

Contents lists available at ScienceDirect

Journal of Cereal Science



journal homepage: www.elsevier.com/locate/jcs

Determining levels of water-extractable and water-unextractable arabinoxylan in commercial Swedish wheat flours by a high-throughput method

Louise Selga^{a,b,*}, Annica A.M. Andersson^a, Annelie Moldin^b, Roger Andersson^a

^a Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07, Uppsala, Sweden
^b Lantmännen, Sankt Göransgatan 160A, Stockholm, 112 17, Sweden

ARTICLE INFO	A B S T R A C T				
<i>Keywords:</i> Arabinoxylan Wheat flour Principal component analysis	A high-throughput method for quantification of water extractable arabinoxylan (WE-AX) and water unextract- able arabinoxylan (WU-AX) was adapted for and evaluated on 197 commercial Swedish wheat flours, collected continuously during harvest years 2018 and 2019. In the method, starch was hydrolysed by alpha-amylase and WE-AX was precipitated with 80% ethanol. AX residues were quantified by gas chromatography after acid hy- drolysis. The method had a good repeatability (2.1% RSD _r for total AX). Spring wheat flour had a higher WE-AX content (0.68%) and lower WU-AX content (1.19%) than winter wheat flour (0.56% and 1.31%). The variation of total AX content was high for winter wheat flour (1.5–2.2%), with no correlation to ash or protein content. Total AX content differed significantly both between harvest years and locations, indicating an impact from envi-				

further used to study how endogenous AX impacts baking quality.

1. Introduction

Wheat is unique in its capacity to yield well aerated bread. Gluten is the main factor impacting loaf volume (Goesaert et al., 2005), but multiple studies indicate that arabinoxylan (AX) also is of importance (Courtin et al., 1999; Gan et al., 1995). While the impact of AX on baking properties may be less than that of gluten, it is still worthwhile to study. Gluten consists of a wide arrange of large insoluble proteins (Goesaert et al., 2005) which makes it difficult to quantify. As AX is easier to quantify, it would be a great asset to the milling and baking industries if AX could improve quality evaluations of wheat flour. Gluten content and structures are heavily influenced by environment and genotype and can vary considerably between harvest years (Johansson et al., 2013). This can lead to fluctuations in quality which are difficult to predict and manage. This in turn may mask the influence of flour components of a smaller quantity, such as fibres, enzymes, and lipids, and makes it difficult to assess their impact on baking quality.

AX is the main cell wall polysaccharide in the endosperm constituting 70% of the cell wall (Mares and Stone, 1973). Other cell wall polymers include beta-glucan, 20%, glucomannan, 7% and cellulose, constituting 2% of the cell wall in wheat endosperm (Mares and Stone, 1973). The total AX content in sieved wheat flour is typically around 2.2%, out of which about 25% is water-extractable AX (WE-AX) (Barron et al., 2007). Still, quite a varying range has been observed with total AX contents of 1.4–2.7% and WE-AX contents of 0.3–1.4% (Saulnier et al., 2007).

ronment on AX composition. Overall, the method enabled high-throughput analysis of wheat flour and can be

AX in wheat is composed of β -d-xylopyranosyl residues linked through 1 \rightarrow 4 glycosidic linkages, partially substituted with α -L-arabinofuranosyl at position 2 or positions 2 and 3 (Izydorczyk and Biliaderis, 1994). AX can also be substituted with ferulic acid, but the levels of ferulic acid are low in the wheat endosperm (Saulnier et al., 2012). The degree of arabinofuranosyl substitution determines the solubility of the AX, with a lower substitution degree in water-unextractable AX (WU-AX) and higher heterogeneity in WE-AX (Stone and Morell, 2009).

Both WE-AX and WU-AX contributes to increasing the water absorption, but the influence on baking properties appears to differ (Courtin et al., 1999). The insolubility of WU-AX causes it to trap water, limiting the water available for gluten development (Wang et al., 2003). WE-AX instead increases the viscosity of the free water in the dough upon absorption (Courtin et al., 1999). Gan et al. (1995) has theorized that when dough expands beyond the capacity of the gluten network the structure is stabilized by a liquid film that surrounds the gas cells and

https://doi.org/10.1016/j.jcs.2022.103608

Received 7 July 2022; Received in revised form 7 November 2022; Accepted 30 November 2022 Available online 30 November 2022 0733-5210/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

^{*} Corresponding author. Department of Molecular Sciences, BioCenter, Swedish University of Agricultural Sciences, P.O. Box 7015, SE-750 07, Uppsala, Sweden. *E-mail address:* louise.selga@slu.se (L. Selga).

