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Enzymatic fingerprinting of arabinoxylan and β-glucan in triticale, barley and tritordeum grains

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ABSTRACT

Enzymatic fingerprinting of arabinoxylan (AX) and β-glucan using *endo*-xylanase and lichenase, respectively, helps determine the structural heterogeneity between different cereals and within genotypes of the same cereal. This study characterised the structural features of AX and β-glucan in whole grains of eight triticale cultivars grown at two locations, 20 barley cultivars/lines with wide variation in composition and morphology and five tritordeum breeding lines. Principal component analysis (PCA) resulted in clear clustering of these cereals. In general, barley and tritordeum had a higher relative proportion of highly branched arabinoxylan oligosaccharides (AXOS) than triticale. Subsequent analysis of triticale revealed two clusters based on growing region along principal component (PC) 1, while PC2 explained the genetic variability and was based on mono-substitution and di-substitution in AX fragments. PCA of β-glucan features separated the three cereals based on β-glucan content. The molar ratio of trisaccharide to tetrasaccharide was 2.5–3.4 in triticale, 2.3–3.3 in barley and 2.8–3.4 in tritordeum. Barley showed a strong positive correlation ($r = 0.86$) between β-glucan content and relative proportion of trisaccharide. The results show that structural features of AX and β-glucan vary between and within triticale, barley and tritordeum grains which might be important determinants of end-use quality of grains.

1. Introduction

Cell walls of cereal starchy endosperm and aleurone are rich in polysaccharides, particularly arabinoxylan (AX) and mixed linkage (1→3) (1→4)-β-D-glucan (β-glucan), where these polymers act as structural components (Cui & Wang, 2009; Fincher & Stone, 1986; Stone, 2006), but may also have metabolic activity related to cell function and development (Cosgrove, 1993). AX dominates in the endosperm cell walls of most cereals (60–70%), with the exception of oats and barley (≈20%) (Fincher & Stone, 1986; Matz, 1991). The backbone of AX consists of (1→4)-linked β-D-xylopyranosyl residues, which can be either mono-substituted by α-L-arabinofuranosyl residues at the O-2 or O-3 position or di-substituted at O-2 and O-3. Mono-substitution by α-L-arabinofuranosyl residues at O-2 is very rare in wheat and rye but frequent in barley (Izydorczyk & Dexter, 2008; Izydorczyk, Macri, & MacGregor, 1998a; Saulnier & Quemener, 2009; Viëtor, Angelino, & Voragen, 1992). Variation in degree and pattern of substitution by α-L-arabinofuranosyl residues along the xylan backbone and degree of polymerisation (DP) of the xylan backbone are important structural features of rye AX and contribute to structural heterogeneity and physico-chemical properties (Vinkx & Delcour, 1996). For example, AX with decreasing α-L-arabinofuranosyl residues become less soluble due to the formation of aggregates (Andrewartha, Phillips, & Stone, 1979). The structural features of wheat flour AX may also determine the end-use quality of cereals, since wheat flour with a higher proportion of di-substitution has good bread-making properties (Cleemput, Roels, Van Oort, Grobet, & Delcour, 1993). Furthermore, the extent of substitution controls the action of hydrolytic enzymes and highly branched AX molecules are less prone to enzymatic degradation (Vinkx & Delcour, 1996). Since the *endo*-xylanase action is dependent on the structure of AX, the hydrolysis products can be used to explore structural diversity between and within different cereals or their botanical parts.

Abbreviations: AX, arabinoxylan; A/X, arabinose/xylose; X, xylose; XX, xylobiose; AXOS, arabinoxylan oligosaccharides; β-glucan, (1→3) (1→4)-β-D-glucan; BGC, β-glucan content; BGE, β-glucan extractability; GOS, gluco-oligosaccharides; BG_3, 3-*O*-β-cellobiosyl-D-glucose; BG_4, 3-*O*-β-cellotriosyl-D-glucose; BG_5, 3-*O*-β-cellotetraosyl-D-glucose; BG_6, 3-*O*-β cellopentaosyl-D-glucose; HPAEC, high performance anion exchange chromatography; PCA, principal component analysis.

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Cereal β-glucan is present as a predominant component of endosperm cell walls in oats and barley (Andersson, Westerlund, & Åman, 2006). Barley starchy endosperm cell walls contain ≈75% mixed linkage β-glucan, while it constitutes ≈26% of the aleurone cell walls (Stone, 2006). However, in wheat, cell walls of starchy endosperm contain only ≈20% β-glucan, while aleurone cell walls contain ≈29%. β-Glucan in oats is more concentrated in the sub-aleurone layers, while in barley and rye it is evenly distributed across the starchy endosperm (Cui & Wang, 2009). The level of β-glucan varies from 0.5–1% in wheat grain to as much as 3–9% in barley grain.

Cereal β-glucan is predominantly composed of (1→4)-linked β-D-glucopyranosyl residues (∼70%) interrupted by (1→3)-linked β-D-glucopyranosyl residues (∼30%) (Wood, 2001). So far no evidence exists for two or more adjacent (1→3) linkages in the β-glucan chain of cereal grains (Izydorczyk & Dexter, 2008). The building blocks, (1→3)-linked cellotriosyl and cellotetraosyl units, constitute over 90% of the polymer (Wood, 2010). The amount of these units varies between cereals and in rye they may account for up to 95% and in oats and barley 91–93% of total polymer. The remaining part is composed of longer cellulose-like sequences ranging from 5 to 20 residues (Cui & Wang, 2009; Izydorczyk, Macri, & MacGregor, 1998b; Wood, Weisz, & Blackwell, 1994). The molar ratio of cellotriosyl to cellotetraosyl is an important determinant of the physicochemical properties of β-glucan such as solubility (Autio, 2006; Brennan & Cleary, 2005; Wood, 2010) and increases in the order oats 1.7–2.4 < barley/rye 2.7–3.6 < wheat 3.7–4.8 (Cui, Wood, Blackwell, & Nikiforuk, 2000; Wood, 2010). Apart from differences among cereals, the structural heterogeneity of β-glucan is also contributed from its location in different tissues of the kernel. β-Glucan in oat pericarp has higher trisaccharide to tetrasaccharide ratio (2.6) compared with β-glucan in whole flour (2.2) (Wood et al., 1994). These differences in molar ratio of cellotriosyl and cellotetraosyl can also be observed in the water extractable and unextractable fractions of cereal β-glucan, where the water extractable β-glucan is reported to have a lower ratio than the water unextractable (Izydorczyk et al., 1998a, 1998b; Johansson, Tuomainen, Ylinen, Ekholm, & Virkki, 2004; Wood et al., 1994).

