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The Influence of Arabinoxylan on the Properties of Sourdough Wheat Bread

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Abstract: Sourdough bread is a traditional product made using lactic acid bacteria (LAB) and yeast. The influence of rye arabinoxylans (AXs) of different molar masses on sourdough wheat bread has not been studied to date. The aim of this study was to research the influence of arabinoxylans of different molar masses on the properties of sourdough wheat bread. The breads were baked using the sourdough method with wheat flour without and with 1% or 2% rye AX with different molar masses, which were unmodified, partially enzymatically hydrolyzed and cross-linked. The addition of all the AX preparations significantly increased the water absorption of the wheat flour. In particular, the addition of the preparation of cross-linked arabinoxylans at an amount of 2% caused the highest increase (by 9.8%) in the addition of water to the wheat flour dough. It was shown that a 2% addition of partially hydrolyzed AXs, with a low molar mass (190,440 g/mol), had the highest influence on increasing (by 23.7%) the volume of the bread and decreasing (by 41%) the crumb hardness of the sourdough bread, determined on the day of baking. The addition of the cross-linked AXs at an amount of 2% had the strongest influence on increasing the moisture content of the crumbs on the day of baking, both in the central (by 2.6%) and peripheral (by 5.1%) parts of the bread compared to the bread without the addition of AXs. The breads with all the AX preparations after the first and third days of storage had a higher crumb moisture content compared to the bread without the AXs.

Keywords: arabinoxylan; sourdough; wheat bread; molar mass

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1. Introduction

Sourdough bread is a traditional product that is made using lactic acid bacteria (LAB) and yeast [1]. Sourdough has a beneficial effect on bread quality characteristics, especially in baked products made from rye flours [2,3]. The acids produced by LAB influence the protein fraction. As a result of this action, gluten proteins have an increased ability to absorb water (swelling) and exhibit improved solubility [4–6]. Physicochemical changes in the protein fraction resulting from sourdough fermentation increase gas retention in the dough structure, which results in bread with a larger volume [6,7].

A reduction in the water absorption of flour and dough has been observed with the sourdough method [8–10], which is explained by the partial hydrolysis of proteins in an acidic environment [10]. The effect of hydrocolloid addition on the water absorption of sourdough has not been investigated.

The volume of sourdough wheat bread is larger compared to wheat bread baked without sourdough [11–13] due to the larger amount of gases produced during fermentation and retained in the dough structure [11,13]. It has also been shown that too much sourdough results in a weaker gluten structure and a consequently lower volume of bread [14]. The effect of hydrocolloid addition on the volume of sourdough bread has not been thoroughly investigated. The studies conducted so far have only shown that the addition of dextran has resulted in a lower volume of bread compared to bread without the addition of dextran, which has been explained by the influence of dextran on the interaction of starch and gluten in the dough [15]. It has also been shown that the amount of oat and rye fiber in sourdough wheat bread does not significantly affect its volume compared to sourdough wheat bread without the addition of fiber [16]. In addition, it has shown that the volume of sourdough wheat bread with water-insoluble arabinoxylans [11] as well as enrichment with oat fiber was higher compared to whole wheat bread [17].

In other studies, the effect of the addition of sourdough on the hardness of wheat bread crumb is ambiguous. It has been shown that the crumb hardness of sourdough wheat bread was either lower [12,18] or higher [13,14,19] compared to bread baked without sourdough. In the research conducted so far with dextran, there has not been a clear effect of hydrocolloid on the hardness of the crumb of sourdough wheat bread [15].

Other studies have shown that the increase in crumb hardness of sourdough wheat breads containing 0.1% dextran of different molar masses was lower compared to breads without added dextran [15]. The authors suggested that dextran delays the staling process of sourdough wheat bread due to its inhibitory effect on amylopectin retrogradation [15].

Studies have shown that the addition of sourdough either has no affect [14,18,20] or results in an increase in the moisture content of the crumb of sourdough wheat bread compared to bread baked using a method without sourdough [12,13,18]. The increased crumb moisture of sourdough bread has been explained by the higher addition of water to the dough for sourdough bread [18]. During sourdough fermentation, metabolites such as exopolysaccharides are produced that increase water retention in bread dough, which can result in a higher moisture content in the crumb of sourdough bread [12]. In the research published to date, information on the effect of hydrocolloids on the moisture content of sourdough wheat bread is scarce. It has been shown that 0.1% dextran, with a molar mass in the range of 9608–2,800,000 g/mol, does not significantly affect the crumb moisture content of sourdough wheat bread compared to bread without dextran [15].

Sourdough wheat breads exhibit less crumb moisture loss compared to bread without sourdough [13,14]. This is due to the addition of sourdough, which has a positive effect on the redistribution of moisture inside the bread, and thus causes less crumb moisture loss during storage. Moreover, sourdough metabolites such as organic acids, dextrins and exopolysaccharides, have affected the interaction between water molecules and bread components, such as starch, resulting in a lower crumb moisture loss of sourdough bread during storage compared to bread without sourdough [13,14].

