Distribution and Structural Variation of Arabinoxylans in Common Wheat Mill Streams

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In 19 wheat-milling fractions total pentosan content, calculated as 0.88 × (% L-arabinose + % D-xylose), varied between 1.44 and 30.66% on dry matter (dm). It increased with ash content once the latter exceeded 0.6% (dm basis). Water-extractable arabinoxylans were recovered by saturating water extracts to 65% ethanol. Their contents in the milling fractions varied between 0.35 and 1.38%, and above 0.6% ash content also increased with this parameter. Their L-arabinose-to-D-xylose ratios ranged between 0.65 and 0.39, with the lowest values found for the fractions with highest ash content, indicating that the ash-rich tissues contain more arabinoxylans that are less branched. ^{1}H NMR spectroscopy revealed that the decrease in L-arabinose-to-D-xylose ratio was accompanied by an increase in unsubstituted xylose residues and a decrease in disubstituted xylose residues, while the contents of monosubstituted xyloses were virtually constant.

Keywords: *Pentosan; nonstarch polysaccharides; water-extractable arabinoxylan; mill streams*

INTRODUCTION

The nonstarch polysaccharides (NSP) in wheat are sometimes referred to as pentosans (PEN) and can be water-extractable (WE) or water-unextractable (WU). WE PEN include WE arabinoxylans (AX) and arabinogalactan peptide (AGP). The latter is probably, unlike the AX, not closely related with cell walls (Mares and Stone, 1973c; Fincher and Stone, 1974). The WU NSP consist of AX, which represent the major part (Mares and Stone, 1973a), β-D-glucan, cellulose, and glucomannan.

Interest in wheat NSP and in wheat AX in particular increased since their functional role in the breadmaking process became clear (Pence et al., 1950; D'Appolonia et al., 1970; Jelaca and Hlynka, 1972; Michniewicz et al., 1992; Cleemput et al., 1993; Roels et al., 1993; Vanhamel et al., 1993). In recent years, insight in the structural characteristics of these polysaccharides, consisting of a (β1−4)-linked D-xylose (Xyl) chain to which L-arabinose (Ara) moieties are attached, was improved (Hoffmann et al., 1991, 1992; Izydorczyk and Biliaderis 1992, 1993; Cleemput et al., 1995).

Several researchers have studied NSP and their structural variation in wheat. Mares and Stone (1973a−c) did elaborate work on wheat endosperm. A higher degree of branching of bran AX than of endosperm AX was reported by D'Appolonia and MacArthur (1976). Ciacco and D'Appolonia (1982) characterized WE NSP of different mill streams of a hard red spring wheat and concluded that AX from the inner portion of the kernel were less branched than those isolated from flour streams containing a greater percentage of the outer portion. Total (TOT) and WE AX contents of wheat and some milled wheat products were listed by Hashimoto et al. (1987).

This study was undertaken not only to investigate the distribution of AX throughout a common wheat kernel but also to gain more insight in the variation of the fine structure of the WE AX originating from different parts of the kernel and hence widely varying in ash contents.

It is indeed our firm belief that, in view of recent findings concerning the difference in functionality of WE AX and WU AX in breadmaking (Courtin et al., 1998), results of the present investigation will be of use in formulating wheat flour of improved functionality.

EXPERIMENTAL PROCEDURES

Materials. A European wheat (Dutch 1995 harvest) with a protein content of 12.5% [N × 5.7, dry matter (dm) basis; analyzed according to AACC method 46-11A (AACC, 1995)] and with a hardness intermediate between those of typical North American hard and soft wheats was milled with a pilot mill (model A, Miag, Braunschweig, Germany) in 19 milling fractions. Moisture and ash contents of the milling fractions were determined in triplicate by ICC methods 110/1 (1976) and 104 (1960).

Total Arabinoxylan and Water-Extractable Pentosan Contents. AX and PEN were estimated by gas chromatography (GC) of the composing monosaccharide residues, following hydrolysis as outlined below.

Hydrolysis. For determination of TOT PEN contents, fractions (0.15 g) were hydrolyzed with 5.0 mL of 2.0 M trifluoroacetic acid (120 min, 110 °C). After cooling, the hydrolysates were filtered (Whatman no. 1).

