HGCA

PROJECT REPORT No. 133

STRUCTURE OF ANTI-NUTRITIONAL POLYSACCHARIDES IN WHEAT IN RELATION TO FEEDING VALUE FOR POULTRY

DECEMBER 1996

PRICE £7.00

PROJECT REPORT No. 133

STRUCTURE OF ANTI-NUTRITIONAL POLYSACCHARIDES IN WHEAT IN RELATION TO FEEDING VALUE FOR POULTRY

by

S. AUSTIN AND A. CHESSON

The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB

This is the final report of a three year project which started in July 1992. The work was funded by a grant of £47,562 from the Home-Grown Cereals Authority (Project No. 0039/2/92).

CONTENTS

SUMMARY

A typical European poultry diet contains approximately 60-65% wheat. At such a high inclusion level it has been found that the nutritional value of wheat, measured as its apparent metabolisable energy (AME), can vary substantially depending on variety and growing conditions. The importance of this can be judged from the fact that a difference of just 0.5MJ/kg in the expected AME can have serious financial consequences for a poultry producer. The wheat samples investigated in this study had a similar gross composition but gave AME values ranging from 8.34 to 13.74MJ/kg dry matter when included as 75% of the poultry diet. Water-soluble, non-starch polysaccharides (NSP), particularly arabinoxylans, derived from the grain cell walls, are considered responsible for the observed depression of AME by increasing digesta viscosity. The increase in the viscosity of digesta is thought in turn to reduce the uptake of all major nutrients in the diet. The aim of this work was to establish whether the amount of soluble NSP alone was responsible for AME depression or whether arabinoxylan structure and size were also determinants.

Arabinoxylans and (1-3)(1-4)-β-D-glucans exist in all anatomical regions of the grain. Their concentration was greatest in the bran which contained about 4% mixed-link glucan and 30% arabinoxylan and least in the endosperm which contained only 0.5% mixed-link glucan and 1.5% arabinoxylan. Structure and solubility of the arabinoxylan varied depending on the anatomical region and the wheat sample from which they originated. On a whole grain basis, the endosperm contained about 34% of the total mixed-link glucan and 14% of the total arabinoxylan but contributed about 50% of the soluble mixed-linkage glucans and about 60% of the soluble arabinoxylan. The bran contained most of the total NSP and contributed most of the remaining soluble NSP. The aleurone layer contributed little to the whole grain NSP because of the very small amount by weight of aleurone cells present in the grain.

No relationships were found between AME and the concentration of total NSP, soluble NSP, insoluble NSP or starch indicating that amount of starch or NSP was not a primary factor in AME depression. Correlations were found between AME and the ratio of soluble arabinoxylan to (1-3)(1-4)-β-D-glucan and between AME and the sum of soluble galactose and mannose residues which illustrated the need to consider interactions between polymers. However, no relationship was found between AME and solution viscosity. This is probably

because the extraction procedure used did not sufficiently represent the digestive conditions in the upper parts of the chick digestive tract which lead to the solubilisation of the NSP. This result was contrary to that obtained by others who have observed good correlations between intestinal viscosity and AME.

Before differences in the structure of the soluble arabinoxylan could be determined, samples were first partially hydrolysed with a cloned endo-xylanase, which was free from any other detectable activities. The oligosaccharides produced were separated by HPLC and the molecular weight of the oligomers determined using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF-MS). Detailed structural information was obtained by one-dimensional proton NMR and the two-dimensional experiments total correlation spectroscopy (TOCSY) and rotating frame nuclear Overhauser enhancement spectroscopy (ROESY). Six branched oligomers were fully characterised in this way. Six other oligomers were also produced by the enzyme digestion, two of these were identified as being linear xylan chains, the other four were not produced in sufficient quantity to be fully characterised. The chromatograms from the HPLC were used as fingerprints of the arabinoxylan structures from the different wheat samples. It was found that the structure of the arabinoxylans varied between the different wheat samples. AME was found to be affected by the content of very highly branched arabinoxylan which was directly correlated with an increase in AME. Extensive branching would be expected to interfere with polymer interaction reducing the likelihood of association and a concomitant increase in solution viscosity. No other structural features were found which influenced AME. Solution viscosity was also found to be dependant on the molecular weight distribution of the soluble NSP as well as the degree of branching of the arabinoxylan. This was a complex relationship which showed that polysaccharides with low degrees of branching increased viscosity as their molecular weight increased however arabinoxylans with high degrees of branching had high viscosity at lower molecular weights.

It was concluded that AME and viscosity were determined by a number of factors including arabinoxylan concentration and molecular weight, the degree and distribution of branching within the molecule and by its interactions with other polysaccharides. The response of all the wheat samples examined to the action of a single xylanase demonstrated that the viscosity-enhancing ability of released arabinoxylans could be readily disrupted. However, application of enzyme as a practical solution to the depression of AME is of value only to

some wheat samples. The work reported here provides information on arabinoxylan structure which may be of use in establishing a routine method of identifying feed wheat which would benefit from enzyme addition.

INTRODUCTION

Wheat is a major world resource which, in 1992, accounted for 29% (565.5 MT) of the 1959 MT of cereals produced world wide (Fig 1). In Britain 33% of the wheat produced is used as animal feed, the largest single use for the cereal in this country (Fig 2).

Wheat plays an important role in the feeding of poultry, for example in many European countries a typical broiler diet contains approximately 63% wheat (Holmes 1992). The nutritional value of most wheat varieties has been taken as being fairly constant. As a result feed compounders have used a single value for wheat metabolisable energy (ME) in their least cost formulations. However, work in the last 10 years (Rogel, *et al*, 1987, Choct & Annison, 1990, Wiseman & Inborr, 1990, Annison, 1991) has brought to attention the fact that different wheat samples have different apparent metabolizable energy (AME) values when fed at the higher inclusion rates now common in broiler diets. This is particularly important in the current UK economic climate where a fairly small change in the AME value (as little as 0.5 MJ/kg) can make the difference between profit and loss for poultry producers (Holmes 1992).

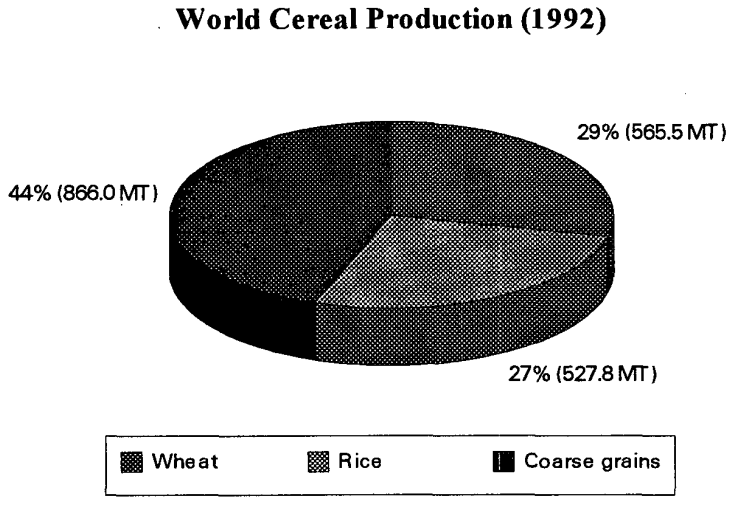


Fig 1: Chart of world cereal production 1992 (figures from *The State of Food & Agriculture*, 1993, FAO of the United Nations)

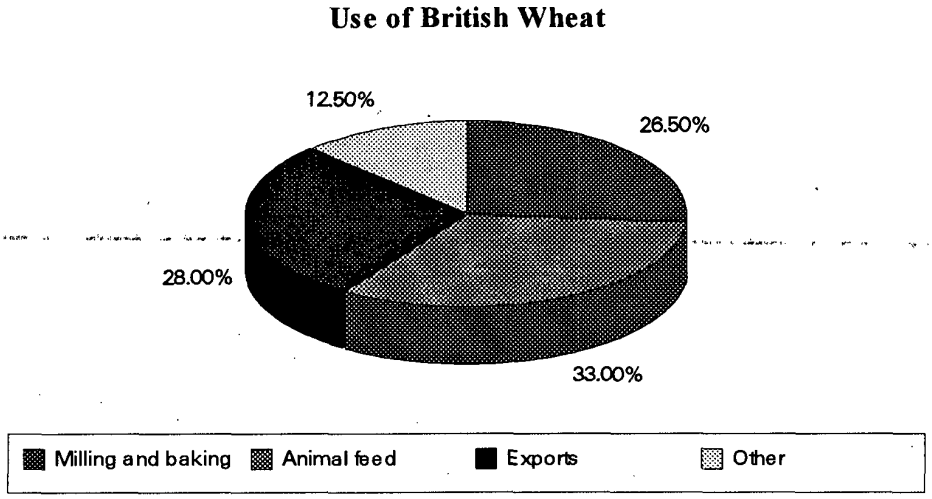


Fig 2: Chart showing use of home-grown wheat in UK (figures from the 1993 HGCA theme review, *Wheat Quality*)

The depression of AME of broiler diets which contain a large proportion of cereal, correlates well with a reduced digestibility of starch (Choct, *et al*, 1995, Annison, 1992, Choct & Annison, 1990, Wiseman *et al*, 1993) but not with the total amount of starch present in the grain (Wiseman & Inborr, 1990, Rogel, *et al*, 1987). It is now fairly well established that lower than expected AME values occur as a result of soluble non-starch polysaccharides (NSP) leached from the grain cell walls increasing the digesta viscosity by aggregating into large networks or mesh-like structures through entanglements of large polymers (Bedford, 1995). Increased viscosity in the gastrointestinal tract reduces the digestibility of dry matter, fat and protein and the absorption of minerals (van der Klis, *et al*, 1995, Choct and Annison, 1992 Fengler & Marquardt, 1988) as well as reducing feed intake and amylase activity (Bedford, 1995, Almirall, *et al*, 1995).

In all grains the soluble NSP is composed primarily of arabinoxylans and mixed-link glucans ((1-3)(1-4)-β-D-glucans), but the ratios differ depending on the cereal. In barley and oats mixed-link glucans predominate while in wheat and rye the arabinoxylans form the greatest part of any released NSP. Several studies (Izydorczyk, *et al*, 1991b, van der Klis *et al*, 1995, Saulnier *et al*, 1995) have shown that the amount of soluble arabinoxylans present in the grain control *in vitro* extract viscosity and/or the viscosity of the digesta. Arabinoxylans with different structural features have different viscosity enhancing properties and gel-forming

abilities. Vinkx *et al* (1993) reported that molecular weight, ferulic acid content and chain stiffness (controlled by degree of branching) all contribute to their physiochemical properties in solution. Izydorczyk & Biliaderis (1995) confirmed that high viscosity polysaccharides had a low degree of branching and high feruloyl content. This was in contrast to the findings of Bengtsson, *et al* (1992) who found that arabinoxylans with a high degree of branching had a strong, positive, non-linear correlation with solution viscosity.

The effect of (1-3)(1-4)-β-D-glucans on barley digestion has been extensively investigated. It is similarly thought to cause an increase in viscosity of the chick digesta and reduce nutrient digestability hence reducing the AME of the grain (Burnett 1966).

The reduction of intestinal viscosity requires that only a few glycosidic bonds are broken to reduce the size of the polymers sufficiently such that they can no longer associate in large entanglements (Bedford 1995). This is achieved in practise by the use of enzyme supplementation. In Europe, about 40% of all diets for broilers and piglets have enzymes added. Studies on broilers fed wheat and rye based diets have shown that the addition of pentosanases and glucanases reduce intestinal viscosity and improves the AME of the feed (Bedford & Classen, 1992, Frigard, *et al*, 1994, Peterson, *et al*, 1991, Choct, *et al*, 1995, van der Klis *et al*, 1995, Brenes, *et al*, 1993, Marquardt, *et al*, 1994, Annison, 1992). Similar results have been found when adding β-glucanases to oat and barley based diets (White, *et al*, 1981, Vranjes & Wenk, 1995, Almirall *et al*, 1995). The increase in AME of the feed depends on the AME value prior to enzyme addition. Choct *et al* (1995) found that wheat samples having low AME could have their energy value increased by up to 24% upon enzyme supplementation but the improvement in normal wheat samples was only about 2%.

In Australia it has been shown that the amount of soluble NSP in the wheat is sufficient information to predict AME (Annison, 1991), but this has not been demonstrated elsewhere. This work was undertaken to establish whether the concentration of soluble NSP alone determined AME in UK wheat or whether structure also had an effect.

OBJECTIVES

The initial objectives of this investigation were to:

- Measure the total soluble and insoluble arabinoxylan and mixed-linked glucan contents of a range of UK wheats selected to show the widest range of metabolisable energy values.
- Measure the average branching distribution and pattern of branching of soluble arabinoxylans.
- Establish the key parameters which relate to nutritional data.
- Use the structural data obtained to optimise endogenous enzyme treatments designed to remove anti-nutritional effects.

Additionally it was found useful to:

- Identify the anatomical source of the anti-nutritional polysaccharides.

The work was used as the basis for a PhD thesis (Austin, 1996), submitted to the University of Nottingham which can be referred to for more detailed information not included in this report.

MATERIALS AND METHODS

Wheat samples and their preparation

Twelve wheat samples were obtained from the University of Nottingham. Most of the wheats were grown at two sites at the university (site 1 and site 2) and harvested in 1992. The exceptions were Galahad and Pastiche which were harvested in 1991 and grown by the National Institute of Agricultural Botany at Headly Hall, near Leeds, and Cambridge respectively. The apparent metabolisable energy (AME) values of the wheat samples were determined at Nottingham University (Table 1) on a dry matter (DM) basis when included as 75% of the broiler diets.

Table 1 - Variety, origin & AME of wheat samples

Sample	Variety	Site	AME ($MJkg^{-1}$ DM)
A	Avalon	1	8.43
B	Haven	1	8.43
C	Hereward	1	9.29
D	Mercia	2	9.43
E	Admiral	1	9.89
F	Hereward	2	10.95
G	Admiral	2	12.00
H	Beaver	1	11.41
I	Avalon	2	11.22
J	Mercia	1	12.63
K	Pastiche	Cambs	13.67
L	Galahad	H. Hall	13.74

Cambs = Cambridge, H. Hall = Headly Hall.

Wheat milling fractions were obtained from the Campden and Chorleywood Food Research Association (CCFRA). Six fractions were obtained from each of two wheat samples, both of the variety Riband from the 1993 UK harvest, one with a high bushel weight the other with a low bushel weight.

Each wheat sample was milled in a Janke & Kunkel A10 analytical mill to produce a fine powder capable of passing through a 500μm sieve. The samples were freeze-dried and stored under vacuum over P_2O_5.

Isolation of non-starch polysaccharides

NSP were isolated in small quantities for quantitative analysis and in larger quantities for molecular weight determination, viscosity experiments and for subsequent hydrolysis to oligosaccharides and structural characterisation. The ground wheat (150mg for the small scale isolation or 5g for the large scale isolation) was weighed out and treated with ethanol (80%) under reflux for 20min. After cooling the mixture was centrifuged (20000*g*, 30min), the ethanol decanted and the residue dried. For small scale isolations the residue was treated with dimethyl sulphoxide (DMSO) at 100°C (total NSP) or 80°C (soluble NSP) for 30 min. Termamyl (300L, Novo Nordisk) solution ($3\%_{(v/v)}$, pH 7, $4cm^3$ for soluble NSP and 1.25%, pH 5.2, $4cm^3$ for total NSP) was added and the mixture extracted at 80°C for 2 hours (soluble) or at 100°C for 10min (total). The samples were equilibrated in a water bath (50°C) for 3 min and an aqueous solution of pancreatin ($10\%_{(w/v)}$, $0.25cm^3$) containing Promozyme (200L, Novo Nordisk, $25\%_{(v/v)}$) was added. The samples were incubated at 50°C for 30min then replaced in the boiling water bath (total) or the 80°C bath (soluble) for 10 min. The samples for determination of soluble NSP were then centrifuged (600g, 10min) and the supernatant was recovered.

For the large scale extraction of soluble NSP, Termamyl (300L, Novo Nordisk) solution ($3\%_{(v/v)}$, pH 7, $50cm^3$) was added and the samples were incubated at 80°C for 1 hour with constant shaking. The mixture was then centrifuged (20000g, 30min), the supernatant collected and the residue treated under the same conditions with a fresh batch of enzyme. The mixture was centrifuged once again and the supernatants were combined. The supernatant was then dialysed (MW cut off 10,000 -12,000 Da) against distilled water for three days.

Ethanol was added to the supernatant (for soluble NSP isolation) or to the mixture left after enzyme treatment (for total NSP) until the concentration was $80\%_{(v/v)}$ ethanol. The samples were cooled in ice for 30 min (small scale) or in the cold room at 4°C overnight (large

scale) to precipitate NSP. The mixture was centrifuged (600g, 5min, small scale, 20,000g, 30min, large scale) and the residue isolated. For small scale isolation the residue was resuspended in ethanol, mixed for 5min and centrifuged (600g, 5min) , the residue isolated and then the same procedure repeated with acetone. After removal of acetone the residue was heated gently with constant stirring until the residue was dry. For large scale isolation the residue was placed under vacuum until dry, it was then resuspended in water and freeze dried.

Carbohydrate Analysis

The carbohydrate content of samples was determined by the method of Dubois *et al* (1956). Total starch was determined by the method of Aman and Hesselman (1984). Neutral sugars were determined as their alditol acetate derivatives by the method of Blakney *et al* (1983). Acidic sugars were determined by the method of Blumenkrantz and Asboe-Hansen (1973). Mixed-linkage glucan was measured according to the procedure of McCleary & Codd (1991) using the mixed-linkage β-glucan and glucose test kits (Megazyme, Australia).

Solution viscosity of wheat extracts

Two approaches o measure the viscosity of solutions of wheat extracts were taken. Firstly, solutions were prepared where the amount of water-soluble material was kept constant to investigate differences in rheological properties of the water-soluble material from different wheat sources. Secondly, samples were prepared where the amount of wheat extracted was kept constant, to determine any correlation with AME.

Constant water-soluble material concentration

Samples of NSP, as prepared earlier, were suspended in water to a desired concentration (0.5mg cm^{-3}) by sonication. The viscosity of the solutions was measured on a Brookfield DVIII rheometer working in LV mode fitted with a CP-40 cone and plate at 30rpm, 25°C.

Constant wheat concentration

Wheat samples (1g) were weighed into screw cap tubes containing magnetic stirring bars. Ethanol (80%, 10cm^3) was added and the tubes heated to 80°C with constant stirring for

15min. After cooling the tubes were centrifuged (350*g*, 5min) and the ethanol decanted. Acetone (5cm^3) was added and left stirring for 5min. The tubes were then gently heated with constant stirring until dry. Sufficient Termamyl solution (3%$_{(v/v)}$, pH 7) was then added to make the wheat concentration 200mg cm^{-3} and the tubes incubated at 80°C for 2h with constant stirring. After cooling the tubes were centrifuged (350 x g, 5min) and the supernatant was filtered into a clean vial through Whatman GF/A filter paper. The viscosity of this liquid was measured on a Brookfield DVIII rheometer working in LV mode fitted with a CP-40 cone and plate at 30rpm, 25°C.

Production of arabinoxylan oligosaccharides

A solution of a cloned xylanase (2%$_{(v/v)}$, 25cm^3, SP628, Novo Nordisk) was added to a tube containing commercially available (Megazyme, Australia) wheat arabinoxylan (1g) and a magnetic stirring bar. The tube was heated at 60°C for 84 hours with constant stirring. After cooling, ethanol (absolute) was added until the total ethanol concentration was 80%. The tube was then cooled in an ice bath for 30min and the precipitate removed by centrifugation (25000*g*, 30min), dried and weighed. The supernatant was transferred to a round bottom flask and dried on a rotary film evaporator. The residue left in the flask was dissolved in water then freeze-dried and the resulting oligosaccharide mixture weighed.

A sample of the oligosaccharide mixture (~500mg) was dissolved in water (3cm^3) and separated on a column of Biorad Biogel P2 (2.5 x 75 cm), eluted with water at 60°C, flow rate 20cm^3 h^{-1}. Fractions (7cm^3) were collected and analysed by the phenol-sulphuric acid method for total carbohydrate. The fractions containing carbohydrate were freeze-dried and weighed. The fractions were analysed by Matrix-assisted laser desorption/ionisation time of flight mass spectrometry (MALDI-TOF-MS) to check for purity and to provide an indication of molecular weight. Fractions found to contain a mixture of oligosaccharides were re-applied to the column and separated under the same conditions. Fractions (5cm^3) were collected and again analysed for carbohydrate. Those indicating the presence of carbohydrate were further analysed using MALDI-TOF-MS and those containing oligosaccharides of the same molecular weight were combined. The purified oligosaccharides were freeze-dried and stored under vacuum over P_2O_5.

Molecular weight determination

Oligosaccharides

A sample of the oligosaccharide was dissolved in water (2mg cm^{-3}) and an aliquot ($0.001cm^3$) was placed on the sample plate and mixed with the matrix solution ($0.001cm^3$, 2,5-dihydroxybenzoic acid (120mg) dissolved in methanol:water (70:30; $10cm^3$)). This was allowed to dry then it was placed into a Finnigan Mat Lasermat MALDI-TOF-MS. The oligosaccharides were vaporised and ionised using the lowest possible laser power which would produce a good mass spectrum.

Polysaccharides

Dextran standards (5mg of each mw = 12,000, 25,000, 50,000, 80,000, 150,000, 270,000 & 410,000Da) were weighed into a vial and dissolved in water ($3cm^3$). An aliquot of this ($1.5cm^3$) was applied to a column of Sephacryl S-400 (1.5 x 112cm) and eluted with sodium azide solution (0.02%) at a flow rate of $18cm^3 h^{-1}$ at 25°C, $5cm^3$ fractions were collected off the column. An aliquot ($0.5cm^3$) of each fraction was analysed for carbohydrate (section 2.9) and a calibration curve was prepared. Samples of soluble NSP (5mg), as prepared earlier, were diluted with water ($3cm^3$) and an aliquot ($1.5cm^3$) was applied to the column and eluted under the same conditions. The molecular weight distribution was calculated from the standard curve.

