

Feruloylation and hydrolysis of arabinoxylan extracted from wheat bran: Effect on bread quality and shelf-life

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ABSTRACT

Arabinoxylan (AX) is a potential health-promoting fiber ingredient that could be used to improve nutritional properties of bread, but is also known to affect bread and dough quality. To identify the role of feruloylation and hydrolysis of wheat bran AX on bread quality and shelf-life, hydrolyzed and unhydrolyzed AX with low and high ferulic acid content were incorporated into wheat bread. Water absorption, visual appearance, specific volume, and crumb structure were evaluated in fresh bread, and texture and moisture content over 14 days of storage. Feruloylated and unhydrolyzed AX breads underwent less moisture loss during storage but none of the AX fractions retarded crumb hardening. Feruloylated and hydrolyzed AX breads were comparable to control bread even at the highest addition level (5%) in terms of volume and crumb structure. The higher quality of these breads was associated with ferulic acid content and lower molar mass based on multivariate analysis. Based on our work, knowledge on specific AX structure can facilitate the use of increased AX levels in breadmaking.

1. Introduction

Wheat bran, a low-value side-stream currently used mostly for animal feed, has an estimated annual global production volume of 150 million tons (Prückler et al., 2014). Wheat bran contains many nutritionally interesting components, such as dietary fiber and bioactive compounds, that could be used in food applications, increasing the value of this by-product (Katilieviciute et al., 2019). One such compound is arabinoxylan (AX), an abundant dietary fiber component in wheat bran composed of a β -(1 \rightarrow 4)-linked β -D-xylopyranose backbone substituted with arabinofuranosyl (Darvill et al., 1980). Arabinose can be further linked to ferulic acid (FA) via an ester bond (Izydorczyk and Biliaderis, 1995). Although increased dietary fiber intake would reduce the risk of various nutrition-related diseases, AX is not widely utilized by the

baking industry, partly due to its reported negative effect on bread quality (Pietiäinen et al., 2022). New solutions are therefore needed to enable the high fiber addition levels required for nutrition benefits.

Feruloylated AX has known beneficial bioactivities and could potentially provide additional health benefits when added to bread (Zhang et al., 2023). The effect of FA on bread quality remains unclear, as existing results are contradictory. For example, Snelders et al. (2014) found that feruloylated AX oligosaccharides increase dough firmness. Other studies suggest that FA is involved in covalent cross-linking and strengthening the gluten network (Courtin and Delcour, 2002; Koh and Ng, 2009; Wang et al., 2021), although most of these studies were performed using free FA. Reduction of molar mass during hydrolysis has been shown to play a key role in AX functionality in breadmaking (Li et al., 2017), but to our knowledge its connection to feruloylation and

Abbreviations: AX, arabinoxylan; BU, Brabender unit; D, dispersity index; DDT, dough development time; FA, ferulic acid; FAX, feruloylated arabinoxylan; H-AX, hydrolyzed unferuloylated arabinoxylan; H-FAX, hydrolyzed feruloylated arabinoxylan; HPAEC-PAD, High performance anion exchange chromatography with pulsed amperometry detection; M_n , number-average molecular weight; M_w , weight-average molecular weight; PLS, partial least squares regression analysis; SWE, subcritical water extraction; TPA, texture profile analysis.

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their combined effect on AX baking quality have not been studied previously.

Staling is a complex process whereby bread gradually loses its freshness, leading to significant amounts of food waste worldwide (Fadda et al., 2014). There are indications that AX could retard staling by increasing crumb moisture content, interacting with gluten, or reducing starch retrogradation (Biliaderis et al., 1995; Wang et al., 2019; Hou et al., 2020). High molar mass AX has been found to prevent long-term retrogradation better than low molar mass AX, due to preferential binding to amylopectin (Hou et al., 2020). To our knowledge, the effect of feruloylated AX on bread shelf-life has not been studied previously and more knowledge on the links between AX feruloylation, molar mass, and bread quality is needed to facilitate use of feruloylated AX as a bread ingredient.

The aim of this study was to determine the effect of hydrolysis and feruloylation of wheat bran AX on wheat bread quality and shelf-life. Unhydrolyzed and hydrolyzed AX with high and low levels of FA were used in baking trials to identify the ideal AX structure for optimal wheat bread quality. Dough properties were studied using a farinograph and bread quality was evaluated by visual evaluation combined with crumb structure evaluation by image analysis and measurement of specific volume. Moisture migration from bread crumb to crust and crumb hardness were measured during a 14-day storage period, to evaluate whether AX can retard staling by preventing moisture migration. The intention with the work was to provide new insights into how feruloylated AX can be used as a functional bread ingredient to improve the technological and nutritional quality of bread, and to reduce the amount of food waste by improving bread shelf-life.

