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#### Review



### Effect of physicochemical properties, pre-processing, and extraction on the functionality of wheat bran arabinoxylans in breadmaking – A review

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#### ABSTRACT

Arabinoxylan (AX) is an abundant hemicellulose in wheat bran and an important functional component in bakery products. This review compares preprocessing and extraction methods, and evaluates their effect on AX properties and functionality as a bread ingredient. The extraction process results in AX isolates or concentrates with varying molecular characteristics, indicating that the process can be adjusted to produce AX with targeted functionality. AX functionality in bread seems to depend on AX properties but also on AX addition level and interactions with other components. This review suggests that the use of AX with tailored properties together with properly optimized baking process could help increasing the amount of added fiber in bread while maintaining or even improving bread quality.

#### 1. Introduction

Wheat is one of the most cultivated crops worldwide, with annual total harvest of around 770 million tons (FAO, 2019). Most wheat intended for human consumption is milled into white flour in a process where the inner starchy endosperm is separated from other grain parts. The purest white wheat flour has only small amounts of minerals and dietary fiber, but is considered the most valuable milling product due to its superior properties, especially in baking applications.

Wheat bran, defined as the outer layers of wheat grain that are separated from the other kernel parts by milling, makes up around 15% of the wheat kernel and has an estimated annual production volume of 150 million tons (Prückler et al., 2014). This huge low-value side-stream is currently used mostly for animal feed and bioenergy production (Hemery et al., 2011). Wheat bran contains many interesting components, such as dietary fiber, minerals, and bioactive compounds, that could be used in food applications to increase the efficiency of cereal processing (Katileviciute et al., 2019).

Arabinoxylan (AX) is an abundant hemicellulose in wheat bran and an important functional component in baked products because it affects water binding, dough rheology, and starch retrogradation (Courtin & Delcour, 2002; Izydorczyk & Biliaderis, 1995; Zhang, Smith, & Li,

2014). Although the health benefits of fiber incorporation in the human diet are widely known, wheat bran AX is not highly utilized by the bakery industry due to its negative effect on sensory and technological quality (Coda et al., 2014). Novel methods are therefore required to facilitate fiber addition to bread without compromising its overall acceptability. Adding dietary fiber in a more purified form could help to overcome some of the undesirable effects of bran addition, because AX isolation removes several components that negatively affect bread quality.

Wheat bran AX has been extensively studied over the past few decades, but comparing results from these studies is often challenging due to differences in extraction methods. In addition, many studies fail to provide information on process parameters that are known to affect the functional properties of AX (Izydorczyk & Biliaderis, 1995; Izydorczyk & Biliaderis, 2006; Zhang et al., 2014). The aim of this literature review was to address this problem by investigating the effect of a variety of preprocessing and extraction methods on AX functionality, and thus provide a more comprehensive understanding of AX as a functional bread ingredient. The focus of the review was on AX extracted from wheat bran but, due to lack of more relevant literature, studies on AX from other sources were also included. The source of AX is always mentioned in the text.

Abbreviations: AX, arabinoxylan; FA, ferulic acid; HMW, high molecular weight; LMW, low molecular weight; WEAX, water-extractable arabinoxylan; SWE, subcritical water extraction.

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#### 2. Arabinoxylan

Arabinoxylan is an important structural component of lignified plant cell walls and makes up around 30% of wheat bran (Maes & Delcour, 2001). It is structurally composed of a  $\beta$ -(1  $\rightarrow$  4)-linked  $\beta$ -D-xylopyranose backbone (Fig. 1). Xylose in the backbone can be unsubstituted, monosubstituted in C2, mono-substituted in C3, or di-substituted in both C2 and C3 by  $\alpha$ -D-arabinofuranosyl (Darvill, McNeil, Darvill, & A. P., 1980). In grasses such as wheat, arabinose can be further cross-linked to ferulic acid at the C5 position via an ester linkage (Izydorczyk & Biliaderis, 2000; Marcia, 2009).