Triticale (X *Triticosecale* Wittmack) is a man-made hybrid of durum wheat (*Triticum durum*) and rye (*Secale cereal* L.) designed to combine the high yield potential and good grain quality of wheat with the disease resistance and environmental tolerance of rye (Varughese, Pfeiffer, & Pena, 1996). Tritordeum is a cereal created by wide hybridisation between durum wheat and *Hordeum chilense*, a wild barley species, in order to combine the biotic and abiotic stress resistance of wild barley and technological features of wheat (Martín, Alvarez, Martín, Barro, & Ballesteros, 1999). In the present study, we sought to analyse and compare the structural features of AX and β-glucan in whole grain of triticale, barley (*Hordeum vulgare*) and tritordeum. The whole grain cereals were selected because of their utility in animal feed or human food. Subsequently we aim to correlate the structural features of the cereals particularly triticale with their rheological properties. The effect of genotypic and environmental variation on the structural features of these two polymers in triticale was also assessed.

2. Materials and methods

2.1. Grain samples

Eight triticale cultivars were grown at two different locations in Sweden, Svalöv (55°55′N, 13°5′E) and Kölbäck (58°26′N, 15°15′E). Two of these cultivars were also grown at a third location, Haga (59°36′N, 17°2′E) (Rakha, Åman, & Andersson, 2011). Rye (cv. Ottarp) and wheat (cv. Harnesk) reference cultivars grown at Svalöv and Kölbäck were also included in the study.

Twenty spring barley cultivars/breeding lines were grown in Vilcun, Chile, during November 2008–February 2009. These barley lines varied widely not only in morphological feature (covered and naked lines) and physico-chemical characteristics, but also in place of origin. The parent material of lines 1–6 was obtained from the Nordic Gene Bank (NGB) of the Nordic Genetic Resource Centre, Sweden; 7, 8 from the Swedish University of Agricultural Sciences (SLU), Sweden; 9–17 from SW Seed AB, Svalöv, Sweden and 18–20 from the University of Copenhagen, Faculty of Life Science (formerly known as KVL), Denmark. The accession numbers of the NGB, SLU, SW and KVL lines are NGB 13701, NGB 20022, NGB 20028, NGB 110008, NGB 114602, NGB 114621, SLU 7, SLU 17, SW 284, SW 2900, SW 3000, SW 18653, SW 28708, SW Cinnamon, SW Gustav, SW Kamrosé, SW Tibet 7B, KVL 301, KVL 1112 and KVL 1113, respectively. Five tritordeum lines, HT 354, HT 361, HT 437, HT 1608 (JB1) and HT 2218 (JB3), grown at Cordoba during 2008–2009 were obtained from Agrasys S.L. Parc Cientific de Barcelona.

2.2. Enzymes

Endo-xylanase M1 (EC 3.2.1.8) from *Trichoderma viride* (*endo*-1,4-β-xylanase) and lichenase (EC 3.2.1.73) from *Bacillus subtilis* [*endo*-(1→3)(1→4)-β-D-glucan 4-glucanhydrolase] were obtained from Megazyme, Bray, Ireland. The specific activity of *endo*-xylanase determined by the supplier was 210 U/mg (40 °C, pH 4.5, wheat flour arabinoxylan as substrate), while the specific activity of lichenase was 336 U/mg (40 °C, pH 6.5, barley β-glucan as substrate). The optimum pH for *endo*-xylanase is 4.5–5.0 and for lichenase 6.5–7.0.

2.3. Grinding/milling

Grinding is considered an important step in ensuring homogeneous sample. Whole grains of triticale, wheat, rye and tritordeum were finely ground in a cyclone sample mill (Retsch, Hann, Germany), while barley grains were milled using Cyclotec™ 1093 mill to pass through a 0.5 mm screen.

2.4. Enzymatic fingerprinting using endo-xylanase M1 and lichenase

Enzymatic mapping of AX and β-glucan structure was carried out according to the method described by Saulnier and Quemener (2009) and Toole et al. (2010), with slight modifications. Precisely weighed 0.5 g sample was boiled with 4 mL 80% ethanol for 10 min. The insoluble residue was separated by centrifugation. The procedure was repeated once and the residue was finally washed with 4 mL 95% ethanol. The supernatant was discarded and residue obtained was oven-dried at 40 °C for 24 h. The dried residue was suspended in 3 mL MilliQ water and 1 mL aqueous solution containing 80 U of *endo*-xylanase M1 and 10 U of lichenase. Since the pH of MilliQ water (≈6) is compatible for both enzymes, digestion of AX and β-glucan was carried out in one run. After thorough mixing, the mixture was incubated at 40 °C for 16 h under continuous stirring. The reaction mixture was then centrifuged and 2 mL of supernatant were removed and boiled for 5 min to inactivate the enzymes. After cooling and centrifugation, the supernatant was recovered, filtered through 0.45 μm filter (Millex Millipore) and diluted 10-fold.

