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ScienceDirect
Food Chemistry 102 (2007) 707–715
Food Chemistry
www.elsevier.com/locate/foodchem

Preparation and analysis of dietary fibre constituents in whole grain from hulled and hull-less barley

S.H. Knutsen [a,*], A.K. Holtekjølen [a,b]

[a] MATFORSK – Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway
[b] Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, P.O. Box 5003, N-1432, Norway

Received 21 January 2006; received in revised form 22 May 2006; accepted 3 June 2006

Abstract

Sixteen different barley samples (7 of which were hull-less; H-L) were milled and subjected to pre-extraction with ethanol and hexane. Water soluble materials (WSM-TP-AI), containing mostly β-glucans, were purified from the hot water extracts by the use of heat-stable amylase. Crude arabinoxylans were extracted by an alkali solution (WUM-B-S) and purified by the use of β-glucanase and amyloglucosidase, giving AX. Finally insoluble fibre residues were obtained (WUM-B-R). GC and NMR analyses revealed no marked quantitative and qualitative differences of β-glucans or the water-soluble arabinoxylans in WSM-TP-AI between the H-L and the hulled (H) varieties. Significant differences among the two barley types were found in the Ara/Xyl ratio of the starting materials as well as the alkali soluble material (WUM-B-S) and the alkali insoluble residue (WUM-B-R). For alkali soluble AX the H samples had the lowest arabinose content. A single-tube preparative isolation procedure for starch-free barley fibre was used. Combined with NMR and GC this is a tool to produce defined fibre fractions for biological testing.

Keywords: Hulled and hull-less barley; Fractionation; Fibre; Arabinoxylans; β-glucans; NMR

1. Introduction

The dominating fibre components in barley are the β-glucans and the arabinoxylans, located mainly in the cell walls of the endosperm and the aleurone layer cell walls (Oscarsson, Andersson, Salomonsson, & Åman, 1996). In general, β-glucans are water soluble whereas only a part of the AX are soluble in water (Izydorczyk, Macri, & MacGregor, 1998a, 1998b). These polysaccharides are important constituents of dietary fibres and influence the nutritional values and functional properties of food. High intakes of cereal fibre (or whole grain) also seem to lower the risk of cancer (Han, Lee, Moon, Jo, & Rhee, 2004), coronary heart disease, and diabetes (Lu, Walker, Muir, & O'Dea, 2004). In the hulled barley genotypes (H) the kernels do not loose their hull during threshing, whereas for the hull-less (H-L) types, the hull is lost during this process. For food uses the hull-less barley varieties therefore are considered beneficial since they are easier to mill and nutrients are not lost due to pearling (Berglund, Fastnaught, & Holm, 1992).

Several advanced preparation methods for water-soluble arabinoxylans in cereals have been proposed (Faurot et al., 1995; Irakli, Biliaderis, Izydorczyk, & Papadoyannis, 2004; Izydorczyk & Biliaderis, 1995; Loosveld, Grobet, & Delcour, 1997; Trogh et al., 2004). For water insoluble components, barium hydroxide has been used as a selective extractant (Gruppen, Hamer, & Voragen, 1991) and sometimes combined with ammonium sulphate fractionations (Izydorczyk et al., 1998a, Izydorczyk, Macri, & MacGregor, 1998b).

A recent work focused on the gross content of different polysaccharides present in a large number of barley varieties (Holtekjølen, Uhlen, Bråthen, Sahlstrøm, & Knutsen, 2006) based on a common method for determination of

* Corresponding author. Tel.: +47 6497 0334; fax: +47 6497 0333.
E-mail address: svein.knutsen@matforsk.no (S.H. Knutsen).

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doi:10.1016/j.foodchem.2006.06.006

non-starch polysaccharides (Englyst & Cummings, 1984). This method has been designed for a quantitative determination of total and water insoluble fibre and is destructive by its application of acidic hydrolysis and hence no fibre fractions are prepared. In this present work 16 barley varieties were selected from the original sample set as noted above, and re-grown at one locality. A simplified procedure for constituent sugar analysis (Blakeney, Harris, Hemry, & Stone, 1983), including glucose from starch, was performed in addition to a single-tube preparative procedure for starch-free fibre fractions. This allowed the handling of a large number of samples simultaneously, and to collect quite detailed quantitative and qualitative information regarding the produced fractions when combining with NMR-spectroscopy. The work does not aim for a detailed investigation of the tissue-induced variation in the material, but for a characterisation of whole grain sources and a selection tools for breeders. Furthermore, by providing reasonable amounts of well-defined fractions, possible biological effects of different cereal constituents can be studied (Knutsen et al., 2004).

2. Experimental

2.1. Biological source

The 16 barley varieties, previously selected from a larger number of barley varieties, originating from different countries (Holtekjølen et al., 2006), were re-grown at the same location in Norway. The different barley varieties with their identifying numbers and characteristics are defined in Table 1. Hulled and hull-less varieties are denoted as H and H-L in the text when appropriate. Initially no pearling has been performed for H-samples. The extraction procedures as well as the corresponding fractions obtained are explained in Fig. 1.