Arabinoxylans (AXs) belong to the group of non-starch polysaccharides that are part of the dietary fiber of cereal grains [21]. The highest amount of total AXs is found in rye grain, at 8–12% [22–24]. However, the content of total AXs in wheat grain has been found to range from 0.8% to 9% [25,26]. The molar mass of natural AXs in rye grain is in the range of 197,800–2,000,000 g/mol [23,24,27–29], while in wheat grain it is 176,000–381,000 g/mol [28–31]. There are indications that the molar mass of water-insoluble AXs decreases as a consequence of acid hydrolysis, and thus their negative effect on bread quality has not been reported, but this mechanism has not been completely investigated [6,32,33].

The effect of AXs on the sourdough bread baking process has not been investigated in the studies conducted so far. On the other hand, it has been shown that AX significantly influences the process of wheat bread baking using the direct method and shapes its quality parameters. However, the amount of added preparation and the molar mass of AXs are crucial in this case [34,35]. The addition of AXs to wheat bread baked using the direct method improves its quality characteristics, such as the volume and porosity of the bread crumb. In addition, AXs support the retention of water in the bread crumb, which consequently causes its lower hardness [34,35].

According to the available knowledge, the influence of rye AXs significantly differing in molar mass, both natural and those with modified structures resulting from cross-linking and partial enzymatic hydrolysis, on the addition of water to the dough and the properties of sourdough wheat bread has not been studied so far. The aim of this study was to investigate the effect of AXs of different molar masses on the properties of sourdough wheat bread. Moreover, the effect of AXs on the aging of wheat bread baked with sourdough was studied.

2. Materials and Methods

The research material was wheat flour type 750 (PZZ Kraków, Podłęże, Poland). Arabinoxylans were isolated from rye flour of the Amilo variety (Danko, Choryń, Poland) produced by laboratory method. Starter cultures LV2 (SAF LEVAIN; Lesaffre, Marcq-en-Barœul, France), salt (POCh, Gliwice, Poland) and yeast (Lesaffre, France) were used for sourdough preparation.

2.1. Chemical Composition of Wheat Flour

Ash content was determined according to AOAC 930.05 [36]. Total protein was determined according to AOAC 950.36 [36]. Fat content was determined according to AOAC 930.05 [36]. Content of dietary fiber (total dietary fiber (TDF) as well as soluble (SDF) and insoluble (IDF) fractions) were determined according to AOAC 991.43 [36]. Starch and arabinoxylan contents were estimated after acid hydrolysis by HPLC/RI analysis according to Buksa et al. [24].

2.2. Isolation and Modification of Water-Soluble Rye Arabinoxylans

The water-soluble AXs were isolated from rye flour and modified according to the methods of Buksa et al. [24].

2.2.1. Isolation of Arabinoxylans

A total of 100 g of wholemeal rye flour was treated with 500 mL of 80% v/v EtOH at 90 °C for 2 h to inactivate cereal enzymes. The ethanol solution was removed, and the sediment was dried at 40 °C for 20 h, followed by extraction with 2 l of water at 25 °C for 6 h. The suspension was centrifuged, and the clear supernatant was boiled to coagulate soluble proteins, then the sample was cooled and incubated with α-amylase at 37 °C for 2 hr. The solution was boiled, then 20 g/L celite was added and filtered via a Buchner flask. The clear filtrate was transferred to a 4-fold solution of ethanol and acetone (1:1). The sediment was centrifuged, then frozen at −18 °C and stored for further modifications or washed twice with ethanol/acetone and twice with acetone alone. After the last centrifugation, the AX sediment was dried at 50 °C for 2 h. The yield of the unmodified AX preparation (designated AX_NM) was 2.7 g/100 g of whole wheat flour.

2.2.2. Modification of Isolated Rye Arabinoxylans by Cross-Linking

The frozen sediment of 15 g was thawed and dissolved in 40 mL of deionized water with intense stirring at 50 °C for 6 h. After this time, the solution was cooled to 25 °C, hydrogen peroxide H_2O_2 at a concentration of 1 µg/g of AXs and peroxidase at a concentration of 5 U/g of AXs were added, and again treated for 15 min. The process was stopped by flooding the solution with a 4-fold volume of ethanol and acetone solution (1:1). AX sediment was centrifuged and washed twice with ethanol/acetone and twice with acetone only to remove all water from the sample. After the last centrifugation, the sediment of AXs was dried at 50 °C for 2 h. The resulting preparation of cross-linked AXs was denoted as AX_CR.

