Hindawi
Journal of Food Quality
Volume 2018, Article ID 5719681, 10 pages
https://doi.org/10.1155/2018/5719681

Research Article

Effect of Differently Extracted Arabinoxylan on Gluten-Free Sourdough-Bread Properties

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Received 27 April 2018; Accepted 10 July 2018; Published 6 August 2018

Academic Editor: Urszula Krupa-Kozak

The role of arabinoxylans (AXs) in bread-making has gained interest due to their positive contribution to bread quality. Therefore, the effect of differently extracted (water, alkaline, or enzymatic) rye AXs on gluten-free (GF) buckwheat and millet batter rheology and bread properties was evaluated. The results showed that the addition of AXs influenced most of the batter and bread properties differently, which depended on the chemical and structural properties of the AXs. All batter systems displayed a typical weak gel behavior. Enzyme- (Pentopan Mono BG-) extracted AXs (PEAXs) were able to strengthen both millet and buckwheat batter structures to a greater degree, as seen by the increase in storage modulus. Regarding bread properties, in buckwheat breads, calcium hydroxide-extracted AX (CEAX) was able to improve the specific volume (from 1.73 to 1.93 cm^3/g) and firmness (from 10.88 to 4.69 N) the most, compared to the control. The AXs extracted successively with water and the enzyme Pentopan Mono BG (WPEAX) produced the highest loaf volume (2.39 cm^3/g) and one of the lowest crumb firmness values (5.51 N) but caused larger pores and a ruptured crust. In millet breads, water-extracted AXs (WEAXs) and CEAX produced lowest crumb hardness (WEAX: 6.94 N; CEAX: 8.53 N). Specific volume was highest in breads with WEAX (2.35 cm^3/g), but CEAX displayed a better pore structure. Overall, water-extracted AXs improved the GF bread properties to a higher extent than alkaline-extracted AXs. Only CEAX displayed a comparable effect in some cases, and considering the fact that alkaline extraction of AX is much more efficient (much higher yield), its application compared to other AXs could be more favorable. Overall, AXs hold great potential as baking improvers for GF bread; the extent of their improvement will be defined by their functional properties.

1. Introduction

Arabinoxylans (AXs) have been widely studied due to their functional properties in bread-making. Water-soluble AXs have been known to benefit wheat or rye dough properties by stabilizing gas pores and reducing crumb hardness and staling rate [1, 2]. Their functionality not only has allowed them to improve the quality of gluten-containing breads but could also make them suitable for application in GF breads [3]. AXs have outstanding gelling properties which allow them to cross-link to other AX molecules or polymers (e.g., proteins and lignin), forming most commonly covalent di- or tri-ferulic acid (FA) linkages, tyrosine-FA linkages, or weak hydrogen interactions [4, 5]. The oxidative coupling is mainly induced by the presence of free radical-generating agents, such as enzymes (e.g., glucose oxidase and pyranose 2-oxidase) or chemicals (e.g., hydrogen peroxide).

Until now, limited information concerning the potential of AXs for application as baking additives in gluten-free bread is available. Korus et al. [6] showed that the addition of linseed mucilage comprised mainly of AXs and was able to improve the technological (i.e., volume and pore structure) and sensory properties of GF bread, compared to the control. Similarly, Ayala-Soto et al. [7] showed that supplementation of maize fiber AXs to a GF bread formulation enhanced the water-binding capacity of the flour and produced GF breads with a higher specific volume and a softer crumb texture.

Previous research has already confirmed that extraction parameters (solvents, pH, or temperature) significantly modify the chemical and functional properties of AXs, which in turn affect the rheological behavior of gluten-free batters [8, 9]. Their effect will closely depend not only on the type and concentration of AX added but also on the selection of GF flour and the presence of oxidants in the batter [3]. Since these factors determine the interaction between polymers, the formation of various hemicellulose networks from different AXs is expected, which will affect the resulting GF bread quality. Therefore, the main aim of this investigation was to determine the effect of differently extracted rye arabinoxylans on the structure and properties of GF buckwheat and millet bread and batter rheology. Hence, information about AX functionality and baking stability of the formed hemicellulose network shall be gained. This study is the first to determine the potential application of different rye AXs as baking additives in GF bread.

2. Materials and Methods

2.1. Materials. For arabinoxylan extraction, rye bran flour was purchased from Good Mills Austria GmbH (Schwechat, Austria). Buckwheat and millet grains were bought from Caj. Strobl Naturmühle GmbH (Linz, Austria). For wholemeal flour production, the grains were grounded at 12,000 rpm in a pin mill (Fa. Pallmann Maschinenfabrik, PXL 18, Zweibrücken, Germany). For baking, instant yeast was donated from Lesaffre Austria AG (Wiener Neudorf, Austria) and salt and sugar were purchased from Salinen Austria (Ebensee, Austria) and Agrana (Vienna, Austria), respectively. A commercial gluten-free sourdough starter culture (Böcker Reinzucht Sauerteig Reis) was bought from Ernst Böcker GmbH & Co. (Minden, Germany). POx was recombinantly expressed in *Phanerochaete chrysosporium* and purified by following the method of Spadiut et al. [10]. All used chemicals and reagents were of analytical grade and purchased from Sigma-Aldrich (Steinheim, Germany).