2.2. Grinding and pre-extraction

The barley grains were milled in a small scale grinding apparatus (Retch, Type ZM1, Germany) with sieve size 0.5 mm. Ground barley materials (GCM) (2.00 g) were initially placed in a 50 ml screw capped glass tube as the starting materials for the following successive extractions. The samples were first dispersed in 25 ml 70% (v/v) ethanol and boiled for 15 min. After cooling and a slow speed centrifugation (10 min, 1500*g*) the residues were washed with another portion of 70% ethanol and finally with 96% ethanol. The remaining alcohol insoluble materials (AIS) were extracted twice with 30 and 15 ml hexane by continuous shaking. In general, all separation of extracts into supernatants and residues were achieved by slow speed centrifugation of the glass tube (15 min, 1500*g*). No supernatants from these steps were analysed during the present work. These former solvent treatments inactivated endogenous enzymes and removed low molecular sugars and fat. To avoid phase separation in the later aqueous extractions, incubation of the tubes with the pre-extracted and de-fatted material (DFM) on a water bath until apparent dryness, was necessary.

2.3. Extraction of polysaccharides

After adding 1 ml of 70% (v/v) ethanol for moistening, DFM was added 50 ml water and pH was monitored to be approximately pH 6 with a pH paper. Samples were heated and 300 μL thermo-stable amylase (T) (Termamyl 120L, Novo Nordisk, Denmark) were added and the samples were boiled for 2 h in a water bath with periodical mixing on a vortex. The supernatant containing the water-soluble amylase treated material (WSM-T) was decanted off and the residue was re-extracted for 15 min in a boiling water bath. The 2 supernatants (WSM-T) were combined and added Pancreatine (P) (porcine pancreas, SIGMA, USA) and filtered through Whatman GF/D and GF/F filters. Polysaccharides were precipitated by adding 2 volumes of isopropanol and left overnight at 4 °C. After washing 2 times with 60% isopropanol, to remove oligosaccharides from the starch degradation, the precipitates were re-solubilised in water and subjected to dialysis (Medicell International, 12 kD cut-off). Finally the protease treated alcohol insoluble (P-AI) polysaccharides were freeze-dried, giving WSM-TP-AI.

The remaining water insoluble material in the tube (WUM), was subjected to an alkali extraction with 20 ml of 1 M NaOH with 1% $NaBH_4$ (w/v) at room temperature for 30 min. After centrifugation the base soluble (B-S) polysaccharides were precipitated by adding 1 volume of isopropanol, washed twice with 60% isopropanol and one time with pure isopropanol, resuspended in water, dialysed and freeze-dried, giving WUM-B-S.

In order to purify the arabinoxylans of WUM-B-S, co-extracted β-glucans and starch were first degraded by lichenase (Megazyme International Ireland, Ltd. Bray, Ireland) at 40 °C in 50 mM phosphate buffer at pH 6.5 and then with amyloglucosidase (Megazyme) at 60 °C in sodium acetate buffer at pH 4.5. The degradation products were removed by precipitation of the remaining polysaccharides with 2 volumes of isopropanol and subsequent washing with 60% isopropanol and dialysis. Arabinoxylans (AX) were obtained after freeze-drying.

2.4. Constituent sugars

GCM, DFM and the obtained fibre fractions were analysed for constituent sugars by gas chromatography (GC) as alditol acetates (Blakeney et al., 1983). After a two step acidic hydrolysis; first in 12 M H_2SO_4 at 35 °C for 60 min and then in 1.5 M H_2SO_4 at 100 °C for 60 min. 2-deoxy-D-Glucose was added as an internal standard before the final reduction and derivatisation steps. Alditol acetates were analysed by using a Hewlett Packard 5890 Gas Chromatograph with a flame ionisation detector, auto-sampler HP 7673 and He as carrier gas. A DB-23 capillary column

Table 1
The barley varieties studied and some characteristics related to their arabinoxylan content