Table 1

Mean values (% of dry matter) and standard deviations for total arabinoxylan content (total AX), water-unextractable arabinoxylan (WU-AX), water-extractable arabinoxylan (WE-AX), unextractable Ara/Xyl-ratio (ara/xyl U), extractable Ara/Xyl-ratio (ara/xyl E), Ara/Xyl-ratio (ara/xyl), WE-AX/total AX, unextractable mannose residues (man U), unextractable galactose residues (gal U), extractable galactose residues (gal E), ash and protein in different wheat flour products, harvest years and milling locations, with n number of samples.

Content (% flour dm)	Product			Harvest year		Milling location	
	$\frac{\text{Winter wheat}}{n = 104}$	$\frac{\text{Spring wheat}}{n = 49}$	Flour blend $n = 43$	2018 n = 92	2019 n = 104	$\frac{Malm\ddot{o}}{n=154}$	$\frac{Strängnäs}{n = 42}$
WU-AX ^a	$1.31\pm0.11a$	$1.19\pm0.10b$	$1.22\pm0.09b$	$1.28\pm0.10a$	$1.25\pm0.13a$	$1.25\pm0.11a$	$1.30\pm0.14a$
WE-AX ^a	$0.56\pm0.06b$	$\textbf{0.68} \pm \textbf{0.07a}$	$\textbf{0.66} \pm \textbf{0.05a}$	$\textbf{0.61} \pm \textbf{0.06a}$	$0.61\pm0.10\text{a}$	$\textbf{0.60} \pm \textbf{0.09a}$	$\textbf{0.63} \pm \textbf{0.06a}$
ara/xyl U	$0.59\pm0.01a$	$0.56\pm0.02c$	$0.58\pm0.01\mathrm{b}$	$0.58\pm0.02a$	$0.57\pm0.02b$	$0.58\pm0.02a$	0.57 ± 0.02a
ara/xyl E	$0.57\pm0.05a$	$0.55\pm0.03b$	$0.57\pm0.03~\mathrm{ab}$	$0.57\pm0.03a$	$0.56 \pm 0.05a$	$0.57\pm0.04a$	$0.54\pm0.04b$
ara/xyl	$0.59\pm0.02a$	$0.55\pm0.02c$	$0.57\pm0.01b$	$0.58\pm0.02a$	$0.57\pm0.03b$	$0.58\pm0.02a$	$0.56\pm0.03b$
WE-AX/total AX	$0.30\pm0.02b$	$\textbf{0.36} \pm \textbf{0.03a}$	$\textbf{0.35}\pm\textbf{0.03a}$	$\textbf{0.32}\pm\textbf{0.03a}$	$0.32\pm0.05\text{a}$	$0.32\pm0.04\text{a}$	$0.33\pm0.04a$
man U	$0.08\pm0.01\mathrm{a}$	$0.07\pm0.01\mathrm{b}$	$0.07\pm0.01\mathrm{b}$	$0.07\pm0.01\mathrm{b}$	$0.08\pm0.01a$	0.08 ± 0.01 a	$0.08 \pm 0.01a$
gal U	$0.04 \pm 0.01a$	$0.04 \pm 0.01a$	$0.04\pm0.00a$	$0.04\pm0.00a$	$0.04\pm0.01\mathrm{b}$	$0.04\pm0.01b$	$0.05\pm0.00a$
gal E	$0.14\pm0.02b$	$\textbf{0.15}\pm\textbf{0.03a}$	$0.14\pm0.02~ab$	$0.14\pm0.02a$	$\textbf{0.14}\pm\textbf{0.03a}$	$0.14\pm0.02a$	$0.15\pm0.02a$
Ash	$0.58\pm0.05c$	$0.64\pm0.04a$	$0.60\pm0.03\mathrm{b}$	$0.60\pm0.05a$	$0.59\pm0.05a$	$0.59\pm0.05b$	$0.64\pm0.03a$
Protein	$11.6 \pm 0.5c$	$13.8 \pm 0.6a$	$13.3 \pm 0.4b$	$12.6 \pm 1.1a$	$12.4 \pm 1.2a$	$12.5 \pm 1.2a$	$12.6 \pm 0.7a$

^a Calculated from arabinose, xylose and galactose residues assuming that the arabinose to xylose ratio was 0.69 in arabinogalactan.

^b Different letters in the same row indicate significant differences (p < 0.05) within categories product, harvest year and milling location.

retains their integrity. WE-AX could stabilise this film by lowering the surface tension and increase its sturdiness by increasing its viscosity (Gan et al., 1995). WE-AX with a higher molecular weight increases the water absorption further (Biliaderis et al., 1995). For these reasons, AX is of interest in breadmaking both through modification using xylanases (Courtin et al., 1999) and as an ingredient (Pietiäinen et al., 2022). The effect of differences in endogenous AX levels are more difficult to study.