For WE PEN contents, samples of the milling fractions (2.0 g) were extracted with deionized water (1:10 v/w) under continuous stirring (30 °C, 120 min). After centrifugation (15 min, 3000*g*), hydrolysis was by incubating 2.5 mL of the extracts with 2.5 mL of 4.0 M trifluoroacetic acid (60 min, 110 °C). The cooled hydrolysates were centrifuged (15 min, 3000*g*).

Derivatization. Alditol acetates were prepared based on the method of Englyst and Cummings (1984). To 3.0 mL of the hydrolysates, 1.0 mL of internal standard solution (100 mg of β-D-allose in 100 mL of a 1:1 diluted saturated benzoic acid

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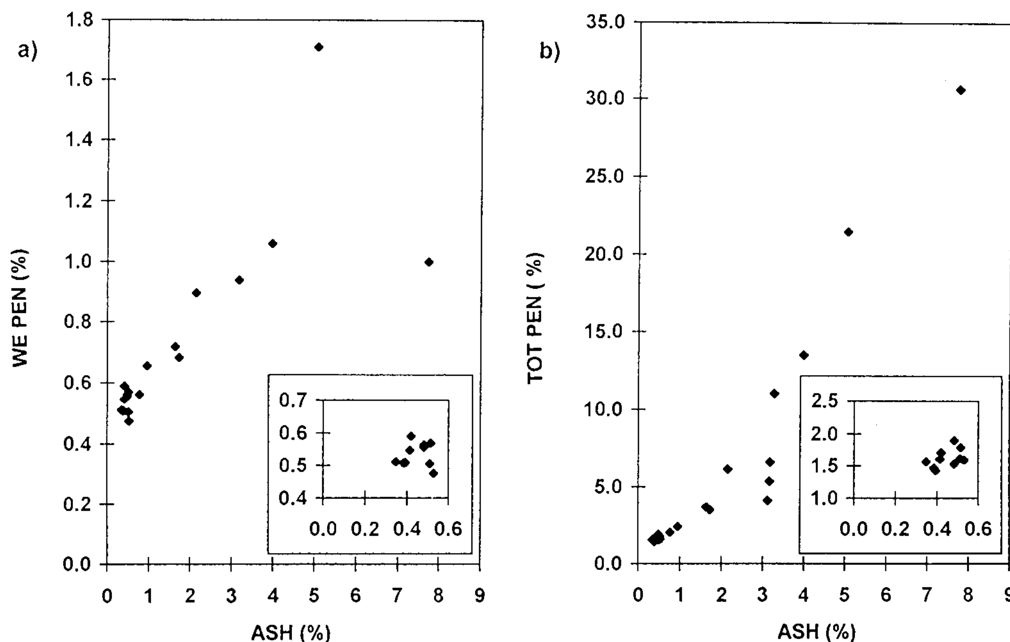

Figure 1. PEN contents of the milling fractions of a common wheat variety as a function of the respective ash contents (expressed on dm): (a) WE PEN and (b) TOT PEN.

solution) was added. The tubes were placed in ice−water, and 1.0 mL of NH_3 (25%), 1 drop of octan-2-ol, and 200 μL of 2.0 M NH_3 containing sodium borohydride (200 mg/mL) were added. After incubation (30 min, 40 °C), the solutions were mixed with 400 μL of glacial acetic acid. To 500 μL of the resulting mixture were added 500 μL of *N*-methylimidazole and 5.0 mL of acetic anhydride. After 10 min, the solutions were mixed with 900 μL of ethanol and left for 5 min. Water (10.0 mL) was added. After addition of 500 μL (0.04%) bof romophenol blue, the tubes were placed in ice−water and 5.0 mL of 7.5 M potassium hydroxide was added twice within a 5-min period. The tubes were mixed, and after phase separation, the upper phase was isolated and dried over anhydrous sodium sulfate.

Gas Chromatography. Separation of the alditol acetates was with a Chrompack 9011 chromatograph (Middelburg, The Netherlands) using a Supelco SP-2380 column (30 m, 0.32 mm i.d., 0.2-μm film thickness) (Bellefonte, PA). Separation was at 225 °C, with injection and detection (flame ionization detector) temperatures of 275 °C.

TOT PEN and WE PEN contents were calculated as 0.88 × (% Ara + % Xyl) on dm. Results given below are means of four to six measurements. Under these conditions, the coefficients of variation were <2% for both WE PEN and TOT PEN contents.