Barley varieties		A/X-ratio (±SD)						Yields				
Name	Type	GCM	DFM	WSM-TP-AI	WUM-B-S	WUM-B-R	AX	WSM-TP-AI	WUM-B-S	WUM-B-R	Total Ara + Xyl	% Recovery
1. Thule	6rd H N	0.49 ± 0.01	0.52 ± 0.04	0.66 ± 0.03	0.41 ± 0.01	0.57 ± 0.01	0.39 ± 0.01	0.54	3.29	3.25	7.08	89
2. Olsok	6rd H N	0.51 ± 0.01	0.41 ± 0.02	0.56 ± 0.01	0.44 ± 0.01	0.48 ± 0.02	0.42 ± 0.01	0.59	2.81	3.52	6.92	91
3. NK96300	6rd H N	0.46 ± 0.08	0.48 ± 0.03	0.68 ± 0.04	0.42 ± 0.01	0.43 ± 0.04	0.41 ± 0.01	0.33	2.60	3.05	5.98	79
4. Åker	6rd H N	0.49 ± 0.06	0.43 ± 0.04	0.58 ± 0.01	0.43 ± 0.01	0.61 ± 0.05	0.42 ± 0.01	0.69	3.47	3.46	7.61	96
5. Tyra	2rd H N	0.47 ± 0.01	0.43 ± 0.02	0.61 ± 0.01	0.44 ± 0.01	0.53 ± 0.01	0.41 ± 0.01	0.54	3.22	3.24	7.00	91
6. Justina	2rd H N	0.46 ± 0.02	0.46 ± 0.03	0.68 ± 0.01	0.42 ± 0.01	0.51 ± 0.02	0.43 ± 0.01	0.36	2.36	3.19	5.91	81
7. Olve	2rd H N	0.51 ± 0.01	0.49 ± 0.03	0.58 ± 0.03	0.46 ± 0.01	0.55 ± 0.06	0.45 ± 0.01	1.06	3.43	3.56	8.05	96
8. Otira	2rd H N	0.42 ± 0.03	0.45 ± 0.02	0.66 ± 0.01	0.43 ± 0.01	0.48 ± 0.04	0.42 ± 0.01	0.26	2.27	3.64	6.17	87
9. CDC Dolly	2rd H N	0.52 ± 0.03	0.38 ± 0.02	0.53 ± 0.02	0.4 ± 0.01	0.59 ± 0.03	0.41 ± 0.04	0.31	2.77	2.90	5.98	97
Average (Hulled)		0.48	0.45	0.62	0.43	0.53	0.42	0.52	2.91	3.31	6.74	90
SD (Hulled)		0.03	0.04	0.06	0.02	0.06	0.02	0.25	0.46	0.25	0.78	6
10. NK95003	2rd H-L N	0.63 ± 0.01	0.64 ± 0.02	0.66 ± 0.03	0.55 ± 0.01	0.91 ± 0.03	0.53 ± 0.01	0.65	2.33	1.84	4.82	80
11. CDC Dawn	2rd H-L N	0.66 ± 0.01	0.66 ± 0.03	0.64 ± 0.01	0.58 ± 0.01	0.81 ± 0.01	0.56 ± 0.01	0.58	2.31	1.85	4.75	91
12. CDC Gainer	2rd H-L N	0.68 ± 0.01	0.62 ± 0.04	0.57 ± 0.01	0.57 ± 0.01	0.77 ± 0.02	0.55 ± 0.01	0.45	2.20	2.01	4.66	79
13. CDC McGwire	2rd H-L N	0.66 ± 0.01	0.68 ± 0.01	0.54 ± 0.01	0.57 ± 0.01	0.92 ± 0.01	0.57 ± 0.01	0.34	1.44	1.59	3.36	62
14. CDC Candle	2rd H-L W	0.71 ± 0.01	0.64 ± 0.01	0.64 ± 0.01	0.55 ± 0.01	0.84 ± 0.05	0.56 ± 0.01	0.41	1.76	1.73	3.90	75
15. CDC Alamo	2rd H-L W	0.67 ± 0.01	0.68 ± 0.01	0.63 ± 0.01	0.55 ± 0.01	0.89 ± 0.04	0.55 ± 0.01	0.64	1.94	1.80	4.39	80
16. SB94897	2rd H-L Ha	0.62 ± 0.01	0.65 ± 0.01	0.47 ± 0.01	0.53 ± 0.01	0.91 ± 0.02	0.53 ± 0.01	0.63	2.66	1.66	4.94	71
Average (Hull-less)		0.66	0.66	0.59	0.56	0.86	0.55	0.53	2.09	1.78	4.40	77
SD (Hull-less)		0.03	0.02	0.07	0.02	0.06	0.02	0.13	0.41	0.14	0.58	9

The ratio between Ara and Xyl (A/X) in the starting materials and in the different fractions are shown as well as average values for the two groups: hulled or hull-less. Yields are given as the crude arabinoxylan content (Ara + Xyl) in each fraction relative to GCM (% w/w). Recovery (%) refers to the amounts of summarised Ara + Xyl in the isolated fractions relative to the Ara + Xyl contents in GCM.
6rd = 6 rowed, 2rd = 2 rowed, H = hulled, H-L = Hull-less, N = normal starch, W = waxy starch, Ha = high amylose starch.