High performance liquid chromatography (HPLC) separation of arabinoxylan oligosaccharides

Water-soluble extract, as prepared earlier (100mg), was weighed into a screw cap tube containing a magnetic stirring bar. Water ($5cm^3$) and xylanase ($0.15cm^3$, SP628) was added and the tube incubated at 60°C for 84h with constant stirring. After cooling, ethanol ($21cm^3$) was added and the tubes placed in an ice bath for 1h to precipitate polymeric material. The mixture was centrifuged (20000 x g) and the supernatant was transferred to a round bottom flask. The residue was placed under vacuum to dry, weighed and stored in a vacuum desiccator over P_2O_5. The supernatant was dried on a rotary film evaporator, the residue was then redisolved in water and freeze-dried to recover the oligosaccharides which were also weighed and stored in a vacuum desiccator over P_2O_5.

Oligosaccharide mixtures were dissolved in water to a concentration of 200μg cm^{-3} and an aliquot (0.025cm^3) was separated on a HPLC (Dionex) using the conditions below. The oligosaccharides prepared in section 2.9 were also analysed under the same conditions and used to identify the peaks on the chromatograms from wheat samples.

HPLC Conditions

Buffer A: Sodium acetate (1mol dm^{-3}) in sodium hydroxide (0.1mol dm^{-3})

Buffer B: Water

Buffer C: Sodium hydroxide (0.5mol dm^{-3})

Column : Dionex Carbopac PA-1 (4 x 250mm)

Flow Rate : 1cm^3 min^{-1}

Detector: Pulsed amperometric detector: E_1 = +0.05V, E_2 = +0.6V, E_3 = -0.6V

Temperature : 20°C

Gradient:

Time	%A	%B	%C
0	5	75	20
0.1	5	75	20
30.0	29	51	20
30.1	0	50	50
35.0	0	50	50
35.1	5	75	20
40.0	5	75	20

NMR of oligosaccharides

Isolated oligosaccharides were treated with D_2O (99.9 atom%) to deuterium exchange hydroxyl protons and freeze-dried. They were then re-dissolved in D_2O (100%) and the ^1H spectra recorded on a Jeol LA-300 spectrometer at a probe temperature of 27°C. Chemical shifts were measured with reference to external 3-(trimethylsilyl)-1-propane-sulphonic acid.

Total correlation spectroscopy (TOCSY) experiments were recorded using the pulse sequence 90°-t_1-SL-acq where SL stands for a multiple of the MLEV-17 sequence (Bax & Davis, 1985 & Davis & Bax, 1985). The MLEV-17 sequence utilised 90° pulse widths of 36μs and 180° pulse widths of 72μs, total mixing time was 112ms. The spectral width was

1000Hz in both dimensions and 512 experiments of 512 data points were recorded. The data matrices were multiplied in each time domain with a phase shifted sine function (shifted $\pi/3$) prior to phase-sensitive Fourier transformation.

Rotating-frame nuclear Overhauser enhancement spectroscopy (ROESY) was carried out using the pulse sequence $90°$-t_1-SL-acq, where SL stands for the DANTE pulse train (Kessler *et al*, 1987). The DANTE pulse train utilised a 45° pulse width of 59μs with a 118μs pulse interval, total mixing time was 200ms. The spectral width was 3200Hz in both dimensions and 1024 experiments of 1024 data points were recorded. The data matrices were multiplied in each time domain with a phase shifted sine function (shifted $\pi/2$) prior to phase-sensitive Fourier transformation.

RESULTS

Origin of NSP within the grain

Six milling fractions were prepared from each of two wheat samples (Table 2). These were pure endosperm (endo), three fractions enriched in aleurone (aleu 1-3) and two bran fractions, one finished (F bran), which was expected to be less contaminated with starchy endosperm and aleurone than the unfinished (U bran). Each fraction was analysed for total and soluble NSP (Table 3).

Table 2: Contribution by weight of each milling fraction to the whole grain

Anatomical region	Proportion of whole grain (%)	
	High bushel weight	Low bushel weight
Endo	76.5	74.5
Aleu (1)	1.6	2.0
Aleu (2)	0.6	0.6
Aleu (3)	0.4	0.4
U Bran	15.3	18.0
F Bran	8.2	7.5

Approximately 40% of the arabinoxylan present in the endosperm of both wheat samples was soluble (Table 3). The low bushel weight wheat had a higher percentage of soluble arabinoxylan in the three aleurone-enriched samples and the two bran fractions than the wheat with the high bushel weight. In both cases the proportion of arabinoxylan which was soluble decreased as fractions contained more of the outer layers of the grain.

The extent of branching of the arabinoxylans was found to vary depending on source. Soluble arabinoxylans from the endosperm were more highly branched than those which were insoluble. In the bran fractions, however, the soluble arabinoxylans were less branched. The unfinished bran from the high bushel weight wheat was an exception, the soluble arabinoxylan in that case being more highly branched, as in the endosperm. It is difficult to predict if this is an anomaly since only two samples have been analysed. In both wheat samples the arabinose : xylose ratio of both total and soluble arabinoxylan decreased as the anatomical region from which it originated approached the outer layers of the grain

Table 3: Total and soluble arabinoxylan (AX) and mixed-linkage glucan (mlG) content of milling fractions

Anatomical region	AX (g/kg)		mlG (g/kg)		Ara / Xyl ratio	
	Total	Soluble	Total	Soluble	Total	Soluble
			Low bushel weight			
Endo	17.99	7.54	5.61	0.76	0.704	0.771
Aleu (1)	12.11	6.02	5.64	1.22	0.696	0.836
Aleu (2)	52.56	12.78	10.23	1.39	0.647	0.630
Aleu (3)	95.23	15.54	13.03	2.01	0.690	0.601
U Bran	283.05	15.96	38.34	2.54	0.524	0.503
F Bran	323.18	15.95	45.61	2.42	0.520	0.472
			High bushel weight			
Endo	13.95	5.83	5.93	1.01	0.774	0.937
Aleu (1)	15.78	5.04	6.05	0.91	0.756	0.848
Aleu (2)	48.75	8.68	7.73	1.47	0.640	0.775
Aleu (3)	88.02	10.35	14.86	2.79	0.657	0.596
U Bran	267.65	9.73	26.91	2.69	0.569	0.670
F Bran	329.60	10.09	31.59	2.63	0.581	0.567

Ara = anhydrous arabinose (i.e. that found in a polysaccharide), Xyl = anhydrous xylose, Endo = milling fraction containing endosperm, Aleu = milling fraction enriched in aleurone cells (1= closest to endosperm, 3 = closest to bran, 2 = middle), U Bran = milling fraction containing unfinished bran, likely to include endosperm and aleurone cells, F Bran = milling fraction containing finished bran, containing little endosperm or aleurone.

The fractions which contribute most to the total and soluble NSP were calculated from the data in Tables 2 and 3 and summarised in Table 4. It is evident from this data that the largest contribution (55-65%) to soluble arabinoxylan came from the endosperm, despite representing only 13-15% of the total arabinoxylan. The endosperm contributed proportionally more to the total mixed-linkage glucan (28-40%) and contributed 45-55% of the soluble mixed-linkage glucan. The bran fractions made up most of the remaining contributions with the aleurone layer only contributing 2-3% of the total and soluble polysaccharides.

The contribution by the bran fraction to soluble NSP was higher than expected and represented a significant source of anti-nutritional polysaccharide. However arabinoxylan of

bran and endosperm origin had different degree of substitution and would be expected to have different physical properties.

Table 4: Proportion of arabinoxylan (AX) and mixed-linkage glucan (mlG) contributed by each fraction for wheat with high and low bushel weight

Anatomical Region	Proportion of grain mlG (%)		Proportion of grain AX (%)	
	Total	Soluble	Total	Soluble
		Low bushel weight		
Endo	28.4	45.4	15.0	56.5
Aleu (1)	0.8	2.0	0.3	1.2
Aleu (2)	0.4	0.7	0.4	0.8
Aleu (3)	0.4	0.6	0.4	0.6
U Bran	46.9	36.7	56.9	28.9
F Bran	23.2	14.6	27.1	12.0
		High bushel weight		
Endo	39.6	53.8	13.4	64.2
Aleu (1)	0.8	1.0	0.3	1.2
Aleu (2)	0.4	0.6	0.4	0.7
Aleu (3)	0.5	0.8	0.4	0.6
U Bran	36.0	28.7	51.5	21.4
F Bran	22.6	15.0	34.0	11.9

NSP content of wheat samples with varying AME

The total and soluble NSP contents of the 12 wheat samples of known AME were determined (Table 5 & 6). As is evident from the results, large quantities of glucose were detected in soluble as well as total NSP. The (1-3)(1-4)-β-D-glucan was determined enzymatically and only contributed a fraction of the detected glucose. For the total NSP determination where the starch was removed by amylase under optimum conditions the glucose, as would be expected, derived from cellulose. However, in the analysis for soluble NSP, the glucose must have arisen from partially digested starch. This probably occurred because the enzyme used to remove starch was used under sub-optimum conditions and the maltodextrins produced remained sufficiently large to be precipitated with ethanol along with the NSP.

Table 5: Total NSP content of wheat samples

Sample	AME (MJ/kg DM)	Ara (g/kg)	Xyl (g/kg)	Man (g/kg)	Gal (g/kg)	Glu (g/kg)	UA (g/kg)	mlG (g/kg)	Total NSP (g/kg)
A	8.43	16.88 *(0.65)*	29.03 *(0.44)*	2.60 *(0.05)*	4.09 *(0.03)*	31.70 *(0.68)*	3.79 *(0.10)*	5.93 *(0.52)*	88.09 *(1.31)*
B	8.43	24.00 *(0.70)*	48.07 *(0.97)*	2.27 *(0.10)*	4.76 *(1.52)*	34.35 *(0.76)*	4.06 *(0.54)*	6.69 *(0.12)*	117.52 *(3.76)*
C	9.29	26.32 *(0.07)*	47.44 *(0.73)*	1.80 *(0.10)*	3.51 *(0.14)*	40.06 *(3.09)*	3.95 *(0.38)*	6.65 *(0.10)*	123.08 *(2.78)*
D	9.43	18.37 *(0.38)*	25.85 *(4.28)*	1.95 *(0.04)*	3.67 *(0.06)*	31.95 *(0.92)*	3.75 *(0.21)*	5.78 *(0.22)*	85.53 *(3.53)*
E	9.89	12.37 *(1.30)*	26.86 *(1.96)*	1.69 *(0.18)*	3.46 *(0.17)*	39.29 *(1.83)*	4.35 *(0.13)*	6.42 *(0.19)*	88.02 *(3.08)*
F	10.95	24.93 *(2.36)*	45.13 *(4.15)*	1.91 *(0.18)*	4.14 *(0.27)*	37.28 *(2.53)*	3.56 *(0.48)*	7.06 *(0.22)*	116.95 *(9.68)*
G	11.22	12.72 *(0.90)*	24.05 *(1.93)*	2.04 *(0.16)*	3.13 *(0.25)*	36.85 *(3.03)*	3.82 *(0.15)*	5.98 *(0.15)*	82.61 *(5.69)*
H	11.41	27.10 *(3.14)*	53.92 *(4.43)*	1.99 *(0.25)*	3.26 *(0.68)*	38.95 *(3.83)*	3.99 *(0.29)*	7.17 *(0.04)*	129.22 *(12.06)*
I	12.00	18.82 *(1.00)*	33.56 *(1.99)*	2.23 *(0.09)*	3.93 *(0.12)*	36.17 *(2.67)*	3.89 *(0.13)*	5.75 *(0.08)*	98.60 *(5.60)*
J	12.63	17.35 *(0.99)*	28.43 *(1.70)*	2.06 *(0.04)*	3.27 *(0.05)*	32.98 *(1.69)*	3.49 *(0.20)*	5.63 *(0.32)*	87.58 *(3.24)*
K	13.67	22.95 *(0.73)*	43.52 *(1.36)*	2.39 *(0.10)*	3.65 *(0.38)*	34.65 *(1.09)*	3.63 *(0.14)*	6.52 *(0.11)*	110.79 *(3.44)*
L	13.74	26.45 *(0.26)*	47.68 *(0.94)*	2.19 *(0.04)*	4.03 *(0.25)*	39.05 *(1.36)*	4.07 *(0.05)*	7.22 *(0.39)*	123.48 *(2.24)*

Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glu = glucose, UA = uronic acids mlG = mixed linkage (1-3)(1-4)-β-D-glucan (all values quoted as anhydrous sugars). Standard deviations shown in parenthesis.

Table 6: Soluble NSP content of wheat samples

Wheat Sample	AME (MJ/kg DM)	Ara (g/kg)	Xyl (g/kg)	Man (g/kg)	Gal (g/kg)	Glu (g/kg)	UA (g/kg)	mlG (g/kg)	Soluble NSP* (g/kg)
A	8.43	3.54 *(0.28)*	4.63 *(0.53)*	0.40 *(0.57)*	3.32 *(0.35)*	28.63 *(4.47)*	4.22 *(0.53)*	1.67 *(0.19)*	17.78 *(1.19)*
B	8.43	5.92 *(0.38)*	10.33 *(0.96)*	1.05 *(0.05)*	1.79 *(0.11)*	41.11 *(3.94)*	2.27 *(0.08)*	2.08 *(0.13)*	23.45 *(1.39)*
C	9.29	4.35 *(0.18)*	7.71 *(0.16)*	0.45 *(0.45)*	1.06 *(0.86)*	26.89 *(13.52)*	2.24 *(0.12)*	2.01 *(0.34)*	17.82 *(1.18)*
D	9.43	3.25 *(0.35)*	4.30 *(0.59)*	0.34 *(0.48)*	3.01 *(0.23)*	29.35 *(7.74)*	4.01 *(0.29)*	1.24 *(0.03)*	16.15 *(1.05)*
E	9.89	3.53 *(0.54)*	4.82 *(0.55)*	0.31 *(0.43)*	2.70 *(0.25)*	28.62 *(6.03)*	3.81 *(0.30)*	1.50 *(0.16)*	16.66 *(1.33)*
F	10.95	4.64 *(0.76)*	8.58 *(1.36)*	0.86 *(0.17)*	1.95 *(0.36)*	39.52 *(2.82)*	2.19 *(0.04)*	2.53 *(0.20)*	20.75 *(2.59)*
G	11.22	3.42 *(0.25)*	4.34 *(0.29)*	0.41 *(0.58)*	2.51 *(0.17)*	27.61 *(3.24)*	3.92 *(0.47)*	1.63 *(0.18)*	16.22 *(0.10)*
H	11.41	5.67 *(0.40)*	10.20 *(1.11)*	0.42 *(0.31)*	1.58 *(0.20)*	38.91 *(5.60)*	2.31 *(0.24)*	1.61 *(0.13)*	21.78 *(1.22)*
I	12.00	3.16 *(0.42)*	4.39 *(0.34)*	0.00 *(0.00)*	2.42 *(0.24)*	26.68 *(6.05)*	4.01 *(0.13)*	1.49 *(0.05)*	15.48 *(1.06)*
J	12.63	3.13 *(0.43)*	3.97 *(0.62)*	0.29 *(0.41)*	2.70 *(0.30)*	27.79 *(6.04)*	3.81 *(0.26)*	1.32 *(0.16)*	15.22 *(1.87)*
K	13.67	3.61 *(0.14)*	6.11 *(0.11)*	0.14 *(0.19)*	1.80 *(0.20)*	31.65 *(1.01)*	2.01 *(0.09)*	2.83 *(0.28)*	16.50 *(0.27)*
L	13.74	3.80 *(0.36)*	6.79 *(0.68)*	0.40 *(0.28)*	1.68 *(0.31)*	35.05 *(2.29)*	2.34 *(0.27)*	2.47 *(0.09)*	17.46 *(1.42)*

Ara = arabinose, Xyl = xylose, Man = mannose, Gal = galactose, Glu = glucose, UA = uronic acids mlG = mixed linkage (1-3)(1-4)-β-D-glucan (all values quoted as anhydrous sugars). * - Soluble NSP excludes glu but includes mlG. Standard deviations shown in parenthesis.

No relationship was found between the total or soluble NSP and AME or between any individual sugar component and AME. When the extent of branching of the arabinoxylan was plotted against AME again no relationship was found. However, a weak relationship was found between the ratio soluble arabinoxylan : (1-3)(1-4)-β-D-glucan and AME (Fig 3) indicating an increasing AME with decreasing arabinoxylan and increasing mixed-linkage glucan. If the outlying value from sample H was omitted from the data this relationship had an $r^2 = 0.54$, including H gave an r^2 of only 0.16. Plotting the sum of the soluble galactose and mannose content against AME (Fig 4) also showed a negative relationship with an $r^2 = 0.58$ if the data from sample C was excluded, including C gave $r^2 = 0.22$. The reason for this apparent relationship is obscure, particularly given the low concentrations of galactose and mannose present in the soluble NSP fraction (Table 6)

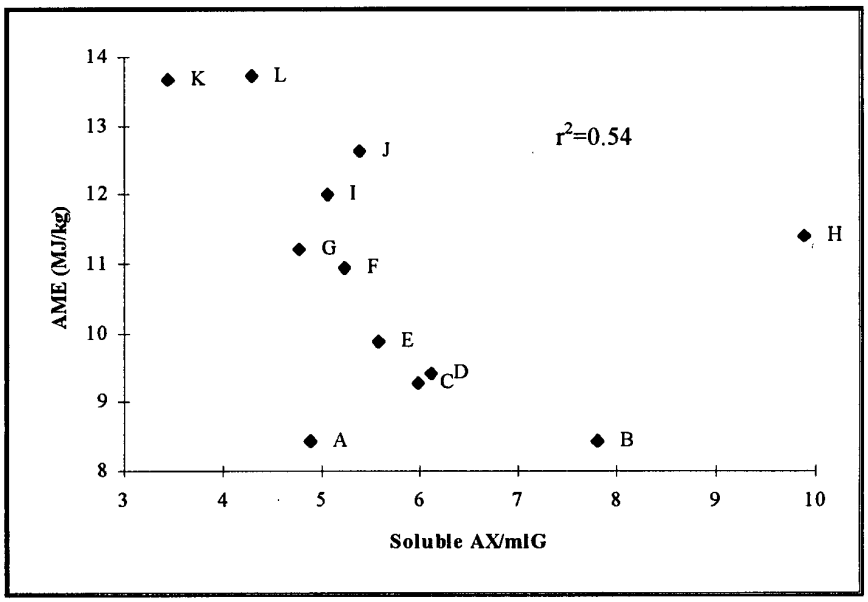


Fig 3: Plot of AME v soluble arabinoxylan / (1-3)(1-4)-β-D-glucan (r^2 value excludes sample H)

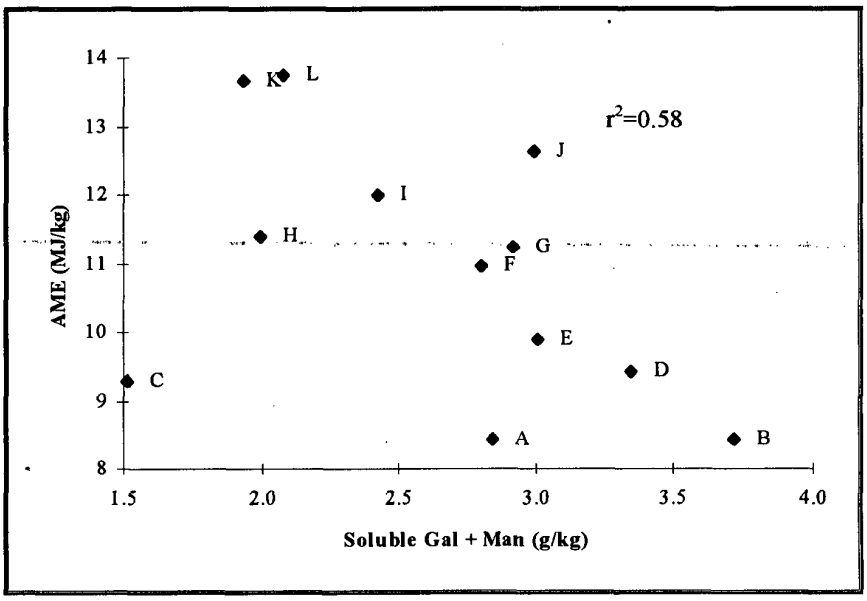


Fig 4: Plot of AME v soluble mannose + galactose
(r^2 value excludes sample C)

The total starch content of the wheat samples (Table 7) also showed no significant correlation with AME.

Table 7: Starch content of wheat samples

Sample	Starch (g/kg)	AME (MJ/kg wheat)	AME (MJ/kg
A	645	8.43	13.06
B	657	8.43	12.83
C	662	9.29	14.04
D	641	9.43	14.71
E	638	9.89	15.49
F	680	10.95	16.11
G	649	11.22	17.29
H	652	11.41	17.49
I	634	12.00	18.93
J	670	12.63	18.84
K	659	13.67	20.73
L	647	13.74	21.33

Viscosity of the soluble extracts of wheat

Two approaches were taken to investigate the rheological properties of wheat extracts. Firstly wheat samples were extracted in such a manner as to keep the ratio of wheat weight : liquid volume constant (extract viscosity) and secondly the viscosity of solutions containing equal concentrations of extracted material (isolate viscosity) was measured (Table 8).

Isolate viscosities were much lower than the extract viscosities. This was due to the partial insolubility of the previously isolated 'soluble' polysaccharides even at elevated temperatures. This loss of solubility has been previously observed, Ebringerova *et.al.* (1994) found that soluble arabinoxylans from rye, which were originally extracted as single or dimerised strands, on precipitation aggregated into clusters which continued to grow even in the dry state and which became increasingly insoluble.

Table 8: Viscosity of wheat extracts and isolate solutions

Wheat Sample	AME (MJ/kg)	Extract Viscosity (mPa s)	Isolate viscosity (mPa s)
A	8.43	5.61 *(0.04)*	1.20 *(0.04)*
B	8.43	2.07 *(0.02)*	0.98 *(0.02)*
C	9.29	2.09 *(0.01)*	0.95 *(0.01)*
D	9.43	5.21 *(0.07)*	1.23 *(0.38)*
E	9.89	8.39 *(0.04)*	1.11 *(0.01)*
F	10.95	2.24 *(0.02)*	*
G	11.22	7.40 *(0.03)*	1.18 *(0.01)*
H	11.41	2.66 *(0.04)*	1.13 *(0.01)*
I	12.00	7.15 *(0.06)*	1.18 *(0.01)*
J	12.63	5.29 *(0.04)*	1.08 *(0.01)*
K	13.67	3.36 *(0.01)*	0.92 *(0.01)*
L	13.74	2.85 *(0.02)*	0.88 *(0.03)*

* denotes missing value due to insufficient sample. Standard deviations are given in parenthesis

No correlation was found between *in vitro* viscosity and AME. This is in apparent contradiction to the findings of Bedford (1995), who found that intestinal viscosity correlated negatively with nutrient digestibility. However, since the conditions in the chick digestive tract are likely to be different from those used here to investigate viscosity, the two sets of data are not strictly comparable.