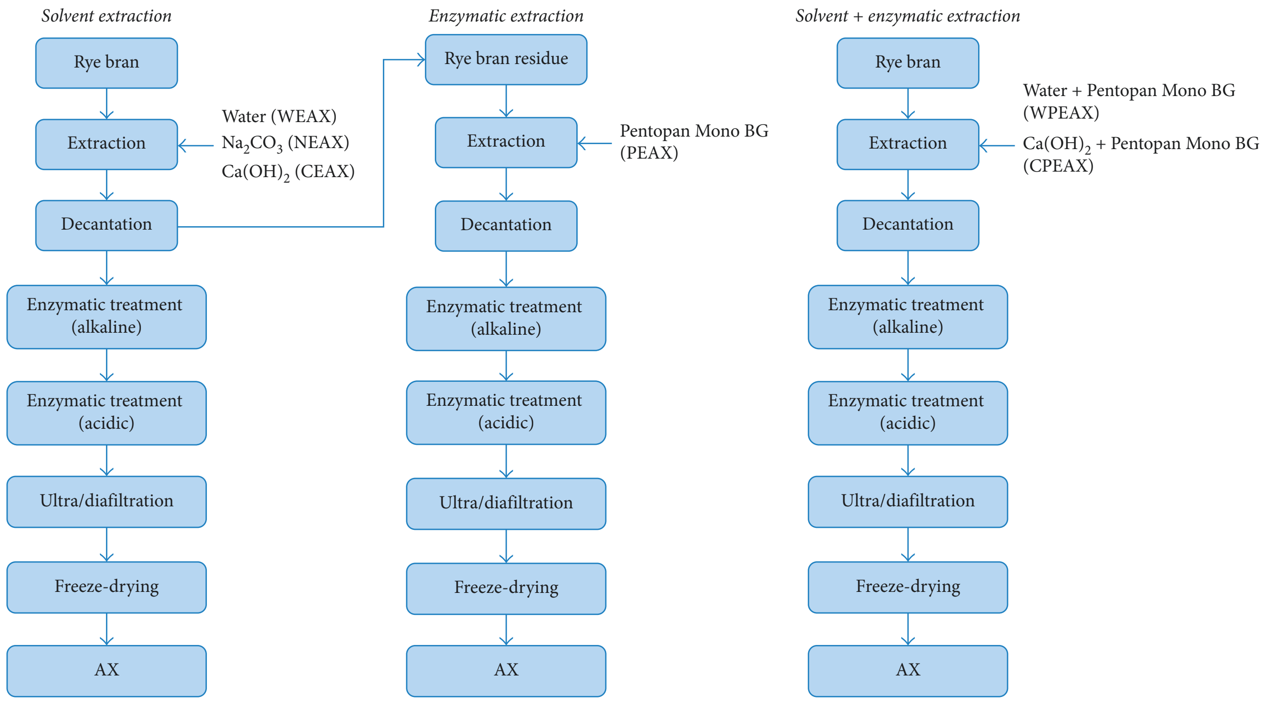
2.2. Arabinoxylan Extraction. Arabinoxylans were extracted from rye bran by following the procedure of Bender et al. [8] using a two-step extraction or a successive extraction as shown in Figure 1. For the solvent extraction, water, calcium hydroxide, and sodium carbonate were selected. Enzymatic extraction was performed using the endo-1,4-β-xylanase Pentopan Mono BG® (Novozymes Ltd., Bagsvaerd, Denmark). In total, six AX extracts were selected according to their extraction yield and availability and tested in GF formulations. Their physicochemical properties are provided in Table 1, in order to understand the potential effect on the functional bread properties. The composition of each concentrate varied significantly amongst arabinoxylans, and therefore, different functionalities in the GF batter were expected. Especially, FA might favor cross-linking to a different degree, but also other properties such as their branching degree (A/X ratio), molecular weight, and the presence of coextracted impurities (i.e., glucose and dextrins, protein, and other dietary fiber components) might determine their behavior in the bread batter as reported in earlier studies [3, 8].

2.3. Physicochemical Characterization of Arabinoxylans. The physicochemical composition of the AXs was assessed by following standard methods. Dry matter was determined by the ICC standard method no. 110/1. Insoluble dietary fiber (IDF) was analyzed by following the standard method of AACC no. 32-07 (Megazyme test kit; Megazyme International Ireland Ltd., Wicklow, Ireland). Protein content was determined by the Dumas combustion principle according to the ICC standard 167. Ferulic acid of AXs was analyzed by following the procedure of Mattila et al. [11]. For the determination of the AX content, monosaccharide composition was measured by gas chromatography with a precolumn derivatization as described by Bender et al. [8]. The AX content was calculated as the sum of arabinose and xylose fractions. All analyses were performed in triplicate, except for monosaccharide composition, which was performed in duplicate.

The water-holding capacity (WHC) of the AXs was determined in triplicate by following the official AACC method no. 56-30 with some modifications. Two hundred fifty milligrams of AX were dissolved in 25 ml of distilled water and stirred for 1 hour. Afterwards, the samples were centrifuged twice at 2,000 g for 10 min, discarding the supernatant each time. The centrifuge tubes were then inverted and drained for 10 min. The remaining sample was weighed, and the WHC was calculated.

2.4. Sourdough and Gluten-Free Bread Preparation. The formulation of the gluten-free sourdough and sourdough-bread is presented in Table 2. For preparing the sourdough, the commercial starter culture was dissolved in water at 30°C in a 1 : 10 ratio and mixed manually with the flour until complete homogenization. The resulting batter was fermented in a fermenter (Model 60/rW; MANZ Backtechnik GmbH, Creglingen, Germany) at 25–27°C and 85% RH (relative humidity) for 16–18 h and immediately used for baking.

For model GF bread baking, a simplified GF recipe was selected (Table 1). In order to exclude any interactions between arabinoxylan, POx, and other additives, no isolated protein, emulsifier, or hydrocolloid was added. The amount of water, sourdough, AXs, and POx in the bread formulation was optimized in pretrials according to each raw material (results not shown). Since formulations containing AXs significantly modify the water absorption and therefore the rheology of GF batters, addition of water was adjusted in accordance to the water-holding capacity of each AX concentrate. The GF batter was made first by homogenizing all dry ingredients with a laboratory dough mixer (Bear Varimixer Teddy 5L; A/S Wodschow & Co., Broendby, Denmark) for 1 min at 78 rpm. Separately, the water was weighed in



(a) (b)

Figure 1: Solvent and enzymatic arabinoxylan extraction from rye bran. (a) Two-step extraction. (b) Successive extraction. Adapted from Bender et al. [9].

Table 1: Physicochemical properties of differently extracted AXs and their respective extraction yield[1].