Isolation and Characterization of WE AX. The Ara contents, used for calculating TOT PEN and WE PEN contents, also partly originate from AGP (Loosveld et al., 1997). It follows that the calculated levels of NSP components do not really correspond to physical reality, although certainly in the case of the TOT PEN data, the overestimation by inclusion of the WE AGP-associated Ara is negligible. That is why WE AX were further purified. To that end, we used an ethanol precipitation procedure allowing for a clear-cut separation between WE AX and WE AGP (Cleemput et al., 1993).

The monosaccharide composition of the isolated preparations was determined by GC analysis as described above. Samples (15 mg) were hydrolyzed with 5.0 mL of 2.0 M trifluoroacetic acid (60 min, 110 °C). Analyses were in duplicate.

^{1}H NMR spectra were recorded on a Bruker 300-MHz FT spectrometer (Bruker, Rheinstetten 4, Karlsruhe, Germany) at 85 °C. Samples were prepared by dissolving in D_2O (99%) (1 mg/mL), lyophilization, and renewed dissolving in D_2O.

Table 1. Yields and Ash Contents of the Milling Fractions

fraction[a]	yield (%)	ash[b] (%)
1R	9.8	0.35
2R	11.4	0.39
3R	5.2	0.39
4R	10.1	0.42
5R	8.0	0.42
6R	1.7	0.48
7R	4.6	0.48
8B	6.8	0.51
9R	4.4	0.52
10B	5.7	0.53
11B	3.7	0.78
12R	2.1	0.95
13R	1.5	1.64
14B	2.7	1.73
15R	0.7	2.15
16R	3.5	3.18
germ	0.7	3.98
shorts	6.8	5.06
bran	10.7	7.75
total	100	

[a] B and R, fractions originating from break and reduction system, respectively. [b] Expressed on dm basis.

RESULTS AND DISCUSSION

Ash Contents and Yields of the Milling Fractions. Table 1 lists the yields and ash contents of the 19 milling fractions. Ash contents of the fractions, a global indication of the origin in the kernel (Hinton, 1959), ranged between 0.35 and 7.75% on dm.

NSP Contents. TOT PEN and WE PEN contents varied between 1.44 and between 30.66% and 0.48−1.71% on dm, respectively. In Figure 1, these data are plotted as a function of the respective ash contents of the milling fractions.

For the fractions with a low ash content (<0.6% on dm), which may be considered to consist mainly of pure starch endosperm (Hinton, 1959), no correlation was found between ash and either WE PEN or TOT PEN contents. Once above this concentration, we observed an increase in these contents which was most pro-

nounced for TOT PEN. For the bran fraction, we measured a lower WE PEN content than for the shorts. This difference is probably due to a reduced accessibility of the bran fraction to water. Furthermore, shorts comprise endosperm attached to seed coats and are very rich in aleurone (where AX are concentrated). Pentosan contents in different milled wheat products reported by Hashimoto et al. (1987) are in line with these results.

The increased amount of the analyzed NSP with ash content is explained by the presence of more cell wall material. The increased difference between TOT PEN and WE PEN indicates a decreased degree of water extractability of the components toward the outer layers of the kernel. Mares and Stone (1973a) concluded that differences in solubility of wheat endosperm AX cannot be ascribed to differences in size or Ara-to-Xyl ratio (A/X) but to chemical interactions with other cell wall components. They found evidence that WE AX are not bound to the other cell wall polymers and that they are located at the surface of the cell wall (Mares and Stone, 1973a). In contrast, the WU portion is held in the cell wall structure by ester linkages.

Structural Characterization of WE AX. Upon saturation of water extracts of the milling fractions to 65% ethanol, WE AX are obtained with little contamination of WE AGP (Fincher and Stone, 1974; Suckow et al., 1983; Cleemput et al., 1993). GC confirmed that the isolated materials contained little D-galactose (Gal, <1% for most fractions). The purity was best for the fractions originating from pure endosperm (75–90% AX for fractions 1–13). The other fractions had a purity of ca. 50%, while the precipitated material from the bran water extract only contained 14% AX. Glucose (Glc) residues were detected in all fractions; in most cases this was less than 10%. At least part of the Glc originated from (β1–3)- and (β1–4)-linked β-D-glucan (see below).