2. Materials and methods

2.1. Materials

Feruloylated arabinoxylan (FAX) and unferuloylated arabinoxylan (AX) were provided by Lantmännen (Stockholm, Sweden). The pilot-scale subcritical extraction process and chemical composition of these fractions have been described previously by Zhang et al. (2023). Briefly, fraction FAX was first extracted from wheat bran using subcritical water extraction (150 °C), and then fraction AX was produced from FAX by removing FA with mild saponification (0.5 M NaOH, 20 °C, 4 h). Bread ingredients were purchased from a local supermarket. These were: wheat flour (Kungsörnen Vetemjöl special), sugar (Garant Strösocker), yeast (Jästbolaget Kronjäst), salt (Jozo Fint salt utan jod), and rapeseed oil (Eldorado Rapsolja). Microencapsulated sorbic acid (MIRCAP® SB 85-G) was provided by Lantmännen and 1,4- β -xylanase (Pentopan Mono BG) was provided by Novozymes (Bagsværd, Denmark).

2.2. Hydrolyzation of arabinoxylan

Hydrolyzed AX (H-AX) and hydrolyzed FAX (H-FAX) were produced by enzymatic hydrolysis of AX and FAX as described by Ruthes et al. (2017). The AX and FAX were first solubilized in water (solid:liquid ratio 1:10, pH 5.0, 60 °C), and then Pentopan Mono BG, a 1,4- β -xylanase (2500 FXU-W/g, Novozymes) was added at 20 U/g AX and the slurries were incubated for 24 h. After incubation, the enzyme was inactivated by heating the slurries to 100 °C for 5 min.

2.3. Characterization of fractions

2.3.1. Monosaccharide composition

The monosaccharide composition of AX fractions was determined using a method developed by Sluiter et al. (2008) with modifications. First, 3 mL 72% H₂SO₄ (Sigma-Aldrich, Stockholm, Sweden) were added to 200 mg of sample, which was kept under vacuum for 15 min and incubated for 1 h at 30 °C with stirring every 20 min. Next, 84 g of water were added and the samples were autoclaved at 125 °C for 1 h. The

samples were then vacuum-filtered and diluted to a total volume of 100 mL. Fucose was added as an internal standard and the samples were filtered (0.45 μ m). Monosaccharide composition was analyzed using HPAEC-PAD (ICS 3000 Dionex, Thermo Scientific, Sunnyvale, USA) equipped with an AEC column (CarboPac PA 1 analytical 4 \times 250 nm, Thermo Scientific, Sunnyvale, USA). All samples were analyzed in duplicate.

2.3.2. Total starch content

Total starch content of AX fractions was determined in duplicate using a total starch assay kit (Total Starch HK Assay Kit, Megazyme Ltd, Wicklow, Ireland).

2.3.3. β -glucan content

The β -glucan content of AX fractions was determined in duplicate using a β -glucan assay kit (Mixed Linkage Assay Kit, Megazyme Ltd, Wicklow, Ireland).

2.3.4. Protein content

The soluble protein content of AX fractions was determined in duplicate by the Bradford method (Bradford, 1976).

2.3.5. Ferulic acid content

Ferulic acid content analysis began with saponification of the phenolic compounds using 2 M NaOH at 37 °C overnight, as previously described by Rudjito et al. (2019). Samples were filtered and analyzed in a high-performance liquid chromatography (HPLC) system (Waters 2695 separation module, USA) equipped with a C18 guard column and an SB-C18 separation column (Zorbax SB-C18 5 μ m particle size, 4.6 \times 250 mm, Agilent, USA), as described by Kasmaei et al. (2022). Concentrations of ferulic acid between 0.005 and 0.1 g L⁻¹ were used for standard calibration.

2.3.6. Molar mass distribution

Molar mass distribution was determined by size exclusion chromatography (SEC) (SECurity 1260, Polymer Standard Services, Mainz, Germany), as previously described by Ruthes et al. (2017).

2.4. Breadmaking

Wheat pan breads were prepared with a farinograph (Brabender GmhH, Duisburg, Germany) using the straight-dough procedure (AACC Approved Methods of Analysis, 1995). The dough ingredients were (baker's percentages): wheat flour 100%, oil 2.5%, sugar 5%, salt 1.5%, yeast 4.6%, and sorbic acid 0.15%. Four different AX fractions were used in test baking: AX, FAX, H-AX, and H-FAX. These fractions were added at three different levels (1.0%, 2.0%, and 5.0% of flour weight) by replacing flour based on their AX content. The fractions were dispersed in water prior to baking by stirring while heating to 80 °C and cooling to room temperature. Dough consistency was kept constant at 500 BU by adding water according to water absorption of flour or flour/AX mixtures. Each dough was baked in duplicate and divided into four breads.

2.5. Water absorption and dough development time

Water absorption and dough development time (DDT) were determined with a farinograph (Brabender GmhH, Duisburg, Germany) according to AACC method 54–21.01 (AACC Approved Methods of Analysis, 1995). Water absorption was determined as the amount of water required to reach 500 BU and expressed as percentage of flour weight. DDT was determined by the test baker as the time from water addition to dough peak consistency.

2.6. Bread measurements

Bread parameters were measured on the day of baking (visual

appearance, specific volume, crumb structure) and after 0, 7, and 14 days of storage at room temperature in closed plastic bags (crumb texture, moisture content). The baker subjectively evaluated the visual appearance of bread crumb immediately after baking from bread slices that were cut for assessments.

2.6.1. Specific volume

Specific volume was determined for all breads after 3 h of cooling, using the rapeseed displacement method (AACC Approved Methods of Analysis, 1995).