A correlation between AME and the isolate viscosity was not expected and was not found. This experiment was designed to determine the difference in the physiochemical characteristics of the isolated polymers and would be unlikely to be representative of the viscosity-enhancing effects of whole wheat extracts.

Both the soluble and total arabinoxylan content correlated with extract viscosity (Fig 5 & Fig 6), the soluble arabinoxylan had a weaker correlation ($r^2 = 0.61$) than the total ($r^2 = 0.82$). Since arabinoxylan was expected to be the main contributor to viscosity this was not unexpected. However both correlations were negative. This was in marked contradiction to the results of van der Klis *et.al.* (1995), Choct & Annison (1990), Annison (1992), Saulnier *et.al.* (1995) and Fengler & Marquardt (1988) all of whom found the expected positive correlations between arabinoxylan concentration and viscosity. The most likely explanation for the unusual result obtained is the increasing solubility of arabinoxylans with decreasing molecular weight. A reduction in the molecular weight of a polysaccharide will make it less likely to form the large intermolecular networks which cause increases in solution viscosity but it may also render them more soluble. This type of phenomenon has been observed in enzyme supplementation trials. When birds have been fed enzyme supplements the intestinal viscosity has been reduced but the concentration of soluble NSP has increased (Choct *et.al.*, 1995). This was due to the enzyme solubilising more of the insoluble NSP from the plant cell wall while simultaneously reducing the molecular weight of the released polysaccharide below the size where they contribute to intestinal viscosity.

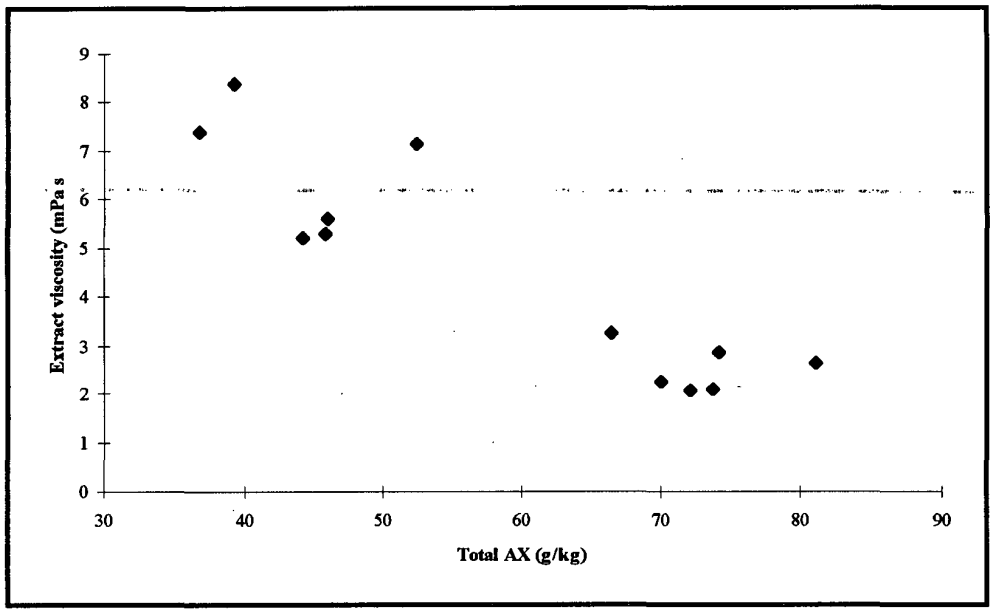


Fig 5: Effect of total arabinoxylan on extract viscosity

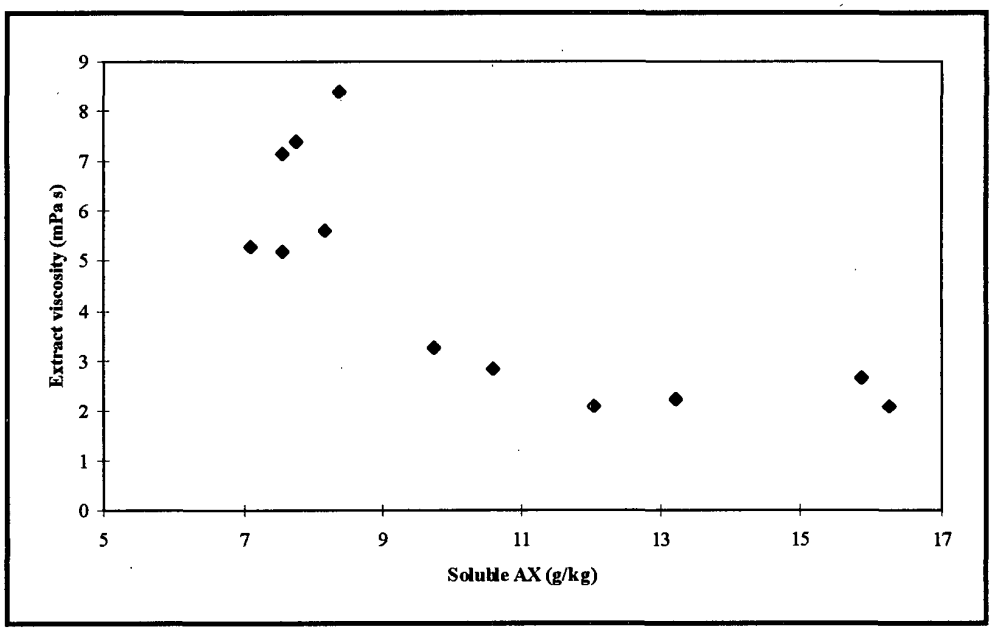


Fig 6: Effect of soluble arabinoxylan on extract viscosity

It was similarly noticed that the isolate viscosity had a negative correlation with the soluble (1-3)(1-4)-β-D-glucan content (r^2 = 0.73, Fig 7) but not with the arabinoxylan content. Although the same argument can be applied to explain the β-glucans negative correlation the relationship was unexpected since the arabinoxylans were the major determinant of viscosity. However, if the arabinoxylans formed insoluble clusters on precipitation, as described by Ebringerova *et.al.* (1994) and could not be re-solubilised, then any (1-3)(1-4)-β-D-glucans which remained soluble would become the major contributor to viscosity.

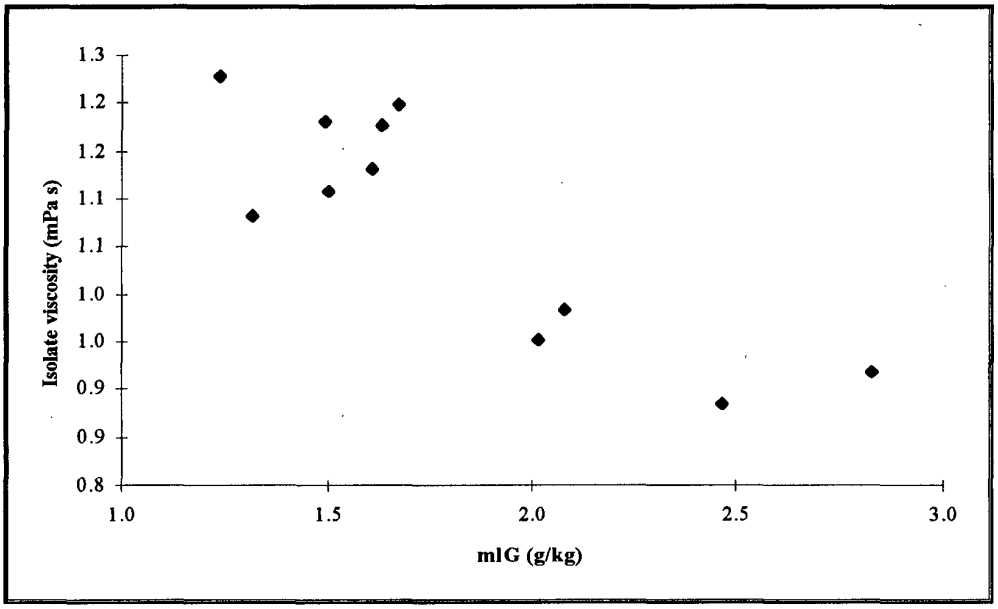


Fig 7: Effect of soluble (1-3)(1-4)-β-D-glucan on isolate viscosity

The degree of branching of the soluble arabinoxylan correlated with extract viscosity (r^2 = 0.75, Fig 8). This was a positive correlation indicating increased viscosity with increased branching. This, again, was unexpected and in contradiction to the work of Izydorczyk *et.al.* (1995 & 1991), but in agreement with that of Bengtsson *et.al.* (1992). However, if the extracts contain polysaccharides which are not large enough to form intermolecular networks then the degree of branching may assume greater importance. A highly branched molecule will have a larger hydrodynamic volume than an unbranched molecule and so would contribute more to solution viscosity.

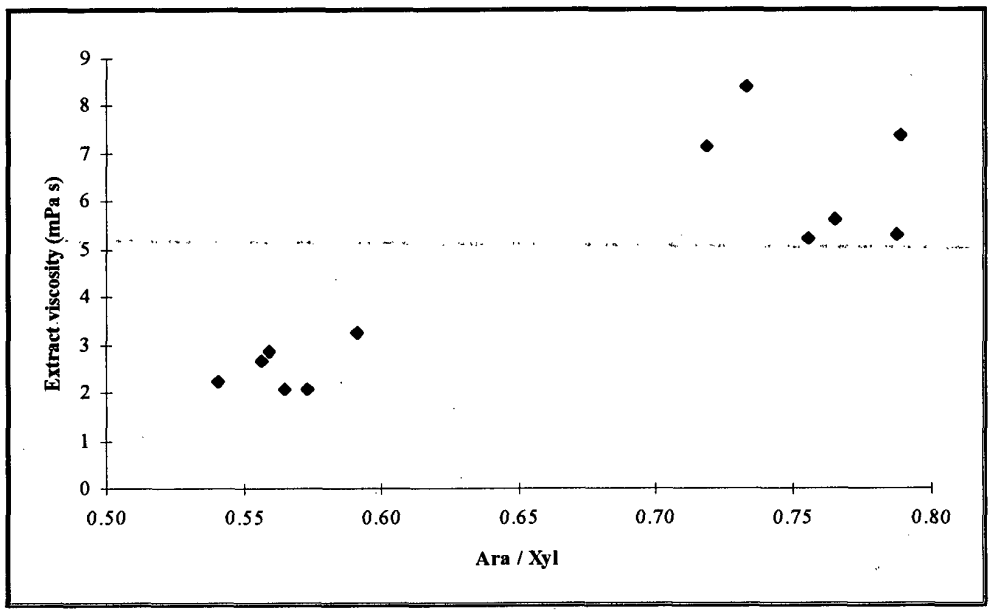


Fig 8: Effect of branching of soluble arabinoxylan on extract viscosity

The molecular weight distribution of the released polysaccharides was determined by size exclusion chromatography (Appendix 1) and the data summarised in Table 9. The results indicated two sets of data (Fig 9). One set, which had low branching, showed an increase in viscosity with an increase in the concentration of high molecular weight carbohydrate. The second set had a higher degree of branching and higher viscosity but showed no correlation with the concentration of high molecular weight carbohydrate. One sample (A in Fig 9) was different and contained a very large proportion of high molecular weight polysaccharide with a high degree of branching but still conformed to the relationship where increased molecular weight increased viscosity. These results indicated that:-

- higher degrees of branching were important for viscosity enhancing ability when the molecular weight was low.

- when the degree of branching is low then molecular size becomes the dominant viscosity-enhancing feature.

Table 9: Summary of the molecular weight distribution of soluble NSP

Wheat	Proportion of soluble NSP (%)					Extract viscosity (mPa s)	Isolate viscosity (mPa s)
	>300 (kDa)	200-300 (kDa)	100-200 (kDa)	20-100 (kDa)	<20 (kDa)		
A	13	32	6	7	42	5.61	1.20
B	0	0	30	41	29	2.07	0.98
C	1	18	15	18	48	2.09	0.95
D	4	0.5	0	2	93.5	5.21	1.23
E	2	7	17	29	44	8.39	1.11
F	1	4	18	46	30	2.24	*
G	8	7	16	18	52	7.40	1.18
H	1	11	22	17	49	2.66	1.13
I	1	6	21	21	51	7.15	1.18
J	4	7	12	25	51	5.29	1.08
K	5	25	34	25	11	3.36	0.92
L	7	15	26	32	20	2.85	0.88

* insufficient material for analysis

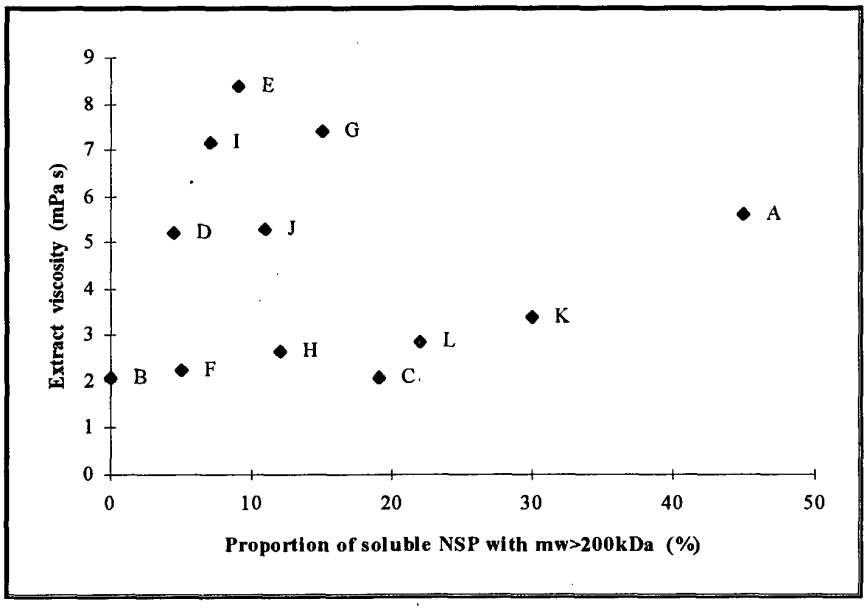


Fig 9: Relationship between viscosity and the molecular weight of soluble NSP

Bedford & Classen (1992) reported that the concentration of carbohydrate having a molecular weight greater than 500kDa correlated with intestinal viscosity in an exponential manner. The results reported here do not wholly agree with this observation. Firstly, the soluble carbohydrate in this investigation contained few polysaccharides having such a high molecular weight, yet a positive relationship between viscosity and concentration of high molecular weight carbohydrate was still noted. Secondly, the relationship was linear not exponential. The results do not necessarily contradict those of Bedford & Classen, since their viscosity measurements were made on the intestinal contents and not with an *in vitro* model. The polysaccharides from this study generally had a much lower molecular weight than those of Bedford & Classens so a different relationship may be true in the absence of material with molecular weight greater than 500kDa.

There were two correlations with isolate viscosity, firstly a negative correlation with the concentration of carbohydrate with molecular weight greater than 200kDa (Fig 10) and secondly a positive correlation with carbohydrate having molecular weight less than 20kDa (Fig 11). Superficially, this appears to be the converse of what might be expected. However, if high molecular weight polysaccharides form large insoluble clusters, as described by Ebringerova *et.al.* (1994), then the high molecular weight material would not be able to contribute to solution viscosity. Increasing the concentration of low molecular weight material, which remains soluble, would increase solution viscosity.

The data suggests that extract viscosity is determined by the molecular weight of the arabinoxylan where the degree of branching is fairly low. However when the degree of branching is increased other factors must also be important in determining viscosity.

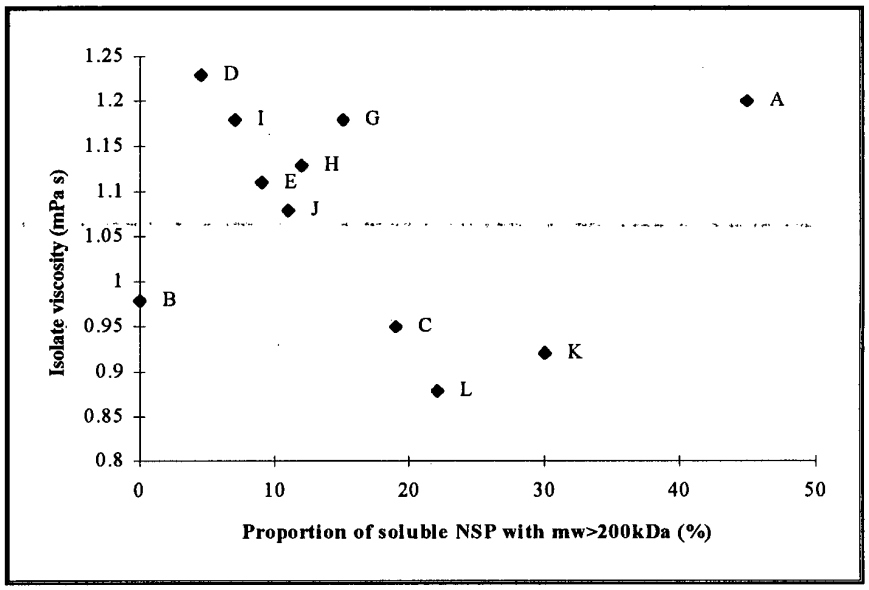


Fig 10: Relationship between isolate viscosity and soluble NSP with high molecular weight

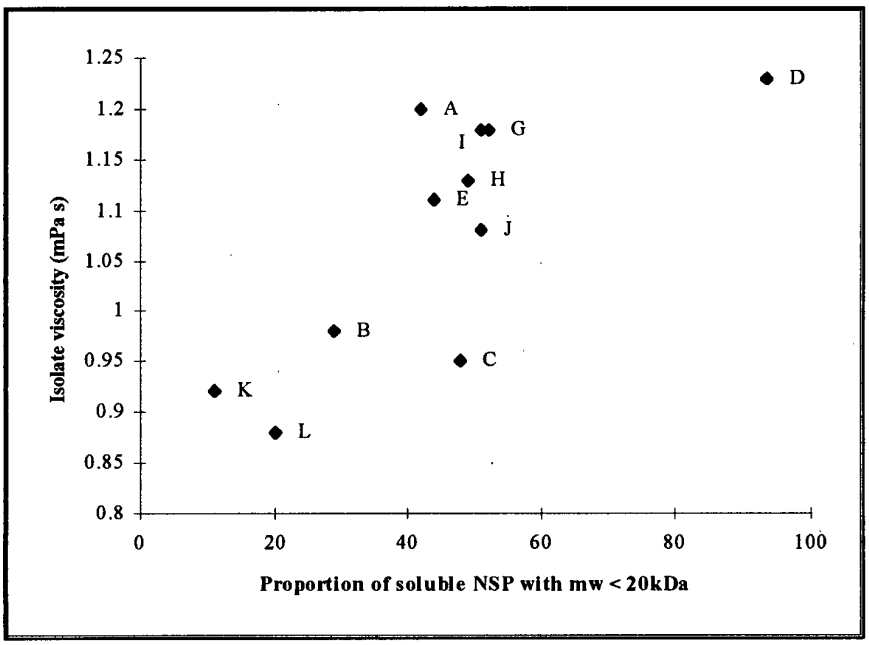


Fig 11: Relationship between isolate viscosity and soluble NSP with low molecular weight

Determination of arabinoxylan structure

A commercially available wheat arabinoxylan was treated with xylanase and the released oligosaccharides separated on a Biogel-P2 column (Fig 12). The fractions obtained from the column were mixtures of several oligosaccharides as observed by MALDI-TOF-MS (Fig 13) and had to be further purified by re-passage through the same column. With oligosaccharides of simmilar molecular weight, little or no separation was observed when the fractions collected were analysed by the method for determining total carbohydrate. However, analysis by MALDI often provided evidence of further separation. This is exemplified in the chromatogram for the separation of fraction f6 (Fig 14), which shows how the single peak detected by the total carbohydrates method was resolved into three when using MALDI detection. This technique enabled the isolation of fairly pure oligosaccharides in milligram quantities. Each oligosaccharide was analysed by proton NMR and the two-dimensional experiments, total correlation spectroscopy (TOCSY) and rotating frame nuclear Overhauser effect spectroscopy (ROESY). The data obtained from these experiments was used to identify the structure of each oligosaccharide