#### 2.1. Arabinoxylan structural features

#### 2.1.1. Substitution pattern and A/X ratio

The arabinose to xylose (A/X) ratio and substitution pattern affect the physico-chemical properties of AX. The A/X ratio is directly related to the amount of di-substituted xylose residues, and increase in unsubstituted residues decreases A/X ratio (Delcour, Van Win, & Grobet, 1999; Dervilly-Pinel, Rimsten, Saulnier, Andersson, & Åman, 2001; Yan et al., 2019). Distribution of arabinose substitutes is not random, and there can be both highly branched and less branched parts in the AX chain (Heikkinen et al., 2013). The A/X ratio and the distribution of arabinose side-chains determine the conformation of AX, and hence affect the solubility and thermal degradation of the molecule (Pavlovich-Abril et al., 2016). The A/X ratio also influences the interactions of AX with other molecules (Yan et al., 2019). The A/X ratio varies in different parts of wheat and even different layers of wheat bran. The aleurone layer has the lowest A/X ratio (0.31), followed by wheat endosperm (0.50-0.71), while the outer bran layers have the highest ratio (1.02-1.14) (Antoine, Peyron, Lullien-Pellerin, Abecassis, & Rouau, 2004; Z. Zhang et al., 2014).

#### 2.1.2. Molar mass

The molar mass of wheat bran AX affects its physico-chemical properties, especially in solution, and it is a strong indicator of the thickening ability of AX (Hou, Zhao, Tian, Zhou, Yang, Gu, & Wang, 2020; Kale, Pai, Hamaker, & Campanella, 2010). Molar mass is also important for some nutritional properties, such as prebiotic potential (Damen et al., 2012). The molar mass of AX is affected by extraction method, with water-extractable AX (WEAX) tending to have lower molar mass than water-unextractable AX (WUAX). Reported values for alkaliextracted wheat bran WUAX lie between 210 and 716 kDa (Anderson & Simsek, 2019; Chen et al., 2019). Molar mass averages for WEAX from wheat bran vary between 30 and 513 kDa, and for AX extracted in subcritical water conditions between 126 and 370 kDa (Chen et al.,

2019; Rudjito, Ruthes, Jiménez-Quero, & Vilaplana, 2019; Ruthes et al., 2020; Wang, Hou, Zhao, Tian, Gu, & Yang, 2019; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). Wheat endosperm AX has higher molar mass than wheat bran AX, and molar mass is reported to vary even between different bran layers (J. Wang, Sun, Liu, & Zhang, 2014). High molar mass AX can be enzymatically decreased to produce low molar mass AX with specific functionality (Boll et al., 2016). An increase in alcohol concentration and in process temperature during extraction have been shown to decrease molar mass (Lu et al., 2020; P; Wang et al., 2019).

#### 2.1.3. Feruloylation

The arabinose in AX is often esterified with ferulic acid (FA) by a linkage between the carboxyl acid group of FA and the primary alcohol on the C5 carbon of the arabinose side-chain (Smith & Hartley, 1983). Ferulic acid has antioxidant, anti-inflammatory, antiviral, antiallergic, antimicrobial, antithrombotic, and anticarcinogenic activity (Kumar & Pruthi, 2014). Hence, addition of feruloylated AX in baking applications could offer additional health benefits besides increasing the fiber content (Koegelenberg & Chimphango, 2017; Pihlajaniemi et al., 2020). Although the organization of lignified cell wall structures remains unclear, ferulic acid seems to be able to cross-link polysaccharide chains by forming ferulate dimers, trimers, and even tetramers, including 8-O-4', 8-5', and 5-5' dehydrodimers, through peroxidase-meditated oxidative coupling (C. Li, Wang, Chen, Li, & Li, 2020; Mnich et al., 2020). Ferulic acid can form a covalent ether linkage between AX and lignin, and it has also been suggested that ferulic acid links AX to proteins via a dehydroferulic acid-tyrosine cross-link (Piber & Koehler, 2005). This crosslinking of esterified ferulic acid ties AX to the cell wall matrix, explaining why ferulic acid in wheat bran is predominantly bound to water-unextractable polymers (Schooneveld-Bergmans, Dignum, Grabber, Beldman, & Voragen, 1999).