2.2.3. Modification of Isolated Rye Arabinoxylans by Partial Enzymatic Hydrolysis

The frozen sediment of 15 g was thawed and dissolved in 40 mL of deionized water with intense stirring at 50 °C for 6 h. The obtained solution was cooled to 30 °C, then xylanase was added at 375 FXU/g arabinoxylan and incubated at 37 °C for 30 min. After boiling, the sample was centrifuged, and the resulting supernatant was transferred to a 4-fold volume of ethanol/acetone solution (1:1). The sediment AXs were centrifuged and washed twice with ethanol/acetone and twice with acetone only. After the last centrifugation, the sediment of AXs was dried at 50 °C for 2 h. The resulting preparation of partly hydrolyzed AX was denoted as AX_HYD.

2.3. Determination of the Monosaccharide Composition of Arabinoxylans, Arabinoxylan Content and Molecular Properties of Arabinoxylans

The monosaccharide composition of AXs and AX content in flour and AX preparations was determined by HPLC/RI method after acid hydrolysis according to Buksa et al. [24].

The distribution of molar mass of AXs was evaluated using HPSEC/RI according to Buksa et al. [37]. In short, the chromatographic system was composed of a Knauer chromatograph (Knauer, Berlin, Germany), equipped with a combination of OHpak SB-806HQ and SB-804HQ columns (Shodex, Tokyo, Japan) and a refractometric detector (Knauer, Germany). As eluent, 100 mM $NaNO_3$ was used at a flow rate of 0.6 mL/min. Separation was carried out at a column temperature of 60 °C. The calibration of the SEC system was carried out using pullulan standards (Shodex Standard, Macherey-Nagel, Düren, Germany) with known molar masses (P-5, 10, 100, 400 and 800) and arabinose (Sigma-Aldrich). The distribution of molar masses and apparent average molar mass Mw (related to pullulan standards) were calculated using Eurochrom (ver. 3.05, Knauer) and Clarity (ver. 4.0.1.700, DataApex, Prague, Czech Republic) software.

2.4. Baking Sourdough Wheat Bread with Arabinoxylans

2.4.1. Sourdough Preparation

To 25 g of wheat flour, 0.125 g of LV2 starter culture was added (i.e., 0.5% in relation to 100 g of flour used to prepare the sourdough) and 30 mL of water (obtaining a dough yield of 220%). After thoroughly mixing all the ingredients, the sourdough samples were incubated in a fermentation chamber at 30 °C for 24 h, with preliminary mixing for about an hour.

2.4.2. Preparation and Fermentation of Wheat Dough

In the case of the control dough, after sourdough preparation, a new portion of wheat flour (75 g) was added to the sourdough to obtain a 25% proportion of flour from the sourdough in the whole dough, yeast 2.5% (2.5 g), salt 1.8% (1.8 g) and water in the amount determined using the farinograph (dough with a consistency of 500 BU, determined according to ICC-Standard No. 115/1 [38]). In order to test the influence of AX on sourdough, wheat AX preparations of 1% and 2% by weight of flour were added in place of flour. All dough samples were mixed for 8 min and then 60 g of dough was kneaded, placed in a 3 cm × 3 cm × 4 cm baking pan and placed in the fermentation chamber for 40 min at 25–27 °C.

2.4.3. Baking Sourdough Wheat Bread and Analyzing the Properties of the Bread

After proofing, dough pieces were baked in a Viva-Meteor convection oven (Victus, Costa di Rovigo, Italy) at 230 °C for 20 min. After baking, loaves were cooled at room temperature for 30 min, and then, final analyses of the bread samples were performed and included the following:

- Bread volume measurement was performed using a three-dimensional laser-based scanner, Volscan Profiler (Stable Microsystems, Godalming, UK), according to the manufacturer's manual.
- Moisture of the bread crumb was measured by drying for 1 h at 130 °C, according to AOAC method 925.10 [39] in two places (central and peripheral part of bread).
- Texture parameters (hardness) of the bread crumb were measured using a texture analyzer TA.XT Plus (Stable Microsystems, Godalming, UK) according to the standard program, at the compression rate 5 mm/s. A sample of bread crumb, taken from the base of the loaf with a height 30 mm (the upper part of the bread was removed), was pressed to reach 10 mm maximum strain by a P/20 aluminum compression plate with a diameter of 15 mm, in two cycles with a 5 s delay. From the resulting parameters of

TPA, only the hardness of the crumb was used as an indicator of textural properties. The calculations were performed using the attached software Texture Exponent (ver. 3.0.5.0, Stable Microsystems, UK).

2.5. Statistical Analysis

All analyses were performed at least in triplicate. Statistical analysis of variance (ANOVA) was performed in order to determine statistical significance of the observed differences among mean values (Tukey's test at significance level 0.05). The statistical analysis was performed using Statistica v. 9.0 software (StatSoft, Inc., Tulsa, OK, USA).