From the GC data, the actual A/X of the WE AX were estimated. To account for AGP present, these ratios were calculated as (% Ara − 0.7 × % Gal)/% Xyl, with the assumption that the Ara-to-Gal ratio (w/w) of AGP is constant (0.7) (Fincher and Stone, 1974; Izydorczyk et al., 1991; Loosveld et al., 1997). By means of these ratios and the Xyl contents of the water extracts, contents of WE AX were calculated for each wheat fraction. The WE AX contents (0.35–1.38% on dm) and respective A/X (0.39–0.65) are plotted in Figure 2 as a function of ash content.

The variation in degree of substitution of WE AX from the fractions consisting of the more pure starch endosperm (ash < 0.6% on dm; A/X 0.59–0.65) was not correlated to ash content. For the fractions contaminated with or mainly composed of aleurone layer and outer layers, a decreasing degree of substitution was found with increasing ash value. These results disagree with the findings of D'Appolonia and MacArthur (1976) and Ciacco and D'Appolonia (1982). However, these authors did not differentiate between WE AX and AGP.

Further information on the substitution pattern of the WE AX was obtained by combining the gas chromatography results with analysis of ^{1}H NMR spectra (Westerlund et al., 1990; Cleemput et al., 1993). Figure 3 shows the relevant regions of the spectra of six milling fractions with increasing ash content. The peaks at δ 5.40, 5.30, and 5.23 represent H1 of Ara linked to the O-3 of monosubstituted Xyl and to O-2 and O-3 of disubstituted Xyl residues, respectively. From the relative peak intensities, a substantial variation in the ratio of mono- to disubstituted Xyl residues (0.47–1.12) is clear. Traces of AGP are indicated by a signal at δ 5.27. This is most pronounced for fraction 4, in accordance with the GC analyses. The doublet at δ 4.7–4.8 originates from β-D-glucan. The ratios of the signals representing Ara to those of β-D-glucan illustrate the differences in purity of the isolated samples.

The proportions of unsubstituted (Xn/X), monosubstituted (Xm/X), and disubstituted (Xd/X) Xyl residues

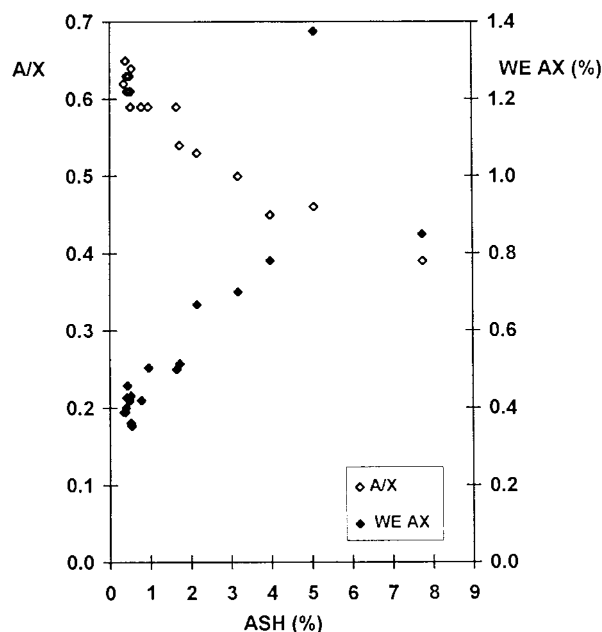

Figure 2. WE AX contents and A/X of the milling fractions of a common wheat variety as a function of ash content (expressed on dm).

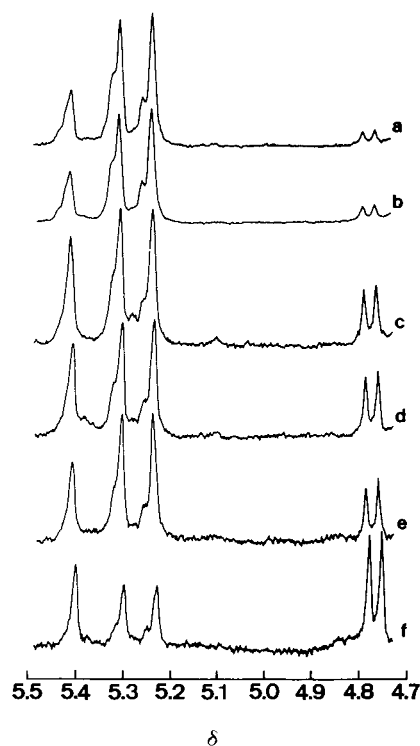

Figure 3. ^{1}H NMR spectra of the WE AX of six milling fractions, with increasing ash content from a to f, of a common wheat variety. Fractions: (a) 4R, (b) 8B, (c) 14B, (d) 16R, (e) germ, and (f) bran.