2.6.2. Crumb texture by texture profile analysis

Crumb texture was determined 0, 7, and 14 days after baking. A 2.5 cm slice cut from the center of the bread was analyzed in duplicate using texture profile analysis (TPA) (TA.XTplusC Stable Micro Systems, UK), with a 50 kg load cell and a 25 mm cylinder aluminum probe. Each bread slice was compressed to 40% of its height at 1.7 mm s⁻¹ in two cycles, with 4 s between compressions. Crumb hardness was determined as the peak force of first compression, cohesiveness as the ratio between force areas of second and first compression, and springiness as the height recovery of the sample between the two compressions. Hardening of bread crumb was expressed as staling rate, i.e., change in crumb hardness (%) between measurement points.

2.6.3. Crumb structure by image analysis

Slices were cut from the middle of the bread loaf and scanned in a color flatbed scanner (Ricoh IM C5500). Scanned images were processed and analyzed with the software ImageJ/Fiji. Images were first converted to 8-bit format, and then segmented by manually adjusting the threshold and subjected to the binary watershed process. A rectangular area covering as large a portion of the bread crumb as possible was selected. The structure of this area was analyzed using the function "Analyze particles", to generate an estimate of mean cell size in number of pixels (px). Image analysis was performed in triplicate.

2.6.4. Moisture content of crumb and crust

Moisture content was determined 0, 7, and 14 days after baking by taking triplicate samples from the center of the bread (crumb) and below the upper crust (crust) using the AACC Approved Methods 44–15.02 (AACC Approved Methods of Analysis, 1995).

2.7. Experimental design and statistical analysis of data

All measurements were performed at least in duplicate, and results are expressed as mean value ± standard deviation. For data on dough and bread properties, type-III analysis of variance (ANOVA) was performed and significant differences at 95% confidence level were determined by Tukey's pairwise comparisons using R (version R 4.3.0, The R Foundation for Statistical Computing, Austria). For repeated measurements of crumb texture and moisture content, measurement day was added as a variable to evaluate interactions between AX fraction, addition level, and time on changes in sample quality. Multivariate analysis was performed using SIMCA 17.02 (Sartorius Stedim Data Analysis AB, Sweden) with partial least squares regression analysis (PLS). Fraction properties were set as predicting X-variables (AX content, β-glucan content, total carbohydrate content, starch content, molecular weight average, A/X-ratio, FA content) and bread and dough properties were set as dependent Y-variables (water absorption, DDT, dough consistency, bread specific volume, average cell size of bread crumb, moisture content of fresh bread crumb, crumb moisture loss, hardness of fresh bread crumb, and staling rate of bread crumb).

3. Results and discussion

3.1. Characterization of fractions

3.1.1. Chemical composition

The chemical composition of the different fractions is presented in Table 1. The AX content was higher for unferuloylated AX (61%) and H-AX (57%) compared with the corresponding feruloylated fraction FAX (53%) and H-FAX (52%). The β-glucan level was slightly lower for hydrolyzed fractions (4.1 and 5.6 for H-AX and H-FAX, respectively) compared with the corresponding unhydrolyzed fractions (6.5 and 6.3, respectively), indicating that the enzymatic hydrolysis process degraded small amounts of β-glucan. The feruloylated fractions FAX and H-FAX contained 4.7 and 10.8 μg mg⁻¹ of FA, respectively, while as expected the unferuloylated fractions contained only traces of FA. The higher ferulic acid content after hydrolysis can be due to FA substitutions affecting xylanase activity, as demonstrated previously by Rudjito et al. (2023). FA is more frequently present even in shorter oligosaccharides, while polymers without FA are more often hydrolyzed in dimers that are washed out in the hydrolysis process (Rudjito et al., 2023), leading to a higher degree of feruloylation in the remaining AX fraction.

3.1.2. Molar mass distribution

Molar mass of the studied fractions is presented in Table 2. For FAX, hydrolysis decreased number-averaged molar mass (M_n) from 317 to 24 kg mol⁻¹. For AX, M_n decreased from 200 to 59 kg mol⁻¹ after hydrolysis. The reduction in molar mass after enzymatic hydrolysis was not as great for AX as for FAX, with M_n reduced by 70% and weight-average molecular weight (M_w) by 72% for AX, compared with 93% and 95%, respectively, for FAX. This might be due to differences in fraction composition (see Table 1) affecting enzyme activity. AX had three-fold higher A/X-ratio, indicating a higher degree of arabinose substitution, which has been shown to affect the action of xylanase on arabinoxylan (Rudjito et al., 2023).

3.2. Water absorption, dough development time, and final dough consistency

Increasing AX inclusion level in bread significantly (p < 0.05) increased water absorption and DDT (Table 3). At an inclusion level of 5%, AX, FAX, H-AX, and H-FAX increased water absorption by 35, 36, 25, and 7 %, respectively, compared with control bread. Among the

Table 1
Carbohydrate, arabinoxylan (AX), starch, mixed linkage β-glucan, Klason lignin, and ferulic acid (FA) content of the different fractions studied. F = feruloylated; H = hydrolyzed.