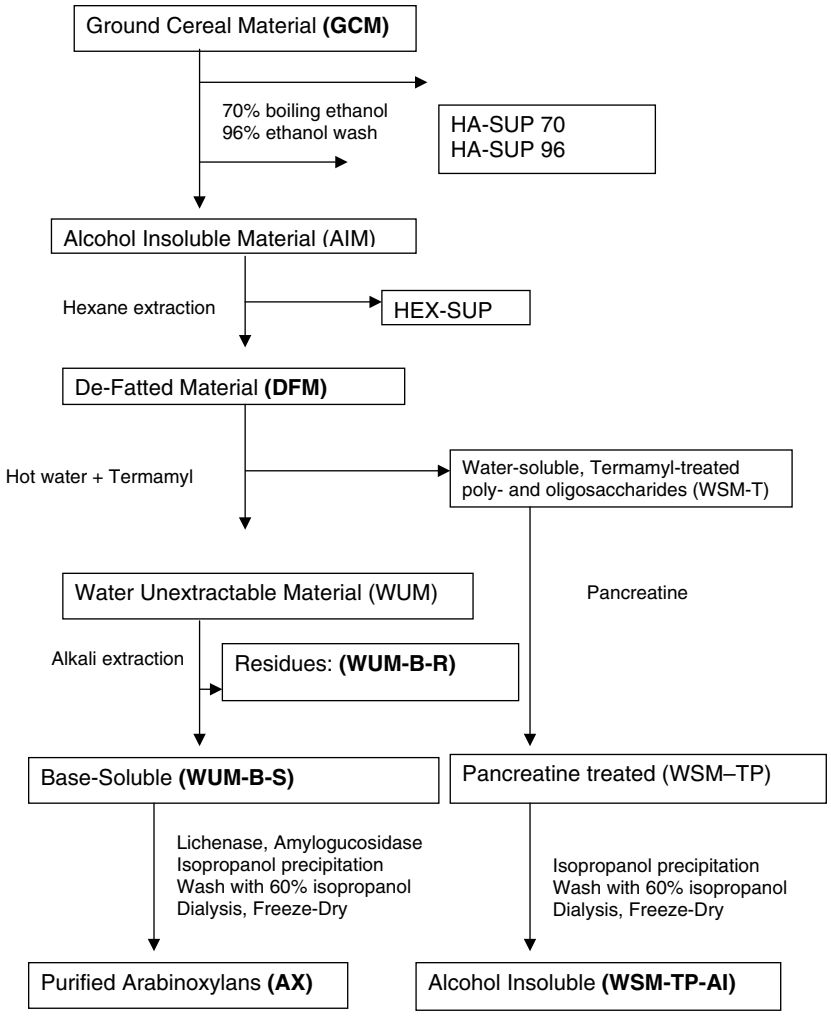

Fig. 1. A fractionation scheme for the preparation of barley fibre. Only fractions in bold letters have been subjected to analysis.

(30 m × 0.25 mm ID, film thickness 0.25 μm) was used. With the injector and detector operating at 250 °C, the following temperature program was used: 100 °C as initial temperature, increased by 10 °C/min to 190 °C and by 4 °C/min to 230 °C and finally held for 20 min. Injection size for samples and standards were 0.5 μl. Samples were run in duplicates with relative standard deviations (RSD) ranging from 0% to 5%.

The predominant sugars; glucose, xylose and arabinose are termed Glc, Xyl and Ara, respectively. A/X is reserved for the Ara/Xyl ratio as estimated by GC whereas AX refers to the purified arabinoxylans (see Fig. 1).

2.5. NMR-spectroscopy

Selected NMR spectra were obtained on a Varian Mercury 300 system. For ^{13}C NMR spectroscopy samples (10–15 mg) were dissolved directly in 0.6 ml D_2O in NMR-glass tubes and spectra were recorded at 80 °C with DMSO as an internal standard (δ 39.6 ppm). Typical ^{13}C NMR proton decoupled spectra were recorded by 30,000 repetitions, with acquisition time of 1.6 s, a pulse delay of 2 s, a 85° pulse and with a 16,000 Hz sweep width.

For ^{1}H NMR spectroscopy samples (5 mg) were dissolved in 0.7 ml D_2O, dried by vacuum centrifugation (SpeedVac ISS110, Thermo Savant), re-dissolved in D_2O and transferred to NMR-tubes. Spectra were recorded at 80 °C or 90 °C with typical 64 repetitions, using an acquisition time 2.7 s, a 45° pulse angle and a pulse delay of 1 s. Chemical shifts (δ) are given in ppm relative to that of TMS, but measured indirectly via acetone (δ 2.225) (Knutsen & Grasdalen, 1992). An in-house starch sample isolated from oat and purified by β-glucanase as well as a commercial arabinoxylan standard (Megazyme International Ireland, Ltd. Bray, Ireland) were used to obtain reference spectra in order to detect these constituents in the fractions. For plotting and presentation, the original free induction decay (FID) data were reprocessed by the software MestRe-C2.3a (http://www.mestrec.com). Before Fourier transformation a 5 Hz line broadening function was applied for the ^{13}C NMR spectra and 0.5 Hz for the ^{1}H NMR spectra.

The arabinoxylan in the purified AX fractions were characterised based on the anomeric region of their ^{1}H NMR spectra and the Ara/Xyl ratio obtained from GC (A_{gc}/X_{gc}). The resonance areas in the ^{1}H NMR spectra

used for the calculation on arabinoxylans were I_h at 5.39 ppm (from the anomeric protons of arabinose linked to the O-3 position of xylose residues) and II_h at 5.29 ppm and III_h 5.22 ppm (from the anomeric protons of arabinose residues linked to O-2 and O-3 of the same xylose residue). For later, the subscripts "h", "gc" and "calc" identify determinations based on ^{1}H NMR data, GC data and calculated areas, respectively (see below and Eqs. (1)–(6)).