3. Results and Discussion

The composition and properties of the flour were typical of wheat flour type 750 (Table 1). The content of ash, starch, protein and fat (Table 1) determined in the tested flour was typical for this type of flour [40–44]. The content of total dietary fiber in the wheat flour was also similar to other studies, as this component constitutes 3.0–3.6% for flours with a similar ash content [42,45,46]. The content of soluble fiber (SDF) in the tested flour was slightly higher, while insoluble fiber (IDF) was lower compared to the other studies regarding flour type 650, in which these values were 1.2% and 1.8%, respectively [46]. The content of AXs and the ratio of arabinose to xylose (Table 1) in the wheat flour studied corresponded to data presented in the literature [47–49].

Table 1. Chemical composition of flour.

Component	Wheat Flour Type 750
Starch [%]	75.3 ± 0.9
Protein [%]	12.2 ± 0.6
Fat [%]	1.7 ± 0.1
TDF *[%]	3.2 ± 0.1
SDF * [%]	1.5 ± 0.0
IDF * [%]	1.7 ± 0.0
AX [%] **	2.2 ± 0.2
A/X **	0.56 ± 0.04
Ash [%]	0.72 ± 0.01

* TDF—total dietary fiber, SDF—soluble dietary fiber, IDF—insoluble dietary fiber. ** AX—the sum of the content of arabinose and xylose after acid hydrolysis and multiplying the result by a factor of 0.88. A/X—the ratio of arabinose to xylose.

Table 2 presents the content and molecular properties of the AXs in the AX preparations isolated from rye grain. The AX preparations were determined as follows: unmodified (AX_NM), partially hydrolyzed (AX_HYD) and cross-linked (AX_CR). The AX content in the preparations at the level of approximately 78% indicated the high purity of the preparations and was typical compared to the content reported in other studies for rye AX preparations, ranging from 68 to 93% [50–52].

Table 2. Arabinoxylan content and molecular parameters of water-soluble arabinoxylan (AX) obtained from rye wholemeal.

Component	AX_NM	AX_HYD	AX_CR
AX [%]	78.2 ± 1.3 [a]	77.7 ± 1.0 [a]	77.6 ± 1.5 [a]
Molecular parameters of AX			
M_w [g/mol]	446,330 [b]	190,440 [a]	574,710 [c]
M_n [g/mol]	12,600 [b]	6290 [a]	13,400 [c]
Đ	35.4 [b]	30.3 [a]	42.9 [c]

AX_NM—non-modified AX preparation; AX_HYD—hydrolyzed AX preparation; AX_CR—cross-linked AX preparation. Mean values in rows marked with the same letters are not statistically significantly different at $p < 0.05$.

The molar mass of unmodified arabinoxylans (AX_NM) was typical of water-soluble rye AXs, which, according to data from other studies, have a molar mass in the range of 200,000–500,000 g/mol [27,52]. The AXs in the AX_HYD preparation modified using partial enzymatic hydrolysis were characterized by a low molar mass, which was similar to that determined as 219,000 g/mol in a study by Buksa [52], where the same method of AX modification was used. The AXs in the preparation of cross-linked arabinoxylans (AX_CR) had the highest molar mass, and this value was higher than the molar mass of 505,000 g/mol determined for AX obtained using the same method [52]. The determined molar masses of the AXs indicate that the process of isolation and modification (cross-linking and partial hydrolysis) of the AXs was carried out correctly.

Table 3 shows the water binding capacity (WBC) of wheat flour, and the dough yield (DY) and baking losses (BL, TBL) of the sourdough wheat bread without and with the AX preparations. The WBC of wheat flour without and with AX preparations was determined at the same dough consistency, specifically at 500 BU (Brabender Units). The WBC of wheat flour without AX addition (Table 3), was similar to data from other studies, where it ranged from 59% to 60% for wheat flour with an ash content of 0.46–0.63% [13,34]. The addition of AX_CR with the highest molar mass (574,710 g/mol) at a proportion of 2% led to the highest increase in the addition of water to wheat flour doughs of 9.8%, achieving a consistency of 500 BU. The addition of a 2% preparation of unmodified arabinoxylans (AX_NM) with a molar mass of 446,330 g/mol resulted in an 8.1% increase in water addition to the dough. A similar effect—an increase in water addition of about 6%—was observed by Koegelenberg and Chimphango [53] after using 1.2% unmodified AXs from wheat bran with a molar mass of 620,000 g/mol. The addition of the preparation of partially hydrolyzed arabinoxylans (AX_HYD) in an amount of 1%, with a molar mass of 190,440 g/mol, resulted in the lowest increase in the WBC of wheat flour (by 3.2%), which could be caused by the lower molar mass of AX.

Table 3. Properties of sourdough wheat bread with addition of AX preparations.