	AX	FAX	H-AX	H-FAX
Total carbohydrates (g 100g ⁻¹) ^a	71.1 (±2.1)	66.4 (±11.8)	70.4 (±0.6)	66.5 (±0.8)
AX (g 100g ⁻¹) ^b	61.1 (±1.9)	52.6 (±10.7)	57.4 (±0.5)	52.2 (±0.5)
A/X-ratio ^c	0.3 (±0.0)	0.1 (±0.0)	0.2 (±0.0)	0.2 (±0.0)
Starch (g 100g ⁻¹)	6.3 (±0.2)	4.8 (±0.7)	7.4 (±0.1)	5.6 (±0.0)
β-glucan (g100g ⁻¹)	6.5 (±0.1)	6.3 (±0.0)	4.1 (±0.0)	5.6 (±0.1)
Protein content (g 100g ⁻¹)	2.8 (±0.2)	2.2 (±0.4)	1.7 (±0.3)	2.9 (±0.4)
FA (μg mg ⁻¹)	0.1 (±0.0)	4.7 (±0.9)	0.2 (±0.1)	10.8 (±0.8)

^a Total carbohydrate content was calculated based on total content of arabinose, rhamnose, galactose, glucose, xylose, and mannose.

^b AX content was calculated based on the total content of arabinose and xylose.

^c Ratio between arabinose and xylose.

Table 2

Molar mass number-average molecular weight (M_n), weight-average molecular weight (M_w), and dispersity index (D) of the different fractions analyzed. F = feruloylated; H = hydrolyzed.

	AX	FAX	H-AX	H-FAX
M_n (kg mol ⁻¹)	200	317	59	24
M_w (kg mol ⁻¹)	714	698	199	29
D	3.6	2.2	3.4	1.2

Table 3

Water absorption (% of flour weight), dough development time (DDT, min), final dough consistency (BU), cell average size (pixel (px)), and specific volume of control bread and breads with different arabinoxylan (AX) fractions and addition levels (1, 2, 5 %). Mean value \pm SD. F = feruloylated; H = hydrolyzed.

	Water absorption (%)	DDT (min)	Final consistency (BU)	Cell average size (px)	Specific volume (mL g ⁻¹)
Control	60 (\pm 0.0)	9 (\pm 0.6)	480 (\pm 20)	16.1 (\pm 3.5)	4.1 (\pm 0.3)
AX 1 %	66.7 (\pm 0.0)	9 (\pm 0.0)	490 (\pm 14)	13.1 (\pm 2.3)	3.9 (\pm 0.1)
AX 2 %	70.5 (\pm 0.7) ^a	11 (\pm 1.4)	510 (\pm 14)	13.8 (\pm 5.1)	3.7 (\pm 0.2)
AX 5 %	80.8 (\pm 1.2) ^a	13 (\pm 4.2)	530 (\pm 28)	9.8 (\pm 2.7) ^a	2.5 (\pm 0.2) ^a
FAX 1 %	65 (\pm 0.0)	11 (\pm 0.0)	470 (\pm 28)	11.8 (\pm 1.0)	3.5 (\pm 0.2) ^a
FAX 2 %	70.8 (\pm 1.2) ^a	11 (\pm 2.1)	485 (\pm 35)	12.3 (\pm 2.2)	3.8 (\pm 0.1)
FAX 5 %	81.7 (\pm 0.0) ^a	10 (\pm 0.0)	500 (\pm 0)	12.0 (\pm 1.8)	3.0 (\pm 0.2) ^a
H-AX 1 %	63.3 (\pm 2.4)	9 (\pm 0.7)	515 (\pm 50)	14.3 (\pm 1.9)	4.0 (\pm 0.2)
H-AX 2 %	70 (\pm 0.0) ^a	10 (\pm 0.0)	490 (\pm 14)	13.9 (\pm 2.3)	4.4 (\pm 0.3)
H-AX 5 %	75 (\pm 4.7) ^a	13 (\pm 0.7)	520 (\pm 28)	12.0 (\pm 2.2)	3.6 (\pm 0.2) ^a
H-FAX 1 %	61.7 (\pm 0.0)	11 (\pm 0.7)	490 (\pm 0)	15.1 (\pm 3.0)	4.1 (\pm 0.2)
H-FAX 2 %	61.7 (\pm 0.0)	10 (\pm 2.1)	485 (\pm 21)	15.9 (\pm 1.8)	4.1 (\pm 0.2)
H-FAX 5 %	64.2 (\pm 1.2)	12 (\pm 2.8)	450 (\pm 42)	14.3 (\pm 6.0)	3.8 (\pm 0.3)

^a $p < 0.05$ compared with control. .

fractions, H-FAX was the only one that did not result in a statistically significant increase in water absorption ($p > 0.05$). Unhydrolyzed fractions FAX and AX with higher molar mass had the highest water absorptions at the highest addition level. High molecular weight AX is known to retain more water than low molecular weight arabinoxylan (Biliaderis et al., 1995), indicating that differences in water absorption might be related to molar mass of fractions. The water absorption values with 5% AX addition differed from those reported in some previous studies. For example, Zhu et al. (2023) observed no increase in water absorption with 5% AX addition of a commercial AX isolate. However, Biliaderis et al. (1995) observed a 12% increase in water absorption on adding only 1.3% of high molecular weight AX extracted from wheat flour and Zhang et al. (2019) observed a 16% increase in water absorption, from 57 to 66 %, on adding 10% AX-enriched fractions from wheat bran. Buksa et al. (2016) achieved water absorption of 85% with 6% fiber addition in a study on AX extracted from rye bran. As even small changes in raw material source and composition are known to affect AX baking properties (Pietiäinen et al., 2022), these conflicting results might be due to differences in AX purity and composition between studies. The different fractions analyzed in the present study did not differ in their effect on DDT ($p > 0.05$).