Due to the partly overlapping of the anomeric signals of β-D-Xyl*p* with the resonance of residual water (HOD), the theoretical area of H-1 anomeric signals of β-Xyl (i.e. IV_{calc}) was calculated from the A_{gc}/X_{gc} and the sum of all anomeric signals of arabinose ($I_h + II_h + III_h$) (Eq. 1).

$$\frac{A_{gc}}{X_{gc}} = \frac{I_h + II_h + III_h}{IV_{calc}} \quad (1)$$

Furthermore, as suggested elsewhere (Trogh et al., 2004; Vinkx, Reynaert, Grobet, & Delcour, 1993), the O-2 substituted xylose can not be analysed directly by its anomer resonance since this overlaps with the signal at 5.29 (II_h). The area contribution of O-2 substituted xylose (V_h) was therefore determined as the difference between II_h and III_h (Oscarsson et al., 1996). In general the arabinose substitution patterns of xylose are given as the fraction of the different possibilities, summarised to unity (see Eq. 2).

$$F_{mono\ 3\text{-}O} + F_{mono\ 2\text{-}O} + F_{2,3\ di\text{-}O} + F_{unsubstituted} = 1 \quad (2)$$

The fractions of the different xylose residues are then calculated as below.

$$F_{mono\ 3\text{-}O} = \frac{I_h}{IV_{calc}} \quad (3)$$

$$F_{mono\ 2\text{-}O} = \frac{II_h - III_h}{IV_{calc}} \quad (4)$$

$$F_{2,3\text{-}di\text{-}O} = \left[\left(\frac{II_h + III_h}{2}\right) - V_h\right] \times \left[\frac{1}{IV_{calc}}\right] \quad (5)$$

$$F_{unsubst} = 1 - (F_{mono\ 3\text{-}O} + F_{mono2\text{-}O} + F_{2,3di\text{-}O}) \quad (6)$$

For the β-glucans the areas of the resonances for the anomeric protons of 3-linked units at 4.75 ppm and of 4-linked units at 4.54 ppm in the ^{1}H NMR spectra were used for the calculations of the linkage ratios (Henriksson et al., 1995).

2.6. Statistical analysis

Analysis of variance and significant differences among means were tested by one-way ANOVA, using Minitab (version 13.3: Minitab Inc. state College, PA, USA). Significant differences were declared at $p < 0.05$.

3. Results

3.1. Extraction and recovering of the samples

The procedure used for the preparation and recovery is outlined in Fig. 1. The RSD of parallels in extraction yields from the same materials did in general not exceed ±10%. A final alcohol precipitation step combined with washing and dialysis were necessary in order to obtain polysaccharide of desired purity from the water and alkali extracts. By omitting precipitation and the following washing, hence only performing dialysis and freeze-drying, the alkali extracts gave rise to ^{1}H NMR spectra (spectra not shown) with several non-carbohydrate specific resonances, probably from proteins (Höije, Gröndahl, Tømmeraas, & Gatenholm, 2005).

By using this preparation procedure, typically eight samples in triplicates were processed in two days, excluding the time for dialysis and freeze-drying. In the present work, the alcohol and the hexane extracts were not investigated, but these can be utilised for analysis of low molecular weights sugars as well as some non-bound phytochemicals. For a larger scale preparation, the same system can be used by combining identical fractions instead of keeping individual parallels apart.

3.2. Yields of the different fractions

The yields of the fractions of the individual samples are shown in Fig. 2. The water soluble, starch-free fraction (WSM-TP-AI) ranged from 2.8% to 7.2% of the starting material with an average of 4.2% (SD = 1.1) for all samples. There were no significant differences between the yields obtained from H (1–9) or the H-L (10–16) samples. The yields of the subsequent alkali extracts (WUM-B-S), showed a smaller total variation, i.e. between 2.8% and 5.6%, with an average for all 16 samples of 4.4 (SD = 0.7). However, significant differences ($p = 0.026$) between WUM-B-S-yields of H (average 4.7%, SD = 0.6) and the H-L samples (average 3.9%, SD = 0.7) were observed. Finally the largest significant differences between the two types of barley were found in the residue weights (WUM-B-R) ($p < 0.000$), i.e. 11.5% (SD = 1.0) and 6.0% (SD = 0.4), for H and H-L, respectively.

3.3. The carbohydrate content of the different fractions

The total contents of carbohydrates in the three fractions of the 16 varieties, as estimated by GC, showed a large variation in the carbohydrate constituents within both barley groups. However, the only significant difference was the higher carbohydrate content of WUM-B-R from H-L samples, i.e. 36.1 (SD = 1.2) compared to 33.2 (SD = 1.2) for H samples. The dominating constituent sugars were Glc, Xyl and Ara. In addition Man and Gal were detected in minor amounts depending on the fraction (data not shown). For the purified AX, mannose was introduced by an excess use of amyloglucosidase that is high in manno-protein (personal communication). The relative distribution of the sugars constituting the different fractions, showed significant ($p < 0.05$) differences between H and H-L samples. Both residues (WUM-B-R) and the alkali extracts (WUM-B-S) varied significantly in their carbohy-

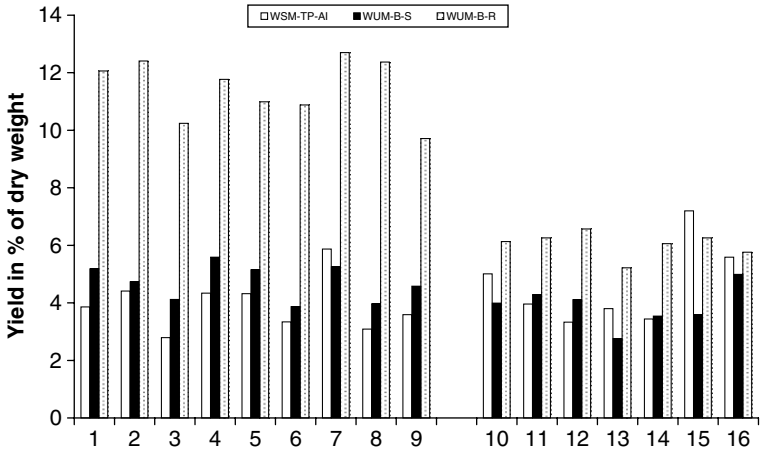

Fig. 2. Yields (% of dry matter) of the 3 fibre fractions of the Barley samples.