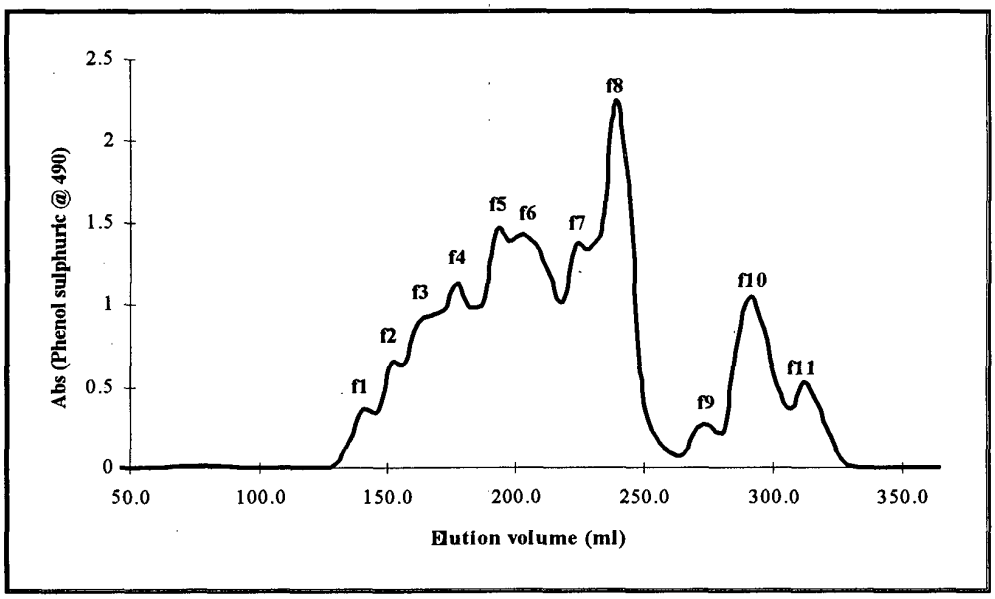


Fig 12: Separation of oligosaccharides by size exclusion chromatography

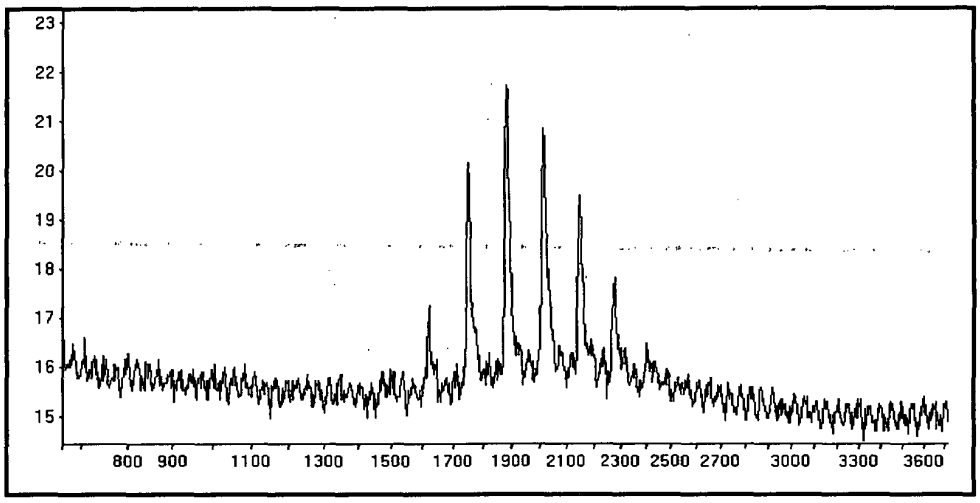


Fig 13: MALDI of fraction f3

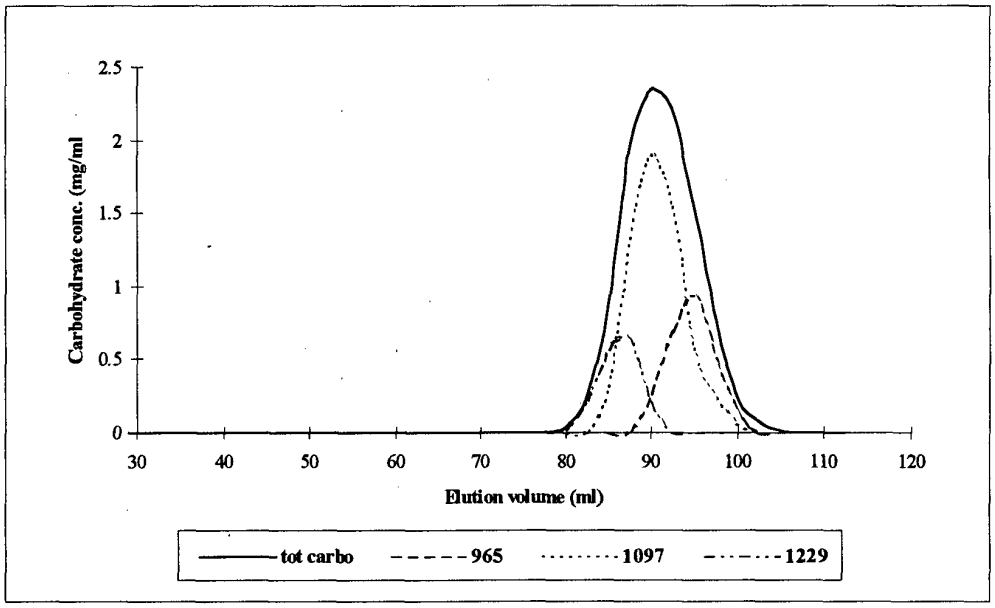


Fig 14: Separation of fraction f6 on Biogel-P2

Tot carbo = peak obtained by phenol sulphuric acid method, 965, 1097 & 1229 are the peaks determined by MALDI having those molecular weights

The structures of the identified oligosaccharides are shown in Appendix 2 along with their MALDI spectra and tables of chemical shifts from the NMR spectra. In some cases full assignment of the NMR spectra was not possible due to overlap of the signals. In these cases it was still possible to identify their structures from the resonances of the anomeric

protons which can be used as structural reporter groups (Vliegenthart *et.al.*, 1992). In such cases only the data for the anomeric protons have been reported. The molecular weights determined by MALDI all represent the $[M+Na]^+$ ion hence the molecular weights were 23Da higher than their calculated molecular weight. After identification, the oligosaccharides were run on the HPAEC system and their retention times were recorded (Table 10).

Table 10: HPAEC retention times for oligosaccharides

Oligosaccharide	Retention time	Oligosaccharide	Retention time
X-1	2.7	X-10	11.2
X-2	3.5	AX-5	11.3
X-3	4.4	X-11	12.0
X-4	5.6	X-12	12.5
X-5	6.6	AX-6	13.0
X-6	7.6	AX-7	17.7
X-7	9.1	AX-8	19.3
X-8	10.0	AX-10	21.5
X-9	10.5	AX-9	22.4

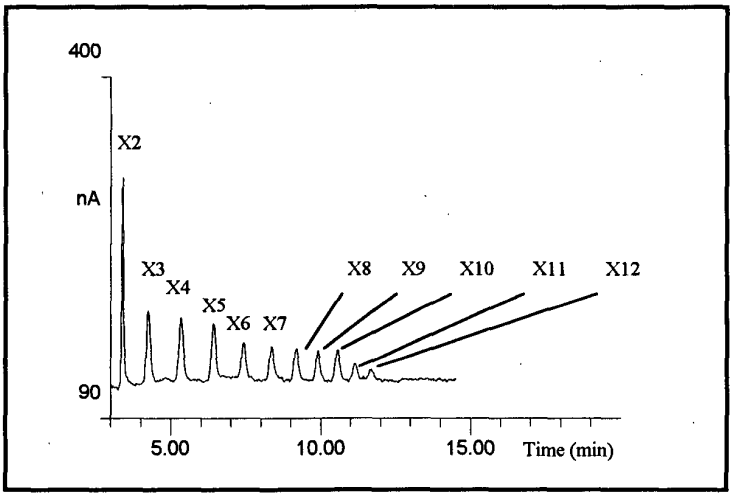


Fig 15: HPLC of linear xylooligosaccharides (X2 represents xylobiose, X3 xylotriose, etc)

Chromatograms from the HPAEC are shown in Figs 15, 16 and 17. The standard chromatogram (Fig 15) was prepared using linear xylans of known size (degree of polymerisation (DP) = 1-12). The wheat chromatograms (Fig 16 & 17) showed that the oligosaccharide patterns and hence the arabinoxylan structures differed between wheat samples. Whilst it was possible to see differences from these chromatograms it was easier to visualise them as bar charts. The data from the chromatograms was normalised such that the total area under the chromatogram was equal to unity, the fraction contributed to the total by each peak was calculated (Table 11) and plotted against retention time as a bar chart (Figs 18-29).

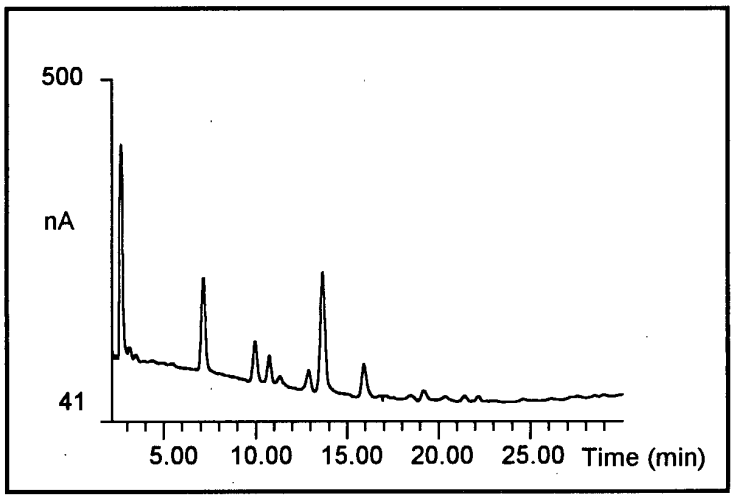


Fig 16: HPAEC of oligosaccharides released from wheat A

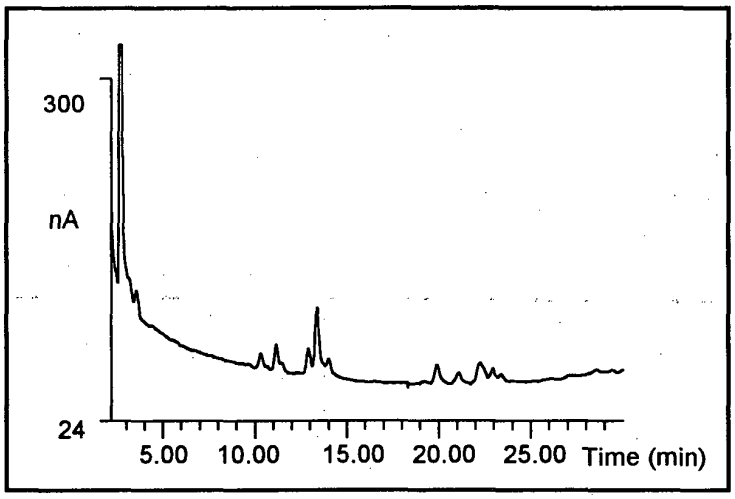


Fig 17: HPAEC of oligosaccharides released from wheat K

Table 11: Relative peak areas in the HPAEC fingerprints

		Relative peak area											
$t_{(R)}$	ID	A	B	C	D	E	F	G	H	I	J	K	L
2.7	X-1	0.28	0.43	0.63	0.40	0.60	0.66	0.51	0.49	0.26	0.59	0.56	0.56
7.1	*	0.16	0.03	0.00	0.10	0.13	0.00	0.11	0.00	0.19	0.08	0.00	0.00
10.0	X-8	0.07	0.06	0.03	0.03	0.04	0.02	0.06	0.03	0.06	0.02	0.03	0.03
10.8	X-9	0.05	0.15	0.09	0.07	0.04	007	0.06	0.12	0.08	0.07	0.04	0.10
11.3	AX-5^{+}	0.02	0.00	0.00	0.00	0.01	0.00	0.02	0.00	0.04	0.00	0.01	0.00
13.0	AX-6	0.04	0.13	0.10	0.12	0.03	0.10	0.04	0.15	0.07	0.08	0.15	0.13
13.7	*	0.25	0.03	0.06	0.10	0.10	0.04	0.12	0.03	0.17	0.07	0.05	0.08
15.8	*	0.08	0.00	0.00	0.00	0.01	0.00	0.04	0.00	0.05	0.00	0.00	0.00
19.3	AX-8	0.02	0.08	0.02	0.03	0.02	0.03	0.02	0.06	0.03	0.02	0.05	0.03
20.4	*	0.01	0.03	0.02	0.04	0.01	0.02	0.01	0.04	0.01	0.02	0.03	0.02
21.5	AX-10	0.01	0.04	0.04	0.07	0.01	0.05	0.01	0.05	0.02	0.03	0.06	0.03
22.4	AX-9	0.01	0.02	0.02	0.04	0.01	0.03	0.01	0.03	0.01	0.02	0.01	0.03

A-L = wheat samples, $t_{(R)}$ = retention time, ID = identification of oligosaccharide, X-1 = xylose, X-2 = xylobiose, etc. AX-5 = oligosaccharide AX-5, AX-6 = oligosaccharide AX-6, etc as identified earlier. * denotes those peaks which have not been identified. + although this peak has been identified here as being AX-5, the linear oligomer X-10 elutes at the same place.