3.3. Visual evaluation of bread

Fiber addition level and type of AX fraction had a clear effect on the visual appearance of the breads (Fig. 1). For all fractions, addition of 1% and 2% of the different fractions had a slight effect on crumb color but crumb structure was similar in appearance to control bread, indicating that lower levels of added AX do not drastically affect bread quality in terms of appearance. At the highest addition level (5%), there were clear differences in crumb structure between the breads containing the different fractions. Addition of 5% of unhydrolyzed AX completely altered crumb structure, but addition of hydrolyzed fractions resulted in bread with structure closer to control bread. Hydrolyzed fractions with lower molar mass tend to have higher solubility than fractions with higher molar mass (Li et al., 2017), which can partly explain the better appearance of breads containing hydrolyzed AX fractions (Fig. 1). Based on visual evaluation, both feruloylated fractions (FAX, H-FAX) resulted in better bread appearance compared with unferuloylated fractions. In particular, H-FAX bread was comparable to control bread in terms of crumb structure even at the highest addition level (5%) (Fig. 1).

3.4. Crumb structure

Results from digital image analysis of bread crumb structure are presented in Table 3. Inclusion of AX at 5% in bread decreased cell average size by 39% compared with the control ($p < 0.05$). For H-AX, FAX, and H-FAX, the decrease in average pore size at the highest fiber addition level (5%) was 25, 25, and 11 %, respectively. At the highest addition level, H-FAX gave the highest cell average size of all fractions, and H-FAX 2% bread had cell average size comparable to that in control bread. These results confirm the high quality in bread with added H-FAX identified by visual evaluation, where H-FAX bread was observed to have structure comparable to control bread even at high addition levels (see Fig. 1). AX fractions with a high FA content have been reported to produce well-developed gel networks (Izydorczyk and Biliaderis, 1995), and feruloylation might help improve the functionality of low molar mass AX in breadmaking.

3.5. Specific volume

Increasing fiber addition level significantly ($p < 0.05$) decreased the specific volume of the breads (Table 3). Compared with the control, AX, FAX, and H-AX decreased the specific volume at the highest addition level ($p < 0.05$), but H-FAX did not give a significant decrease ($p > 0.05$). For unhydrolyzed AX and FAX, specific volume decreased at the highest addition level (5%) by 39 and 27 %, respectively. For the hydrolyzed fractions H-AX and H-FAX, the decrease was only 12 and 7 %, respectively, at the highest addition level. Excess water has been shown to decrease specific volume by slowing down dough development, and prevents proper crumb structure formation (Buksa et al., 2016). Higher molecular weight AX is also known to increase dough viscosity which in turn lowers doughs capacity of expansion and reduces specific volume (Zhu et al., 2023). This was most evident for AX 5% bread, which had extremely low specific volume (2.5 mL g⁻¹). Compared with FAX 5% bread, which had similar water absorption but 23% shorter DDT and significantly higher specific volume, the combination of high water absorption and long DDT indicates that fiber-gluten interactions might have prevented protein hydration and increased DDT for AX 5%. This might have in turn hindered dough formation, leading to both low specific volume and low cell average size.

3.6. Bread shelf-life

3.6.1. Moisture content

Changes in crumb moisture content are presented in Fig. 2A. Increasing addition level significantly increased ($p < 0.05$) crumb moisture content of fresh bread (0 h). However, FAX 5% was the only