Aliquots (15 μL) of the filtrate were analysed on high performance anion exchange chromatography. Separation of AX and β-glucan hydrolysis products was carried out on Carbopac™ PA-200 (5 × 250 mm) analytical column (Dionex, Sunnyvale, USA). The elution was carried out using ultrapure water (A), 1 M NaOAc (B) and 0.5 M NaOH (C). CarboPac™ PA 200 was run at 25 °C with a flow

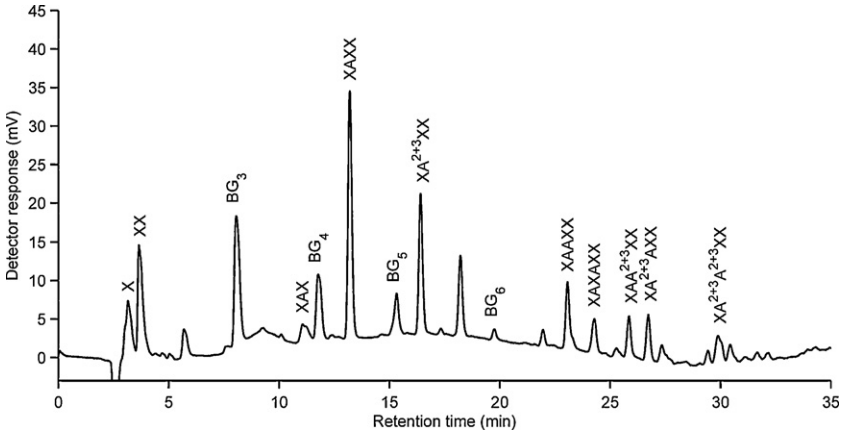

Fig. 1. Typical HPAEC profile of arabinoxylan and β-glucan standard (Dervilly, Saulnier, Roger, & Thibault, 2000) used to identify peaks. AXOS containing letter (A) without any superscript denotes β-D-xylopyranosyl residue with α-L-arabinofuranosyl residues at position *O*-3 in xylan chain while A^{2+3} denotes β-D-xylopyranosyl residue with α-L-arabinofuranosyl residues at positions *O*-2 and *O*-3.

rate of 0.4 mL/min and a linear gradient elution: 0 min (A: 80%, C: 20%), 30 min (A: 63%, B: 17%, C: 20%), 31 min (A: 80%, C: 20%) held up to 60 min. Detection was carried out with a TSP EC2000 pulsed amperometric detector (PAD, Thermo Separation Products) using the following pulse potentials: $E_1 = +0.05$ V, $E_2 = +0.6$ V, $E_3 = -0.6$ V.

Peak identification was based on the retention times of compounds previously isolated by Saulnier et al. (2009) and Ordaz-Ortiz, Devaux, and Saulnier (2005). For PCA the peak areas of each chromatogram were normalized as follows: $S_N Peak_i = (SPeak_i / \sum_{i=1}^{i=n} SPeak_i) \times 100$, with $S_N Peak_i$ being the normalized area of peak i and $SPeak_i$ being the area of peak i. Due to the absence of commercial standards for quantification, the response factors for PAD could not be calculated and the relative proportion of each fragment was based on the peak area (Irakli, Biliaderis, Izydorczyk, & Papadoyannis, 2004). Some unidentified peaks were also observed (Fig. 1), and it has been reported previously that variation in these unknown peaks is associated with the variation in the identified peaks of AXOS and gluco-oligosaccharides (GOS) (Toole et al., 2010).

2.5. Analysis of AX content and β-glucan content and extractability

AX in samples of barley and tritordeum grain was analysed according to the Uppsala method by Theander, Åman, Westerlund, Andersson, and Pettersson (1995). AX content was calculated from the arabinose, xylose, and galactose residues values obtained through the Uppsala method, assuming an arabinose/galactose ratio of 0.69 in arabinogalactan (Loosveld, Grobet, & Delcour, 1997). Total β-glucan was measured by an enzymatic method (McCleary & Codd, 1991), while the extractability of β-glucan was calculated by taking into account the area under the curve during molecular weight determinations, as described by Rimsten, Stengberg, Andersson, Anderssn, and Åman (2003).

2.6. Statistical analysis

The data obtained were analysed by PCA using The Unscrambler X 10.0.1 (CAMO Software AS, Norway) software. The similarity maps drawn from score plots help to group samples with comparable features and loading plots are useful to find the relationship between variables. Different conclusions drawn from PCA regarding location effects on the structural features of triticale grain were confirmed by Analysis of Variance (ANOVA) using Statistical Analysis System Software 9.2 (SAS Institute, Cary, NC).

3. Results and discussion

An overview of AX and β-glucan contents of triticale, rye and wheat reference cultivars (Rakha et al., 2011), barley and tritordeum is presented in Table 1. Both triticale and tritordeum cultivars/lines exhibited a narrow variation in AX content and arabinose/xylose (A/X) ratio compared with barley. This can partly be explained by the huge diversity in barley lines, as it included both covered and naked type. The average content (5.9%) and extractability (69.6%) of β-glucan in barley were much higher than for triticale (0.7% and 15%, respectively). The β-glucan content of tritordeum lines (0.6%) was similar to that in the reference wheat cultivar (0.6%). Differences also arose in the AX and β-glucan contents of triticale cultivars grown at the two different locations, Svalöv and Kölbäck. Furthermore, the triticale cultivars grown at Kölbäck had significantly higher AX extractability (15.2%) than those grown at Svalöv (13.8%) (Rakha et al., 2011).

3.1. Enzymatic fingerprinting of cereals

The relative proportion and properties of AX and β-glucan fragments generated after hydrolysis with *endo*-xylanase and lichenase, respectively, were determined on HPAEC. The digestion of AX with *endo*-xylanase M1 generates xylose (X), xylobiose (XX) and a series of AXOS, the proportions of which vary according to structure of AX. The oligosaccharides generated include XAX (DP 4), XAXX (DP 5), $XA^{2+3}XX$ (DP 6), XAAXX (DP 7), XAXAXX (DP 8), $XAA^{2+3}XX$ (DP 8), $XA^{2+3}AXX$ (DP 8), $XA^{2+3}A^{2+3}XX$ (DP 9) (Ordaz-Ortiz et al., 2004; Saulnier & Quemener, 2009). AXOS containing letter (A) without any superscript denotes β-D-xylopyranosyl residue with α-L-arabinofuranosyl residues at position three in xylan chain while A^{2+3} denotes β-D-xylopyranosyl residue with α-L-arabinofuranosyl residues at positions two and three. For identification and nomenclature of different *endo*-xylanase hydrolysis products, see Fig. 1 and Fauré et al. (2009). Fragments with monosubstitution by α-L-arabinofuranosyl residues at O-2 which are frequent in barley AX were not analysed in this study due to lack of references. Not only the structure of AX but also substituents such as ferulic acid residues and acetyl groups can influence the AX availability for enzymatic degradation by xylanases and thus influence the released fragment pattern. In cereal grain, AXOS essentially originate from endosperm tissues (aleurone layer and starchy endosperm), while the majority of X and XX originates from outer tissues (nucellar epidermis), which contain linear xylans (Saulnier & Quemener, 2009; Saulnier, Sado, Branlard, Charmet, & Guillon, 2007). *Endo*-(1→4)-β-xylanase M1 belongs to family 11

Table 1
Averages and ranges (in brackets) of arabinoxylan content (AX), arabinose/xylose ratio (A/X), β-glucan content (BGC) and β-glucan extractability (BGE) in triticale[a] grown at two locations, rye[a], wheat[a], barley and tritordeum grains. Contents are given as % of dry matter.