AX extraction	Yield[2] (%)	Ferulic acid (mg/100 g AX)	AX purity (%)	A/X ratio	WHC (g H_2O/g AX)	Glucose (%)	IDF (%)	Protein (%)
Two-step extractions[3]								
WEAX	2.92	356.01 ± 52.82^{d}	38.57 ± 0.8^{c}	0.48	2.24 ± 0.00^{b}	22.46 ± 0.54^{d}	2.46 ± 0.07^{a}	10.99 ± 0.86^{a}
NEAX	3.85	259.15 ± 34.59^{c}	41.80 ± 0.18^{d}	0.53	6.52 ± 0.06^{c}	25.98 ± 1.00^{e}	5.40 ± 0.30^{b}	12.46 ± 0.17^{b}
CEAX	4.71	254.89 ± 2.83^{c}	63.82 ± 0.42^{e}	0.57	1.64 ± 0.12^{a}	10.29 ± 0.12^{a}	2.32 ± 0.00^{a}	11.37 ± 0.00^{a}
PEAX	2.69	25.90 ± 1.20^{a}	37.89 ± 2.44^{c}	0.49	11.84 ± 0.17^{d}	14.38 ± 0.03^{b}	17.80 ± 1.22^{e}	13.14 ± 0.13^{b}
Successive extractions								
WPEAX	1.17	177.78 ± 4.25^{b}	33.82 ± 0.38^{b}	0.61	6.62 ± 0.21^{c}	17.47 ± 0.10^{c}	7.74 ± 0.84^{c}	20.43 ± 0.00^{c}
CPEAX	2.02	32.10 ± 2.57^{a}	22.76 ± 0.02^{a}	0.61	16.07 ± 0.01^{e}	9.50 ± 0.01^{a}	11.63 ± 0.09^{d}	10.83 ± 0.09^{a}

WHC: water-holding capacity; IDF: insoluble dietary fiber; WEAX: water-extracted AX; NEAX: Na_2CO_3-extracted AX; CEAX: $Ca(OH)_2$-extracted AX; PEAX: Pentopan Mono BG-extracted AX using the rye bran residue after $Ca(OH)_2$ extraction; WPEAX: water and Pentopan Mono BG-extracted AX; CPEAX: $Ca(OH)_2$ and Pentopan Mono BG-extracted AX. [1]Mean value of triplicate determinations ± standard deviation. Values associated with different lowercase letters in the same column denote significant differences ($p < 0.05$). [2]Yield is expressed as g AX/100 g rye bran. [3]WEAX, NEAX, and CEAX extractions as reported by Bender et al. [3, 9].

a beaker and mixed with POx. Then, sourdough was added to the dry ingredients and the water was slowly poured into the bowl and mixed at 150 rpm for 1 minute. Afterwards, mixing speed was increased to 300 rpm for 1 minute to ensure homogenization and reduced to 78 rpm for 4 minutes. The batter was rested at 30°C and 85% RH in a fermenter for 10 min (Model 60/rW; MANZ Backtechnik GmbH, Creglingen, Germany). Afterwards, 400 g of batter was accurately weighed into a baking tin and subsequently fermented at 30°C and 85% RH for 30 and 35 min for millet and buckwheat, respectively. Breads were baked at 180°C for 35 min in case of millet and 40 min for buckwheat (Model 60/rW; MANZ Backtechnik GmbH, Creglingen, Germany) and then stored at 20°C and 50% RH for 24 h before being analyzed. Baking trials were performed in duplicate measurements.

2.5. Bread Quality Determination. For crumb firmness and relative elasticity determination, a compression test using a texture analyzer (Model TA-XT2i; Stable Microsystems™ Co., Godalming, UK) equipped with a 5 kg load cell and an SMS 100 mm diameter compression probe (P/100) was carried out. Rectangular samples of $3 \times 3 \times 2$ cm ($L \times W \times H$) were cut from the center of the loaves as described by Bender et al. [12], removing the top crust and bottom crust, and subjected to an uniaxial compression test of 25% strain at

Table 2: Formulation of gluten-free sourdough and sourdough-bread added with arabinoxylans and POx.

Ingredients	Buckwheat		Millet	
	Amount (g)	% of total flour weight	Amount (g)	% of total flour weight
Commercial sourdough formulation				
Flour	100		30	
Water	100	100	30	100
Starter culture	10	10	3	10
Model GF bread batter				
Flour	121		195	
Sourdough (flour : water = 1 : 1)	162	82	38	18
Salt	4	1.9	4	1.9
Sugar	4	1.9	4	1.9
Yeast	4	1.9	4	1.9
Water	121	100	170	80
Additional ingredients	Buckwheat		Millet	
POx (nkat/g flour)	1 nkat/g flour		—	
Arabinoxylan[1]	% of total flour weight	Additional water[2] (g)	% of total flour weight	Additional water[2] (g)
Two-step extractions				
WEAX	3	13.58	1	4.81
NEAX	3	39.54	1	13.99
CEAX	3	9.94	1	3.52
PEAX	3	71.74	1	24.93
Successive extractions				
WPEAX	3	40.14	1	14.21
CPEAX	3	97.42	1	34.48

[1]WEAX: water-extracted AX; NEAX: Na_2CO_3-extracted AX; CEAX: $Ca(OH)_2$-extracted AX; PEAX: Pentopan Mono BG-extracted AX using the rye bran residue after $Ca(OH)_2$ extraction; WPEAX: water and Pentopan Mono BG-extracted AX; CPEAX: $Ca(OH)_2$ and Pentopan Mono BG-extracted AX. The amount added was calculated according to the purity of each AX. [2]Water adjustment of the formulations containing AXs, calculated according to the WHC of each AX.

0.5 mm/s speed followed by a relaxation time of 120 s. Pre- and posttest speeds were 5 mm/s and 10.0 mm/s, respectively. The crumb firmness represented the maximum force (F_{max}) in N required to deform each cube. The relative elasticity (REL) in percent was calculated by dividing the residual force (F_{res}) at the end of the relaxation time by the maximum force (F_{max}) multiplied by 100. For each loaf, quadruplicate measurements were carried out, obtaining 8 values for each formulation.

