of the extract was primarily related to its content of WEAX. Microscopic examination revealed that kernels

of high extract viscosity rye (R95) had somewhat larger aleurone cells and thicker endosperm cell walls than did kernels of low extract viscosity rye (R5).

A chick feeding experiment demonstrated that the inclusion of rye, particularly high extract viscosity rye, in the diet seriously impeded growth performance and feed efficiency, and that part of the WEAX survived breadmaking and exerted its effect in chicks. Despite the relatively small reductions in the extract viscosities of diets containing breads compared to those containing wholemeals, substantially lower digesta viscosities were observed in chicks fed bread diets. However, the digesta viscosity of chicks fed the high extract viscosity rye bread diet was still twenty times that of chicks fed a wheat bread diet.

Extract viscosities of rye flours were higher than those of corresponding wholemeals, indicating shifting of WEAX into flour during roller milling. Falling numbers of flours in the presence and absence of enzyme inhibitor correlated positively with their extract viscosities. High amylase activity or xylanase activity, or both in flours from low extract viscosity rye (R5) and commercial rye was indicated by the significant increase in their falling numbers in the presence of the inhibitor. Farinograms revealed the weakness of rye and triticale flours compared to wheat flour. Extract viscosity of rye flours and rye/wheat blends was positively correlated with dough stability, indicating higher strength for flours from higher extract viscosity ryes; extract viscosity was negatively correlated with loaf volume of rye/wheat bread.

Water-extractable arabinoxylan was isolated from three experimental ryes differing in their extract viscosities (high, R95; intermediate, R30; and low R5 extract viscosity). Contaminating polysaccharides were removed from the crude extract by enzymatic

degradation, followed by dialysis; a clay suspension was effective in absorbing most of the protein present in the extract. Ethanol (65%, v/v, ethanol/extract) precipitation yielded preparations of approximately 90% purity.

Structural analysis using H-NMR indicated that the WEAX from R95 was a less branched macromolecule having a lower degree of disubstituted Xylp residues and a higher degree of unsubstituted Xylp residues as compared to WEAX from R30 or R5. Structural analysis using size exclusion HPLC/triple detection revealed that the WEAX from R95 had a higher molecular weight (MW = 495,000), a larger radius of gyration (Rg = 55 nm) and hydrodynamic radius (Rh = 42 nm) and a higher intrinsic viscosity (η = 10 dL/g) compared to the WEAX from R30 or R5. Corresponding values for R30 and R5, respectively, were 280,000 and 199,000 for MW, 37 and 29 nm for Rg, 29 and 22 nm for Rh and 6 and 4 dL/g for intrinsic viscosity.

In conclusion, the present study revealed that both the concentration and the molecular weight of WEAX in rye impacted its extract viscosity. Water-extractable arabinoxylan from ryes differing in extract viscosity also exhibited structural differences, in that higher viscosity was associated with a higher molecular weight, a larger radius of gyration and hydrodynamic radius and a lower degree of branching. Extract viscosity in rye was negatively correlated with the loaf volume of 30:70 (w/w) rye/wheat flour blends. Extract viscosity was also negatively associated with the nutritional value of rye for chicks. The relatively high digesta viscosity of chicks fed rye bread diets suggests a potential benefit of high extract viscosity rye on human health when consumed as bread or related products. Accordingly, ryes varying in extract viscosity may have different applications in food, feed,

etc. High extract viscosity ryes would be preferred in fibre-enriched human foods, or its WEAX may be useful in pharmaceutical, nutraceutical or cosmetic products. Low extract viscosity ryes would be preferred in animal feeds.

Further research on the functional properties of WEAX from rye would be useful for determining potential applications of WEAX differing in structure and molecular weight. The identification of commercial uses for rye WEAX would lead to the development of larger-scale processes for the recovery of WEAX from rye and eventually, perhaps, to the establishment of a commercial-scale plant.

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