The levels of total AX and WE-AX in wheat endosperm are mainly affected by genotype (Finnie et al., 2006), but can also be influenced by the environment (De Santis et al., 2018; Li et al., 2009; Rakszegi et al., 2014). Decreases in arabinose substitution have been observed during grain maturation (Toole et al., 2010) and differences in AX composition may reflect different grain developmental rates among varieties (Marion and Saulnier, 2020). Lower WE-AX levels have been observed in winter wheat after induced drought (Rakszegi et al., 2014). Xylanase activities in wheat are higher after cold and rainy summers compared to hot and dry summers, but the correlation to WE-AX content is low (Dornez et al., 2009). Studies of AX variation have mainly been done on grain from field trials, and the AX variation has to our knowledge not been studied in industrially produced flours to any significant extent.

The method for AX quantification is based on the analytical procedure AOAC 994.13 by Theander et al. (1995), which was developed to accurately determine the total dietary fibre content in a wide range of foods. The method was modified by Andersson et al. (1999) to quantify soluble and insoluble fibres separately. Further modifications are presented in this study to speed up the method for sieved wheat flour. Cellulose and lignin were not quantified as they are only present in low levels in sieved wheat flour (Saulnier et al., 2012). Beta-glucan in wheat has a low solubility (De Paula et al., 2017; Rakha et al., 2011) and remains water-unextractable at 65 °C (Beresford and Stone, 1983). For this reason, beta-glucan was not quantified as it is unlikely to have an impact on baking properties. Additionally, the beta-glucan levels present in wheat endosperm are low compared to AX (Andersson et al., 1993; Mares and Stone, 1973; Saulnier et al., 2012).

The impact of AX on baking properties has mainly been studied by addition of AX, enzymatic treatment, and correlation studies (Gan et al., 1995). However, the source of the added AX or the enzyme levels studied may not be suitable for discovering what impact AX has at

endogenous levels. For this, correlations studies on large samples set are needed, to ensure sufficient variation in AX levels. This in turn requires a high-throughput method. The aims of this study were to evaluate an adapted high-throughput method for WE-AX and WU-AX quantification, and to screen the AX levels in commercial sieved wheat flours.

2. Experimental

2.1. Material

Commercial Swedish wheat flours produced by Lantmännen Cerealia in Malmö and Strängnäs, Sweden, were collected during harvest years 2018 and 2019 (Table 1). A total of 197 samples were collected continuously during this period to give a sample set fully representative of Swedish flour production. Three different products were analysed: i) flour composed of winter wheat, ii) flour of spring wheat, and iii) a high protein blend composed of 0–15% winter wheat, 15–35% spring wheat and 50–70% of a high protein German winter wheat. All products used were sieved flours without any additions, such as malt or ascorbic acid, and all samples were stored at -20 °C. Protein content (determined by NIT measurements) and ash content were provided by the mill.

The variety or blend of varieties used in individual products were not known. Information on winter wheat supplied to the mills showed that varieties used in Strängnäs did not differ much between years but varied more for Malmö. The variety Julius was the most common winter wheat in both Malmö and Strängnäs during both harvest years. Julius constituted roughly half of the winter wheat milled in Strängnäs and the composition and proportion of varieties milled were similar for 2018 and 2019. For Malmö, about one fourth of milled winter wheat was Julius, h Brons was second most common in 2018 and Linus was second most common in 2019.

2.2. Determination of water-extractable and water-unextractable AX

AX analysis was based on the method for dietary fibre analysis by Theander et al. (1995) with modifications by Andersson et al. (1999) to separate soluble and insoluble fractions. The protocol was modified to speed up fibre extraction and allow for analysis of a larger number of samples. Reagents and apparatus used are listed by Theander et al. (1995). Dry matter was determined by oven drying for 16 h at 105 $^{\circ}$ C.

Duplicate samples of approximately 200 mg were weighed in test tubes, noting the exact weight. These were vortexed with 5 ml 0.1 M acetate buffer of pH 5.0 and 40 μ l thermostable α -amylase (3000 U/mL, purified from *Bacillus licheniformis*, product code E-BLAAM, Megazyme, Bray, Ireland). The samples were capped and placed in a boiling water bath, vortexed to disperse the sample particles, and incubated for 1 h with occasional mixing. The samples were cooled and centrifuged for 10 min at $1000 \times g$. The pellet was kept for determination of water-unextractable AX, while the supernatant was used for determination of water-extractable AX.

Two ml of the supernatant were transferred to a new test tube and 8 ml 99.5% ethanol was added. The samples were mixed and kept at 4 °C for 1 h to precipitate water-extractable AX. After refrigeration the samples were centrifuged for 10 min at $1000 \times g$. The supernatant was discarded, and the pellet was resuspended in 5 ml acetone using a spatula. The samples were centrifuged for 15 min at $1000 \times g$, and the acetone was removed.