Cultivar (*n*)	AX	A/X	BGC	BGE
Triticale (18)	6.7 (5.9–7.5)	0.62 (0.59–0.67)	0.7 (0.5–1.0)	15.0 (12.2–18.2)
Svalöv (8)[b]	6.7[B] (5.9–7.4)	0.62[A] (0.59–0.65)	0.7[A] (0.6–1.0)	15.3[A] (12.2–18.2)
Kölbäck (8)[b]	6.9[A] (6.2–7.5)	0.63[A] (0.60–0.67)	0.6[B] (0.5–0.7)	15.1[A] (13.1–17.2)
Rye	8.6	0.60	1.9	11.4
Wheat	6.2	0.50	0.6	18.6
Barley (20)	8.1 (4.6–11.5)	0.53 (0.45–0.78)	5.9 (2.3–10.5)	69.6 (42.3–86.1)
Tritordeum (5)	6.9 (6.7–7.5)	0.58 (0.54–0.62)	0.6 (0.6–0.7)	ND[c]

[a] Data from Rakha et al. (2011).
[b] The uppercase letters (A and B) as a superscript on the average values of triticale grown at Svalöv and Kölbäck indicate the location effect. Values carrying different letters are significantly different from each other ($p < 0.05$).
[c] Not determined.

of the glycoside hydrolases, and requires at least three unsubstituted xylopyranosyl units in the xylan backbone to split the glycosidic bonds (Bach Knudsen & Lærke, 2010). Since our objective was to study the variation in grain, all AX fragments were included in the PCA. Furthermore, about 70% of the AX (in wheat) is extractable under the conditions used in this study and therefore also includes part of the water-unextractable AX (Ordaz-Ortiz & Saulnier, 2005).

Lichenase is a specific, *endo*-(1→3) (1→4)-β-D-glucan 4-glucanhydrolase that cleaves only β-(1→4) linkages on the reducing end of a 3-*O*-linked β-D-glucopyranosyl residue in β-glucans. The major GOS produced after lichenase hydrolysis are DP 3 (3-*O*-β-cellobiosyl-D-glucose, BG_3) and DP 4 (3-*O*-β-cellotriosyl-D-glucose, BG_4), while small quantities of DP 5 (3-*O*-β-cellotetraosyl-D-glucose, BG_5) and DP 6 (3-*O*-β-cellopentaosyl-D-glucose, BG_6) are also detected (Saulnier & Quemener, 2009; Tosh, Brummer, Wood, Wang, & Weisz, 2004). The longer cellulose-like sequences with DP > 6 were hardly detectable and not analysed in this study.

PCA applied to the relative areas of AX and β-glucan peaks of triticale, rye, wheat, barley and tritordeum gave an overall picture of the different whole grain cereals (Fig. 2). The clear grouping observed in the score plot of the different cereals seemed to be mainly controlled by the content of β-glucan. Barley cultivars with the highest β-glucan content and extractability were found on the left-hand side of PC 1, while triticale and tritordeum cultivars grouped together on the right-hand side of PC1.

3.2. Enzymatic hydrolysis of AX

The similarity map of AX features grouped the different cereals (Fig. 3a). The first two PCs explained 65% of the variation. PC1 was mainly controlled by the proportion of XX, which dominates in triticale, while variation in X was explained by PC2 (Fig. 3b). Larger variation (19.8–29.1%) in relative proportion of X in barley (Table 2) helped spread these across PC2, while tritordeum cultivars, which had a smaller proportion of X, lay on the negative co-ordinate of PC2 and closer to each other. The XX/X ratio was different for barley (1.4) compared with triticale (2.3), tritordeum (2.2), rye (2.4) and wheat (2.5). Tritordeum, wheat, rye and triticale had a greater relative proportion of XX compared with barley. Barley was comparatively more dominant in XAXX (19.1%) and $XA^{2+3}XX$ (11.6%) compared with triticale (13.8% and 5.0%, respectively). Furthermore, barley and tritordeum had a greater relative proportion of highly branched AXOS compared with triticale. This observation is supported by the fact that barley endosperm AX has more α-L-arabinofuranosyl residues than other cereals (Fincher & Stone, 1986). However, barley husks contain sparsely substituted AX (A/X ≈ 0.2) (Höije et al., 2006), which resulted in the low average A/X values reported in this study. The barley lines selected for this study were of diverse morphological and physico-chemical characteristics. Rye tended to stand out due to its much higher proportion of XAAXX (13%), while the reference wheat cultivar was similar to triticale.