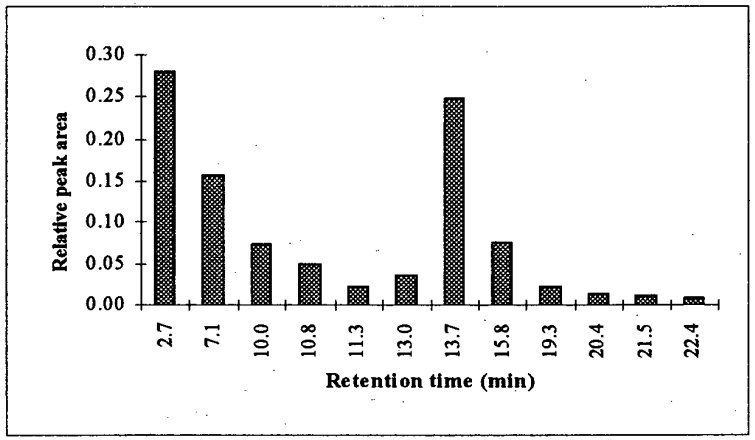


Fig 18: HPAEC of oligosaccharides released from wheat A

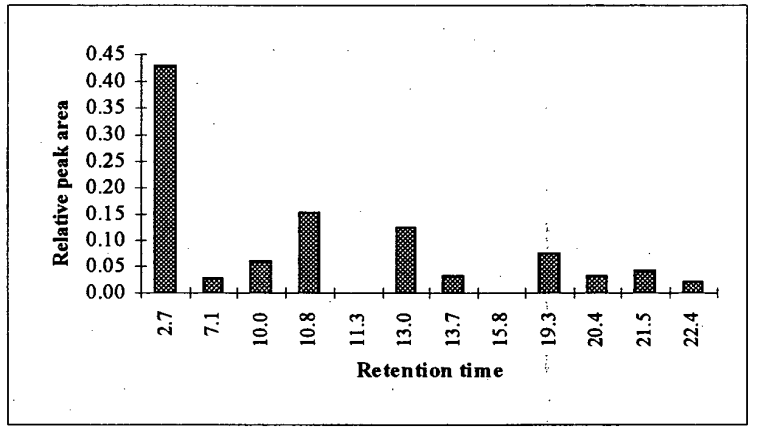


Fig 19: HPAEC of oligosaccharides released from wheat B

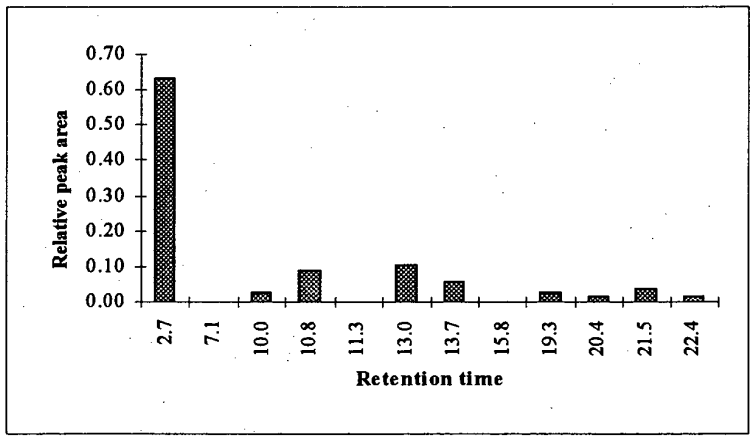


Fig 20: HPAEC of oligosaccharides released from wheat C

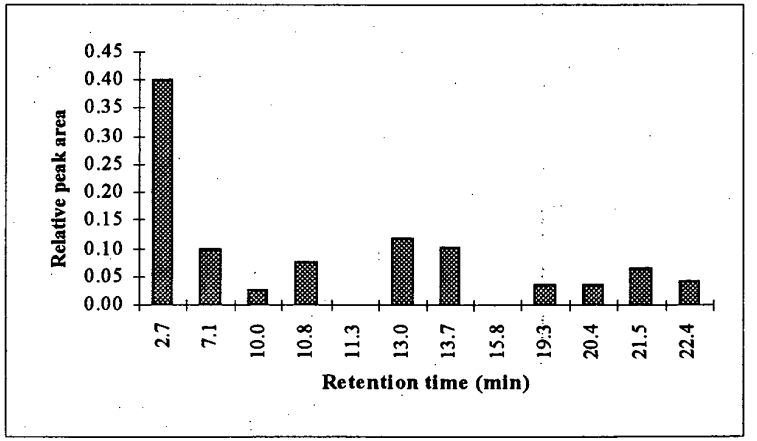


Fig 21: HPAEC of oligosaccharides released from wheat D

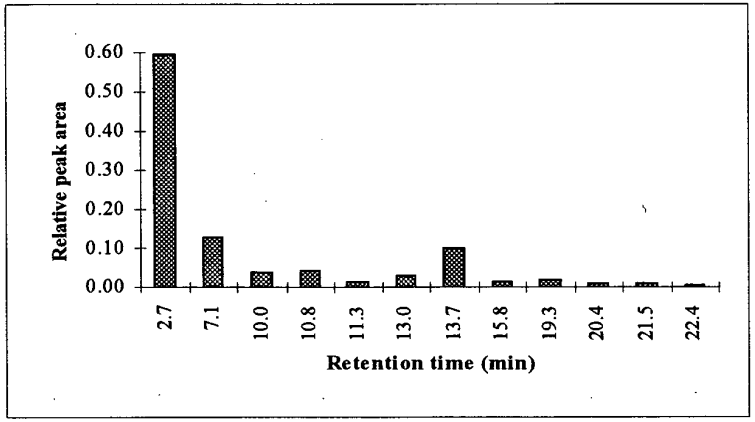


Fig 22: HPAEC of oligosaccharides released from wheat E

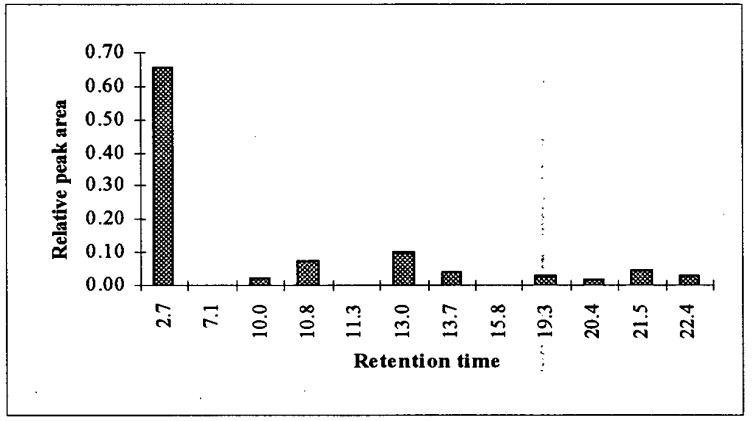


Fig 23: HPAEC of oligosaccharides released from wheat F

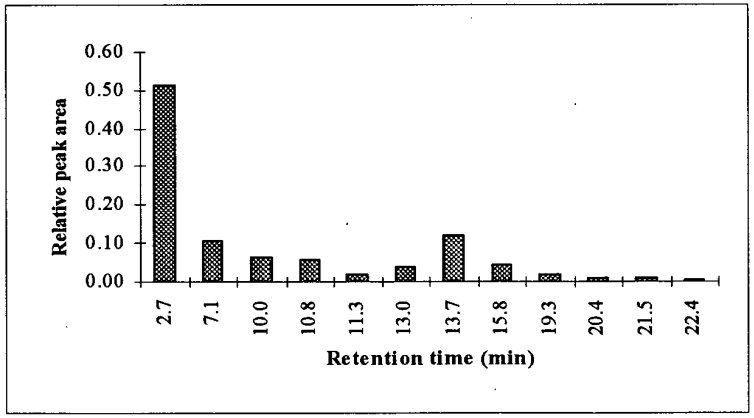


Fig 24: HPAEC of oligosaccharides released from wheat G

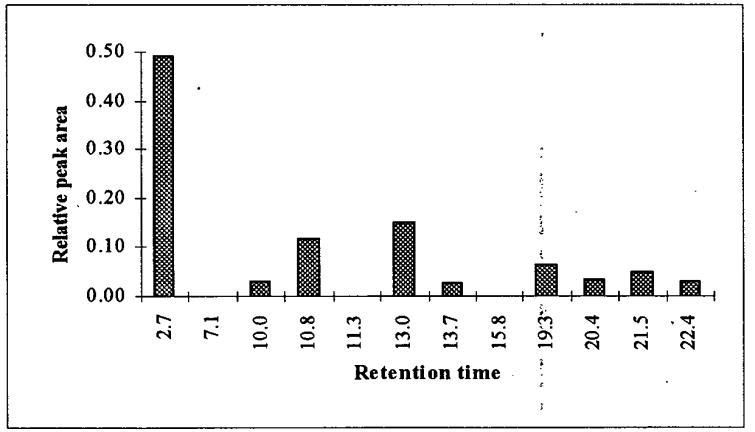


Fig 25: HPAEC of oligosaccharides released from wheat H

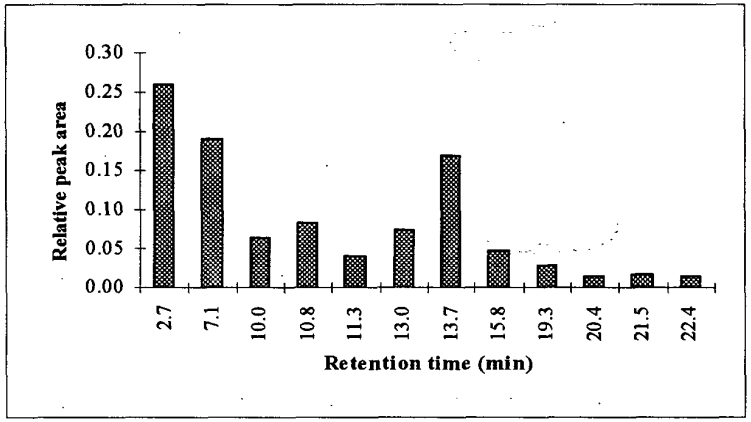


Fig 26: HPAEC of oligosaccharides released from wheat I

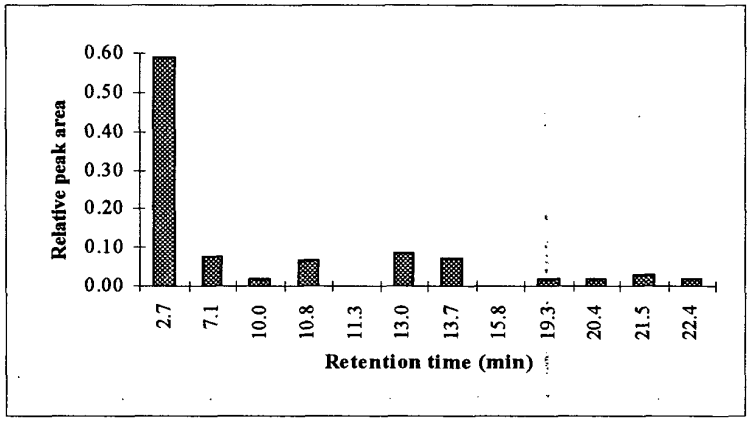


Fig 27: HPAEC of oligosaccharides released from wheat J

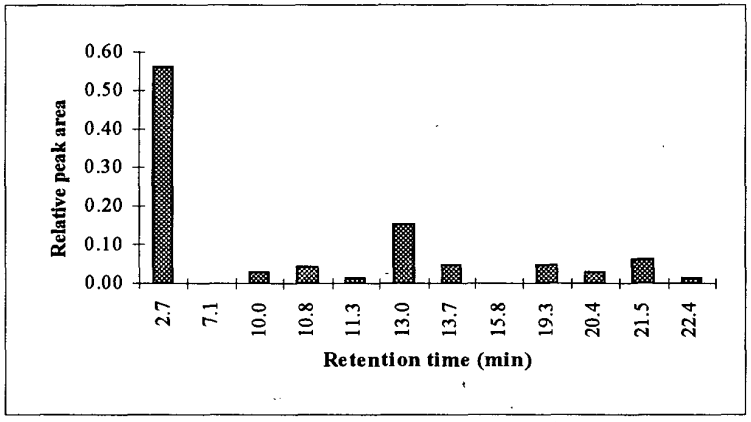


Fig 28: HPAEC of oligosaccharides released from wheat K

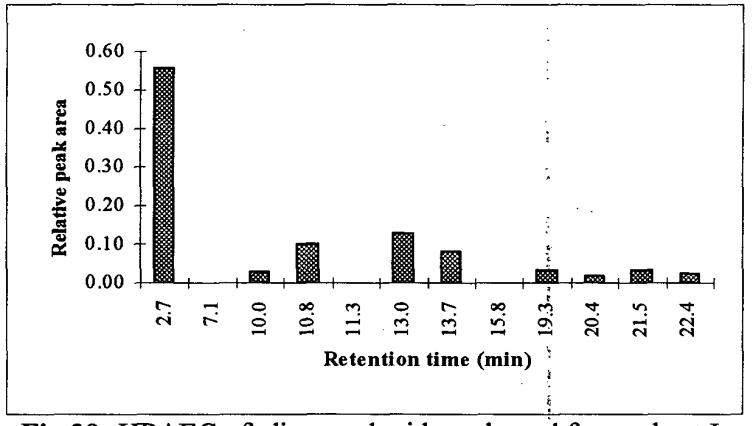


Fig 29: HPAEC of oligosaccharides released from wheat L

The charts showed that there were evident differences in the structure of arabinoxylans from different wheat sources. All the chromatograms were dominated by the peak at 2.7min, known to be xylose which contributed between 25-70% to the total area. The other peaks were present in varying, but smaller, amounts, although there were a few peaks (at 7.1, 11.3 and 15.8min) which were present only in some of the chromatograms. Most of the peaks were identified (Table 10) and it is likely that the unidentified peaks are structural isomers of the oligosaccharides already identified. One oligosaccharide, AX-7, isolated from the commercial sample did not appear in the chromatograms of any of the wheat samples. This is probably because the commercial arabinoxylan was isolated from an Australian wheat variety which may be different from any of the UK varieties analysed.

Any differences between the chromatograms which correlated with differences in AME was investigated by plotting the relative area under each peak against the AME and testing the relationships by linear regression. This confirmed that there were no correlations between AME and any individual oligosaccharide or combination of oligosaccharides. The peak areas were similarly tested for correlations with the viscosity data. No correlations were found with isolate viscosity but a potential correlation was found with extract viscosity (Fig 30). The correlation was with the relative size of the peak eluting at 13.0 min, identified as AX-6, the correlation was quite weak ($r^2 = 0.25$) but when the data for wheat A, which appeared to be outlying, was excluded the relationship was strengthened ($r^2 = 0.58$). The relationship is a negative one suggesting that a high inclusion of the structural feature AX-6, interferes with the ability of the arabinoxylans to increase solution viscosity.

MALDI-TOF-MS was also used as a technique for identifying the oligomers produced by xylanase digestion. MALDI has the advantage over HPAEC in that it takes much less time to run a sample (approx. 5min including sample preparation). Unfortunately the method does not currently have the capability to produce accurate quantitative results and the matrix interferes with oligosaccharides having m/z below 500.

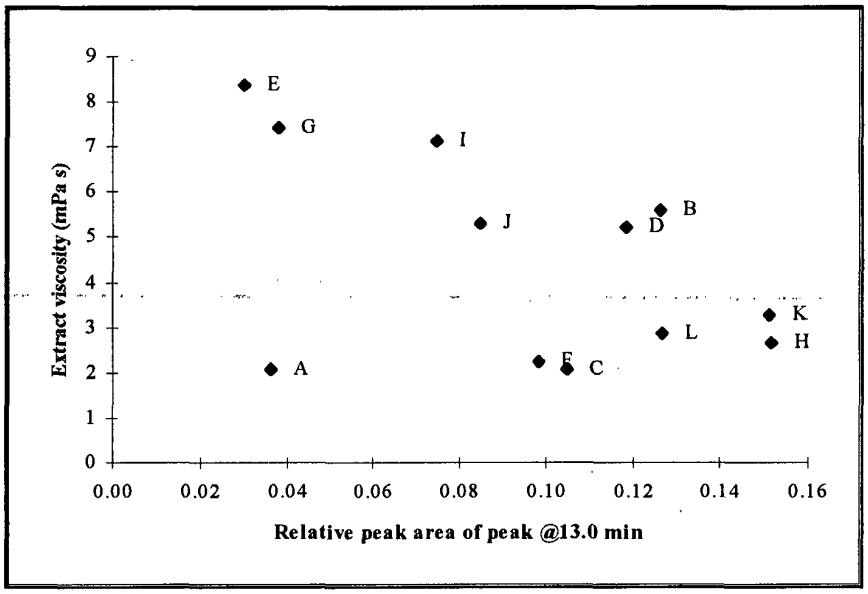


Fig 30: Plot of extract viscosity v relative peak area for the 13.0 min peak (AX-6).

The MALDI spectra have been represented as bar charts (Fig 31-42). Like the HPAEC results they also indicated that arabinoxylans from different wheat samples had different structures. In addition they yielded information about the molecular weight of the oligosaccharides produced. As expected a range of oligomers were found containing multiples of pentose residues (Table 12), however there were two signals which did not. These occurred at m/z = 1025 and m/z = 1172 and represent oligomers consisting of 5 pentoses + 2 hexoses and 6 pentoses + 1 hexose + 1 uronic acid or ferulic acid respectively. Such oligosaccharides did not occur in the xylanase digest of the commercially available arabinoxylan. These oligosaccharides may account for two of the unidentified peaks present in the chromatograms.

Table 12: Oligosaccharide molecular weight and signal observed by MALDI

No of pentose units	calculated molecular weight (Da)	MALDI m/z $[M+Na]^+$
5	678	701
6	810	833
7	942	965
8	1074	1097
9	1206	1229
10	1338	1361
11	1470	1493
12	1602	1625
13	1734	1757
14	1866	1889
15	1998	2021
16	2130	2153
17	2262	2285

The peak areas from the MALDI experiments were also compared to the AME and viscosity data. Weak relationships were found between AME and the oligosaccharide with m/z = 965 (Fig 43) and the oligosaccharides with m/z = 1493 (Fig 44). Both relationships were positive with r^2 = 0.41 and 0.44 respectively. If the data for points H and L were excluded for the oligosaccharide with m/z = 965, r^2 increased to 0.73. No other correlations were found. However, correlations found using the MALDI should be treated with caution. As indicated previously the results may not be quantitative and oligosaccharides with m/z below 500 cannot be detected

The occurrence of a peak at m/z = 965 in the MALDI was unexpected. Neither AX-7 nor X7 were detected by HPAEC in any of the 12 wheat samples. Another isomer containing 7 pentose units must be responsible for this peak and is probably responsible for one of the unidentified peaks in the HPAEC traces. The signal at m/z = 1493 had a larger molecular weight than any of the peaks identified on the HPAEC traces. It is possible that this and small amounts of other larger oligosaccharides were not eluted from the HPAEC.