drate constituents when comparing the H or H-L varieties. These fractions were dominated by arabinoxylans. However, it can be noted that after purification into AX the content of Ara + Xyl were very similar and not significantly different between H and H-L samples. Similarly, for the WSM-TP-AI fractions, which contained mostly β-glucans and some co-extracted arabinoxylans (see below), no significant differences were detected regarding any sugar components between H and H-L barley samples.

3.4. The distribution of soluble and insoluble arabinoxylans and A/X ratios

The yields of the three fractions (Table 1) together with their actual content of Ara and Xyl provided an opportunity for a quantitative estimation of crude arabinoxylan in the different barley samples. All the H-samples had higher contents of crude arabinoxylans ($p < 0.000$) with an average of 6.7% compared to 4.4% in the H-L samples.

Based on the ratio between Ara and Xyl calculated from the GC-analyses, it is shown that the A/X-ratios in most cases were highest for the H-L samples (see Table 1). This indicated a higher Ara substitution of the xylan backbone in the H-L varieties.

3.5. Structural characteristics determined by NMR-spectroscopy

In general ^{13}C NMR spectroscopy was only used for qualitative investigation of the heterogeneity of the fractions (Hoffmann, Leeflang, Debarse, Kamerling, & Vliegenthart, 1991). It is clearly shown in Fig. 3 that a typical WSM-TP-AI fraction was dominated by β-glucans, while the WUM-B-S contained arabinoxylans, co-extracted starch and β-glucans.

The ratio between 1–4 and 1–3 linked β-D-Glc*p* of WSM-TP-AI was estimated by ^1H NMR (see Fig. 4) without any further purification. The calculations were based on the areas of the anomeric resonances of 3-linked (4.75 ppm) and 4-linked β-D-Glc*p* (4.54 ppm) in the ^1H NMR spectra. The linkage ratios of 4-linked/3-linked units were in the range from 2.3 to 2.7. The average ratio for the H and the H-L samples were 2.6 (SD = 0.1) and 2.5 (SD = 0.1), respectively. All values are given in Table 2 and the apparent higher contents of 3-linked units in the H-samples were not significant, although individual variations occurred.

After enzymic removal of β-glucans and starch from WUM-B-S, the purified arabinoxylans (AX) were analysed for their arabinose substitution pattern by combining A/X obtained from GC (A_{gc}/X_{gc}) and the anomeric signals of arabinose in the 5.2–5.4 ppm region in the ^1H NMR spectra (see Section 2: (1)–(6) and Fig. 4). This method is favourable since a HDO signal from residual water at approximately 4.25 ppm (80 °C) disturbs the baseline and hence gives rise to ambiguous area determinations for the anomeric signals of β-D-Xyl*p* residues at 4.65 and 4.47 ppm. Based on this calculation method, only the purified arabinoxylan fractions were analysed and showed some variation in the different substitution possibilities (Eqs. (2)–(6) in Section 2, see Table 2). The H samples had the highest content of non-substituted xylan residues ($p < 0.000$). On the other hand significant higher contents of 3-O substitution ($p < 0.000$), 2-O substitution ($p = 0.019$) and 2,3-di O-substitutions ($p < 0.000$) were found for the H-L samples. For the ratio between di- and mono-substitution no significant differences were detected.

4. Discussion

WSM-TP-AI fractions are in general devoid of starch and have a low level of arabinoxylans (8–15%), calculated as the sum of Ara and Xyl. With an average 10% correction for co-extracted arabinoxylan, the content of soluble β-glucans were calculated and varied from about 2.5% (sample 3; NK96300) to about 6.3% (sample 15; CDC Alamo) (data not shown). Also taking into account the presence of some water insoluble β-glucan, as recovered in the base extract

WUM-B-S, these values are in agreement with values estimated ezymically (Andersson, Elfverson, Andersson, Regner, & Åman, 1999; Holtekjølen et al., 2006; Storsley, Izydorczyk, You, Biliaderis, & Rossnagel, 2003). β-glucan fractions can be obtained in good yields both from hulled (e.g. Olve) as well as hull-less varieties (e.g. NK95003, CDC Alamo and SB94897). The highest ratios between 1–4 and 1–3 linked β-D-Glc*p* in β-glucans were found in the hulled 6-rowed types, but no significant differences in the linkage types in H and H-L barley types were detected. The variation in the calculated ratio (range; 2.3–2.7) is comparable to results obtained elsewhere (Henriksson et al., 1995; Irakli et al., 2004; Storsley et al., 2003), but very different from the ratio (range; 5–14) found in some special alkali solubilised fractions (Izydorczyk & MacGregor, 2000).