Subsequent analysis of AX fragments in triticale grains was carried out to visualise the variation generated by location and cultivar (Fig. 3c, d). The first two PCs accounted for 67% of the variation in

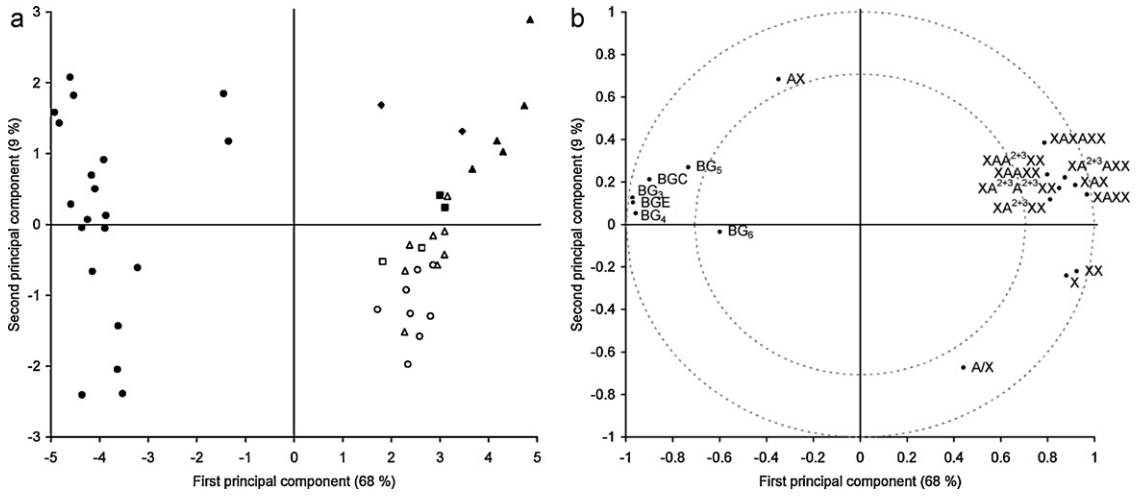

Fig. 2. Score plot (a) and loading plot (b) of cereals and variables studied. The peak areas of arabinoxylan and β-glucan fragments were normalised. Relative proportions of each fragment are expressed as percentage of the total area of identified arabinoxylan and β-glucan peaks (● = barley, Δ = triticale Svalöv, ○ = triticale Kölbäck, □ = triticale Haga, ▲ = tritordeum, ■ = wheat, ♦ = rye).

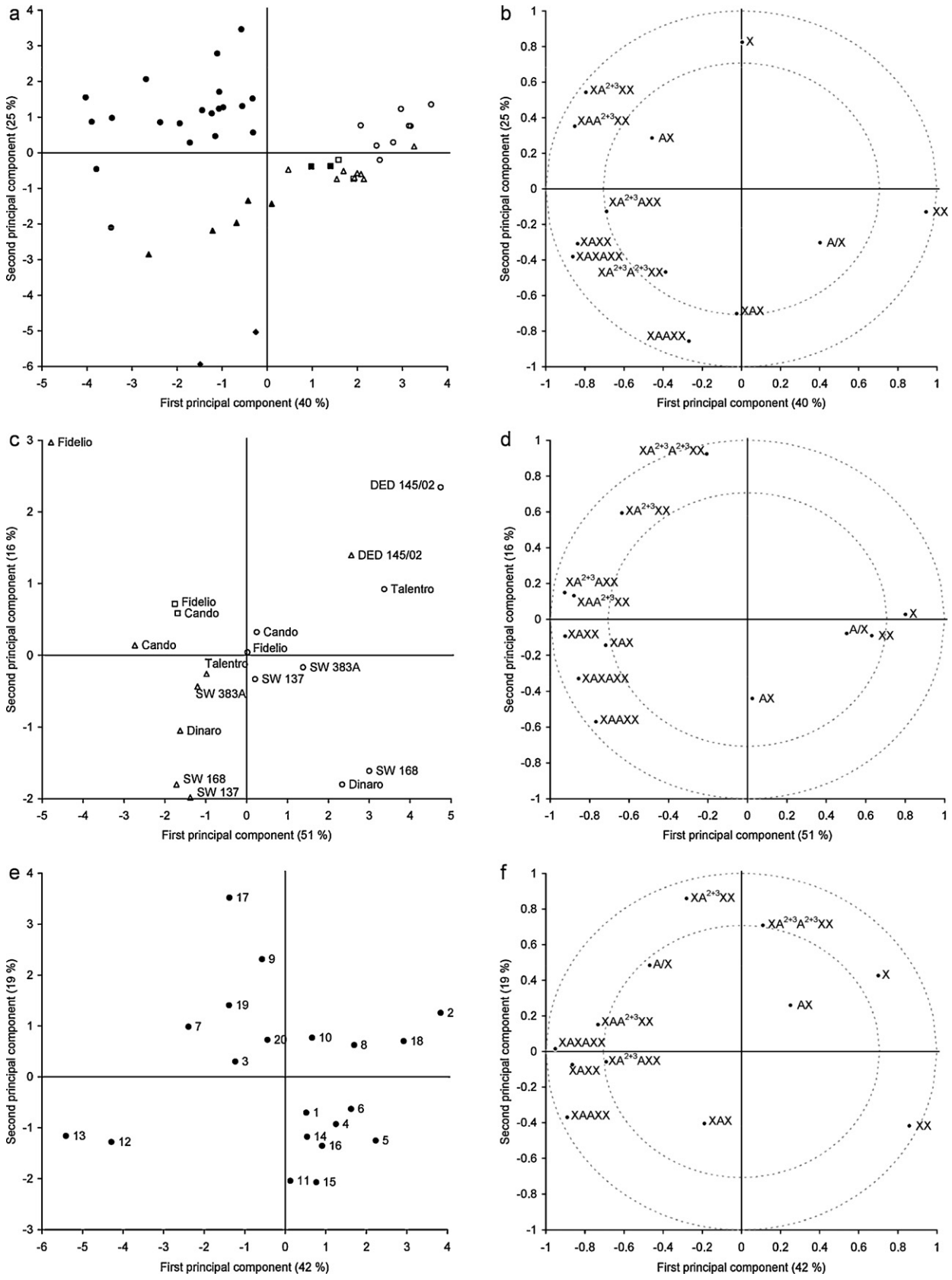

Fig. 3. Score plot and loading plot of arabinoxylan (AX) content, A/X ratio and AX hydrolysis fragments: (a, b = all cereals), (c, d = triticale), (e, f = barley). Relative proportions of each fragment are expressed as percentage of the total area under identified arabinoxylan peaks (● = barley, Δ = triticale Svalöv, ○ = triticale Kölbäck, □ = triticale Haga, ▲ = tritordeum, ■ = wheat, ♦ = rye).

Table 2
Averages and ranges (in brackets) of relative proportion (%) of arabinoxylan fragments in triticale, rye, wheat, barley and tritordeum grains. The area is normalised based on area under arabinoxylan fragment peaks.