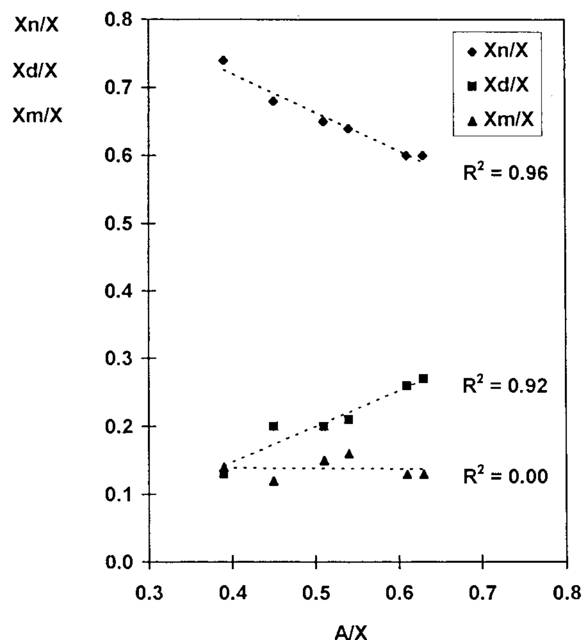

Figure 4. Proportions of unsubstituted xylose (Xn), disubstituted xylose (Xd), and monosubstituted xylose (Xm) in total xylose (X) of isolated WE AX of six milling fractions of a common wheat variety, as a function of ash content (on dm) of the fractions.

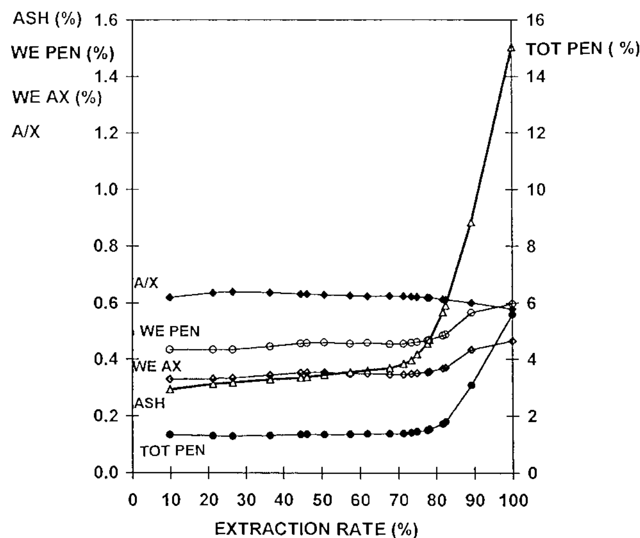

Figure 5. Contents of ash, TOT PEN, WE PEN, and WE AX with respective A/X as a function of the extraction rate of a common wheat variety.

of the six milling fractions are plotted against A/X in Figure 4. A lower degree of substitution (higher ash content) was accompanied by an increase in Xn/X residues (60 → 74%) and a decrease in Xd/X residues (27 → 13%), while Xm/X remained virtually constant. This may be of significance for understanding the biosynthesis of AX of different A/X. For Xn/X (13−16%), no correlation with overall A/X was found. Evidence for the existence of paired disubstituted Xyl residues in all samples is shown by the shoulders at the left side of the peaks at δ 5.30 and 5.27 (Hoffmann et al., 1992; Cleemput et al., 1993).

Ash and NSP Data as a Function of Extraction Rate (ER). In Figure 5, the results are cumulatively plotted as a function of ER (as is). Up to an ER of approximately 70%, a slight linear increase in ash content can be observed (0.29% ash at 10% ER, 0.38% ash at 70% ER), without considerable variation of the other characteristics (WE PEN 0.43−0.46, TOT PEN 1.33−1.38). An increase of the ER with 10% clearly influences ash content (increase of 42%) and to a minor degree also TOT PEN (increase of 17%). Above an ER of 80%, TOT PEN content, like ash content, strongly increases. Whole meal contains approximately 4 times the amount of NSP of the endosperm. Increase in WE PEN or WE AX is only obtained by adding bran material to the milling product (increase of 25 and 30% respectively, compared to the 80% ER product). These observations are in line with the results of Nyman et al. (1984), who found that the soluble fiber content was independent of ER, while the insoluble part increased rapidly for ER above 80%.