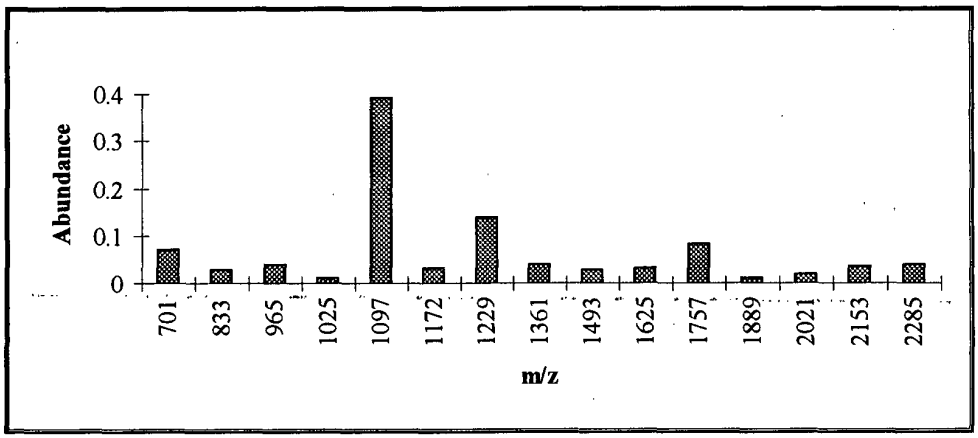


Fig 31: MALDI of oligosaccharides released from wheat A

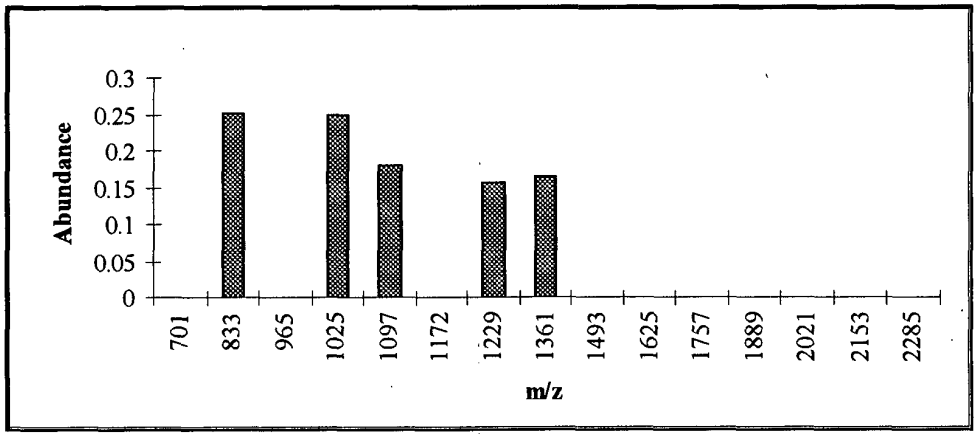


Fig 32: MALDI of oligosaccharides released from wheat B

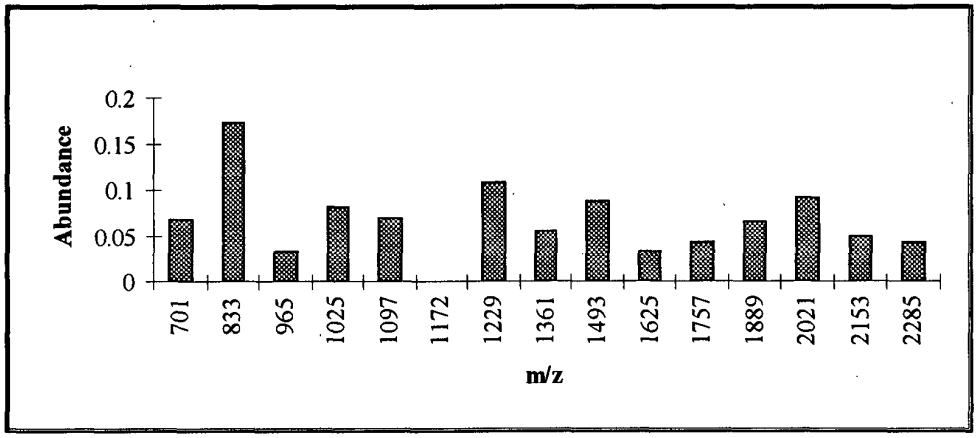


Fig 33: MALDI of oligosaccharides released from wheat C

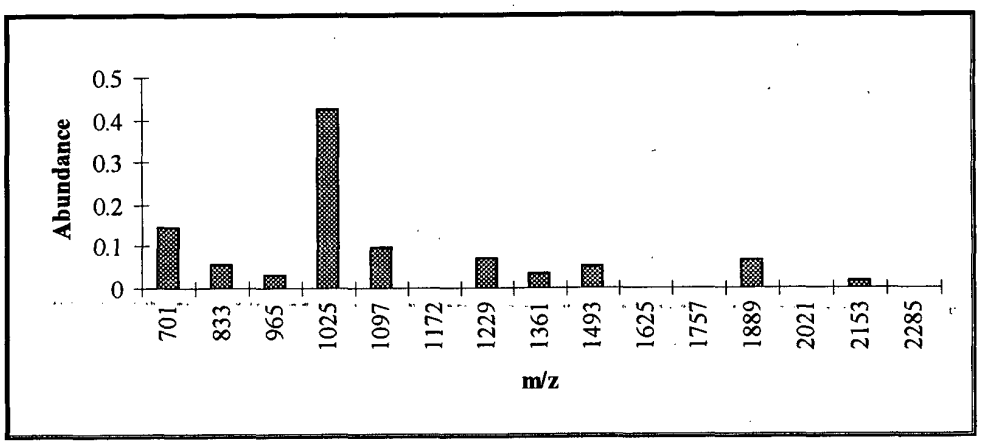


Fig 34: MALDI of oligosaccharides released from wheat D

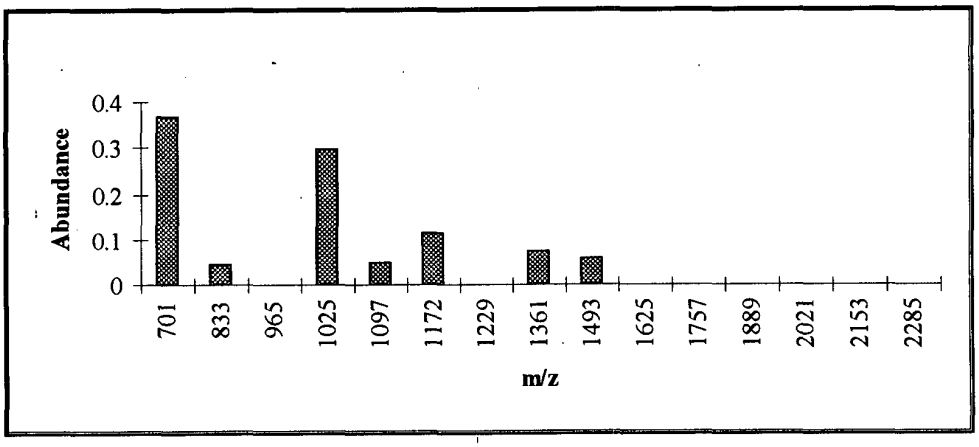


Fig 35: MALDI of oligosaccharides released from wheat E

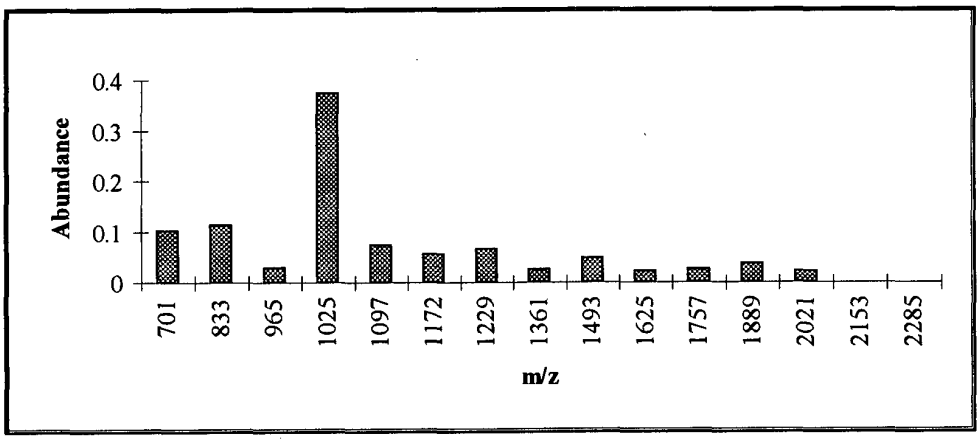


Fig 36: MALDI of oligosaccharides released from wheat F

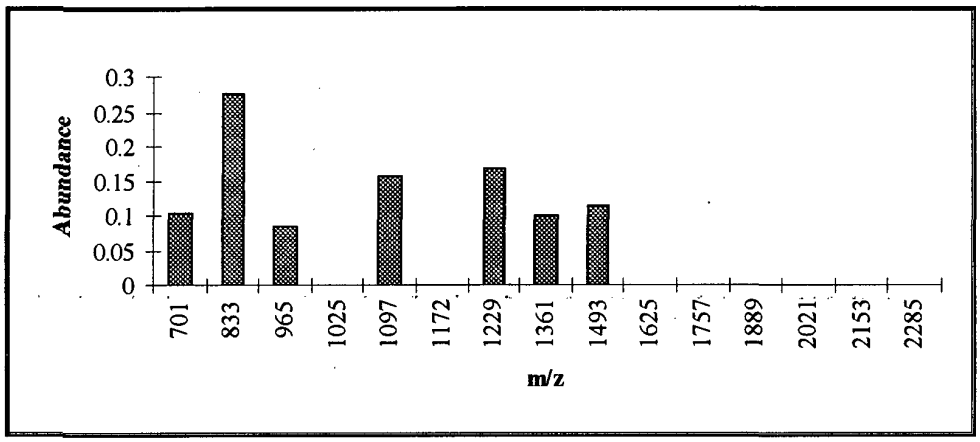


Fig 37: MALDI of oligosaccharides released from wheat G

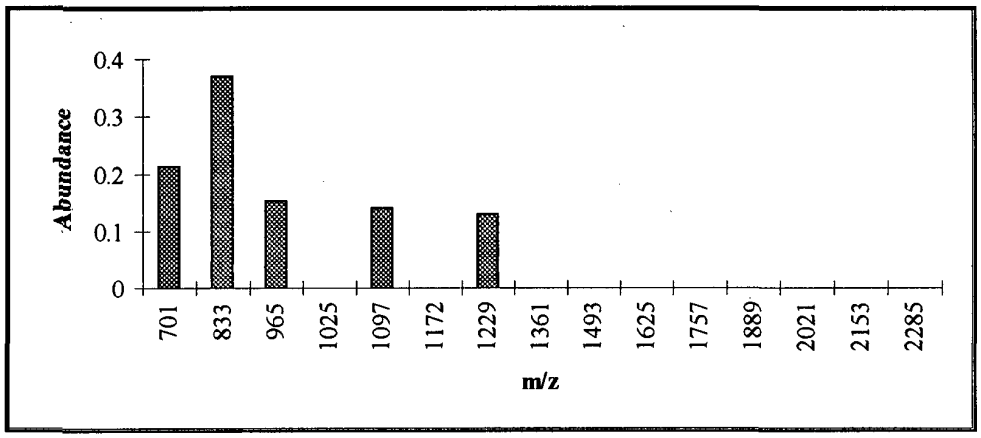


Fig 38: MALDI of oligosaccharides released from wheat H

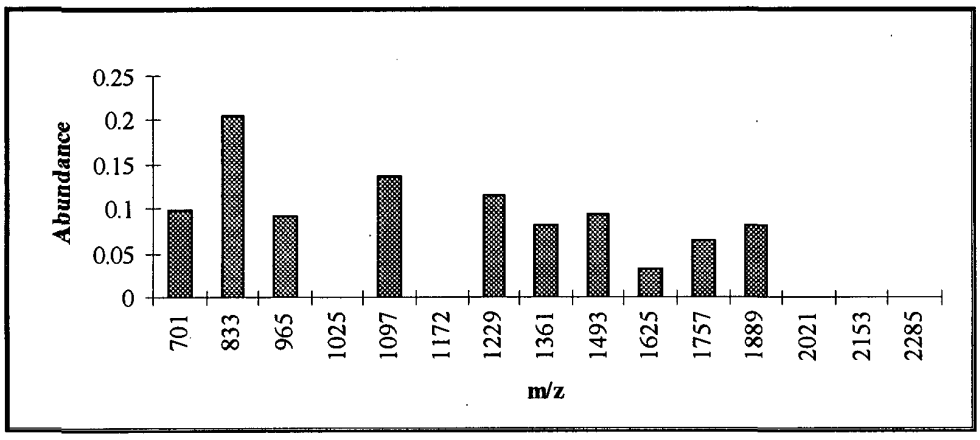


Fig 39: MALDI of oligosaccharides released from wheat I

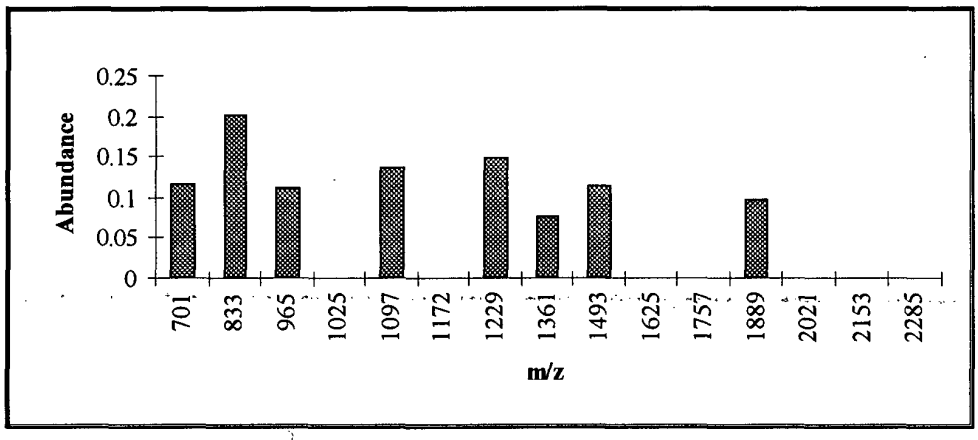


Fig 40: MALDI of oligosaccharides released from wheat J

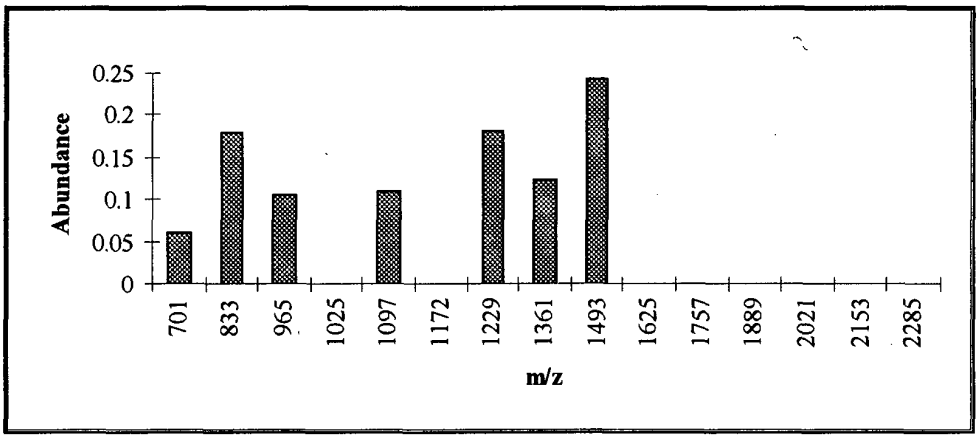


Fig 41: MALDI of oligosaccharides released from wheat K

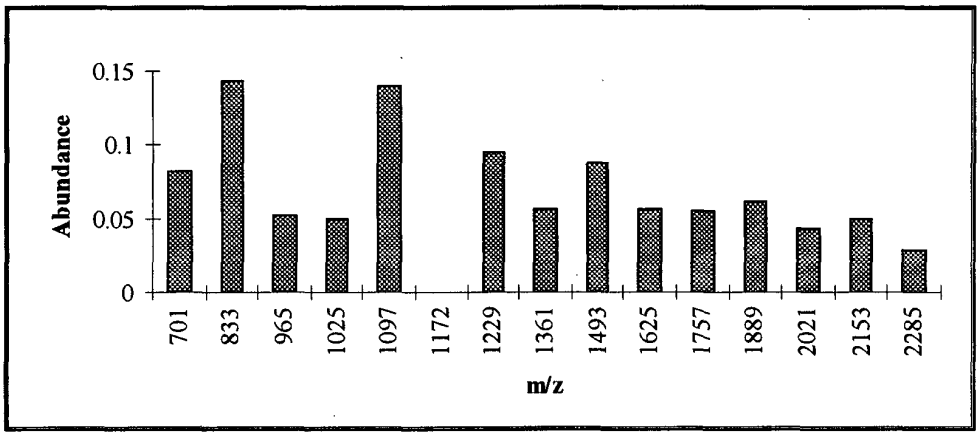


Fig 42: MALDI of oligosaccharides released from wheat L

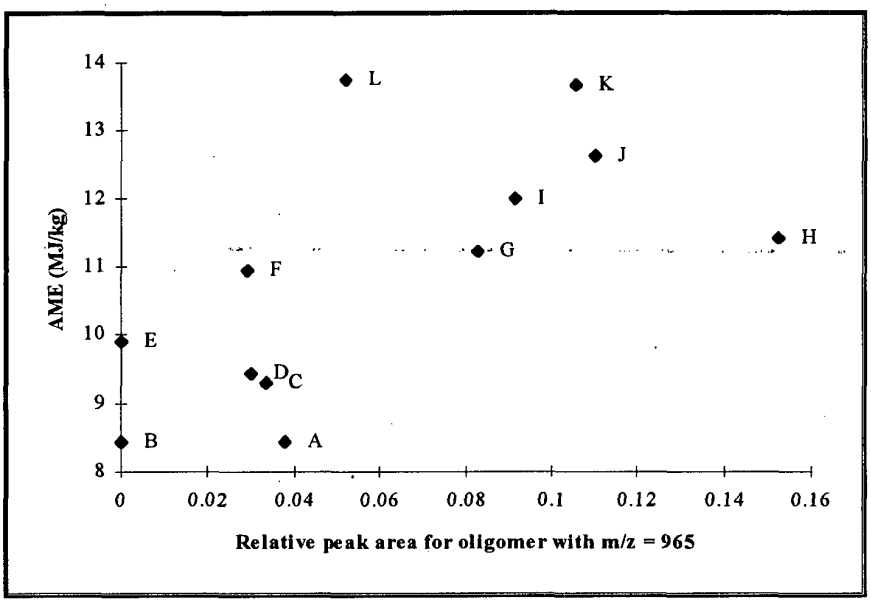


Fig 43: Relationship between AME and the area of the MALDI peak for oligosaccharides with m/z = 965

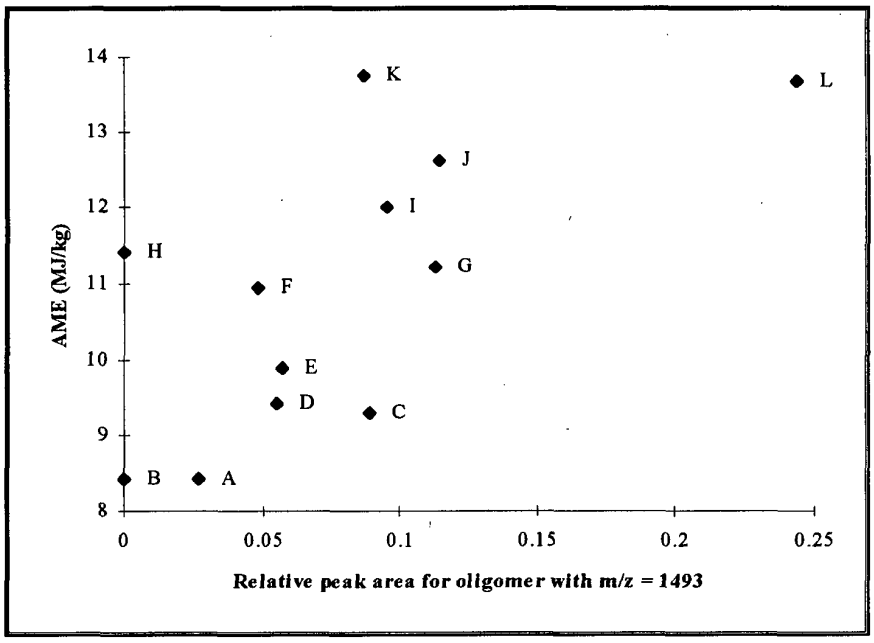


Fig 44: Relationship between AME and the area of the MALDI peak for oligosaccharides with m/z = 1493

Material resistant to xylanase

The soluble material extracted from the wheat was not completely digested by the xylanase. The undigested material was composed of non-arabinoxylan polysaccharides and some arabinoxylan resistant to the enzyme. This was analysed to determine how much of the original arabinoxylan had been left undigested (Table 13).

Table 13: Xylanase digestible and resistant fractions of wheat arabinoxylans

Sample	XDAX (g/kg wheat)	XRAX (g/kg wheat)	A/X for XRAX	Proportion of AX digested (%)
A	11.12	0.52	1.70	95
B	11.97	0.48	1.96	96
C	8.12	0.46	2.43	95
D	10.68	0.36	2.01	97
E	9.85	0.50	1.51	95
F	8.72	0.60	2.32	94
G	9.59	0.70	1.52	93
H	11.91	0.53	1.95	96
I	9.07	0.71	1.57	93
J	9.81	0.72	2.01	93
K	8.38	0.57	2.98	94
L	9.27	0.59	2.31	94

AX = arabinoxylan, XDAX = xylanase digestible arabinoxylan, XRAX = xylanase resistant arabinoxylan, A/X = ratio of arabinose to xylose.

The small quantity of arabinoxylan left undigested had a very high degree of branching; in some cases exceeding two substitutions per xylose unit. There are two possible explanations for this. Firstly, arabinose residues may be present which are not a part of arabinoxylan but linked to other polysaccharides. Secondly, it is possible that instead of their being a single terminal arabinose unit attached to the xylan backbone chains of two or more residues may occur.

The amount of soluble arabinoxylan prone to xylanase digestion and that which was resistant to xylanase digestion in the wheat was plotted against AME, isolate viscosity and extract viscosity to test for any correlations. A weak positive correlation was found between AME and the amount of soluble arabinoxylan resistant to xylanase (Fig 45, r^2 = 0.37) . No other correlations were observed.

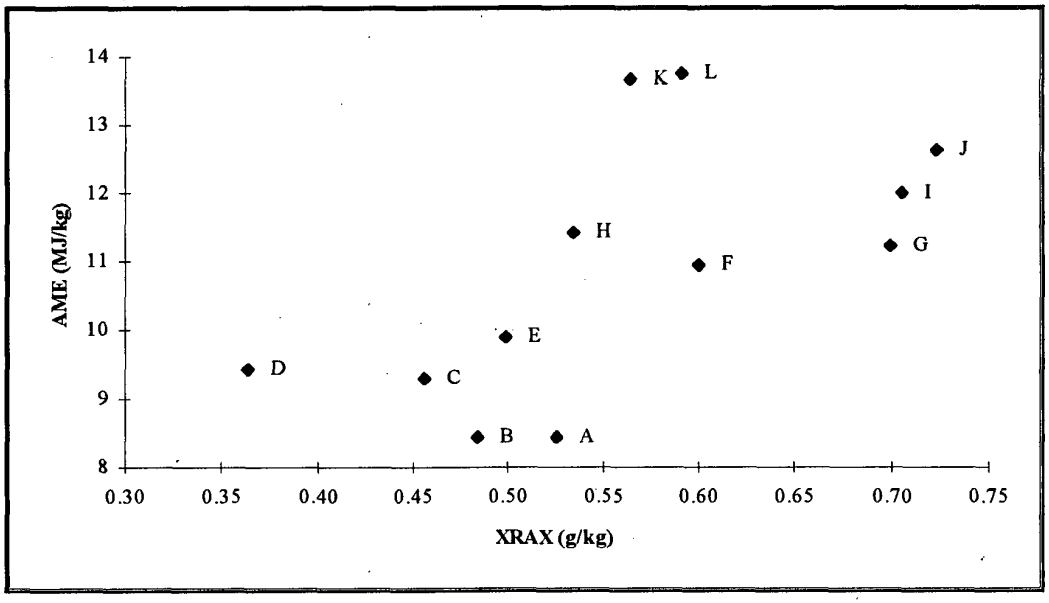


Fig 45: Plot of AME against soluble XRAX content of wheat

The xylanase-resistant arabinoxylan has a high degree of branching. Inclusion of this as part of an arabinoxylan would imply the occurrence of large areas of unbranched xylan in the chain which may account for the depression of AME. Large areas of unbranched xylan are required to allow the close proximity of polysaccharides needed for the formation of the large inter-molecular networks which increase viscosity (and depress AME).

CONCLUSIONS

The basic premise for this work was that the depression of AME shown by some samples of wheat fed to broiler chicks related not only to the amount of NSP solubilised during digestion, but was also a product of the structure of the released polymer(s). Twelve wheat samples were selected from a larger number of samples whose AME had been previously determined at Nottingham University (Wiseman *et.al.* 1993) to evenly cover a wide range of AME values. Any simple relationship existing between AME, the amount of soluble NSP and solution viscosity should have been readily apparent with these samples. However, unlike the earlier observations made with Australian wheat (Annison 1991, Choct & Annison 1990 and 1992), no such relationships were seen with the UK-grown samples. It is probable that varietal differences and the range of growing conditions were more extreme in Australia than currently are encountered in the UK. Certainly the inclusion threshold above which nutritional problems materialise seems lower in Australia and appreciably below the 60-65% inclusion level at which anti-nutritional effects appear with some UK wheat.

The lack of any clear relationship with *in vitro* viscosity was surprising in the light of the commonly held view that soluble NSP produces its anti-nutritional effects by increasing the viscosity of the unstirred layer overlying the intestinal wall of the upper gut, delaying uptake of nutrients and secretion of digestive enzymes. The wheat extracts evidently did not sufficiently represent the gut contents. The absence of soluble starch, selectively hydrolysed and removed during extraction, probably accounted for the major difference. Starch and NSP may well combine to produce the increased viscosity observed in the upper digestive tract *in vivo*. Protein-polysaccharide interactions did not appear important.

Several factors were identified which contribute to the depression of AME produced by some wheat samples. The amount of arabinoxylan and mixed-linked glucan present in the grain, which directly reflected the amount of soluble NSP that could be extracted, was a factor. However, the contribution of amount to the overall variation was small. Of greater importance were the chain length (molecular weight) of the polymers and, in the case of the branched arabinoxylan, the extent and distribution of

branching. In general, branching mitigates against inter-chain interaction and so prevents the formation of gels. The more highly branched arabinoxylan would not be expected to greatly increase viscosity and consequently not to depress NSP. While this held true for high molecular weight arabinoxylan, the reverse was found for arabinoxylans of lower chain-length. The space-filling properties of highly-branched oligomers dominated and led to an increase in viscosity although not by a process of gel formation. Many forms of processing of grain, particularly heat processing, are likely to reduce molecular weight and to lead to an increase in digesta viscosity by this second route. Multivariate analysis is needed to determine the relative importance of these various interlocking factors. The 12 samples used her allowed only a maximum of three variables to be considered which proved insufficient to account for all of the variation in AME.

MALDI, although accurately measuring mass, is not generally used to quantify the relative abundance of each mass fragment present. However this capacity proved particularly valuable when MALDI was used in conjunction with the xylanase digests. The enzyme cleaved the arabinoxylan polymers adjacent to a branch point and, apparently, needed at least three contiguous unbranched units to cleave between branch points. This gave rise to a series of characteristics oligomers whose molecular weight allowed each arabinoxylan extract to be fingerprinted in terms of the structural units contributing to the polymer and their relative abundance. Using this method, substantial differences in structure were demonstrated between the 12 wheat samples examined. MALDI itself is rapid, taking only one or two minutes to perform, and it is possible that this method could be further developed to rapidly characterise wheat samples, probably in conjunction with discriminate analysis to distinguish problem wheat from those which do not depress AME.

Although most arabinoxylan was solubilised from the thin endosperm walls, a substantial proportion derived from the lignified tissues of the pericarp and testa. Arabinoxylans of primary cell walls such as the juvenile wall of the endosperm are characteristically highly-branched with arabinose to xylose ratio approaching unity. Such polymers, providing they remain intact and of high molecular weight are less likely to contribute to digesta viscosity. In contrast, the xylans of secondary-thickened

tissues are less extensively branched and more likely to interact in solution. Increasingly severe forms of grain processing, in addition to solubilising NSP from the endosperm, will solubilise proportionally more xylan and glucan from the outer seed layers. The more linear xylan from these layers may increase the likelihood of gelation and consequently the need for enzyme supplementation to reduce molecular weight.

The cloned xylanase used was essentially a single enzyme product free from any de-branching activities. The ability of this enzyme alone to substantially reduce the molecular size of wheat arabinoxylan demonstrated that only a single activity with the same or similar mode of action is necessary to overcome any anti-nutritional effects of wheat arabinoxylan. In practice, a supplementary enzyme preparation could be further improved by the addition of a β-glucanase and α-arabinofuranosidase, although this is probably not essential.

Although the importance of the structural features of wheat arabinoxylans was confirmed by this work, a number of issues remain unresolved. Interactions between mixed-linkage glucan and arabinoxylan and the galactose/mannose component of the grain were observed to affect AME and would warrant further investigation. While *in vivo* viscosity is closely related to AME, the viscosity of *in vitro* extracts did not provide any predictive capacity. A better *in vitro* method of estimating gut viscosity is required, perhaps by retaining any solubilised starch in polymeric form. Finally, although arabinoxylan solubility, size, degree of branching and the distribution of those branches along the polymer backbone contribute to any anti-nutritional properties demonstrated, their manner of interaction has not been sufficiently defined to allow the characteristics of a wheat to be evaluated *in vitro*. This remains an important objective since it is evident that enzyme supplementation is not required for all wheat samples or for all processing conditions. Broiler producers can ill afford the additional loss of margin represented by the cost of unnecessary enzyme addition.

REFERENCES

Almirall, M, Francesch, M, Perezvendrell, AM, Brufau, J, and Estevegarcia, E (1995) The differences in intestinal viscosity produced by barley and beta-glucanase alter digesta enzyme-activities and ileal nutrient digestibilities more in broiler chicks than in cocks. *Journal of Nutrition* **125**: 947-955.

Aman, P, and Hesselman, K (1984) Analysis of starch and other main constituents of cereal-grains. *Swedish Journal of Agricultural Research* **14**: 135-139.

Annison, G (1992) Commercial enzyme supplementation of wheat-based diets raises ileal glycanase activities and improves apparent metabolizable energy, starch and pentosan digestibilities in broiler-chickens. *Animal Feed Science and Technology* **38**: 105-121.

Annison, G (1991) Relationship between the levels of soluble nonstarch polysaccharides and the apparent metabolizable energy of wheats assayed in broiler- chickens. *Journal of Agricultural and Food Chemistry* **39**: 1252-1256.

Austin, S. (1996) *Structural Characterisation of Anti-nutritional Polysaccharides in Wheat.* PhD Thesis, University of Nottingham, UK.

Bax, A, and Davis, DG (1985) MLEV-17-based two-dimensional homonuclear magnetization transfer spectroscopy. *Journal of Magnetic Resonance* **65:** 355-360.

Bedford, MR (1995) Mechanism of action and potential environmental benefits from the use of feed enzymes. *Animal Feed Science and Technology* **53**: 145-155.

Bedford, MR, and Classen, HL (1992) Reduction of intestinal viscosity through manipulation of dietary rye and pentosanase concentration is effected through changes in the carbohydrate-composition of the intestinal aqueous phase and results in improved growth-rate and food conversion efficiency of broiler chicks. *Journal of Nutrition* **122**: 560-569.

Bengtsson, S, Andersson, R, Westerlund, E, and Aman, P (1992) Content, structure and viscosity of soluble arabinoxylans in rye grain from several countries. *Journal of the Science of Food and Agriculture* **58**: 331-337.

Blakeney, A.B., Harris, P.J., Henry, R.J. & Stone, B.A. (1983) A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydrate Research* **113**: 291-299.

Blumenkrantz, N., and Asboe-Hansen, G. (1973) New Method for Quantitative Determination of Uronic Acids. *Analytical Biochemistry* **54**: 484-489.

Brenes, A, Smith, M, Guenter, W, and Marquardt, RR (1993) Effect of enzyme supplementation on the performance and digestive- tract size of broiler-chickens fed wheat-based and barley-based diets. *Poultry Science* **72**: 1731-1739.

Burnett, G.S. (1966), Studies of viscosity as the probable factor involved in the improvement of certain barleys for chickens by enzyme supplementation. *British Poultry Science* **7:** 55-75

Choct, M, and Annison, G (1992) The inhibition of nutrient digestion by wheat pentosans. *British Journal of Nutrition* **67**: 123-132.

Choct, M, and Annison, G (1990) Antinutritive activity of wheat pentosans in broiler diets. *British Poultry Science* **31**: 811-821.

Choct, M, Hughes, RJ, Trimble, RP, Angkanaporn, K, and Annison, G (1995) Nonstarch polysaccharide-degrading enzymes increase the performance of broiler-chickens fed wheat of low apparent metabolizable energy. *Journal of Nutrition* **125**: 485-492.

Davis, DG, and Bax, A (1985) Assignment of complex h-1-nmr spectra via two-dimensional homonuclear hartmann-hahn spectroscopy. *Journal of the American Chemical Society* **107:** 2820-2821.

Dubois, M.,, Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956) Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**: 350-356.

Ebringerova, A., Hromadkova, Z., Burchard, W., Dolega, R. & Vorwerg, W. (1994) Solution properties of water-insoluble rye-bran arabinoxylan. *Carbohydrate Polymers* **24**: 161-169.

Fengler, A.I., Marquard, R.R. (1988) Water soluble pentosans from rye: 2 effects on rate of dialysis and on the retention of nutrients by the chick. *Cereal Chemistry* **65**: 298-302.

Frigard, T., Pettersson, D. & Aman, P. (1994) Fibre-degrading enzyme increases body weight and total serum cholesterol in broiler chicks fed a rye-based diet. *Journal of Nutrition* **124**: 2422-2430.

Holmes, B. (1992), The compounders viewpoint on cereal quality. *Proceedings of the Cereals R & D Conference*, 135-152, Home-Grown Cereals Authority

Izydorczyk, M, Biliaderis, CG, and Bushuk, W (1991) Physical-properties of water-soluble pentosans from different wheat- varieties. *Cereal Chemistry* **68**: 145-150.

Izydorczyk, MS, and Biliaderis, CG (1995) Cereal arabinoxylans - advances in structure and physicochemical properties. *Carbohydrate Polymers* **28**: 33-48.

Kessler, H., Griesinger, C., Kerssebaum, R., Wagner, K., and Ernst, R.R. (1987) Separation of cross-relaxation and j-cross-peaks in 2d rotating-frame nmr-spectroscopy. *Journal of the American Chemical Society* **109:** 607-609.

Marquardt, RR, Boros, D, Guenter, W, and Crow, G (1994) The nutritive-value of barley, rye, wheat and corn for young chicks as affected by use of a *Trichoderma reesei* enzyme preparation. *Animal Feed Science and Technology* **45**: 363-378.