The pellet obtained after the amylase treatment was washed with 5 ml 0.1 M acetate buffer and centrifuged for 10 min at $1000 \times g$ to obtain water-unextractable AX. The pellet was washed twice with 5 ml acetone by vortexing and centrifugation for 15 min at $1000 \times g$. Acetone was removed by suction to keep the pellet intact. All samples were air dried. Glass rods were placed in each sample for stirring to prevent formation of compact residue.

Hydrolysis was conducted once the samples had dried. For waterunextractable fibres, 0.3 ml 12 M sulfuric acid was added and distributed with the glass rod. Samples were then placed in a 30 °C water bath for 1 h. After this 7.8 ml H₂O and 0.6 ml *myo*-inositol standard solution (2.0 mg/ml) were added. For the water-extractable fibres 2.6 ml H₂O was added with 0.1 ml 12 M sulfuric acid and 0.2 ml *myo*-inositol standard solution (2.0 mg/ml). All samples were then covered with aluminium foil and hydrolysed at 125 °C for 1 h in autoclave. Acetylation and quantification by GC were done in accordance with Theander et al. (1995). Extractable and unextractable residues of the sugars arabinose, xylose, galactose and mannose were quantified.

2.3. Statistical analysis

Significant differences were calculated at 95% confidence by Tukey's pairwise comparisons in Minitab. Principal component analysis was done using SIMCA 16.

3. Results and discussion

3.1. Modification of the method

Adjustments to Theander et al. (1995) and Andersson et al. (1999) were done to allow a high throughput of samples while still achieving a reliable quantification of water-extractable and water-unextractable AX. The repeatability of the method can be observed by the relative standard deviation of repeatability (RSD_r). The RSD_r was calculated for the method by repeat analysis of a sample at 15 separate occasions. The RSD_r was 3.4% for WU-AX, 2.5% for WE-AX and 2.1% for total AX, respectively. These values are in the same range as those obtained by Theander et al. (1995). Further, the results obtained agreed well with those obtained by Andersson et al. (1993) when analysing Swedish wheat flour using the method of Theander et al. (1995).

The main adjustment to the method was that the overnight incubation with amyloglucosidase was removed. This led to residual dextrins originating from starch remaining in the samples throughout quantification. This excluded the quantification of cellulose and beta-glucan from the method. This exclusion was negligible as the overall project aim was to study AX in relation to baking properties. The impacts on baking properties of wheat endosperm cellulose and beta-glucan are

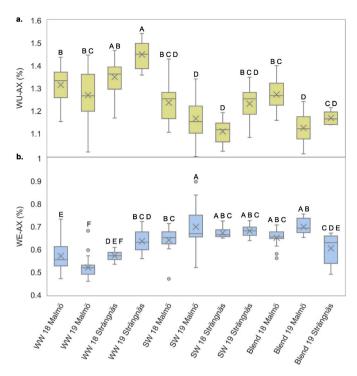


Fig. 1. WU-AX content (% dry matter) (a) and WE-AX content (% dry matter) (b). "WW" refers to winter wheat flour and "SW" to spring wheat flour milled during harvest years 2018 (18) or 2019 (19). Whiskers span highest and lowest samples, boxes span 25–75% percentiles, circles mark outliers (50% higher or lower than these percentiles), and cross marks show the mean values. Different letters indicate significant differences within figures (p < 0.05).

likely not of significance, as the solubilities of both cellulose and betaglucan are low (Beresford and Stone, 1983; De Paula et al., 2017; Rakha et al., 2011), and both are very low in content compared to AX (Saulnier et al., 2012).

3.2. Differences in products, harvest years and milling locations

The total AX content was around 1.9% and did not differ between products but differed significantly both between harvest years and milling locations (Table 1). Total AX content showed no correlation with ash content, indicating that AX content was not affected by differences in milling conditions between locations. It was unexpected that winter wheat and spring wheat did not differ in total AX content, as they differ considerably in genetics. While Finnie et al. (2006) observed total AX to be more impacted by genetics than environment, Li et al. (2009) and Tremmel-Bede et al. (2020) observed environment to have a greater impact. Total AX content was significantly (p < 0.05) higher during the drought affected harvest year 2018 (1.89%) than during 2019 (1.85%) and varied more during 2019. Winter wheat flour had a significantly higher WU-AX content (1.31%) and lower WE-AX content (0.56%) than the other products. The Ara/Xyl-ratios calculated all differed significantly between products, with low variations. This could indicate that the AX structure is more influenced by the different genetic origin of spring and winter wheat. However, Tremmel-Bede et al. (2020) observed genotype to have a lower effect on extractable Ara-Xyl ratio than on WE-AX. The extractable Ara/Xyl-ratio (ara/xyl E) showed a slightly higher variation than the other ratios calculated, which is in line with WE-AX having a higher heterogeneity, as suggested by Stone and Morell (2009). The content of mannose and galactose varied very little. Ash content differed significantly between products and between milling locations, with higher contents for spring wheat and products milled in Strängnäs.