AX Preparation *	WBC [%] **			DY [g] **			BL ** [%]			TBL ** [%]		
	0%	1%	2%	0%	1%	2%	0%	1%	2%	0%	1%	2%
Control	56.2 ± 0.2 a			156.2 ^{a}			9.8 ± 1.6 ^{a}			13.6 ± 0.4 a		
AX_NM		60.6 ± 0.2 c	64.3 ± 0.2 f		160.6 ^{c}	164.3 ^{f}		11.0 ± 0.9 ab	12.4 ± 0.9 bc		14.6 ± 0.6 ab	16.1 ± 0.6 cd
AX_HYD		59.4 ± 0.5 b	61.2 ± 0.2 d		159.4 ^{b}	161.2 ^{d}		11.9 ± 0.8 ab	13.9 ± 1.1 c		15.4 ± 0.5 bc	16.9 ± 0.6 d
AX_CR		63.0 ± 0.3 e	66.0 ± 0.4 g		163.0 ^{e}	166.0 ^{g}		12.3 ± 0.8 bc	11.7 ± 0.7 ab		15.0 ± 0.5 bc	14.6 ± 0.7 ab

* Control—wheat bread without AXs; AX_NM—wheat bread with non-modified AX; AX_HYD—wheat bread with hydrolyzed AX; AX_CR—wheat bread with cross-linked AX. ** WBC—the water binding capacity, DY—the yield of dough obtained from 100 g of flour, BL—baking loss of hot bread, TBL—total baking loss after cooling. Mean values marked with the same letters are not statistically significantly different at $p < 0.05$.

The consequence of the increased water absorption of flour and the increased addition of water to the dough for the sourdough wheat bread was a higher dough yield. The addition of the AX_CR preparation, especially at 2%, had the highest effect on increasing the dough yield (Table 3), raising it by as much as 6.3% compared to the dough without AX addition. The addition of AX_NM in amounts of 1% and 2% also increased the dough yield by 2.8% and 5.2%, respectively. There was also an increase of 2.1% in the dough yield when AX_HYD was applied at 1%, but the effect of this preparation was the lowest. A higher dough yield is advantageous to the baking industry from an economic point of view because it allows for more bread to be baked from the same amount of flour [54].

During the bread baking process, there is a loss of weight due to the evaporation of water and other volatile substances, and this parameter is expressed as the baking loss (BL). However, after the bread has cooled, the total baking loss (TBL) is determined, which includes the evaporation of water during cooling [55,56]. The parameters influencing the baking loss of bread are the type, shape, size and volume of the bread, as well as the method of baking, the technological additives and the type of oven used [55]. The baking loss (BL) and total baking loss (TBL) are presented in Table 3. Baking loss measured immediately after removal from the oven in sourdough wheat bread without AXs accounted for 9.8%. The application of arabinoxylans AX_NM, AX_HYD at 1% and AX_CR at 2% did not significantly ($p < 0.05$) affect the baking loss of sourdough bread when compared to bread without sourdough. Similar results were reported in the other studies, where the addition of 0.1% dextran with a molar mass of 9608–750,000 g/mol to sourdough wheat bread did not significantly affect the parameter under investigation [15]. The AX_HYD preparation at 2% had the highest effect on increasing the baking loss of the sourdough wheat bread, increasing it by about 4.1% compared to the bread without AX addition. Probably, the partially hydrolyzed AX, due to its lowest molar mass, was not able to bind and retain as much water as the other AXs. In addition, the volume of bread with AX_HYD and the associated evaporation area of the breads was the highest (Table 4), which also contributed to the evaporation of larger amounts of water [57,58].

Table 4. Volume of sourdough wheat bread with addition of AX preparations.

AX Preparation *	BV ** [cm^3]			SBV ** (cm^3/100 g of Flour)		
	0%	1%	2%	0%	1%	2%
Control	121.7 ± 1.4 [a]			316.7 ± 3.6 [a]		
AX_NM		123.8 ± 1.5 [ab]	138.8 ± 1.3 [d]		331.2 ± 4.0 [b]	379.9 ± 3.4 [e]
AX_HYD		138.3 ± 1.5 [d]	145.8 ± 1.5 [e]		367.3 ± 4.0 [d]	391.6 ± 4.0 [f]
AX_CR		127.3 ± 0.5 [c]	125.5 ± 1.0 [abc]		345.7 ± 1.4 [c]	347.2 ± 2.8 [c]

* Abbreviations of sample names as in Table 3. ** BV—bread volume; SBV—specific bread volume. Mean values marked with the same letters are not statistically significantly different at $p < 0.05$.

The total baking losses measured for all the variants after the sourdough wheat bread cooled were higher compared to the losses measured immediately after removal from the oven. The addition of AX_NM at 1% and AX_CR at 2% had the lowest effect on increasing (by only 1% in both examples) the total baking loss of the sourdough wheat bread compared to the bread without AX addition (Table 3). The addition of the preparation of partially hydrolyzed arabinoxylans (AX_HYD) at 1% and 2% increased the total baking loss by 1.8% and 3.3%, respectively.