To determine the specific volume of the breads, the rapeseeds replacement method of the AACC-approved method 55-50.01 [13] was used. Specific volume (cm^3/g) was calculated as the ratio of the volume (cm^3) and the mass of the bread (g). For each bread, measurements were carried out in duplicate, obtaining 4 values for each formulation.

The color measurement of the crust was performed in duplicate using a Digi-Eye® system (Carl von Gehlen Spezialmaschinen und Zubehör GmbH & Co. KG, Mönchengladbach, Germany) integrated with a digital camera D-90 Nikon (Tokyo, Japan). $L*$ (0 = black, 100 = white), $a*$ (+value = red, −value = green), and $b*$ (+value = yellow, −value = blue) values according to the CIELAb system definition were recorded, and the total color difference (ΔE) was calculated as follows:

$$\Delta E = \sqrt{(\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})}, \tag{1}$$

where ΔL^*, Δa^*, and Δb^* represent the differences between the L^*, a^*, and b^* values of the bread added with AXs and the reference (control bread). A larger ΔE denoted a greater color change from the reference.

For the crumb porosity measurement, pictures of two 2 cm thick slices were taken by a digital camera D-90 Nikon (Tokyo, Japan) from the Digi-Eye system (Carl von Gehlen Spezialmaschinen und Zubehör GmbH & Co. KG, Mönchengladbach, Germany) and analyzed using an image analyzer software (ImageJ 1.47v; National Institute of Health, Bethesda, USA). This software uses the contrast between two phases (pores and solid part). First, the pixel values are converted into millimeters by using a known length. A single 20 mm × 20 mm square crumb field was selected from the center of each slice and converted into a 16-bit greyscale. After adjusting the threshold, the average pore size diameter and pore area were determined by the software.

2.6. Rheological Properties of GF Batters. To gain knowledge about microstructural changes occurring during fermentation in the GF batters and follow the gelation of differently extracted AX, rheological properties of GF batters were measured using a Kinexus rheometer pro+ (KNX 2001; Malvern Instruments GmbH, Herrenberg, Germany). A strain sweep was carried out at 25°C with a shear strain of 0.001–100% and a constant frequency of 1 Hz to determine the linear viscoelastic region of the batter systems. Afterwards, a frequency sweep was performed at 25°C and 0.01% shear strain using a plate measuring geometry (PU 20, diameter 20 mm) with a 2 mm gap. For the model systems, a 0.05 M phosphate citrate buffer (pH 5.5) with 20 mM of glucose was employed. In order to imitate the GF batter used for baking, the optimal concentrations of AXs (buckwheat: 3%; millet: 1%) and POx (buckwheat: 1 nkat/g flour) were used. The ratio of flour to buffer (buckwheat: 100%, millet: 80%) was also taken from the baking formulations (Table 1) and additional buffer was added for formulations containing AXs. Batters were prepared by weighing 2.5 g flour and 1 or 3% AXs accurately and mixing them manually for 30 s until complete homogenization. For the systems containing POx, the enzyme was homogenized with the buffer (buckwheat: 2.5 g; millet: 2.0 g) before addition to the solids. The resulting batter was mixed manually for 60 s and rested for 30 min at 25°C. Then, the batter was poured onto the base of the rheometer and covered with silicone oil to prevent drying out of the batter. All measurements were carried out in triplicate and rheological parameters were evaluated using the manufacturer's supplied computer software (rSpace for Kinexus; Malvern Instruments GmbH, Herrenberg, Germany).

2.7. Data Analysis. Statistical analyses were performed using STATGRAPHICS Centurion XVII, version 17.1.04 (Statpoint Technologies, Inc., Warrenton, Virginia, USA). All measured parameters are expressed as mean ± standard deviation. To determine statistical significant differences between formulations, one-way ANOVA (analysis of variance with $\alpha = 0.05$) and Fisher's least significance tests were used. Significant differences were indicated by different letters when the p value was lower than or equal to 0.05. Pearson's correlation coefficients and multiple regressions between AX characteristics and bread properties were also determined.

3. Results and Discussion

3.1. Effect of AX Extraction on Gluten-Free Batter Rheology. It is known that a lower batter viscosity enhances the volume of GF breads, since it allows gas cells to expand during fermentation [14]. Nevertheless, if the viscosity is too low, this could weaken the batter structure and decrease gas retention, leading to a compact bread crumb. Finding the optimal viscosity of the batter is therefore challenging. The mechanical spectra of buckwheat and millet batters added with differently extracted AXs are shown in Figure 2. All batter systems displayed a typical weak gel behavior, characterized by higher storage (G') than by loss moduli (G''), which suggested a solid elastic-like behavior. Commonly, loss tangent values ($\tan\delta$) of gluten-free doughs, which represent the ratio between viscous and elastic properties, range between 0.3 and 0.45 [6, 15]. In this study, most of the $\tan\delta$ values were found to be lower (buckwheat: 0.19–0.48; millet: 0.14–0.31). Ronda et al. [16] reported similar values in GF rice dough supplemented with oat and barley β-glucans. These authors attributed the low $\tan\delta$ values to the addition of these structuring agents. It can be observed in Figure 2 that viscoelastic properties slightly increased with angular frequency, which has been reported for GF batter before [6, 17]. The storage and loss moduli were significantly affected by the AXs added.

In buckwheat model systems (Figure 2(a)), the addition of CEAX and PEAX strengthened the structure of the batter as seen by the higher storage moduli, compared to the control, while other AXs reduced this property. G'' was significantly improved when most of the AXs were added to the batter, except for WPEAX and CPEAX. The increase in viscoelastic properties has been attributed to AX-AX, AX-protein, or protein-protein interactions that were formed during the resting period of the dough. The difference in storage moduli at different angular frequencies (e.g., $\Delta G'$ between 0.1 and 10 Hz) is considered as another indicator for polymer cross-linking. The smaller the $\Delta G'$, the lower the susceptibility of the batter structure to the applied stress. The limit of the polymer's chain mobility is more easily reached by smaller deformations due to the greater rigidity of the cross-linked structure. CPEAX showed the lowest $\Delta G'$, suggesting that the polymer was more rigidly cross-linked than other AXs.