Finally, variation of the ER has little impact on the overall degree of substitution of the WE AX in the milling end product.

CONCLUSIONS

No correlation between the measured NSP fractions and ash content was found for the more pure endosperm milling fractions (ash content below 0.6% on dm). At higher ash levels, TOT PEN and WE PEN contents increased with increasing ash content, which implies the existence of a pentosan gradient toward the outer layers of the wheat kernel. This was most pronounced for the TOT PEN content and only to a minor degree for the water-extractable portion. Isolation of WE AX enabled further characterization of their degree and mode of Ara substitution. WE AX from the outer layers were less substituted with Ara than those from the inner endosperm. ^{1}H NMR spectroscopy revealed that this was due to a higher proportion of unsubstituted xylose residues and a lower proportion of disubstituted xylose residues. TOT PEN contents can change above an extraction rate of 70%, while WE AX content and A/X are practically independent of the ER, unless bran is included in the milling end product.

While the present results are (strictly speaking) only valid for the particular wheat milled and the mill scheme used, it is to be expected that the results to a significant degree can be extrapolated to other wheats and milling processes and hence can be useful in the development of wheat flours with a desired functionality.

ABBREVIATIONS USED

Ara, L-arabinose; Gal, D-galactose; Glc, D-glucose; Xyl, D-xylose; AGP, arabinogalactan peptide; AX, arabinoxylan; A/X, L-arabinose-to-D-xylose ratio; Xd/X, ratio of disubstituted D-xylose to total D-xylose; Xm/X, ratio of monosubstituted D-xylose to total D-xylose; Xn/X, ratio of unsubstituted D-xylose to total D-xylose; dm, dry matter; ER, extraction rate; GC, gas chromatography; ^{1}H NMR, proton nuclear magnetic resonance; NSP, nonstarch polysaccharides; PEN, pentosan; TOT, total; WE, water-extractable; WU, water-unextractable.

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LITERATURE CITED

American Association of Cereal Chemists. *Approved Methods of the AACC*, 9th ed.; AACC: St. Paul, MN, 1995; Method 46-11A.

Ciacco, C. F.; D'Appolonia, B. L. Characterisation of pentosans from different wheat flour classes and of their gelling capacity. *Cereal Chem.* **1982**, *59*, 96−99.

Cleemput, G.; Roels, S. P.; van Oort, M.; Grobet, P. J.; Delcour, J. A. Heterogeneity in the structure of water-soluble arabinoxylans in European wheat flours of variable breadmaking quality. *Cereal Chem.* **1993**, *70*, 324−329.

Cleemput, G.; van Oort, M.; Hessing, M.; Bergmans, M. E. F.; Gruppen, H.; Grobet, P. J.; Delcour, J. A. Variation in the degree of D-xylose substitution in arabinoxylans extracted from a European wheat flour. *J. Cereal Sci.* **1995**, *22*, 73−84.

Courtin, C. M.; Roelants, A.; Delcour, J. A. Fractionation-reconstitution experiments provide for insight in the role of endoxylanases in breadmaking. *J. Agric. Food Chem.*, submitted for publication.

D'Appolonia, B. L.; MacArthur, L. A. Comparison of bran and endosperm pentosans in immature and mature wheat. *Cereal Chem.* **1976**, *53*, 711−718.

D'Appolonia, B. L.; Gilles, K. A.; Medcalf, D. G. Effect of water-soluble pentosans on gluten-starch loaves. *Cereal Chem.* **1970**, *47*, 194−204.

Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total nonstarch polysaccharides by gas−liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937−942.

Fincher, G. B.; Stone, B. A. A water-soluble arabinogalactan-peptide from wheat endosperm. *Aust. J. Biol. Sci*. **1974**, *27*, 117−132.

Hashimoto, S.; Shogren, M. D.; Pomeranz, Y. Cereal pentosans: their estimation and significance. I. Pentosans in wheat and milled wheat products. *Cereal Chem.* **1987**, *64*, 30−34.