Some potential inconsistency is noted; Two representatives of waxy barley investigated in this present study showed a large difference in their yields in WSM-TP-AI, i.e. 3.4% for CDC Candle and 7.2% for CDC Alamo. This suggests a large difference in their amounts of soluble β-glucans. In an ongoing study similar β-glucan contents were determined (Megazyme, enzyme kit) to 6.1% and 6.4% for these specific samples, respectively. Previously, the total β-glucan contents of CDC Candle and CDC Alamo grown in Canada have been estimated to similar values, i.e. 6.9% and 6.1% (Storsley et al., 2003) and 6.4 and 7.2% (Megazyme enzyme kit) (Holtekjølen et al., 2006), respectively. As shown by NMR-spectroscopy WSM-TP-AI fractions are in general dominated by solubilised β-glucan. CDC Alamo differs due to a relative low content of total carbohydrates (data not shown) as well as a high content of arabinoxylans in this fraction (Table

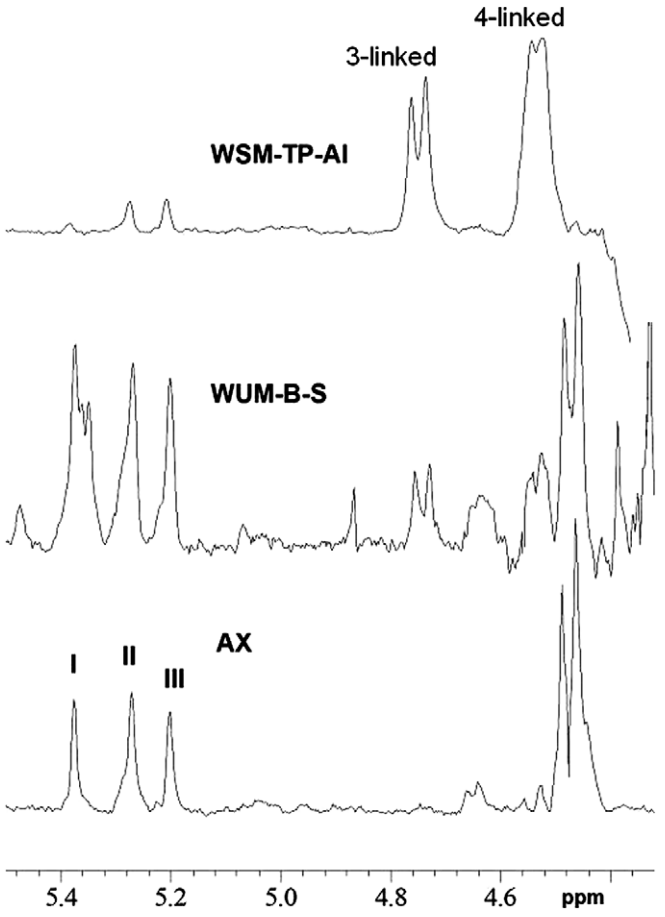

Fig. 4. Spectral regions of ^{1}H NMR spectra obtained from the solubilised fractions of the variety Olsok. Annotations on upper spectrum, 3- and 4-linked, refer to 3- and 4-linked β-D-Glc in β-glucan. For explanation of I, II and III, see Section 2.

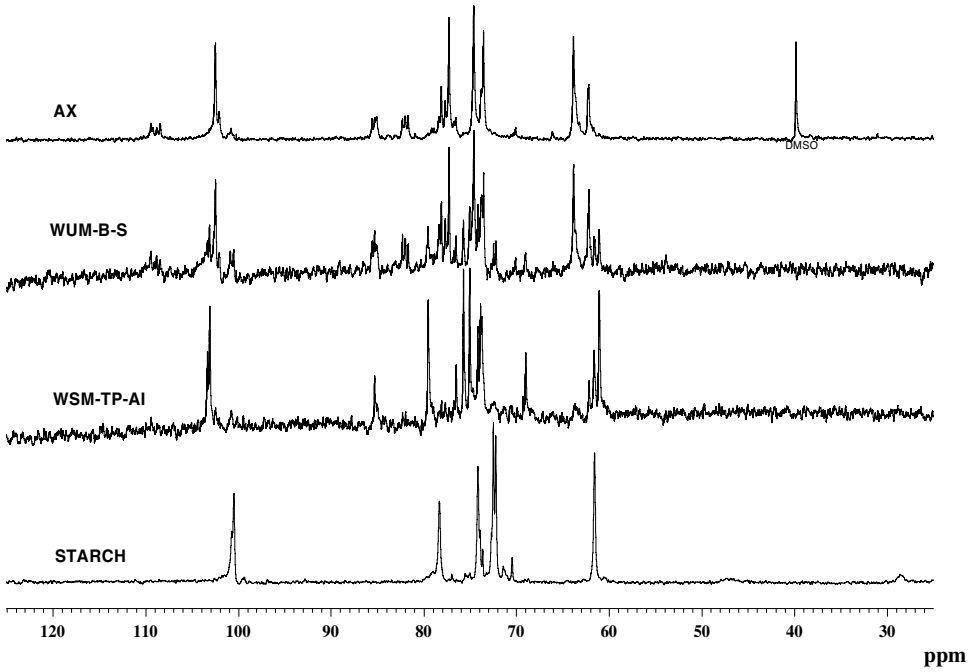

Fig. 3. Typical ^{13}C NMR spectra of the two soluble fractions and their common co-extracts; starch and arabinoxylans.