In case of millet, PEAX and CPEAX showed higher viscoelastic properties than the control, while these properties increased the least (i.e., $\Delta G'$) during the frequency sweep (Figure 2(b)). Differences in behavior in millet and buckwheat systems might also be related to the interaction between different flour components and AX molecules. Next to chemical cross-linking, physical entanglements of AX chains have been reported to occur at concentrations above 0.2–0.4% AXs [18], which also might have contributed to the rheological behavior of these polymers in the batters.

3.2. Effect of Differently Extracted Arabinoxylans on Buckwheat Sourdough-Bread Properties. The influence of differently extracted rye AXs on the technological properties of buckwheat sourdough-breads is presented in Table 3. The optimal AX addition was determined in pretrials (results not shown) by varying the AX concentration between 0 and 6% (w/w). POx was tested in concentrations of 0–2 nkat/g flour to promote further AX cross-linking. The optimal dosage was observed at a concentration of 3% AX and 1 nkat/g flour.

Evaluation of buckwheat breads in Table 3 showed that most of the AXs could improve the bread properties compared to the control. The extent of the improvement mainly depended on differences in chemical composition and functionality of the AX as suggested previously (Table 1) and supported by the Pearson correlation coefficients (Table S1). Specific volumes of the breads added with AXs ranged between 1.53 and 2.39 cm^3/g. Only CEAX and WPEAX reached significantly higher loaf volumes than the control. The specific volume of the bread loaves moderately correlated (0.659) with the protein content of the AX concentrate. Probably, the presence of POx in the batter promoted cross-linking between proteins from the concentrate and AX molecules or flour proteins, resulting in a strengthened network and therefore a higher specific volume. The results are consistent with a previous finding, in which an increased viscoelasticity of a buckwheat batter was seen. This behavior was explained by the formation of inter- and intramolecular cross-links between

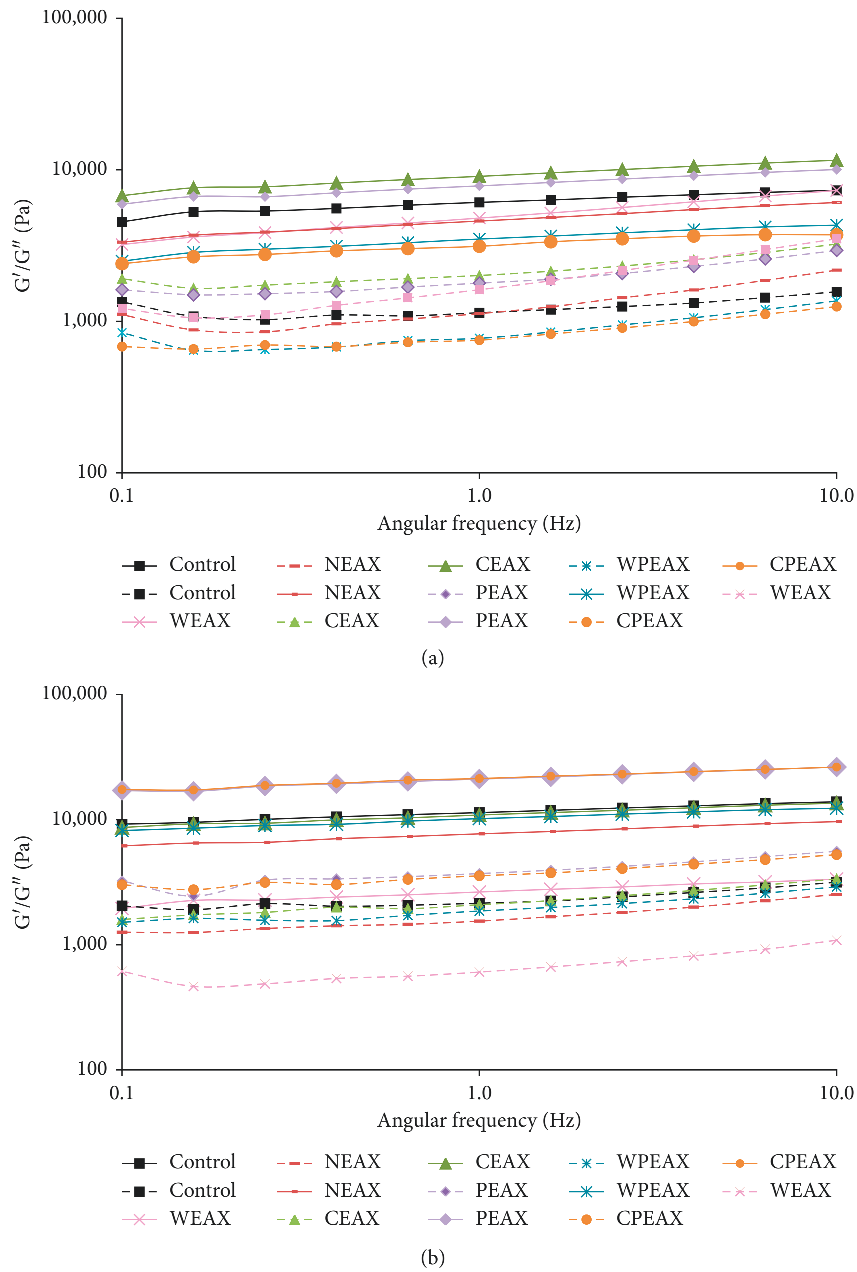


Figure 2: Mechanical spectra of GF model systems added with differently extracted AXs at 25°C and 0.01% strain after 30 min resting. (a) Buckwheat model systems. (b) Millet model systems. Continuous line: G'; discontinuous line: G''.

flour proteins and/or added AXs, which were strongly promoted by the addition of POx in the batter model system [3].