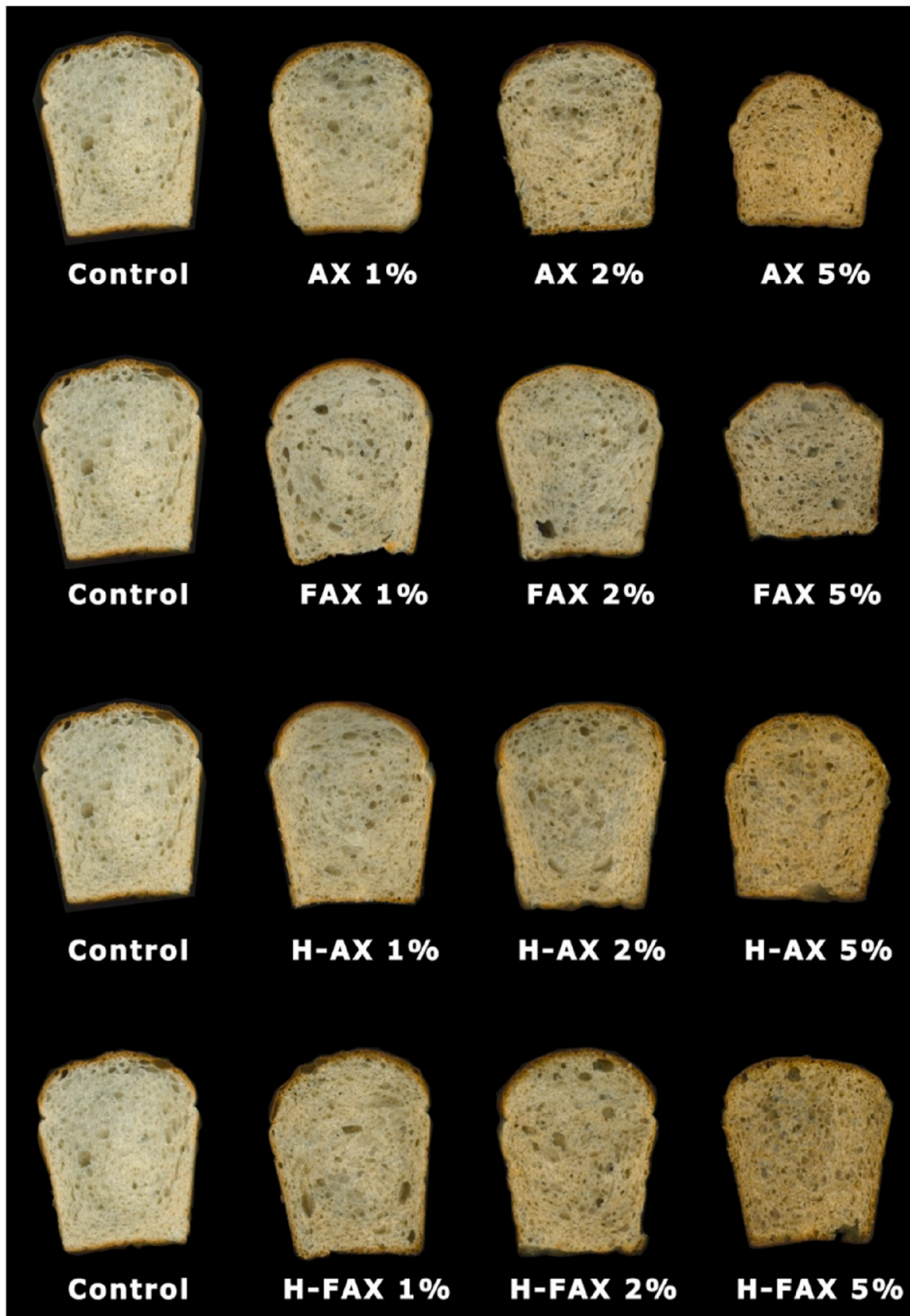


Fig. 1. Images of slices of control bread (left) and breads with different arabinoxylan (AX) fractions at different addition levels (1%, 2%, 5%). F = feruloylated; H = hydrolyzed.

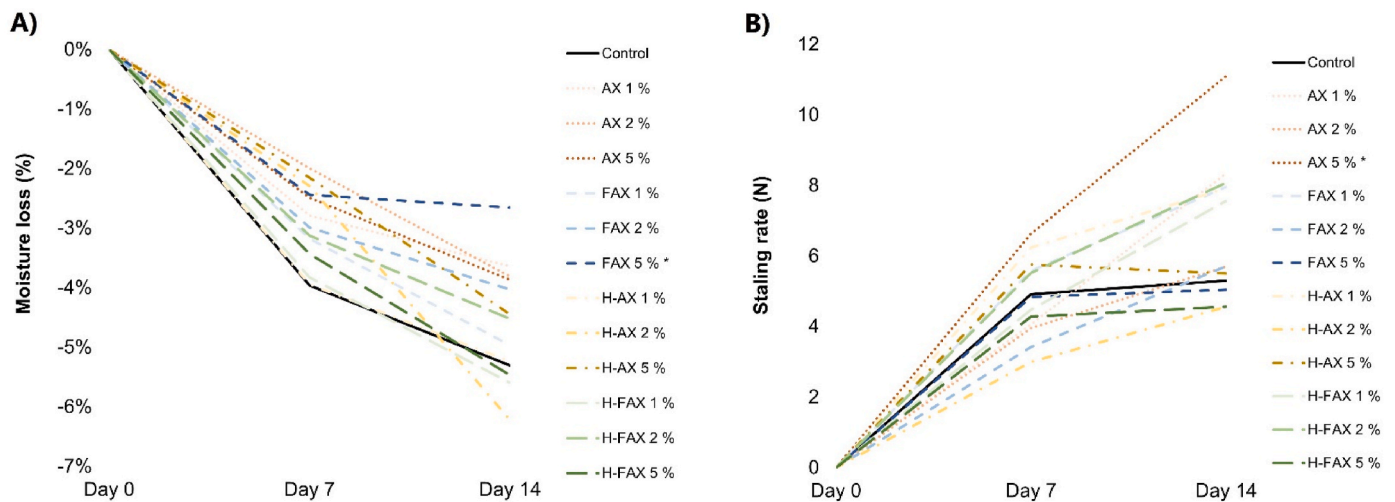


Fig. 2. Changes during the 14-day storage period in (A) crumb moisture content (%) ($n = 3$) and (B) staling rate of bread crumb ($n = 2$) in breads made with different arabinoxyylan (AX) fractions at different addition levels (1%, 2%, 5%). F = feruloylated; H = hydrolyzed. * $p < 0.05$ compared with control.

addition that was able to significantly ($p < 0.05$) prevent crumb moisture loss during the 14-day storage period compared with control bread. Fiber addition did not significantly affect crust moisture content of fresh bread or alter crust moisture content during storage.