During harvest years 1987 and 1988 Andersson et al. (1993)

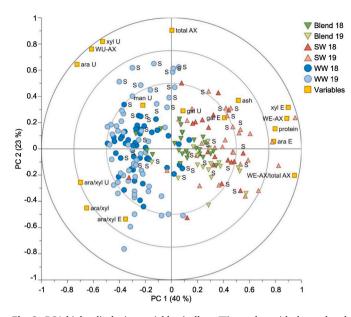


Fig. 2. PCA biplot displaying variables (yellow, ■) together with the analysed samples: flour blend (Blend, green, \checkmark), spring wheat (SW, red, \blacktriangle), and winter wheat (WW, blue, ●). Harvest year 2018 is coloured darker and harvest year 2019 is coloured lighter. Letters denote milling location Strängnäs (S), while all other samples were milled in Malmö. Total AX = total arabinoxylan content, WU-AX = water-unextractable arabinoxylan, WE-AX = water-extractable arabinoxylan, ara U = unextractable arabinose residues, xyl U = unextractable xylose residues, man U = unextractable arabinose residues, gal U = unextractable arabinose residues, gal U = unextractable arabinose residues, gal U = unextractable xylose residues, gal E = extractable galactose residues, ara/xyl U = unextractable Ara/Xyl-ratio. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

collected 49 flours of Swedish spring wheat and winter wheat and the WE-AX and WU-AX contents determined (0.60% and 1.24%) agree well with those obtained in this study. They did however observe a higher variance in AX content compared to this study, likely due to their use of pure varieties, field trial design and sample selection (Andersson et al., 1993). As in this study, they observed higher WE-AX in spring wheat flour but the contrast between spring and winter wheats were not as clear (Andersson et al., 1993). Harvest year 1987 had unfavourable weather conditions during the summer, which yielded lower WU-AX and higher WE-AX, and a higher variation of AX content between different varieties (Andersson et al., 1993). This can be compared to the lower variation in AX content observed during harvest year 2018, during which there was a drought. Overall, Swedish wheat appears to be unchanged in regard to AX content and composition since 1988, but it is possible that atypical varieties could be masked in this study due to the wheat being blended during milling.

3.3. Water-extractable and water-unextractable AX in products, years and locations

There were significant differences in WE-AX and WU-AX contents between the products, years, and locations (Fig. 1). For winter wheat flour, the WU-AX content was highest in flours from Strängnäs during 2019 (Fig. 1a). Harvest year 2019 had significantly (p < 0.05) lower WE-AX in flours from Malmö and higher WE-AX in the Strängnäs flours (Fig. 1b). The flours from Malmö showed a higher variation in WU-AX content during 2019 and a higher variation in WE-AX content during 2018.

For spring wheat flour, WU-AX content did not differ significantly but was somewhat lower in flours from Strängnäs during 2018 (Fig. 1a). The variation in WE-AX content was highest for flours from Malmö during 2019 (Fig. 1b). The flour blends differed in WU-AX content, with highest levels during 2018. This was probably caused by a higher proportion of winter wheat being added to the blend in 2018, due to a lower spring wheat harvest. The WE-AX content was significantly lower for the flour blend from Strängnäs than those from Malmö during 2019.

Previous studies have shown that while environment and genotype both influence AX content in grain (Marion and Saulnier, 2020), AX content in the endosperm is mainly influenced by genotype (Finnie et al., 2006; Li et al., 2009). WE-AX content is mainly influenced by genotype but can also be affected by the environment (De Santis et al., 2018), and differences have been observed between harvest years (Andersson et al., 1993). Rakszegi et al. (2014) observed lower WE-AX content in winter wheat after induced drought. There was a high temperature drought in Sweden during the summer in 2018, which led to a low harvest. Flours milled in 2018 mainly seemed to have a lower variation in AX content (Table 1). One reason could be that the drought generated a consistent environment which led to more similar wheat quality and AX levels nationally. The higher variation in AX content during 2019 could be caused by a more varied environment with local weather differences.

3.4. Overall variation patterns

The overall variation of the data set was explored using Principal Component Analysis (PCA) (Fig. 2). Principal component 1 (PC 1) accounted for 40% of the overall variation in the data set and Principal component 2 (PC 2) for 23%, leaving 37% of the variation unexplained. It could be noted that PC 1 separated samples according to the genetic and seasonal differences between spring and winter wheat, while PC 2 distributed winter wheat samples based on differences in harvest year and location. WE-AX, WE-AX/total AX and protein all had high values on PC 1 and were therefore the main source of variation in the data set. Winter wheat formed a separate cluster with negative values on PC 1 and there was very little overlap between winter wheat and the other products, displaying distinct product differences in WE-AX and protein content.