Compared to the control, firmness of breads was significantly reduced in all cases, except when PEAX was added. Verwimp et al. [19] reported that a decrease in crumb firmness and an increase in specific volume have been associated with a high amount of water-extractable AXs, while a higher share of water-unextractable AXs is responsible for an opposite effect (i.e., increased firmness and a compact bread). This might explain the poor technological properties of the PEAX (IDF: 17.8%) in contrast to the positive crumb firmness lowering effect of CEAX (IDF: 2.32%). These observations were also confirmed by the Pearson correlation coefficient as a moderate negative correlation (−0.530) between IDF of the AX concentrate and specific volume, and a positive correlation (0.762) between IDF of the AX and firmness of the bread was detected.

The relative elasticity of buckwheat breads ranged between 56.98 and 64.53% and was less affected by the type of AX added. This property was negatively correlated (−0.795) with the A/X ratio of the AXs, which further corroborates that branching could affect the interactions between AX molecules differently. This would lead to the formation of differently stable AX networks and hence dough properties during proofing, as already seen before [3].

Table 3: Influence of differently extracted rye arabinoxylan on the technological properties of buckwheat sourdough-breads[1].

Type of arabinoxylan	Specific volume (cm^3/g)	Firmness (N)	REL (%)	ΔE crust	Pore diameter (mm)	Porosity (%)
Control	1.73 ± 0.05^{b}	10.88 ± 0.37^{e}	64.02 ± 0.62^{d}	—	2.79 ± 0.32^{a}	$37.4 \pm 1.86^{b,c}$
Two-step extractions						
WEAX	2.04 ± 0.16^{d}	$5.38 \pm 0.20^{b,c}$	64.53 ± 0.90^{d}	$7.50 \pm 0.57^{a,b}$	3.47 ± 0.36^{b}	$39.65 \pm 2.34^{c,d}$
NEAX	$1.86 \pm 0.01^{b,c}$	8.79 ± 0.76^{d}	$63.17 \pm 0.39^{b,c,d}$	9.40 ± 0.42^{c}	2.26 ± 0.20^{a}	35.18 ± 0.90^{b}
CEAX	$1.93 \pm 0.08^{c,d}$	4.69 ± 0.23^{a}	$62.20 \pm 0.96^{b,c}$	9.10 ± 0.71^{c}	3.68 ± 0.33^{b}	$42.35 \pm 2.22^{d,e}$
PEAX	1.53 ± 0.06^{a}	12.27 ± 1.34^{f}	$63.60 \pm 0.18^{c,d}$	6.60 ± 0.70^{a}	2.25 ± 0.17^{a}	30.13 ± 3.04^{a}
Successive extractions						
WPEAX	2.39 ± 0.12^{e}	$5.51 \pm 0.50^{a,b}$	61.85 ± 1.61^{b}	9.60 ± 0.35^{c}	4.90 ± 0.26^{d}	39.73 ± 3.46^{e}
CPEAX	$1.83 \pm 0.06^{b,c}$	$6.04 \pm 0.46^{b,c}$	56.98 ± 3.98^{a}	$8.50 \pm 0.50^{b,c}$	4.37 ± 0.46^{c}	$42.50 \pm 2.40^{c,d,e}$

IDF: insoluble dietary fiber; WEAX: water-extracted AX; NEAX: Na_2CO_3-extracted AX; CEAX: $Ca(OH)_2$-extracted AX; PEAX: Pentopan Mono BG-extracted AX using the rye bran residue after $Ca(OH)_2$ extraction; WPEAX: water and Pentopan Mono BG-extracted AX; CPEAX: $Ca(OH)_2$ and Pentopan Mono BG-extracted AX. [1]Mean value ± standard deviation. Values associated with different lowercase letters denote significant differences ($p < 0.05$).

Regarding the color measurements, results display a significant browning of the bread crust in formulations containing AXs. The color difference of the crusts might be related to the presence of coextracted impurities in the AX extracts, which are responsible for the browning reactions throughout baking. Glucose and other reducing sugars (e.g., galactose) present in the AX and bread batter, together with the free amino group of lysine, react during baking to form Maillard reaction products, responsible for the browning in bread [20]. This reaction is especially favored at a slightly acidic pH, which is given by the sourdough-bread batter. Another factor that could influence the color of the breads is the presence of insoluble dietary fiber. Especially, lignin is known to induce color changes in food, as observed by Koegelenberg and Chimphango [21]. Since a negative correlation (−0.666) between IDF and color change was seen, the presence of lignin in the AX isolate cannot be excluded.

As for the crumb properties, the pore size and porosity were also affected by the AX added, which could probably be explained by the different networks formed during the fermentation stage. Crumb properties such as cell size and cell uniformity significantly influence the mouthfeel, elasticity, and softness of bread. Therefore, uniform, medium-sized average pores were favored [22]. In general, addition of AXs facilitated the formation of a medium-sized pore structure, except for WPEAX and PEAX. Similar to the control, the breads added with PEAX formed the most dense crumb structure. Bread formulations with WPEAX allowed the greatest expansion of pores. This was also evident in Figure 3(f).

In general, AXs obtained by water extraction (WEAX and WPEAX) enhanced the functional bread properties to a higher extent than alkaline-extracted AXs (CEAX, NEAX, and CPEAX). This partly relied on the FA and protein content of the polymers but could also be related to their molecular weight. Among the water extractions, the addition of WPEAX showed a slightly better effect on the bread properties, than WEAX. Several investigations have already established a positive correlation between FA content and molecular weight of the AXs [3, 23, 24]. WPEAX possessed a lower FA content and therefore probably lower molecular weight than WEAX. These AXs were treated with the xylanase Pentopan Mono BG, which is known to partially solubilize water-insoluble AXs but also depolymerize water-soluble AXs [25]. Therefore, it is suggested that middle-sized molecular weight polymers were extracted using this approach. Regarding the alkaline extractions, a similar relationship between FA of the AXs and GF bread quality was seen. Previous research has already shown that CEAX is mostly constituted of middle-sized polymers smaller than WEAX [3], due to the partial hydrolization that these undergo during alkaline processing. Since CPEAX was treated with the xylanase Pentopan Mono BG, it is proposed that these AXs have smaller molecular sizes than the CEAX. This is also confirmed by the low FA content. Overall, both middle-sized AXs (CEAX and WPEAX) were considered as most suitable AX for enhancing the quality of buckwheat bread. This was probably attributed to their better solubility in comparison to depolymerized or high molecular weight AXs [2, 26]. Among these AXs, breads with CEAX displayed more homogeneous middle-sized pores and no crust defects in contrast to WPEAX. Additionally, extraction with calcium hydroxide provided higher AX yields during the process (Table 1), which would further favor its application.