Hinton, J. J. C. The distribution of ash in the wheat kernel. *Cereal Chem.* **1959**, *36*, 13−31.

Hoffmann, R. A.; Roza, M.; Maat, J.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural characteristics of the cold-water-soluble arabinoxylans from the white flour of the soft wheat variety Kadet. *Carbohydr. Polym*. **1991**, *15*, 415−430.

Hoffmann, R. A.; Kamerling, J. P.; Vliegenthart, J. F. G. Structural features of a water-soluble arabinoxylan from the endosperm of wheat. *Carbohydr. Res.* **1992**, *226*, 303−311.

ICC Standards. *Standard Methods of the International Association for Cereal Chemistry*; Verlag Moritz Schäfer: Detmold, 1960, 1976; Methods 110/1 and 104.

Izydorczyk, M. S.; Biliaderis, C. G. Influence of structure on the physicochemical properties of wheat arabinoxylan. *Carbohydr. Polym.* **1992**, *17*, 237−247.

Izydorczyk, M. S.; Biliaderis, C. G. Structural heterogeneity of wheat endosperm arabinoxylans. *Cereal Chem.* **1993**, *70*, 641−646.

Izydorczyk, M.; Biliaderis, C. G.; Bushuk, W. Comparison of the structure and composition of water-soluble pentosans from different wheat varieties. *Cereal Chem.* **1991**, *68*, 139−144.

Jelaca, S. L.; Hlynka, I. Effect of wheat flour pentosans in dough, gluten and bread. *Cereal Chem.* **1972**, *49*, 489−495.

Loosveld, A.-M. A.; Grobet, P. J.; Delcour, J. A. Contents and structural features of water-extractable arabinogalactan in wheat flour fractions. *J. Agric. Food Chem.* **1997**, *45*, 1998−2002.

Mares, D. J.; Stone, B. A. Studies on wheat endosperm. I. Chemical composition and ultrastructure of the cell walls. *Aust. J. Biol. Sci.* **1973a**, *26*, 793−812.

Mares, D. J.; Stone, B. A. Studies on wheat endosperm. II. Properties of the wall components and studies on their organization in the wall. *Aust. J. Biol. Sci*. **1973b**, *26*, 813−830.

Mares, J. D.; Stone, B. A. Studies on wheat endosperm. III. Galactose-rich polysaccharides. *Aust. J. Biol. Sci.* **1973c**, *26*, 1005−1007.

Michniewicz, J.; Biliaderis, C. G.; Bushuk, W. Effect of added pentosans on some properties of wheat bread. *Food Chem.* **1992**, *43*, 251−257.

Nyman, M.; Siljeström, M.; Pedersen, B.; Bach Knudsen, K. E.; Asp, N.-G.; Johansson, C.-G.; Eggum, B. O. Dietary fiber content and composition in six cereals at different extraction rates. *Cereal Chem.* **1984**, *61*, 14−19.

Pence, J. W.; Elder, A. H.; Mecham, D. K. Preparation of wheat flour pentosans for use in reconstituted doughs. *Cereal Chem.* **1950**, *27*, 60−66.

Roels, S. P.; Cleemput, G.; Vandewalle, X.; Nys, M.; Delcour, J. A. Bread volume potential of variable-quality flours with constant protein level as determined by factors governing mixing time and baking absorption levels. *Cereal Chem.* **1993**, *70*, 318−323.

Suckow, P.; Abdel-Gawad, A.; Meuser, F. Versuche zur Aufklarung des Anomalen Technologischen Verhaltnis nicht Backfahiger Weizen. Schriftenreihe aus dem Fachgebiet Getreidetechnologie. Technische Universität Berlin: Berlin, 1983; Heft 6.

Vanhamel, S.; Cleemput, G.; Delcour, J. A.; Nys, M.; Darius, P. L. Physicochemical and functional properties of rye nonstarch polysaccharides. IV. The effect of high molecular weight water-soluble pentosans on wheat-bread quality in a straight-dough procedure. *Cereal Chem.* **1993**, *70*, 306−311.

Westerlund, E.; Andersson, R.; Åman, P.; Theander, O. Effects of baking on water-soluble polysaccharides in white bread fractions. *J. Cereal Sci.* **1990**, *12*, 33−42.

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