The volume of the breads (BV) and the specific volume of the breads (SBV) are shown in Table 4. The addition of 2% of the AX_HYD preparation had the highest effect on increasing (by 19.8%) the volume of sourdough wheat bread compared to the bread without AX addition. The 1% addition of AX_HYD also significantly increased the bread volume, by 14.1%, compared to the bread without AX addition, and a similar effect on this parameter was observed when a 2% addition of AX_NM was applied. This result is in agreement with data from the other studies, where a 1% addition of water-insoluble wheat pentosans also influenced the increase in the volume (BV) of sourdough bread [11], and a similar correlation was reported with the use of oat fiber [17]. The addition of the AX_CR preparation at 1% also increased (by 4.6%) the volume of the sourdough wheat bread compared to the bread without AXs. The addition of the AX_NM preparation at 1% and AX_CR at 2% did not significantly ($p < 0.05$) affect the volume (BV) of the sourdough wheat bread.

The specific volume (SBV) is the total volume of bread obtained from 100 g of flour, which takes into account not only the structure-forming properties, but also the water absorption of the flour and the associated dough yield [37]. The addition of the 2% AX_HYD preparation had the highest effect on increasing (by 23.7%) the specific volume of the sourdough wheat bread compared to the bread without AX addition (Table 4). The addition of AX_NM at both levels of substitution increased (by 4.6% and 20%, respectively) the volume of the sourdough wheat breads compared to the breads without AX addition. The addition of a preparation of cross-linked arabinoxylans (AX_CR) at both levels of substitution also increased (by 9.2% and 9.6%, respectively) the specific volume of the sourdough wheat breads. The observed correlation is not confirmed by other studies, where the addition of another hydrocolloid—dextran—affected the reduction in the specific volume of sourdough wheat breads compared to bread without dextran [15]. The differences In the results may have been due to the use of preparations with different molar masses. The molar mass of the AX preparations was 192,320–535,630 g/mol (Table 2), and their level of addition to the sourdough bread was 1% and 2%. In contrast, Zhang et al. [15] used dextran with a molar mass in the range of 9608–2,800,000 g/mol, and the level of addition to sourdough bread was 0.1%.

Table 5 shows the results regarding the hardness of the crumb of the wheat bread without and with AXs on the day of baking and after 1 and 3 days of storage. On the day of baking, the 1% and 2% addition of AX_HYD had the highest effect on reducing (by approximately 33% and 41%, respectively) the hardness of the crumb of the sourdough wheat bread compared to the bread without AX addition. The 2% share of AX_NM and 1% and 2% share of AX_CR also reduced the crumb hardness of the sourdough bread (by approximately 26%, 9% and 12%, respectively) on the day of baking compared to the bread without AXs. The AX_NM preparation at 1% did not significantly ($p < 0.05$) affect the hardness of the crumb of the sourdough wheat bread on the day of baking compared to the bread without AX addition (Table 5). In another study, a 0.1% share of a different hydrocolloid—dextran with a molar mass in the range of 9608–2,800,000 g/mol—also did not significantly influence the hardness of the bread crumb on the day of baking compared to bread without dextran [15].

Table 5. Hardness (N) of sourdough wheat bread with addition of AX preparations on the day of baking and after 1 and 3 days of storage.

		Storage Time [Days]		
AX Preparation *	**Share [%]**	**0**	**1**	**3**
Control	0%	9.7 ± 0.4 ^{cA}	17.5 ± 0.1 ^{eB}	28.9 ± 0.2 ^{deC}
AX_NM	1%	9.7 ± 0.4 ^{cA}	17.8 ± 1.7 ^{eB}	31.2 ± 2.2 ^{eC}
	2%	7.2 ± 0.3 ^{aA}	11.8 ± 0.1 ^{aB}	21.8 ± 0.8 ^{aC}
AX_HYD	1%	6.7 ± 0.3 ^{aA}	12.5 ± 0.1 ^{bB}	25.4 ± 2.3 ^{bcC}
	2%	6.5 ± 0.4 ^{aA}	11.8 ± 1.4 ^{abcB}	22.7 ± 1.2 ^{abC}
AX_CR	1%	8.8 ± 0.3 ^{bA}	14.3 ± 1.1 ^{cdB}	28.0 ± 0.7 ^{cdeC}
	2%	8.5 ± 0.4 ^{bA}	13.5 ± 0.1 ^{dB}	23.2 ± 1.4 ^{abC}

* Abbreviations of sample names as in Table 3. Capital letters indicate differences in results at day 0, 1 and 3 of analysis. Lowercase letters indicate differences in results with various arabinoxylan preparations. Mean values marked with the same letters are not statistically significantly different at $p < 0.05$.