3.6.2. Crumb texture

Hardening of bread crumb during the storage period, expressed as staling rate, is presented in Fig. 2B. Increasing AX addition level did not significantly affect staling rate during the 14-day storage period. In terms of overall change in staling rate during storage, only AX 5% significantly ($p < 0.05$) increased staling rate compared with control bread. Some previous studies have found that high molar mass of unhydrolyzed AX can improve crumb softness during storage better than low molar mass AX, by increasing the amount of water in bread (Biliaderis et al., 1995; Zhang et al., 2019). The hardening observed for AX-fortified bread (Fig. 2B) might be due to high water absorption, which has been shown to lower dough foam stability and therefore result in a denser bread crumb (Zhang et al., 2019). Increasing addition level of AX significantly ($p < 0.05$) increased (crumb hardness of fresh bread (day 0), but fiber addition did not significantly affect the cohesiveness or springiness of bread crumb (Table S1 in Supplementary Material).

3.7. Impact of fraction composition on dough and bread properties

A PLS model is presented as a biplot in Fig. 3. PLS component 1 explained 58.4% of the variation and PLS component 2 explained 19.8%, and the model had a Q^2 value of 0.382. Based on variable importance in projection, AX content, β -glucan content, and molar mass were the most important fraction properties (X variables). In ANOVA on the cross-validated residuals of Y-variables, water absorption, specific volume, and crumb moisture content showed significantly different values ($p < 0.05$). Most separation was seen for samples with 5% fiber addition, confirming findings in visual evaluations that most differences arose in breads with the highest fiber addition level (Fig. 1).

Water absorption by optimal wheat bran AX levels has been observed to improve bread quality in some studies (Biliaderis et al., 1995; Buksa et al., 2016). However, based on multivariate analysis, increased water absorption was associated with negative impact on bread quality in the present study (Table 3). Water absorption was positively correlated with the carbohydrate content of fractions, crumb moisture content, crumb hardness, and DDT, and these variables were in turn negatively correlated with specific volume and cell average size. This indicates that excess amounts of water and increased dough viscosity may prevent formation of proper crumb cell structure and hence decrease specific

volume and produce harder bread crumb. Molar mass was negatively correlated with specific volume, suggesting that higher molar mass of AX might further increase the amount of excess water in dough and therefore decrease volume. However, differences in water absorption might not be only related to AX structure, as impurities in AX extracts possibly contributed to the baking properties of the fractions (see section 3.2). This theory was supported by the results of multivariate analysis, where β -glucan content was one of the most important fraction properties according to variable importance in projection. This, combined with the high water-holding capacity of β -glucan (Lazaridou and Biliaderis, 2007), indicates that the extreme increase in water absorption might be due to impurities in the AX extracts used in this study.

Water absorption and molar mass were negatively correlated with change in moisture content (Table 3), suggesting that increased water-holding ability, especially of high molar mass AX, might prevent moisture loss during storage. Hydrocolloids such as AX have previously been shown to form a network that acts as gas diffusion barrier during baking, resulting in bread with a higher moisture content (Bell, 1990). Unexpectedly, however, changes in moisture content were not correlated with changes in hardness. This shows that the increase in bread softness from the amount of added water was not sufficient to overcome the hardness caused by AX disturbing formation of gluten network, as suggested previously by Zhang et al. (2019).

For H-FAX 5% samples, high FA content and low molar mass were the main contributors to bread properties. A previous study by Wang et al. (2021) observed that addition of FA to dough decreases water absorption, an effect they attributed to dough breakdown induced by FA. The H-FAX fraction contained twice as much FA as FAX (Table 1), and the lower FA content in FAX might explain why FA did not contribute significantly to bread properties.

4. Conclusions

Effects of wheat bran AX addition on dough and bread properties were found to be modulated by hydrolyzation and feruloylation of AX. Based on multivariate analysis, water absorption was the most important property, and was positively correlated with AX molar mass, bread crumb hardness, decreased specific volume, and crumb cell size. Increased water absorption by high molar mass AX was therefore associated with reduced quality of fresh bread, but also to slower moisture loss during storage. However, differences in water absorption might not derive solely from AX structure. Fraction β -glucan content was one of the most important properties according to variable importance in projection, indicating that impurities in AX extracts possibly also contributed