3.3. Effect of Differently Extracted Arabinoxylans on Millet Sourdough-Bread Properties. Due to the poorer suitability of millet as a raw material for GF baking, compared to buckwheat, the bread formulations had to be adjusted more thoroughly (results not shown). One optimization concerned the amount of sourdough in the formulation. In general, a reduction of sourdough from 82% to 18% led to an improved specific volume from 1.80 to 1.88 cm^3/g, reduced the crumb firmness from 36.68 to 12.30 N, and enhanced the relative elasticity from 29.5 to 41.58%, respectively. AX addition was also optimized similarly to buckwheat, varying AX and POx concentrations in the same ranges (AX: 0–6%; POx: 0–2 nkat/g flour). In this case, AX did not seem to act synergistically with POx. The optimal dosage was observed at a concentration of 1% AXs. Therefore, 1% AXs and 18% of sourdough were used for all millet bread formulations.

The influence of differently extracted rye AXs on the technological properties of millet sourdough-breads is displayed in Table 4. Similar to buckwheat breads, AX affected the bread properties depending on the structural and chemical properties of the polymer (Table 1). This was also

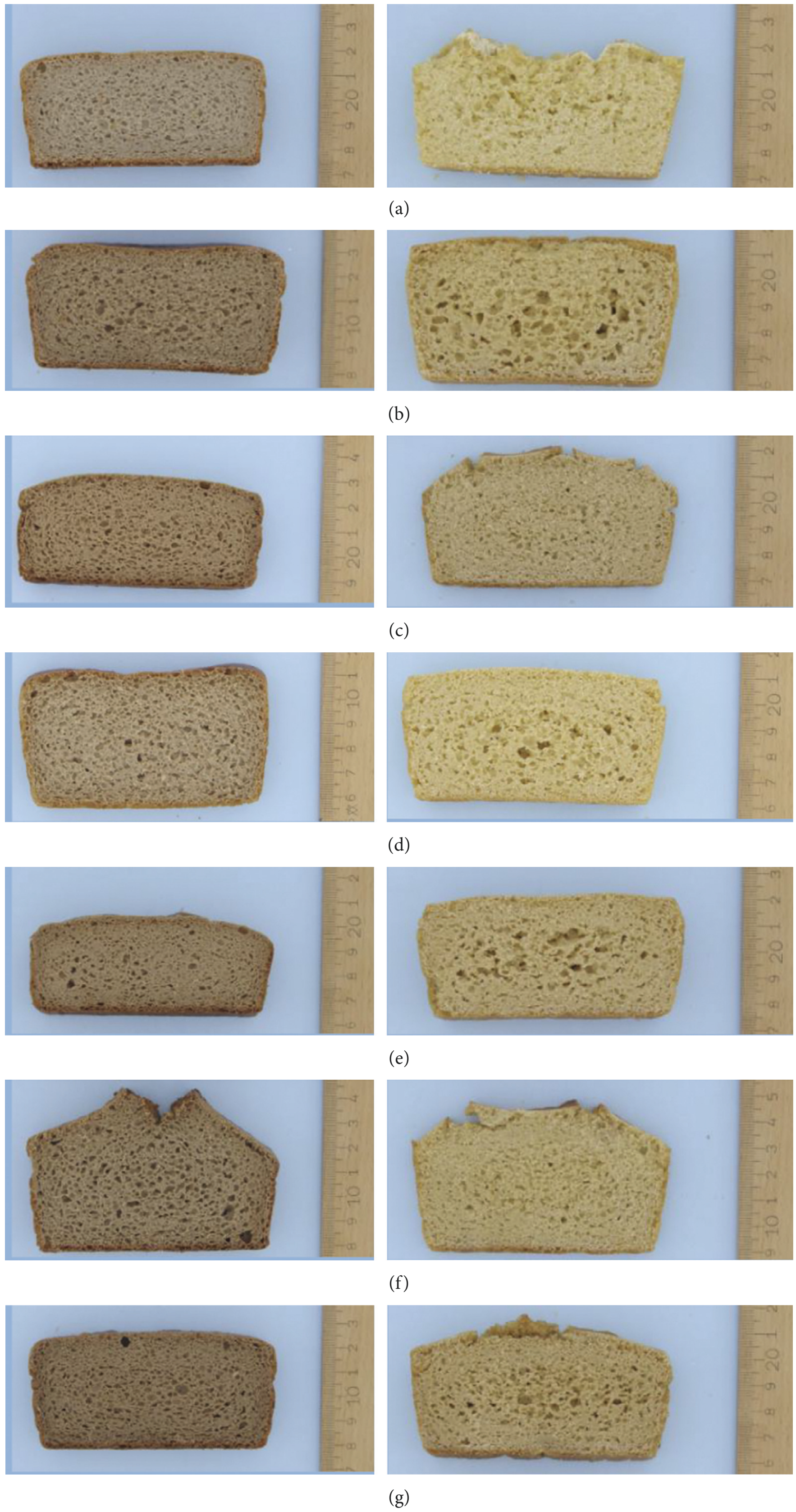


Figure 3: Slices of sourdough-bread made from whole meal buckwheat (left) and millet (right) flour added with differently extracted arabinoxylans. (a) Control. (b) WEAX. (c) NEAX. (d) CEAX. (e) PEAX. (f) WPEAX. (g) CPEAX.

Table 4: Influence of differently extracted arabinoxylan on the technological properties of millet sourdough-breads[1].