Cultivar (n)	X	XX	XAX	XAXX	$XA^{2+3}XX$	XAAXX	XAXAXX	$XAA^{2+3}XX$	$XA^{2+3}AXX$	$XA^{2+3}A^{2+3}XX$[a]
Triticale (18)	22.1 (19.1–26.5)	50.0 (45.7–53.3)	1.0 (0.5–1.3)	13.8 (11.5–15.3)	5.0 (3.8–7.1)	3.7 (2.0–4.6)	1.1 (0.6–1.4)	1.4 (0.8–2.3)	0.8 (0.4–1.3)	1.0 (0.7–1.5)
Svalöv (8)[b]	20.4^{B} (19.3–22.1)	49.8^{A} (45.7–53.3)	1.2^{A} (1.0–1.3)	14.6^{A} (12.7–15.3)	5.2^{A} (4.5–7.1)	4.1^{A} (2.5–4.6)	1.1^{A} (0.7–1.3)	1.6^{A} (1.1–2.3)	0.9^{A} (0.6–1.3)	1.0^{A} (0.8–1.5)
Kölbäck (8)[b]	24.4^{A} (21.5–26.5)	50.1^{A} (46.6–51.6)	0.8^{B} (0.5–1.2)	12.8^{B} (11.5–14.2)	4.8^{A} (3.8–5.5)	3.4^{B} (2.0–3.8)	0.9^{B} (0.6–1.0)	1.2^{B} (0.8–1.6)	0.6^{B} (0.4–0.7)	1.0^{A} (0.7–1.3)
Rye	16.5	39.3	1.7	19.7	2.9	13.0	2.9	0.6	0.6	2.8
Wheat	19.6	48.7	1.0	15.6	5.8	4.0	1.3	1.8	1.1	1.1
Barley (20)	24.3 (19.8–29.1)	33.0 (24.7–38.3)	0.8 (0.1–2.2)	19.1 (14.1–25.4)	11.6 (9.8–14.0)	3.8 (1.6–6.7)	2.0 (1.4–3.1)	2.9 (2.0–4.4)	1.1 (0.6–1.7)	1.4 (0.7–2.0)
Tritordeum (5)	18.1 (16.9–18.8)	40.2 (34.4–43.8)	1.6 (1.3–1.9)	20.8 (18.6–25.3)	7.2 (6.2–8.5)	5.3 (4.1–7.9)	2.0 (1.4–3.1)	2.1 (1.9–2.5)	1.3 (1.0–1.5)	1.6 (1.0–2.0)

[a] Impure peak.
[b] The uppercase letters (A and B) as a superscript on the average values of triticale grown at Svalöv and Kölbäck indicate the location effect. Values carrying different letters are significantly different from each other ($p < 0.05$).

the data. Variation along PC1 was mainly driven by the differences between the locations (Svalöv and Kölbäck), which experienced different growing conditions. Extreme weather with high rainfall was reported at Kölbäck during the growing season. Cultivars grown at Kölbäck had more X compared with those grown at Svalöv. This was confirmed by ANOVA, where the average proportion of X at Kölbäck (24.4%) was significantly higher than at Svalöv (20.4%) (Table 2). On the other hand, triticale cultivars grown at Svalöv were significantly higher in mono-substituted and di-substituted AXOS, with the exception of $XA^{2+3}XX$ and $XA^{2+3}A^{2+3}XX$. The relatively higher proportion of branched fragments in Svalöv is not supported by A/X ratio of total AX, which was similar for both regions. Pre-harvest action of AX-degrading enzymes in kernels can be one explanation, since the bad weather with high rainfall at Kölbäck might have induced enzyme activity. Strong positive correlations between A/X ratio and the proportion of di-substituted β-D-xylopyranosyl residue in water extractable AX ($r = 0.96$) and xylanase extractable AX ($r = 0.88$) of wheat have been reported previously (Ordaz-Ortiz et al., 2005). Those authors also reported a negative correlation between mono-substituted β-D-xylopyranosyl residue and A/X ratio of water-extractable AX. Another explanation might be differences in the extractability of AX in triticale cultivars grown at the two locations, with Kölbäck having significantly higher AX extractability (15.2%) than Svalöv (13.8%). It is known that highly substituted AX are often difficult to extract, perhaps due to their stronger embedment in cell walls (Cyran, Courtin, & Delcour, 2003).

The second component dealt with genetic variation among cultivars (Fig. 3c, d). By visualising the loading plot it can be observed that PC2 dealt with mono-substitution and di-substitution and was not governed by the growing region. The cultivars on the positive co-ordinate of PC2 had more di-substitution than those on the negative co-ordinate.

A similarity plot of barley AX fragments is presented in Fig. 3e and f. The first two PCs accounted for 61% of variation in the data. The samples along PC1 were separated based on relative proportion of X and XX. Barley lines 2 and 18 had the highest proportion of X (29.1% and 28.0%, respectively). The proportion of XX was also relatively higher in lines 2 and 18 (37.1 and 36.7, respectively) compared with the average for barley (33%). These two lines also contained the highest AX content (11.5%). However, the correlation between AX content and relative proportion of X was not established for barley. The two barley lines 12 and 13, lying on the extreme end of PC1, are hull-less, waxy type, with low AX content and had the highest XAAXX (6.4% and 6.7%, respectively) compared with the average value for barley (3.8%). The cultivars along PC2 were separated based on extent of mono-substitution and di-substitution.