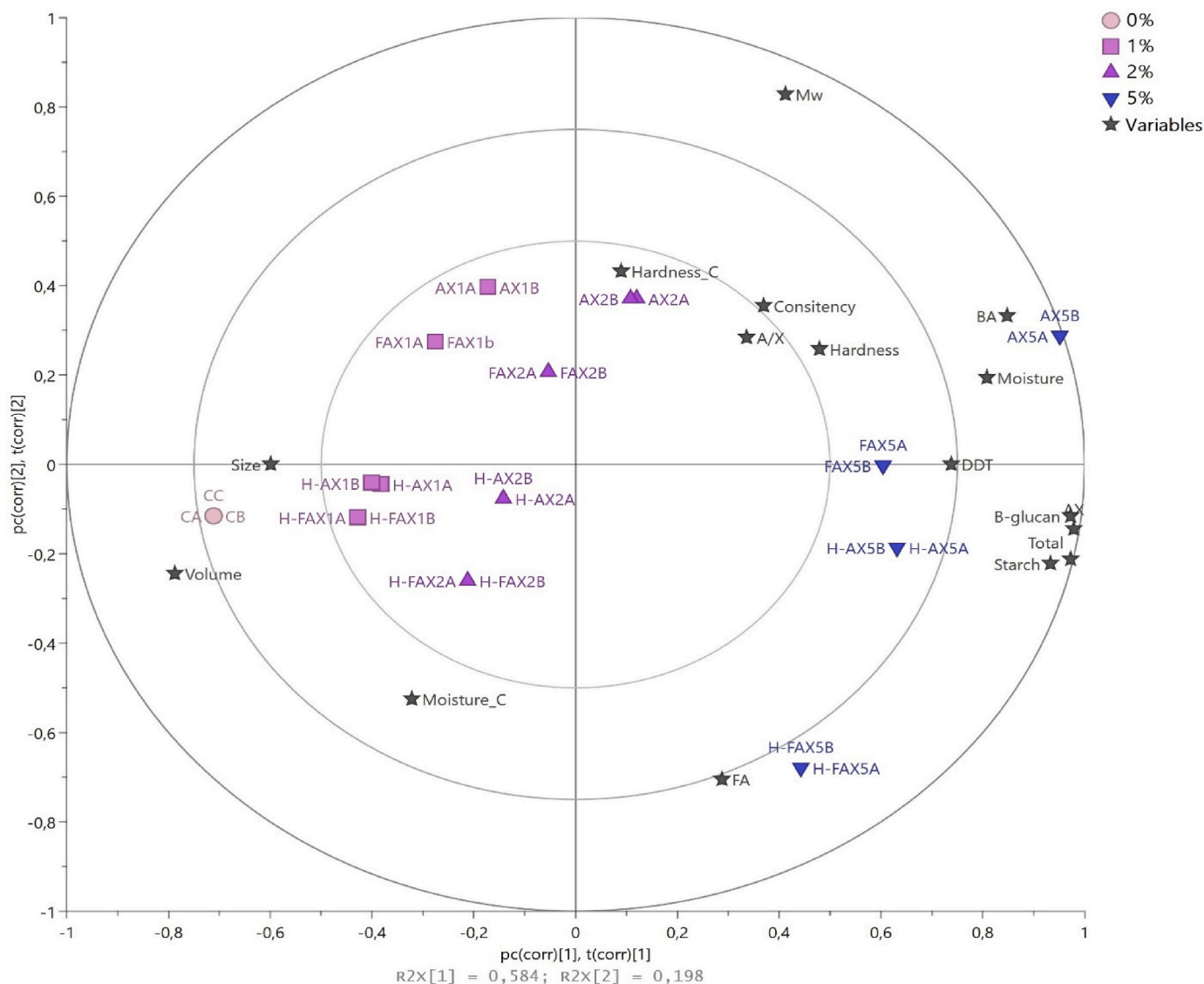


Fig. 3. Partial least squares (PLS) biplot showing variables (★) together with the different samples analyzed, colored based on arabinoxylan (AX) addition level (0% = ●; 1% = ■; 2% = ▲; 5% = ▼, F = feruloylated, H = hydrolyzed). Total = total carbohydrate content, A/X = A/X-ratio, Mw = molecular weight average of AX, FA = ferulic acid content, B-glucan = β -glucan content, Starch = starch content, BA = water absorption, DDT = dough development time, Consistency = final dough consistency, Volume = specific volume of bread, Size = average cell size of bread crumb, Moisture = moisture content of fresh bread crumb, Moisture_C = moisture loss, Hardness = hardness of fresh bread crumb, Hardness_C = staling rate.

to the baking properties. Among the fractions and addition rates analyzed, only FAX 5% prevented crumb moisture loss during storage. Change in moisture content was not linked to hardening of bread crumb and none of the fractions retarded crumb hardening. Addition of 2% AX or less produced a bread comparable to control bread, indicating that addition of lower amounts of AX is possible without adjusting AX structure by hydrolysis or feruloylation. The effects of 5% addition rate were related to fraction composition, and H-FAX bread was comparable to control bread in visual appearance, specific volume, and crumb structure. Multivariate analysis indicated that the higher quality of breads containing H-FAX was associated with the FA content and lower molar mass of H-AX. This indicates that optimization of AX structure can facilitate use of higher fiber levels in breadmaking.

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Availability of data and material

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Solja Pietiäinen: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. **Amparo Jimenez-Quero:** Investigation, Writing – review & editing. **Annelie Moldin:** Conceptualization, Supervision, Writing – review & editing. **Anna Ström:** Conceptualization, Supervision, Writing – review & editing. **Kati Katina:** Supervision, Writing – review & editing. **Maud Langton:** Writing – review & editing, Conceptualization, Funding acquisition, Resources.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jcs.2024.103920>.

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