Mccleary, B.V., and Codd, R. (1991) Measurement of (1-3),(1-4)-beta-d-glucan in barley and oats - a streamlined enzymatic procedure. *Journal of the Science of Food and Agriculture* **55:** 303-312.

Pettersson, D, Graham, H, and Aman, P (1991) The nutritive-value for broiler-chickens of pelleting and enzyme supplementation of a diet containing barley, wheat and rye. *Animal Feed Science and Technology* **33**: 1-14.

Rogel, A.M., Annison, E.F., Bryden, W.L., Balnave, D. (1987) The digestion of wheat starch in broiler chickens. *Australian Journal of Agricultural Research*, **38**: 639-649.

Saulnier, L, Peneau, N, and Thibault, JF (1995) Variability in grain extract viscosity and water-soluble arabinoxylan content in wheat. *Journal of Cereal Science* **22**: 259-264.

van der Klis, JD, Kwakernaak, C, and de Wit, W (1995) Effects of endoxylanase addition to wheat-based diets on physicochemical chyme conditions and mineral absorption in broilers. *Animal Feed Science and Technology* **51**: 15-27.

Vinkx, CJA, Reynaert, HR, Grobet, PJ, and Delcour, JA (1993) Physicochemical and functional-properties of rye nonstarch polysaccharides .5. variability in the structure of water-soluble arabinoxylans. *Cereal Chemistry* **70**: 311-317.

Vliegenthart, J.F.G., Hoffmann, R.A. and Kamerling, J.P. (1992), A ^{1}H-NMR spectroscopic study on oligosaccharides obtained from wheat arabinoxylans. In: *Xylans and Xylanases*, Visser, J., Beldman, G., Kusters van Somern, M.A. and Voragen, A.G.J. (eds), 17-37, Elsevier, The Netherlands.

Vranjes, MV, and Wenk, C (1995) The influence of extruded vs untreated barley in the feed, with and without dietary enzyme supplement on broiler performance. *Animal Feed Science and Technology* **54**: 21-32.

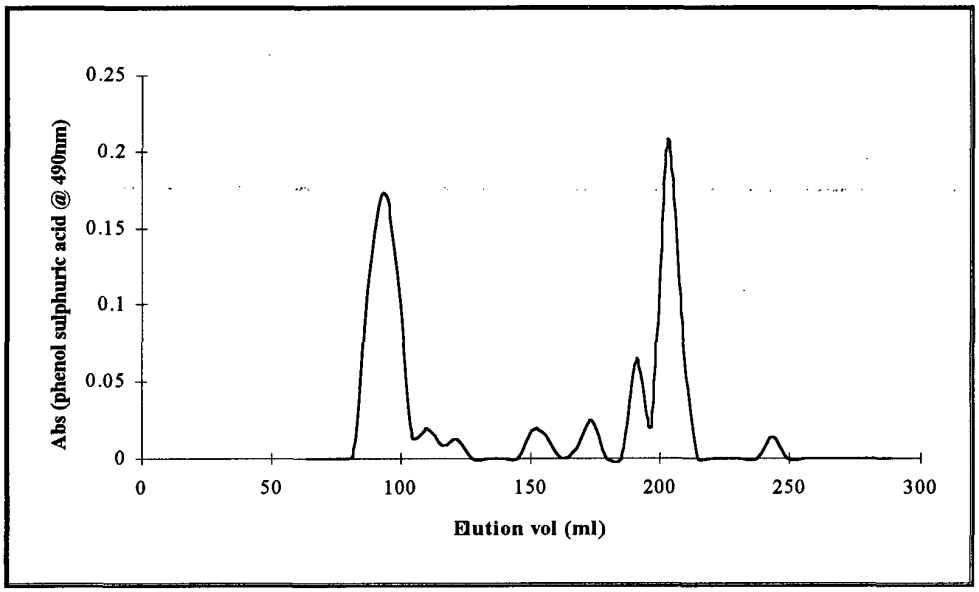
White, W.B., Bird, H.R., Sunde, M.L., Prentice, N., Burger, W.C. & Marlett, J.A. (1981) The viscosity interaction of barley beta-glucan with *Trichoderma viride* cellulase in the chick intestine. *Poultry Science* **60**: 1043-1048.

Wiseman, J. & Inborr, J. (1990) The nutritive value of wheat and it's effect on broiler performance. In: *Recent Advances in Animal Nutrition*, Haresign, W. and Cole, D.J.A. (eds), 79-102, Butterworths, London.

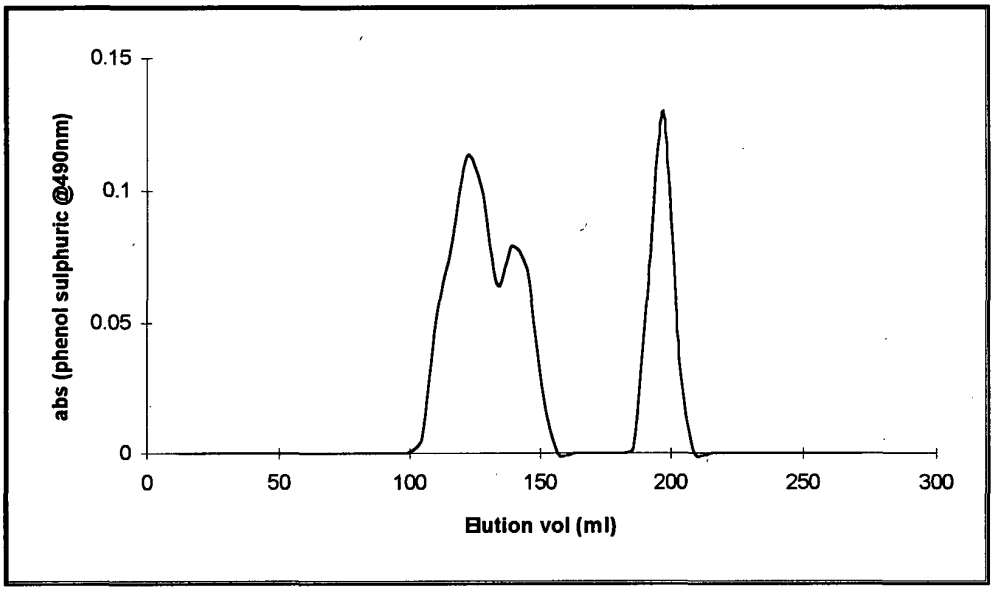
Wiseman, J., Nicol, N., and Norton, G. (1993) Biochemical and nutritional characterisation of wheat varieties for young broilers. *Aspects of Applied Biology* **34**: 329-334.

APPENDIX 1

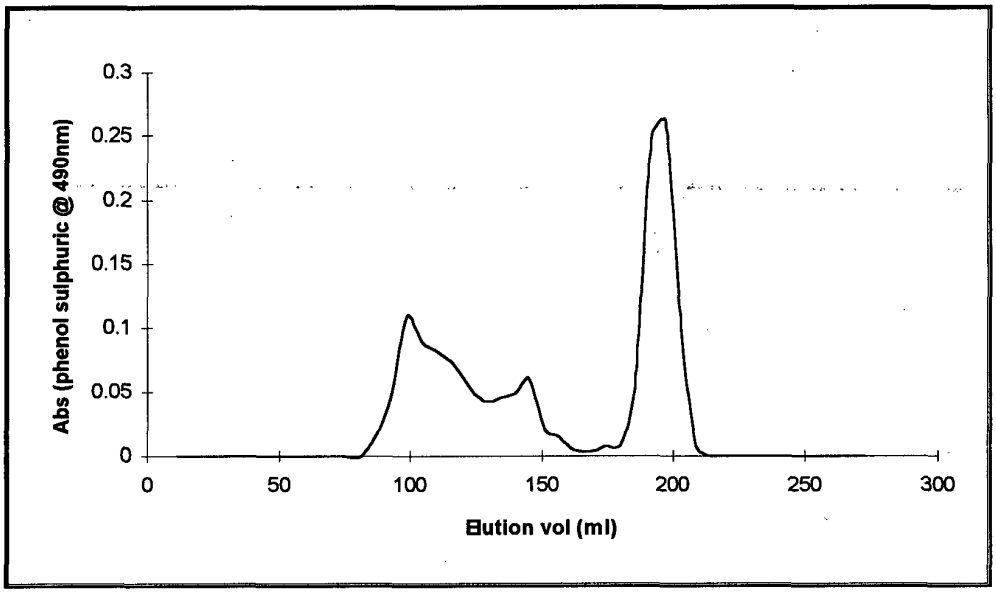
Size exclusion chromatograms of soluble NSP



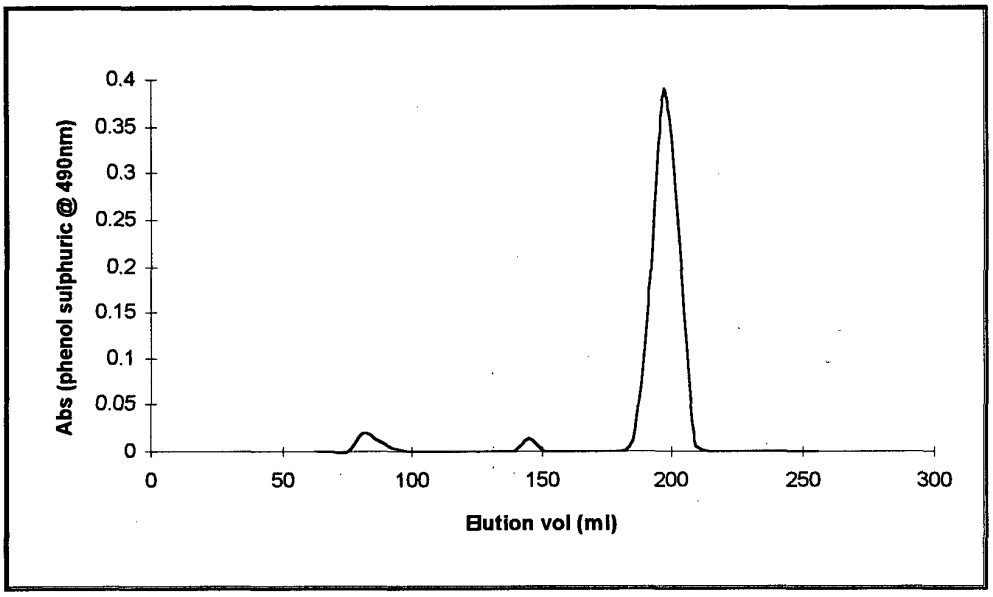
Elution profile of soluble NSP from wheat A



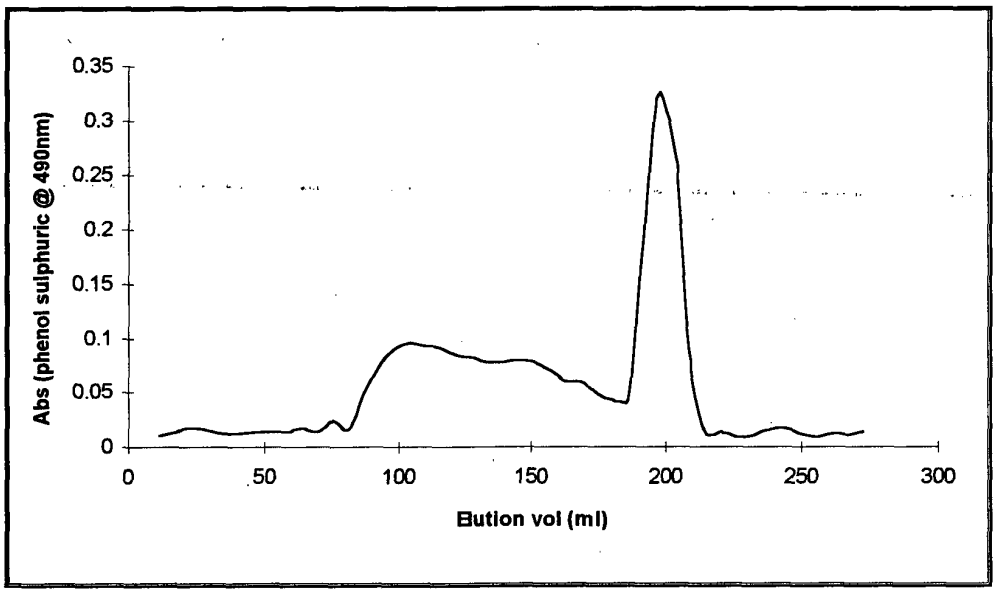
Elution profile for soluble NSP from wheat B



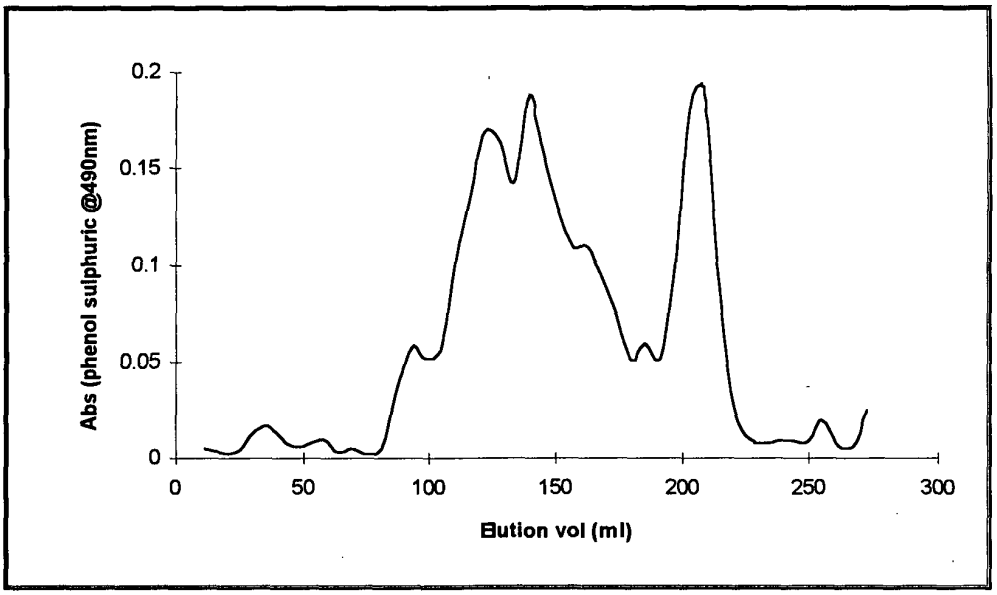
Elution profile of soluble NSP from wheat C



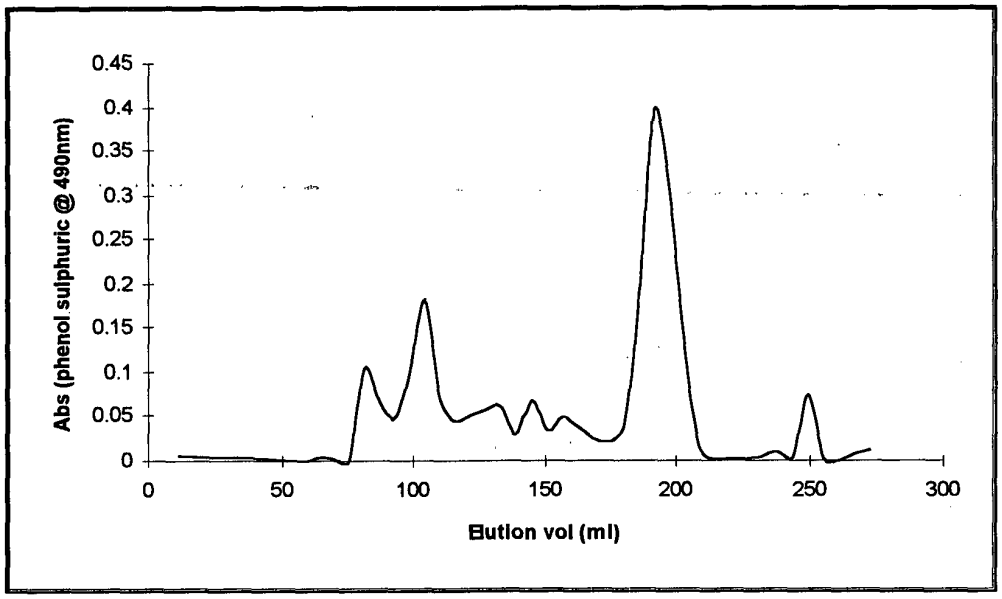
Elution profile of soluble NSP from wheat D



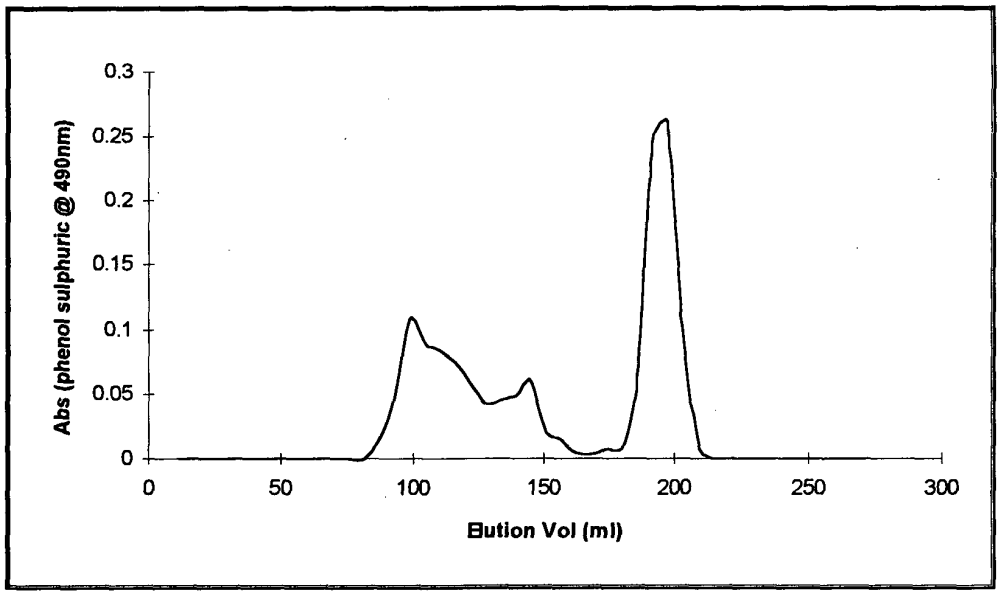
Elution profile for soluble NSP from wheat E



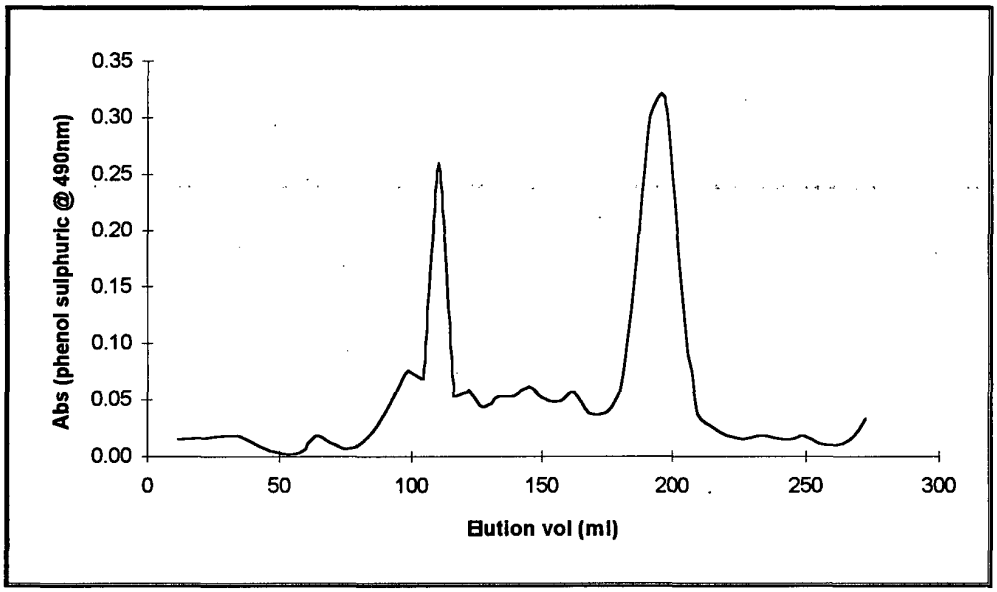
Elution profile of soluble NSP from wheat F



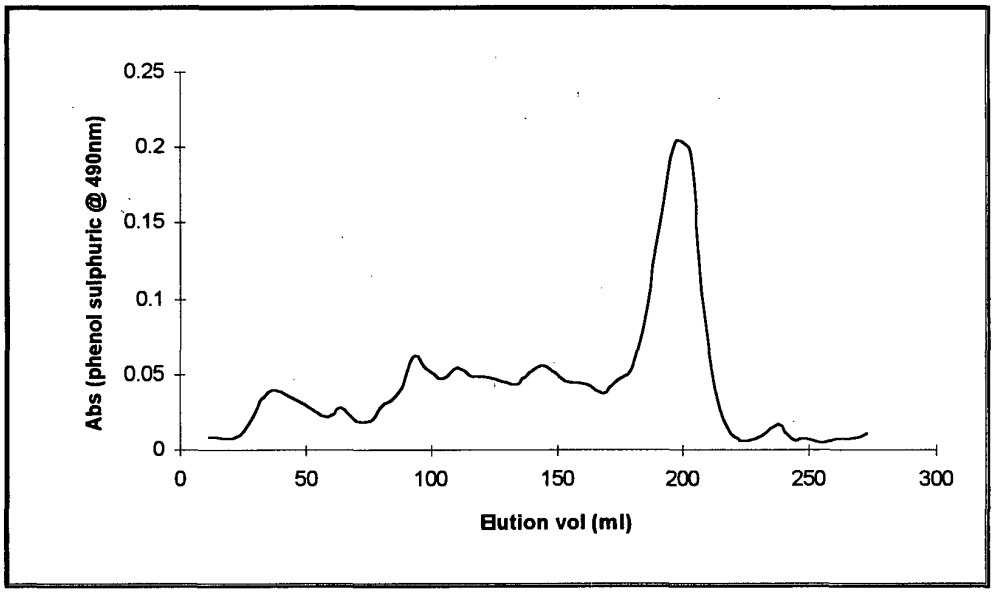
Elution profile for soluble NSP from wheat G