Total AX had the highest value on PC 2 and differences between winter wheat samples was the main pattern identified. Total AX content showed no correlation with ash content, which can be seen by their orthogonal placement in Fig. 2. The lack of a correlation to ash content indicates that the total AX content was not influenced by varying levels of aleurone layer or bran being incorporated during milling. Winter wheat flours milled in Strängnäs during 2019 formed a group with high PC 2 values and had notably high WU-AX and WE-AX content (Fig. 1). These differences between winter wheat milled in Strängnäs during 2018 and 2019 appear to be caused by differences in weather conditions between harvest years rather than genetic differences, as the wheat varieties supplied to Strängnäs barely differed between the years.

The high PC 1 values for WE-AX content and protein indicated that they could be correlated, but this was caused by them both being present in higher concentrations in spring wheat. Correlations between protein and AX were not observed when further studying winter wheat and spring wheat separately. It could be expected that a higher protein content would lead to a relatively lower total AX content, in the same way as a higher starch content in the grain can lead to a relatively lower amount of protein. This was however not the case, which could be an indication of stable cell sizes. Dunstone and Evans (1974) have studied endosperm cell sizes in wild and cultivated wheats and they did not detect a larger endosperm cell size in larger grain. Increases in starch or protein content seemed to influence the number of endosperm cells, not the cell sizes. This would then not influence the AX content in the endosperm.

The mill in Malmö receives wheat from southern Sweden where there is a lower demand for winter hardiness in cultivars and longer daylight hours during winter, which allows for cultivars with earlier grain development. Marion and Saulnier (2020) speculate that different

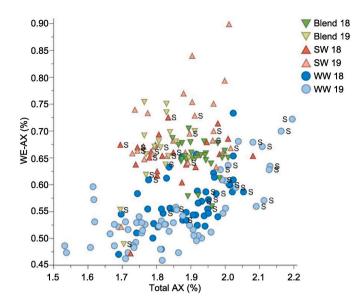


Fig. 3. Total arabinoxylan content (% dry matter) plotted against WE-AX content (% dry matter): flour blend (Blend, green, ▼), spring wheat (SW, red, ▲), and winter wheat (WW, blue, ●). Harvest year 2018 is coloured darker and harvest year 2019 is coloured lighter. Letters denote milling location Strängnäs (S), all other samples were milled in Malmö. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

grain developmental rates could lead to differences in arabinose substitution between cultivars. During grain development, the substitution of arabinose decreases and this occurs more rapidly in plants under drought conditions (Toole et al., 2007). This could be seen in samples milled in Malmö, where the extractable Ara/Xyl-ratio was somewhat lower in 2018 in all product categories. This was however not seen for samples milled in Strängnäs.

According to the PCA, the WE-AX content and total AX content cover the overall variation in the dataset, as they align with PC 1 and PC 2. The WE-AX content and total AX content appear to be inherently different in winter wheat and spring wheat (Fig. 3). Both the spring wheat and the flour blend show a low variation in total AX. Winter wheat had a larger variation in total AX, especially flours milled in Malmö. This could be due to a higher diversity in varieties being grown in the Malmö area compared to Strängnäs, as previous studies indicate that differences in genotype have a larger influence on AX content compared to differences in environment (Gebruers et al., 2010; Marion and Saulnier, 2020). The flour blend showed the lowest variation out of the different products, which is in line with the stable quality of this product.

4. Conclusions

A high-throughput analysis of AX was used to screen a large number of commercially produced Swedish wheat flours. The method showed a good repeatability, and the obtained results were in line with those obtained by Andersson et al. (1993) when using the original method by Theander et al. (1995) on sieved wheat flour. Continuous sampling ensured an accurate representation of Swedish flours during harvest years 2018–2019. Milling conditions did not seem to have an effect on the total AX content, as there was no correlation between this and ash content. WE-AX and WU-AX levels were similar to those measured on Swedish wheat during harvest years 1987–1988. According to previous studies, genotype influences AX content and composition more than environment. There was however a drought during 2018 which appears to have reduced the variation in AX content. In addition to this, flours milled in Strängnäs displayed large differences in total AX content between years, despite a similar assortment of varieties being used. Overall, a larger variation was seen for winter wheat compared to spring wheat, and further studies will examine if this variation can be linked to fluctuations in baking quality. This screening of AX content may be useful for evaluating the impact of xylanases, which are commonly used in bakeries to affect the baking properties.

Author statement

Louise Selga: Investigation, Formal analysis, Visualization, Writing -Original Draft, Review & Editing. Annica A.M. Andersson: Methodology, Validation, Writing - Review & Editing. Annelie Moldin: Supervision, Resources, Writing - Review & Editing. Roger Andersson: Conceptualization, Methodology, Supervision, Writing - Review & Editing.