3.3. Enzymatic hydrolysis of β-glucan

An overview of score plot shows a clear clustering of cereals along PC1 (Fig. 4a, b). The relative proportions of BG_5 and BG_6 were negatively correlated to the relative proportions of BG_3 and BG_4, as well as content and extractability of β-glucan. Barley cultivars stretched out on the right-hand side of PC1, which was mainly driven by high β-glucan content and relative proportion of trisaccharides and tetrasaccharides. Barley had a higher (95.0%) proportion of these two oligomers compared with triticale (88.3%) and tritordeum (86.5%) (Table 3). Trisaccharides and tetrasaccharides comprised 95.1% of the oligomers in rye (cv. Ottarp), which is similar to previous values (95%) reported by Wood et al. (1994), while in wheat (cv. Harnesk) it comprised 88.0% of the oligomers. Both tritordeum and triticale had a relatively high proportion of long-chain cellulose-like sequences with DP6. The reference wheat cultivar (cv. Harnesk) tended to associate with triticale and tritordeum grains, while rye was closer to barley. The

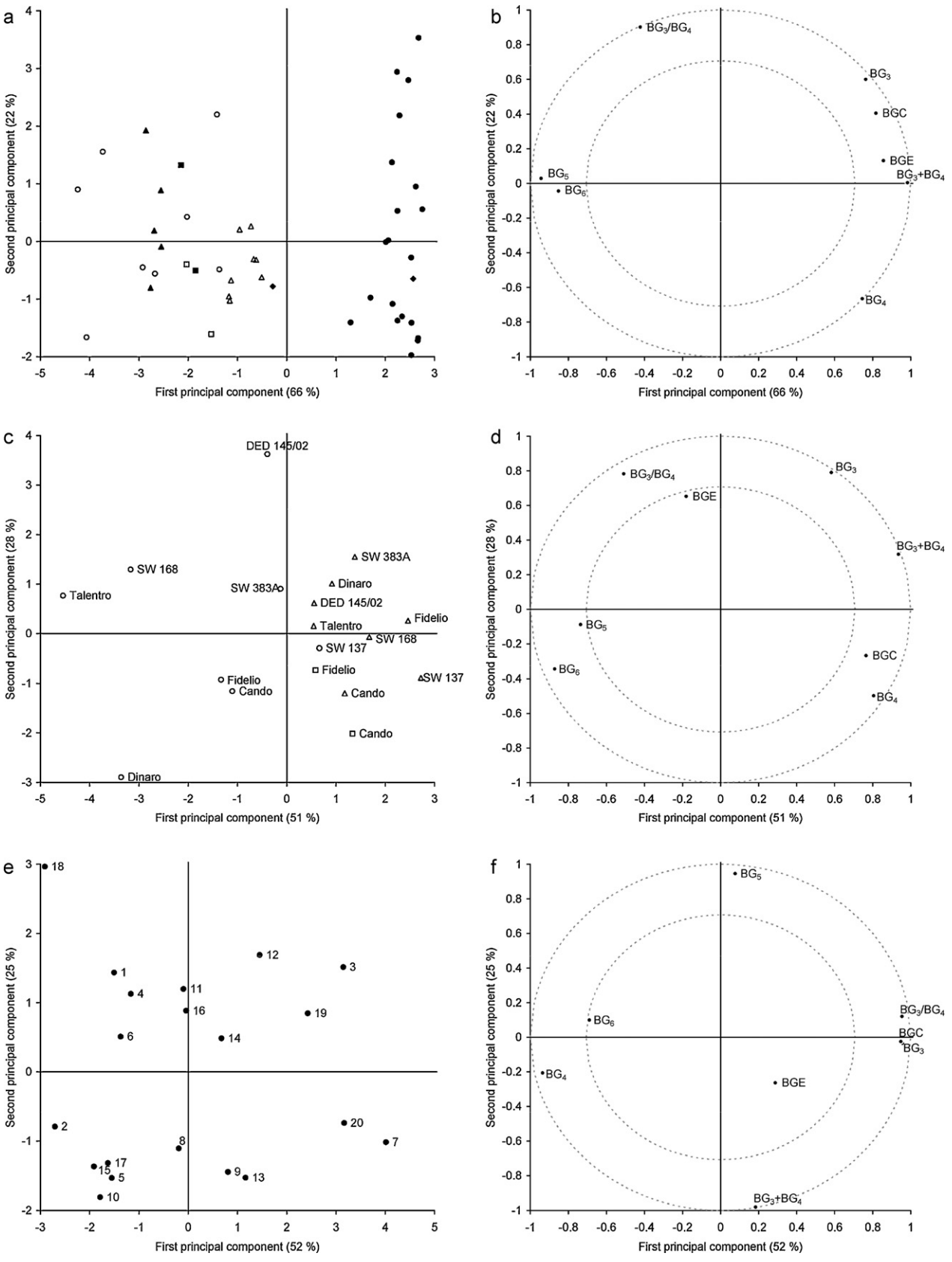

Fig. 4. Score plot and loading plot of β-glucan content (BGC), extractability (BGE) and β-glucan hydrolysis fragments: (a, b = all cereals), (c, d = triticale), (e, f = barley). Relative proportions of each fragment are expressed as percentage of the total area under identified β-glucan peaks (● = barley, Δ = triticale Svalöv, ○ = triticale Kölbäck, □ = triticale Haga, ▲ = tritordeum, ■ = wheat, ♦ = rye).

Table 3
Averages and ranges (in brackets) of relative proportion (%) of β-glucan fragments in triticale, rye, wheat, barley and tritordeum grains. The area is normalised based on area under β-glucan fragment peaks.

Cultivar (*n*)	BG_3	BG_4	BG_5	BG_6[a]	$BG_3 + BG_4$	BG_3/BG_4
Triticale (18)	65.3 (63.3–69.6)	23.0 (19.4–25.0)	7.5 (6.6–9.4)	4.2 (1.5–7.9)	88.3 (82.9–91.2)	2.9 (2.5–3.4)
Svalöv (8)[b]	66.4^{A} (65.0–67.4)	24.1^{A} (23.2–24.7)	7.4^{A} (6.8–7.8)	2.2^{B} (1.5–2.9)	90.4^{A} (89.5–91.2)	2.8^{A} (2.6–2.9)
Kölbäck (8)[b]	64.5^{A} (59.8–69.6)	21.6^{B} (19.4–23.7)	7.8^{A} (6.7–9.4)	6.0^{A} (2.5–8.8)	86.2^{B} (82.5–90.2)	3.0^{A} (2.6–3.4)
Rye	68.8	26.3	3.1	1.8	95.1	2.6
Wheat	65.9	22.1	8.0	4.0	88.0	3.0
Barley (20)	69.0 (66.5–73.5)	26.0 (22.0–28.9)	3.4 (2.5–4.1)	1.6 (1.4–2.3)	95.0 (93.8–95.6)	2.7 (2.3–3.3)
Tritordeum (5)	65.0 (63.0–67.0)	21.6 (19.8–22.9)	9.2 (8.4–9.6)	4.3 (3.8–4.9)	86.5 (85.9–87.0)	3.0 (2.8–3.4)