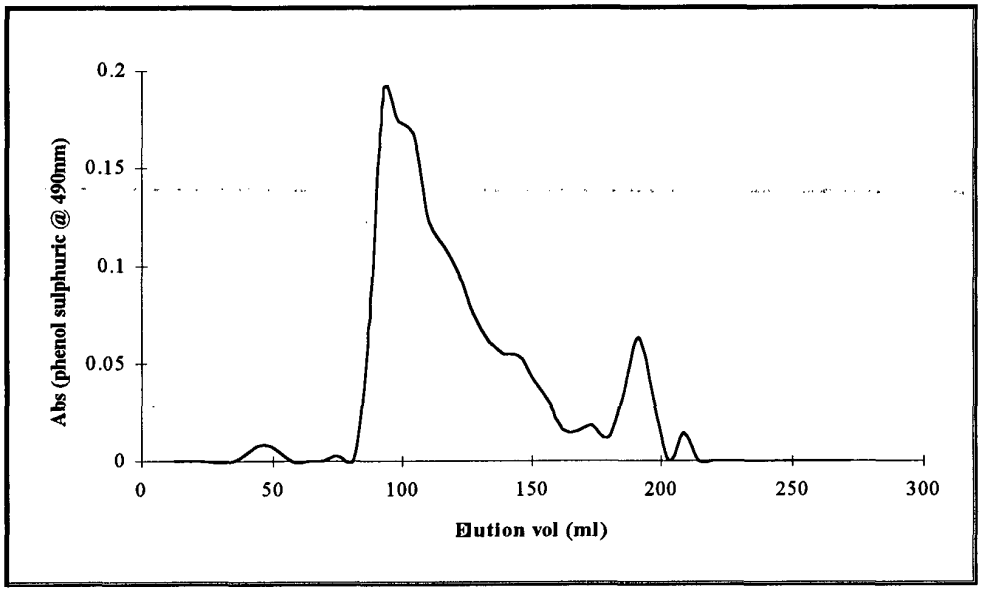
Elution profile for soluble NSP from wheat H



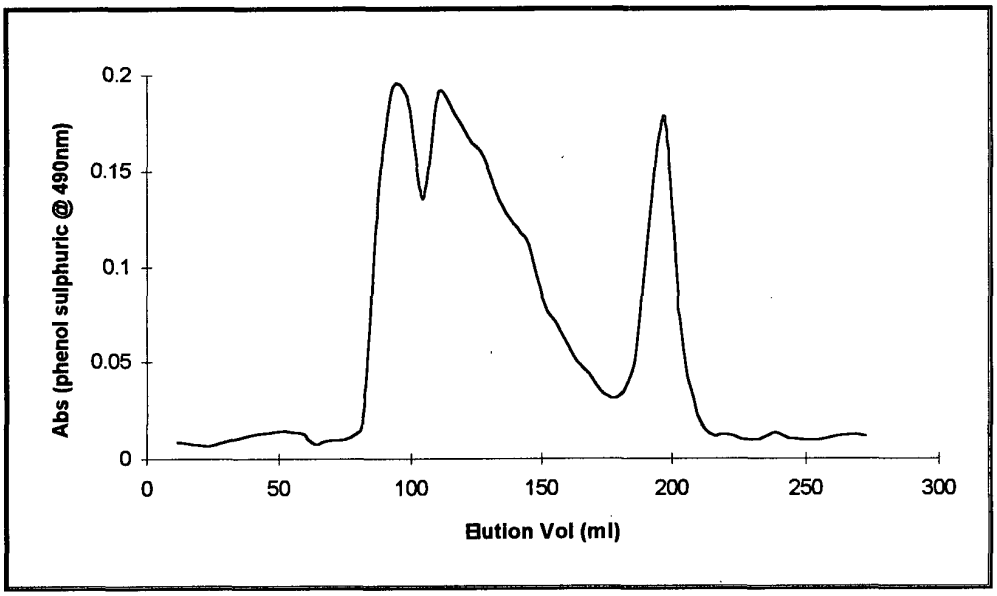
Elution profile for soluble NSP from wheat I



Elution profile for soluble NSP from wheat J



Elution profile of soluble NSP from wheat K



Elution profile of soluble NSP from wheat L

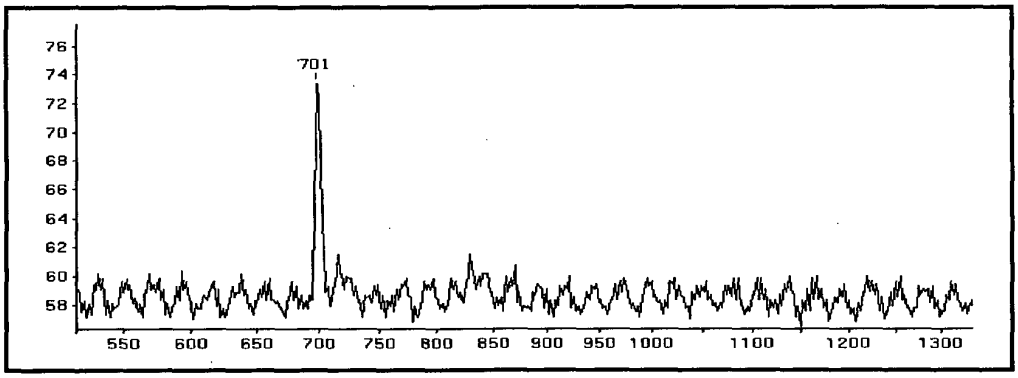
APPENDIX 2

Identification of arabinoxylan oligosaccharides

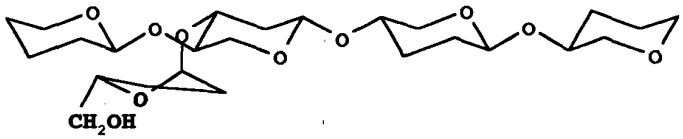
^{1}H-NMR chemical shifts for protons of oligosaccharide AX-5

Residue	H-1	H-2	H-3	H-4	H-5e/R	H-5a/S
α-Xyl*p*-1	5.183	*	*	*	*	*
β-Xyl*p*-1	4.583	3.235	3.539	3.768	4.042	3.365
β-Xyl*p*-2	4.475	3.260	3.539	3.783	4.096	3.365
β-Xyl*p*-3	4.512	3.430	3.736	3.810	4.114	3.260
β-Xyl*p*-4	4.440	3.235	3.401	3.583	3.903	3.257
α-Ara*f*$_{3x3}$	5.385	4.146	3.883	4.260	3.783	3.710

Xylp-1 denotes \xx at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc. Ara*f*$_{3x3}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p*-3. Chemical shifts are expressed in ppm relative to external DSS. An * means that the signals for the α anomer were not resolved from those of the β anomer.



MALDI of oligosaccharide AX-5

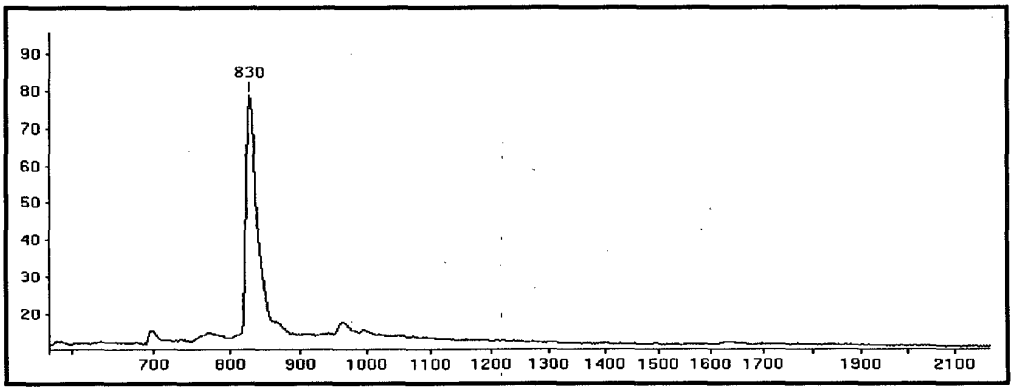


Structure of oligosaccharide AX-5

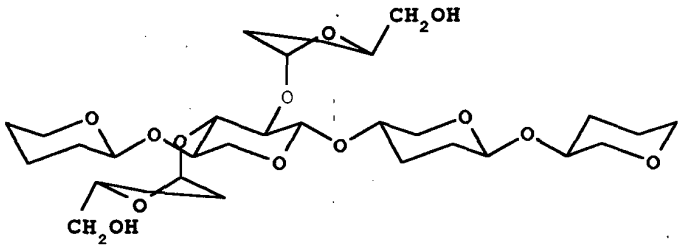
^{1}H-NMR chemical shifts for protons of oligosaccharide AX-6

Residue	H-1	H-2	H-3	H-4	H-5e / H-5R	H-5a / H-5S
α-Xyl*p*-1	5.167	*	*	*	*	*
β-Xyl*p*-1	4.568	3.233	3.545	3.764	4.036	3.361
β-Xyl*p*-2	4.449	3.263	3.547	3.777	4.114	3.398
β-Xyl*p*-3	4.623	3.577	3.769	3.824	4.134	3.426
β-Xyl*p*-4	4.421	3.277	3.422	3.602	3.926	3.251
α-Araf_{2X3}	5.208	4.093	3.944	4.134	3.805	3.701
α-Araf_{3X3}	5.257	4.147	3.906	4.287	3.763	3.692

Xyl*p*-1 denotes xylopyranose at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc. Araf_{2X3} denotes arabinofuranose side chain joined at position 2 of Xyl*p* 3, Araf_{3X3} denotes arabinofuranose side chain joined at position 3 of Xyl*p* 3. Chemical shifts are expressed in ppm relative to external DSS. An * means that the signals for the α anomer were not resolved from those of the β anomer.



MALDI of oligosaccharide AX-6

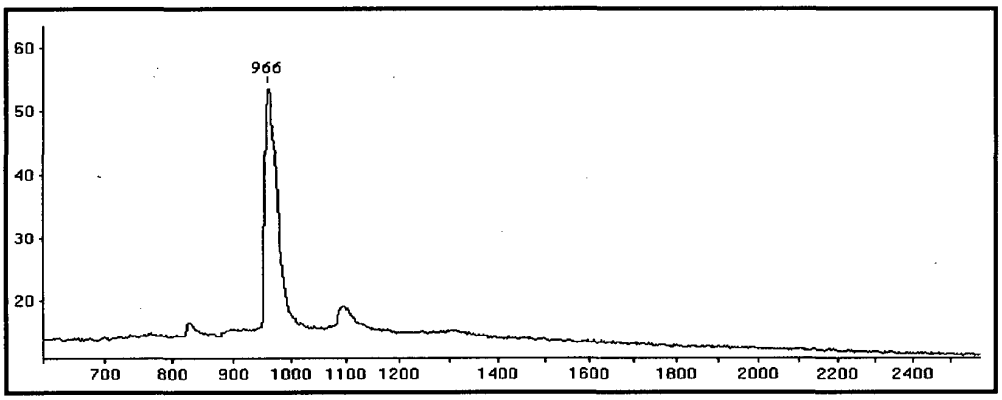


Structure of oligosaccharide AX-6

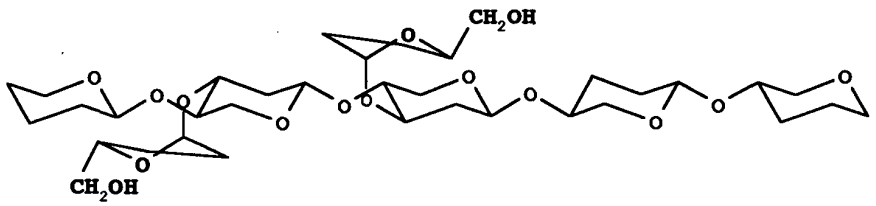
^{1}H-NMR assignments for oligosaccharide AX-7

Residue	H-1	H-2	H-3	H-4	H-5e / H-5R	H-5a / H-5S
α-Xyl*p*-1	5.178	*	*	*	*	*
β-Xyl*p*-1	4.579	3.243	3.542	3.763	4.033	3.369
β-Xyl*p*-2	4.472	3.286	3.545	3.783	4.083	3.359
β-Xyl*p*-3	4.509	3.434	3.732	3.820	4.110	3.396
β-Xyl*p*-4	4.483	3.424	3.733	3.784	4.074	3.359
β-Xyl*p*-5	4.426	3.230	3.404	3.588	3.903	3.269
α-Ara*f*$_{3X3}$	5.382	4.160	3.912	4.267	3.804	3.719
α-Ara*f*$_{3X4}$	5.392	4.154	3.903	4.267	3.804	3.709

Xyl*p*-1 denotes xylopyranose at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc., Ara*f*$_{3X3}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p* 3, Ara*f*$_{3X4}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p* 4. Chemical shifts are expressed in ppm relative to external DSS. An * means that the signals for the α anomer were not resolved from those of the β anomer.



MALDI of oligosaccharide AX-7

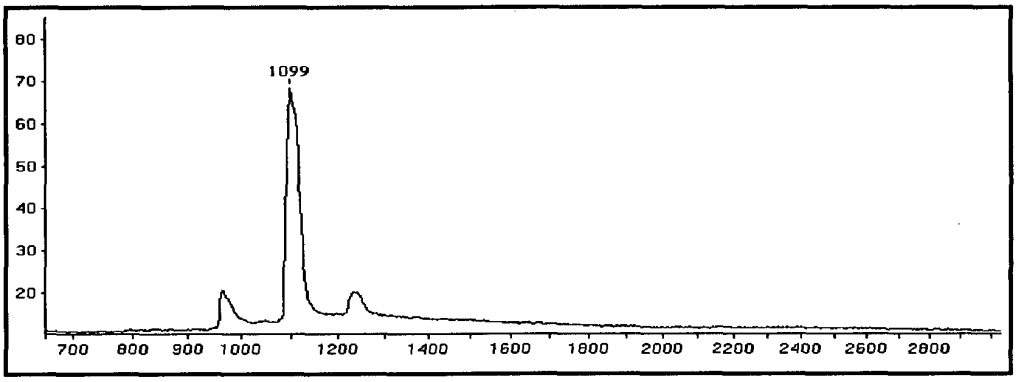


Structure of oligosaccharide AX-7

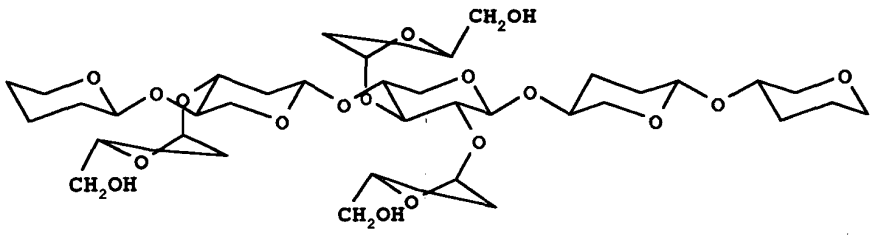
^{1}H-NMR assignments for the structure reporting groups of oligosaccharide AX-8

Residue	H-1
α-Xyl*p*-1	5.179
β-Xyl*p*-1	4.580
β-Xyl*p*-2	4.474
β-Xyl*p*-3	4.635
β-Xyl*p*-4	4.487
β-Xyl*p*-5	4.432
α-Ara*f*$_{2X3}$	5.222
α-Ara*f*$_{3X3}$	5.268
α-Ara*f*$_{3X4}$	5.393

Xyl*p*-1 denotes xylopyranose at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc., Ara*f*$_{2X3}$ denotes arabinofuranose side chain joined at position 2 of Xyl*p* 3, Ara*f*$_{3X3}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p* 3, etc. Chemical shifts are expressed in ppm relative to external DSS.



MALDI of oligosaccharide AX-8

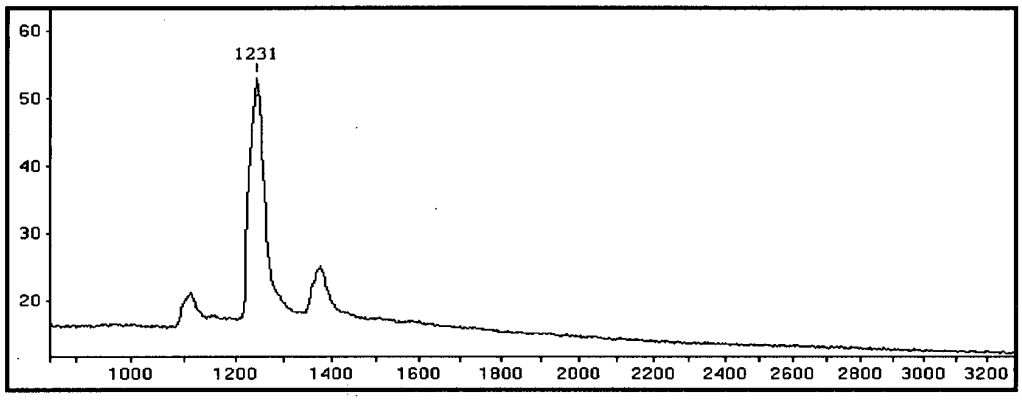


Structure of oligosaccharide AX-8

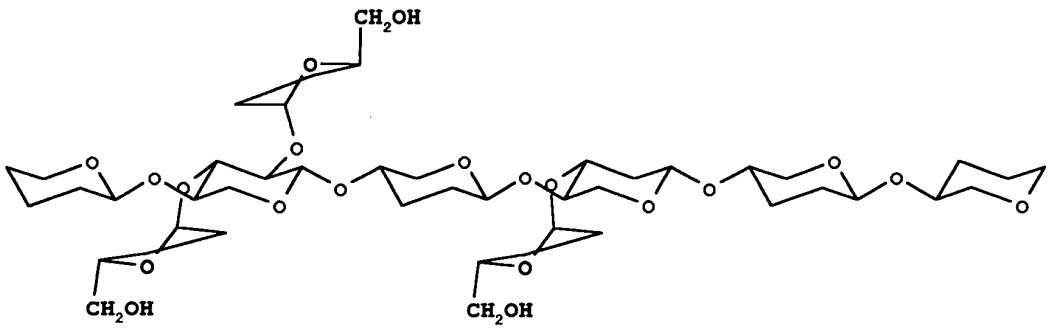
^{1}H-NMR assignments for the structure reporting groups of oligosaccharide AX-9

Residue	H-1
α-Xyl*p*-1	5.179
β-Xyl*p*-1	4.580
β-Xyl*p*-2	4.477
β-Xyl*p*-3	4.509
β-Xyl*p*-4	4.463
β-Xyl*p*-5	4.634
β-Xyl*p*-6	4.437
α-Ara*f*$_{3X3}$	5.387
α-Ara*f*$_{2X5}$	5.218
α-Ara*f*$_{3X5}$	5.267

Xyl*p*-1 denotes xylopyranose at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc., Ara*f*$_{3X3}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p* 3, Ara*f*$_{2X5}$ denotes arabinofuranose side chain joined at position 2 of Xyl*p* 5, etc. Chemical shifts are expressed in ppm relative to external DSS.



MALDI of oligosaccharide AX-9

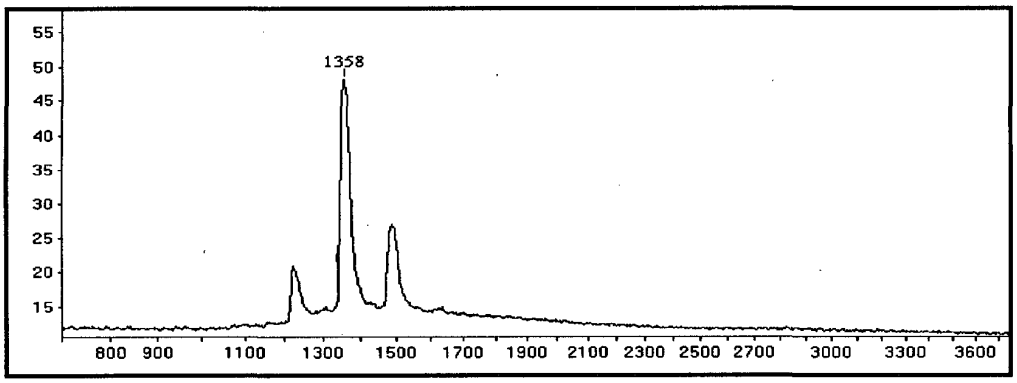


Structure of oligosaccharide AX-9

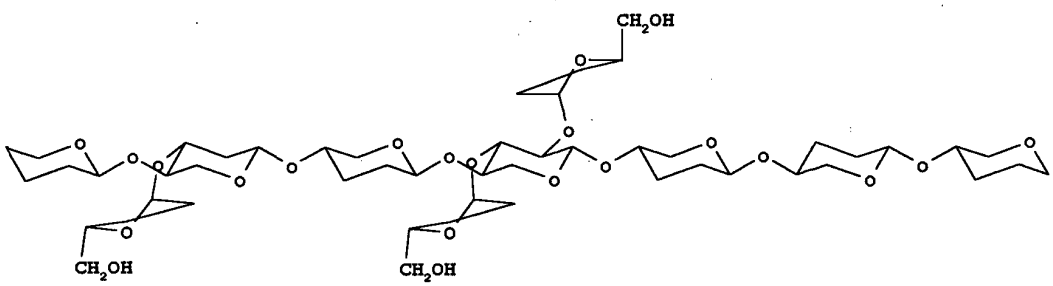
^{1}H-NMR assignments for the structure reporting groups of oligosaccharide AX-10

Residue	H-1
α-Xyl*p*-1	5.176
β-Xyl*p*-1	4.577
β-Xyl*p*-2	4.482
β-Xyl*p*-3	4.469
β-Xyl*p*-4	4.631
β-Xyl*p*-5	4.459
β-Xyl*p*-6	4.506
β-Xyl*p*-7	4.432
α-Ara*f*$_{2X4}$	5.215
α-Ara*f*$_{3X4}$	5.264
α-Ara*f*$_{3X6}$	5.385

Xyl*p*-1 denotes xylopyranose at the reducing end, Xyl*p*-2 denotes the next xylose in the chain etc., Ara*f*$_{2X4}$ denotes arabinofuranose side chain joined at position 2 of Xyl*p* 4, Ara*f*$_{3X4}$ denotes arabinofuranose side chain joined at position 3 of Xyl*p* 4, etc. Chemical shifts are expressed in ppm relative to external DSS.



MALDI of oligosaccharide AX-10



Structure of oligosaccharide AX-10