The moisture content of the crumb of the breads was determined in the central (Table 6) and peripheral (Table 7) parts of the bread. This examination aimed to assess the effect of arabinoxylan preparations on reducing the moisture loss in the crumb and water migration in the bread. On the day of baking, the preparation of cross-linked arabinoxylans (AX_CR) at both levels had the strongest effect on increasing the crumb moisture in the central part of the sourdough wheat bread compared to the bread without AX addition (Table 6) and this parameter increased by 1.9% and 2.6%, respectively. This was due to the highest molar mass of the AXs of those used in the research and the highest amount of water added to the dough, as indicated by data from other studies [18]. On the day of baking, the addition of the AX_NM preparation at both levels also increased (by 1.5% and 1.9%, respectively) the moisture content of the crumb in the central part of the sourdough wheat bread (Table 6) compared to the bread without AX addition. On the day of baking, AX_HYD at 1% also had a significant ($p < 0.05$) effect on increasing the crumb moisture in the central part of the sourdough wheat bread compared to the bread without AX addition, but to the least extent. In another study, where a 0.1% share of dextran with a molar mass of 9608–2,800,000 g/mol was used, there was no significant effect on the crumb moisture of the sourdough wheat bread [15], which could be due to the different structure of the natural and modified AXs used. In addition, the AX substitution levels were 1% and 2%, while dextran was only added at a proportion of 0.1%.

Table 6. Moisture of the crumb in the central part of sourdough wheat bread with AX preparations on the day of baking and after 1 and 3 days of storage.

		Storage Time [Days]		
AX Preparation *	**Share [%]**	**0**	**1**	**3**
Control	0%	41.7 ± 0.3^{aC}	41.2 ± 0.1^{aB}	40.1 ± 0.0^{aA}
AX_NM	1%	43.2 ± 0.5^{bcB}	43.1 ± 0.4^{cdB}	40.9 ± 0.4^{bA}
	2%	43.6 ± 0.2^{cB}	43.5 ± 0.2^{dAB}	42.9 ± 0.5^{dA}
AX_HYD	1%	42.8 ± 0.2^{bC}	42.2 ± 0.2^{bB}	40.1 ± 0.0^{aA}
	2%	43.4 ± 0.5^{bcC}	42.4 ± 0.2^{bB}	41.3 ± 0.1^{bcA}
AX_CR	1%	43.6 ± 0.4^{cB}	43.5 ± 0.5^{dB}	41.8 ± 0.4^{cA}
	2%	44.3 ± 0.3^{dB}	44.1 ± 0.1^{eB}	43.2 ± 0.5^{eA}

* Abbreviations of sample names as in Table 3. Capital letters indicate differences in results at day 0, 1 and 3 of analysis. Lowercase letters indicate differences in results with various arabinoxylan preparations. Mean values marked with the same letters are not statistically significantly different at $p < 0.05$.

Table 7. Moisture of the crumb in the peripheral part of sourdough wheat bread with AX preparations on the day of baking and after 1 and 3 days of storage.

		Storage Time [Days]		
AX Preparation *	**Share [%]**	**0**	**1**	**3**
Control	0%	37.9 ± 0.6^{aC}	32.4 ± 0.6^{aB}	31.0 ± 0.5^{aA}
AX_NM	1%	40.9 ± 0.1^{cB}	34.2 ± 0.3^{bA}	34.0 ± 0.1^{cA}
	2%	42.8 ± 0.2^{eB}	36.4 ± 0.5^{dA}	35.7 ± 0.3^{dA}
AX_HYD	1%	39.8 ± 0.1^{bC}	34.0 ± 0.6^{bcB}	32.6 ± 0.4^{bA}
	2%	40.8 ± 0.2^{cC}	34.8 ± 0.2^{cB}	33.3 ± 0.7^{bA}
AX_CR	1%	42.6 ± 0.2^{deB}	36.4 ± 0.0^{dA}	36.1 ± 0.1^{dA}
	2%	43.0 ± 0.5^{eC}	37.3 ± 0.4^{eB}	36.7 ± 0.1^{eA}

* Abbreviations of sample names as in Table 3. Capital letters indicate differences in results at day 0, 1 and 3 of analysis. Lowercase letters indicate differences in results with various arabinoxylan preparations. Mean values marked with the same letters are not statistically significantly different at $p < 0.05$.

On the day of baking, the AX_CR preparation at the amount of 2% had the highest effect on increasing the moisture content of the peripheral part (crust) of the sourdough wheat bread, with an increase of 5.1% compared to the bread without AXs (Table 7). This could be due to the overall highest addition of water to the dough, as well as the low volume and surface area of the bread and the consequently lower intensity of water evaporation at the baking stage and immediately after it compared to the bread without AXs. On the day of baking, the AX_NM preparation in amounts of 1% and 2% also increased (by 3% and 4.9%, respectively) the crumb moisture in the peripheral part of the sourdough wheat bread compared to the bread without AXs. The 1% addition of AX_HYD also had a significant ($p < 0.05$) effect on increasing (by 1.9%) the crumb moisture in the peripheral part of the sourdough wheat bread.