Type of arabinoxylan	Specific volume (cm^3/g)	Firmness (N)	REL (%)	ΔE crust	Pore diameter (mm)	Porosity (%)
Control	$1.88 \pm 0.02^{b,c,d}$	12.30 ± 0.89^{d}	41.58 ± 1.83^{a}	—	7.48 ± 0.50^{b}	$39.74 \pm 1.19^{a,b}$
Two-step extractions						
WEAX	2.35 ± 0.06^{e}	6.94 ± 1.07^{a}	44.94 ± 3.38^{b}	12.50 ± 0.99^{b}	7.23 ± 0.60^{b}	41.40 ± 2.41^{b}
NEAX	$1.95 \pm 0.13^{c,d}$	14.40 ± 1.06^{e}	$45.87 \pm 2.02^{b,c}$	15.22 ± 0.50^{c}	4.17 ± 0.72^{a}	$39.16 \pm 1.79^{a,b}$
CEAX	$1.86 \pm 0.07^{b,c}$	8.53 ± 1.06^{b}	44.22 ± 1.86^{b}	6.30 ± 1.98^{a}	4.69 ± 0.46^{a}	$39.57 \pm 0.82^{a,b}$
PEAX	$1.79 \pm 0.06^{a,b}$	10.50 ± 1.29^{c}	50.17 ± 2.84^{d}	12.46 ± 0.80^{b}	4.69 ± 0.73^{a}	38.10 ± 2.20^{a}
Successive extractions						
WPEAX	1.74 ± 0.11^{a}	$11.40 \pm 1.04^{c,d}$	$45.08 \pm 0.85^{b,c}$	$13.29 \pm 0.42^{b,c}$	4.80 ± 0.61^{a}	$39.53 \pm 2.74^{a,b}$
CPEAX	1.99 ± 0.05^{d}	16.20 ± 0.79^{f}	47.61 ± 1.17^{c}	17.59 ± 0.42^{d}	4.95 ± 0.63^{a}	$38.66 \pm 1.19^{a,b}$

IDF: insoluble dietary fiber; WEAX: water-extracted AX; NEAX: Na_2CO_3-extracted AX; CEAX: $Ca(OH)_2$-extracted AX; PEAX: Pentopan Mono BG-extracted AX using the rye bran residue after $Ca(OH)_2$ extraction; WPEAX: water and Pentopan Mono BG-extracted AX; CPEAX: $Ca(OH)_2$ and Pentopan Mono BG-extracted AX. [1]Mean value ± standard deviation. Values associated with different lowercase letters denote significant differences ($p < 0.05$).

supported by the Pearson correlation coefficients in Table S2. Most AXs improved the properties of millet breads more evidently than in case of buckwheat (Figure 3). Highest specific loaf volume was reached when WEAX was added to the sourdough-breads, while the addition of WPEAX adversely affected this property. This was probably attributed to the presence of a higher amount of IDF, as seen with buckwheat. Courtin and Delcour [27] reported that IDF reduces film formation during fermentation, which lowers the gas retention and loaf volume, leading to breads with coarser crumb textures. Specific loaf volume was positively correlated (0.786) with the FA content of the AXs, except for CPEAX, indicating a more stable network formation with higher FA content in AXs. Compared to the control, firmness was improved by adding the AXs from the two-step extraction, except for NEAX. Values ranged between 6.94 and 16.20 N. The ability of AXs to reduce crumb firmness was already seen in some investigations [27, 28]. Relative elasticity lay between 44.94 and 50.17% and was improved by all added AXs. Color differences between control and breads with added AXs were more evident in millet than in buckwheat breads, although in this case only a moderate correlation (0.414) between IDF and color could be established, attributing the color change mainly to the presence of impurities. In regard to the crumb properties, all AXs except WEAX were able to improve the crumb structure by forming middle-sized pores. The control showed big holes in the crumb and a noncontinuous fractured crust surface. Crumb properties need to be further improved with other additives such as egg albumin or emulsifiers, to increase the homogeneity of the pores and avoid crust defects [29]. In general, most millet breads presented the same crust defects, disregarding the type of AX, and were rather related to the lack of elasticity of the millet batter. The poor suitability of millet for GF baking has already been reported before [12, 30].

Similar to that obtained in buckwheat, water-extracted AXs displayed in general a better effect on the bread properties of millet than alkaline-extracted AXs. In this case, higher molecular weight AXs with high FA content improved the functional bread properties. In this occasion, both two-step extracted AXs, WEAX and CEAX, improved most of the bread properties to a higher extent than the successively extracted WPEAX and CPEAX. However, CEAX was still more suitable as a baking additive for millet bread than WEAX, as it induced a more homogeneous crumb structure in the breads. Its alkaline extraction yielded 60% more AXs than the water extraction (Table 1), which is also an important factor for its application.

4. Conclusion

This study demonstrated the potential of AXs as baking improvers for GF bread. It was seen that the extraction conditions of AXs significantly affected their functionality and interactions in GF batters. Water-extracted AXs (WEAX and WPEAX) generally improved the bread properties to a higher extent than alkaline-extracted AXs (CPEAX and NEAX), except CEAX which displayed in some cases a comparable effect to water-extracted AXs. Considering that the AX yield of the alkaline extraction is much higher, its application compared to other AXs could be more favorable. The strength and stability of the network formed was mostly delimited by the structural and chemical properties of the AXs, such as their FA content and probably their molecular weight, but was also influenced by other ingredients (e.g., flour) used for bread-making. In conclusion, most AXs were able to improve the bread properties and are therefore promising to be used as natural structure-building agents for GF bread.

Data Availability

The authors confirm that the data supporting the findings of this study are available within this article and/or its supplementary materials.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was financially supported by the Austrian Scientific Research Fund (FWF) (Project 11842-N28) and Hungarian Scientific Research Fund (OTKA) (ANN-114554). The authors would like to thank Edina Jaksics (Budapest, Hungary) for her kind collaboration.

Supplementary Materials

The tables from the supplementary material summarize the calculated Pearson correlation coefficients for the investigated physical and chemical variables of AXs and each GF bread (buckwheat and millet). (*Supplementary Materials*)

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