Table 2
The characteristic arabinose substitution pattern of enzyme purified alkali soluble arabinoxylans (AX) and the ratio between 1–4 and 1–3 linkages in water-soluble β-glucans (WSM-TP-AI) as estimated by ^{1}H NMR

Variety	$F_{mono3\text{-}O}$	$F_{mono2\text{-}O}$	$F_{2,3\text{-}di\text{-}O}$	$F_{unsubst}$	(1–4)/(1–3) ratio
1. Thule	0.12	0.06	0.11	0.72	2.73
2. Olsok	0.13	0.07	0.11	0.69	2.73
3. NK96300	0.11	0.06	0.12	0.71	2.76
4. Åker	0.11	0.03	0.14	0.72	2.59
5. Tyra	0.12	0.06	0.11	0.70	2.48
6. Justina	0.12	0.04	0.13	0.70	2.46
7. Olve	0.13	0.08	0.12	0.67	2.57
8. Otira	0.12	0.06	0.12	0.70	2.56
9. CDC Dolly	0.13	0.04	0.12	0.72	2.33
Hulled; Average	0.12	0.06	0.12	0.70	2.58
SD	0.01	0.02	0.01	0.02	0.14
10. NK95003	0.16	0.08	0.15	0.62	2.57
11. CDC Dawn	0.15	0.09	0.16	0.60	2.56
12. CDC Gainer	0.15	0.05	0.18	0.63	2.59
13. CDC McGwire	0.16	0.09	0.16	0.59	2.33
14. CDC Candle	0.15	0.07	0.17	0.61	2.35
15. CDC Alamo	0.16	0.08	0.15	0.60	2.50
16. SB94897	0.15	0.07	0.16	0.63	2.30
Hull-less; Average	0.15	0.07	0.16	0.61	2.46
SD	0.01	0.01	0.01	0.01	0.13

1). Moreover, quantitative and qualitative differences other than β-glucans in the hot water-soluble components, such as arabinoxylans and proteins, most probably exist between CDC Candle and CDC Alamo.

As noted above, the base solubilised fractions (WUM-B-S) contained starch and β-glucans in addition to the major component, arabinoxylan. These findings show that all β-glucans are not completely solubilised in hot water and that the amylase used does not penetrate the tissue efficiently. No quantitative or qualitative investigations were performed with respect to the starch components in this work. Although individual differences among the varieties were detected for the content of Ara and Xyl in WSM-TP-AI (Table 1), no systematic difference between H- and H-L samples were found. This was also the case for A/X of these water-soluble arabinoxylans (Fig. 4). Trials to characterise the arabinoxylans present in such fractions with ^{1}H NMR will be undertaken in future works. Based on the yields of the three fractions (see Table 1) and their Ara and Xyl content, the hulled samples contained on average 6.7% and the hull-less samples 4.4% total arabinoxylans. This is in agreement with other findings (Andersson et al., 1999; Storsley et al., 2003).

Approximately 90% (SD = 6) and 77% (SD = 9) of the total crude arabinoxylans in the starting materials were recovered in fractions obtained from H and H-L, respectively. This calculation is based on comparing the Ara and Xyl content of GCM with the Ara and Xyl content in the three corresponding fractions after correction for their actual yields. The apparent losses were attributed to that some of the Ara residues in the starting material might occur in arabino-galactan peptide moieties. These may be washed off during extraction (HA-SUP 70 and -96). In addition mechanical losses of polysaccharides during the isolation procedures will most likely appear. A small decrease in the A/X ratio due to solvent treatment, i.e. from GCM to DFM, may be caused by removal of arabino-galactan peptides. Similar A/X ratios of crude alkali soluble arabinoxylans (WUM-B-S) and the purified arabinoxylans (AX) suggest that negligible amount of arabinoxylans, if any were removed during purification. The very similar A/X-ratios found in the WSM-TP-AI fractions of H and H-L suggest that the water-soluble arabinoxylans of the barley varieties have a relative uniform structure.

The residual fractions (WUM-B-R) were not studied in detail except from the GC analysis. However, large differences in weights were observed when comparing H and H-L. A relative high content of Ara and Xyl indicated that the carbohydrate part of this fibre fraction was dominated by arabinoxylans, but the low contents of total carbohydrates were most probably due to water insoluble fibrous proteins and lignin.

5. Conclusions

It has been shown that a quite simple experimental design can handle a large number of samples. Although giving less detailed information as compared to methodology based on selective alkali extraction and ammonium sulfate precipitation important information was still obtained. Information regarding variation in the important fibre constituents of the individual barley varieties as well as some group characteristics of H and H-L barley were obtained. In addition the methodology is well suited for the concomitant preparation of well-characterised fibre fractions for study of biological activities.

Acknowledgements

The authors are grateful for the financial support from the Norwegian Research Council (Oslo, Norway) by grants NFR 140507 (AKH) and NFR 167863 (SHK). Stein Bergersen at Graminor AS (Bjørke Research Farm, Ilseng, Norway) is gratefully acknowledged for supplying barley samples.

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