This study, for the first time, examined the effect of the molar mass of AXs on the reduction in the hardness increase and moisture loss of sourdough wheat bread. The statistical analysis of the moisture content and crumb hardness of the sourdough breads without and with arabinoxylans showed ($p < 0.05$) that the hardness of the breads progressively increased with storage time, while the moisture content in the central and peripheral parts decreased.

After 1 and 3 days of storage (Table 5), the addition of all the AX preparations at the amount of 2% affected the increase in the hardness of the crumb of the sourdough wheat bread compared to the hardness of the control sample (bread without addition of AXs). A similar correlation was observed by Zhang et al. [15], where a 0.1% share of dextran with a molar mass of 9608–2,800,000 g/mol influenced the reduction in the increase in the crumb hardness of sourdough wheat bread compared to bread without the addition of dextran [15]. In general, the 1% addition of AX_HYD slightly reduced the increase in the bread crumb hardness, while the 1% addition of AX_NM and AX_CR had no significant effect ($p < 0.05$) on the reduction in the increase in the crumb hardness of the sourdough wheat bread after 1 and 3 days of storage compared to the hardness of the bread without AXs (Table 5). This may have been due to insufficient AXs in the bread to effectively affect the retrogradation of starch, which is responsible for the staling of the bread [15].

Tables 6 and 7 show the results of the moisture loss of the crumb during the storage of the sourdough breads without and with the AX preparations. On the first day of storage, the addition of 1% and 2% of the AX_NM and AX_CR preparations had an influence on reducing the moisture loss of the crumb in the central part of the sourdough bread compared to the breads with the AX preparations on the day of baking ($p < 0.05$), which was not observed for the bread with AX_HYD and the bread without AXs (Table 6). On the third day of storage, a significant loss of moisture in the central part of the bread crumb was observed in all the baked breads. In the peripheral part of the breads (crust), the moisture loss was observed on the first as well as the third day of storage in all the variants of the breads (Table 7). The crust drying occurred with crumb moisture retention in the central part of the AX sourdough bread. The water migration from the crumb to the crust did not occur or was delayed.

In general, the addition of all the preparations at both levels resulted in a higher moisture content of the crumb in the central (Table 6) and peripheral (Table 7) parts of the bread on the first and third days of storage compared to the bread without AXs. The most effective in reducing the crumb moisture loss was the addition of 2% of the cross-linked AX preparation, and, to a lower extent, the addition of 2% of AX_NM. The application of 1% AX_HYD resulted in a higher crumb moisture content in the central part of the bread after the first day of storage compared to the bread without AXs. However, after three days of storage, the moisture content of the crumb in the central part of bread with 1% AX_HYD was the same as the bread without AXs. The unmodified and cross-linked AXs had the strongest influence on reducing the moisture loss of the crumb in the central and peripheral parts of the bread, which was probably due to the high molar mass of AX (446,330–574,710 g/mol). In other studies, data have been reported on the influence of sourdough on reducing moisture loss in the crumb of wheat bread during storage [13,14]. However, the influence of hydrocolloids on reducing moisture loss in the crumb of sourdough wheat bread has not been studied to date.

4. Conclusions

The addition of AXs increased the water absorption of the wheat flour and dough yield proportionally to the amount and molar mass of the AXs. The addition of 2% of the AX_CR preparation, which contains AXs with the highest molar mass, caused the highest (by 9.8%) increase in the water absorption of the wheat flour dough. The addition of 2% of AX_NM increased the water absorption by 8.1%, while the addition of 2% of AX_HYD increased the water absorption of the wheat flour dough by 5%.

The partially hydrolyzed AXs in the AX_HYD preparation, with the lowest molar mass of 190,440 g/mol, was the most effective in increasing (by about 20%) the volume of the bread, especially the addition of 2% compared to the bread without added AXs. The addition of all the AX preparations had the effect of increasing the bread volume per 100 g of flour compared to the bread without AXs.

The addition of the partially hydrolyzed arabinoxylans (AX_HYD), especially at 2% most effectively reduced (by 41%) the hardness of the bread crumb on the day of baking compared to the bread without AXs. The reduction in the hardness of the bread crumb was observed as a consequence of the application of all the AX preparations, except for the addition of 1% of unmodified AXs.

On the day of baking, the AX_CR preparation at the amount of 2% had the strongest effect on increasing the moisture content of the crumb in the central (by 2.6%) and peripheral (by 5.1%) parts of the bread compared to the bread without AXs. Also, the other AX_NM and AX_HYD preparations at the amounts of 1% and 2% had an effect on increasing the moisture content of the bread crumb on the day of baking compared to the bread without AX addition.

On the first day of storage, the addition of the unmodified and cross-linked AX resulted in the inhibition of crumb moisture loss in the central part of the sourdough bread compared to the bread from the day of baking.

In general, the addition of all the preparations resulted in a higher moisture content of the crumb in the central and peripheral parts of the bread on the first and third days of storage compared to the bread without AXs.

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