Data availability

Data will be made available upon project completion.

Acknowledgements

Funding: This study was financed through an industry PhD program of the Swedish Government (governmental decision N201703895), SLU (grant number SLU.ua.2017.1.1.1–2416) and Lantmännen. Louise Selga and Annelie Moldin are employed by Lantmännen. Annica Andersson and Roger Andersson have no interests to declare.

References

- Andersson, A.A.M., Merker, A., Nilsson, P., Sörensen, H., Åman, P., 1999. Chemical composition of the potential new oilseed crops Barbarea vulgaris, Barbarea verna and Lepidium campestre. J. Sci. Food Agric. 79, 179–186. https://doi.org/10.1002/ (SICI)1097-0010(199902)79:2<179::AID-JSFA163>3.0.CO:2-N.
- Andersson, R., Westerlund, E., Tilly, A.-C., Åman, P., 1993. Natural variations in the chemical composition of white flour. J. Cereal. Sci. 17, 183–189. https://doi.org/ 10.1006/jcrs.1993.1018.
- Barron, C., Surget, A., Rouau, X., 2007. Relative amounts of tissues in mature wheat (Triticum aestivum L.) grain and their carbohydrate and phenolic acid composition. J. Cereal. Sci. 45, 88–96. https://doi.org/10.1016/j.jcs.2006.07.004.
- Beresford, G., Stone, B.A., 1983. (1→3), (1→4)-β-D-glucan content of Triticum grains. J. Cereal. Sci. 1, 111–114. https://doi.org/10.1016/S0733-5210(83)80028-9.
- Biliaderis, C.G., Izydorczyk, M.S., Rattan, O., 1995. Effect of arabinoxylans on breadmaking quality of wheat flours. Food Chem. 53, 165–171. https://doi.org/10.1016/ 0308-8146(95)90783-4.
- Courtin, C.M., Roelants, A., Delcour, J.A., 1999. Fractionation–Reconstitution experiments provide insight into the role of endoxylanases in bread-making. J. Agric. Food Chem. 47, 1870–1877. https://doi.org/10.1021/jf981178w.
- De Paula, R., Abdel-Aal, E.-S.M., Messia, M.C., Rabalski, I., Marconi, E., 2017. Effect of processing on the beta-glucan physicochemical properties in barley and semolina pasta. J. Cereal. Sci. 75, 124–131. https://doi.org/10.1016/j.jcs.2017.03.030.
- De Santis, M.A., Kosik, O., Passmore, D., Flagella, Z., Shewry, P.R., Lovegrove, A., 2018. Comparison of the dietary fibre composition of old and modern durum wheat (Triticum turgidum spp. durum) genotypes. Food Chem. 244, 304–310. https://doi. org/10.1016/j.foodchem.2017.09.143.
- Dornez, E., Gebruers, K., Delcour, J.A., Courtin, C.M., 2009. Grain-associated xylanases: occurrence, variability, and implications for cereal processing. Trends Food Sci. Technol. 20, 495–510. https://doi.org/10.1016/j.tifs.2009.05.004.
- Dunstone, R.L., Evans, L.T., 1974. Role of changes in cell size in the evolution of wheat. Funct. Plant Biol. 1, 157–165. https://doi.org/10.1071/pp9740157.
- Finnie, S.M., Bettge, A.D., Morris, C.F., 2006. Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. Cereal Chem. 83 (6), 617–623. https://doi.org/10.1094/CC-83-0617.
- Gan, Z., Ellis, P.R., Schofield, J.D., 1995. Gas cell stabilisation and gas retention in wheat bread dough. J. Cereal. Sci. 21, 215–230. https://doi.org/10.1006/jcrs.1995.0025.
- Gebruers, K., Dornez, E., Bedō, Z., Rakszegi, M., Frás, A., Boros, D., Courtin, C.M., Delcour, J.A., 2010. Environment and genotype effects on the content of dietary fiber and its components in wheat in the HEALTHGRAIN diversity screen. J. Agric. Food Chem. 58, 9353–9361. https://doi.org/10.1021/jf100447g.
- Goesaert, H., Brijs, K., Veraverbeke, W.S., Courtin, C.M., Gebruers, K., Delcour, J.A., 2005. Wheat flour constituents: how they impact bread quality, and how to impact their functionality. Trends Food Sci. Technol. 16, 12–30. https://doi.org/10.1016/j. tifs.2004.02.011.
- Izydorczyk, M.S., Biliaderis, C.G., 1994. Studies on the structure of wheat-endosperm arabinoxylans. Carbohydr. Polym. 24, 61–71. https://doi.org/10.1016/0144-8617 (94)90118-X.
- Johansson, E., Malik, A.H., Hussain, A., Rasheed, F., Newson, W.R., Plivelic, T., Hedenqvist, M.S., Gällstedt, M., Kuktaite, R., 2013. Wheat gluten polymer structures: the impact of genotype, environment, and processing on their functionality in

L. Selga et al.

various applications. Cereal Chemistry Journal 90, 367–376. https://doi.org/ 10.1094/CCHEM-08-12-0105-FI.