[a] BG_6 was not always pure for triticale cultivars.
[b] The uppercase letters (A and B) as a superscript on the average values of triticale grown at Svalöv and Kölbäck indicate the location effect. Values carrying different letters are significantly different from each other ($p < 0.05$).

relative proportion of different oligomers in five tritordeum cultivars spanned a very narrow range and was quite similar to that of the wheat cultivar tested. The former was higher in relative proportion of long-chain oligomers (BG_5 and BG_6) and lower in cumulative proportion of trisaccharides and tetrasaccharides compared with barley. The second PC accounted for 22% of the data variation and was driven by BG_3/BG_4 ratio. It described the inter-cultivar variation, since the barley cultivars tended to align vertically based on differences in BG_3/BG_4 ratio. The first two PCs explained 88% of the variation in data.

The β-glucan extractability (BGE) of cereals was positively correlated ($r = 0.75$) with the proportion of $BG_3 + BG_4$. In contrast to this, the extractability of β-glucan was slightly negatively correlated with DP5 ($r = -0.79$) and DP6 ($r = -0.57$). The present results corroborate previous findings, since the long cellulose-like sequences of (1→4)-linked β-D-glucose residues may form strong aggregation through hydrogen bonding, thus making β-glucan less soluble (Izydorczyk et al., 1998a; Lazaridou & Biliaderis, 2007). Apart from this, BG_3/BG_4 is also important in determining β-glucan solubility. However, in this study no apparent correlation between β-glucan extractability and ratio of BG_3/BG_4 could be established ($r = -0.25$). Higher ratio of BG_3/BG_4 gives a greater prospect of consecutive cellotriosyl units, which can form junction zones, as do longer cellulose-like sequences of (1→4)-linked β-D-glucose residues, which result in aggregation and lower extractability of β-glucan (Cui et al., 2000; Doublier & Wood, 1995; Lazaridou & Biliaderis, 2007; Tosh et al., 2004).

Triticale cultivars grown at the two locations exhibited significant variation in β-glucan structural features (Table 3, Fig. 4c, d). Cultivars grown at Svalöv were significantly higher in relative proportion of BG_4 (average 24.1%) compared with triticale grown at Kölbäck (21.6%). Conversely, cultivars grown at Kölbäck were significantly higher in longer GOS with DP 6 (average 6.0%) than cultivars grown at Svalöv (average 2.2%). However, the location effects on BG_3 and BG_5 were non-significant in triticale. The cumulative proportion of trisaccharides and tetrasaccharides in triticale was 88.3%, with significant differences between the two locations, whereas the ratio of BG_3/BG_4 was not significantly different for the two growing regions. These differences in triticale cultivars grown at the two locations are visualised by score plot in Fig. 4c, where triticale cultivars grown at Svalöv grouped together on the positive co-ordinate of PC1. PC1 described the location effects and separated the two regions into distinct clusters by explaining 51% variation. The greater variation within cultivars grown at Kölbäck is quite apparent from the score plot and can be ascertained from Table 3.

The first two principal components explained 77% of the variation in barley β-glucan data (Fig. 4e, f). PC1 was mainly driven by the content of β-glucan and proportion of BG_3. Barley lines 3, 7, 19 and 20 had the highest β-glucan content and stretched out on the right-hand side of the score plot. Conversely lines 2 and 18, lying on the extreme left-hand side of the score plot, contained the lowest amount of β-glucan (2.3% and 2.4%, respectively). A positive correlation ($r = 0.86$) between the content of β-glucan in barley and the relative proportion of BG_3 is visible from the loading plot. Cumulative proportion of trisaccharides and tetrasaccharides in barley β-glucan ranged from 93.8 to 95.6% (average 95.0%). This is slightly higher than in a previous study (Irakli et al., 2004), where 91 to 92% of the total oligomers (DP3–DP11) consisted of $BG_3 + BG_4$. The slightly higher values observed in our study are probably due to the fact that we accounted for GOS up to DP6 and normalised the area under each peak.

The molar ratio of trisaccharides to tetrasaccharides is an important determinant of structural and functional properties of β-glucan, for example solubility (Cui et al., 2000). The molar ratio in the barley cultivars studied here varied from 2.3 to 3.3 (average 2.7), which is a wider variation than previously reported (2.3–2.8) (Irakli et al., 2004). The molar ratio of trisaccharide/tetrasaccharide in water extractable β-glucan can also vary depending on extraction temperature, since the barley β-glucan extracted at 65 °C is reported to have slightly higher (2.13) ratio than that extracted at 40 °C (1.76) (Izydorczyk et al., 1998a). Differences in molar ratio of BG_3/BG_4 can also be observed in soluble and insoluble β-glucan (Izydorczyk et al., 1998a, 1998b). Alkali extractable barley β-glucan was also reported to have a higher proportion of long-chain (1→4)-linked oligomers, a higher ratio of cellotriosyl/cellotetraosyl units and higher ratios of β-(1→4)/(1→3) linkages than its water-extractable counterpart.

4. Conclusions

The structural features of AX and β-glucan in different cereals are important determinants of their end-use quality. The present study revealed structural heterogeneity of AX and β-glucan in whole grains of triticale, barley and tritordeum, with the different cereals grouped together based on distinct structural features of AX and β-glucan. The average ratio of XX/X was lower for barley (1.4) than for triticale (2.3) and tritordeum (2.2). Barley and tritordeum normally had a higher relative proportion of branched AXOS than triticale. Significant location effects were observed for triticale. Structural features of β-glucan were also quite diversified among cereals and within genotypes of the different cereals. BGE was positively correlated ($r = 75$) with the proportion of $BG_3 + BG_4$, whereas β-glucan content in barley was positively correlated ($r = 0.86$) with relative proportion of BG_3.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.06.054.

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