- Li, S., Morris, C.F., Bettge, A.D., 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the U.S. Pacific northwest. Cereal Chem. 86, 88–95. https://doi.org/10.1094/CCHEM-86-1-0088.
- Mares, D.J., Stone, B.A., 1973. Studies on wheat endosperm I. Chemical composition and ultrastructure of the cell walls. Aust. Jnl. Of Bio. Sci. 26, 793–812. https://doi.org/ 10.1071/bi9730793.
- Marion, D., Saulnier, L., 2020. Minor components and wheat quality: perspectives on climate changes. J. Cereal. Sci. 94, 103001 https://doi.org/10.1016/j. jcs.2020.103001.
- Pietiäinen, S., Moldin, A., Ström, A., Malmberg, C., Langton, M., 2022. Effect of physicochemical properties, pre-processing, and extraction on the functionality of wheat bran arabinoxylans in breadmaking – a review. Food Chem. 383, 132584 https://doi.org/10.1016/j.foodchem.2022.132584.
- Rakha, A., Åman, P., Andersson, R., 2011. Dietary fiber in triticale grain: variation in content, composition, and molecular weight distribution of extractable components. J. Cereal. Sci. 54, 324–331. https://doi.org/10.1016/j.jcs.2011.06.010.
- Rakszegi, M., Lovegrove, A., Balla, K., Láng, L., Bedő, Z., Veisz, O., Shewry, P.R., 2014. Effect of heat and drought stress on the structure and composition of arabinoxylan and β-glucan in wheat grain. Carbohydr. Polym. 102, 557–565. https://doi.org/ 10.1016/j.carbpol.2013.12.005.
- Saulnier, L., Guillon, F., Chateigner-Boutin, A.-L., 2012. Cell wall deposition and metabolism in wheat grain. J. Cereal. Sci. 56, 91–108. https://doi.org/10.1016/j. jcs.2012.02.010.

- Saulnier, L., Sado, P.-E., Branlard, G., Charmet, G., Guillon, F., 2007. Wheat arabinoxylans: exploiting variation in amount and composition to develop enhanced varieties. J. Cereal. Sci. 46, 261–281. https://doi.org/10.1016/j.jcs.2007.06.014.
- Stone, B., Morell, M., 2009. Carbohydrates. In: Khalil, K., Shewry, P. (Eds.), Wheat: Chemistry and Technology, fourth ed. American Association of Cereal Chemists, St. Paul, Minnesota, pp. 322–323.
- Theander, O., Åman, P., Westerlund, E., Andersson, R., Pettersson, D., 1995. Total dietary fiber determined as neutral sugar residues, uronic acid residues, and Klason lignin (the uppsala method): collaborative study. J. AOAC Int. 78, 1030–1044. https://doi.org/10.1093/jaoac/78.4.1030.
- Toole, G.A., Le Gall, G., Colquhoun, I.J., Nemeth, C., Saulnier, L., Lovegrove, A., Pellny, T., Wilkinson, M.D., Freeman, J., Mitchell, R.A.C., Mills, E.N.C., Shewry, P. R., 2010. Temporal and spatial changes in cell wall composition in developing grains of wheat cv. Hereward. Planta 232, 677–689. https://doi.org/10.1007/s00425-010-1199-5.
- Toole, G.A., Wilson, R.H., Parker, M.L., Wellner, N.K., Wheeler, T.R., Shewry, P.R., Mills, E.N.C., 2007. The effect of environment on endosperm cell-wall development in Triticum aestivum during grain filling: an infrared spectroscopic imaging study. Planta 225, 1393–1403. https://doi.org/10.1007/s00425-006-0448-0.
- Tremmel-Bede, K., Szentmiklóssy, M., Tömösközi, S., Török, K., Lovegrove, A., Shewry, P.R., Láng, L., Bedő, Z., Vida, G., Rakszegi, M., 2020. Stability analysis of wheat lines with increased level of arabinoxylan. PLoS One 15. https://doi.org/ 10.1371/journal.pone.0232892.
- Wang, M., Hamer, R.J., van Vliet, T., Gruppen, H., Marseille, H., Weegels, P.L., 2003. Effect of water unextractable solids on gluten formation and properties: mechanistic considerations. J. Cereal. Sci. 37, 55–64. https://doi.org/10.1006/jcrs.2002.0478.