#### 2.2. Arabinoxylan properties

#### 2.2.1. Extractability

In addition to covalent crosslinking via ferulic acid, AX can also bind to other cell wall polysaccharides like cellulose. The mechanism of this interaction is not completely clear, but hydrogen-bonding has been suggested to play a crucial role in it (Mnich et al., 2020). These interactions between different cell wall components create an almost impermeable and non-wettable strong phenolic copolymer, which makes up around 95% of wheat bran AX unextractable with water (Escarnot, Aguedo, Agneessens, Wathelet, & Paquot, 2011). Due to its higher ferulic acid content and higher molar mass, WUAX forms covalent ester bonds between the carboxylic acid group of uronic acids and

Fig. 1. Schematic diagram of the structure of arabinoxylan, showing the xylose backbone (black), arabinose substitutions (green), and ferulic acid (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the hydroxyl groups of AX, or diferulic acid bridges between AX chains (Zhou et al., 2010). This ties the molecule tightly into the cell wall matrix and decreases the water solubility of AX. WEAX is located on the cell wall surface and lacks these linkages between polymers, making it more easily extractable (Escarnot et al., 2011).

#### 2.2.2. Solubility

Solubility and the solution conformation of wheat bran AX have been observed to be influenced by arabinose side-groups, because a lower degree of substitution decreases AX solubility (Izydorczyk & Biliaderis, 1995; Q. Li, Liu, Wu, & Zhang, 2017; Pitkänen, Virkki, Tenkanen, & Tuomainen, 2009). Insolubility of fractions with low arabinose substitution level can be attributed to increased aggregation of unsubstituted regions of wheat bran AX stabilized by hydrogen bonds. These interactions may also contribute to an increase in viscosity or precipitation of polymer chains (Heikkinen et al., 2013). However, A/X ratio alone is not sufficient to explain differences in the solubility of wheat endosperm AX (Mares & Stone, 1973). Solubility depends also on other factors, such as molar mass, substitution pattern of AX and degree of diferulate crosslinking (Izydorczyk & Biliaderis, 1995). According to Q. Li et al. (2017), higher molar mass also increases aggregation and lowers the solubility of AX extracted from wheat bran. AX solubility seems to have a crucial effect on its functionality in baking applications because the insoluble AX aggregates have been shown to cause uneven dough mixing and destroy bubble interface in dough (Koegelenberg & Chimphango, 2017; Xiao, Zhang, Niu, Xiang, Chang, Zhao, Xiong, Zhao, Rong, Tang, & Wu, 2021).

#### 2.2.3. Viscosity

The physical conformation, molecular weight, and solubility of wheat bran AX strongly affect the viscosity of AX dispersions. The viscosity and rheological behavior of AX dispersions are directly manifested in their processing behavior in foods and baked products (Kale, Yadav, Hicks, & Hanah, 2015). Izydorczyk and Biliaderis (1992) were among the first to study the rheological properties of AX. They found a typical random coil behavior of AX extracted from wheat endosperm, with a clear critical overlap concentration separating the dilute and concentrated regime. All three different extracted fractions in that study fell onto a master curve, in line with typical polymeric behavior. In addition, they found that extracted AX shows a Newtonian plateau at low shear rates, followed by a shear thinning regime at higher shear rates for fractions with intrinsic viscosities >3.2 dl/g (Izydorczyk & Biliaderis, 1992). Yan et al. (2019) further explored viscosity dependence of AX from wheat bran at different pH values.

The physical conformation of AX is defined by its monosaccharide composition, e.g., results in one study suggested that high intrinsic viscosity is correlated with low A/X ratio and high elongational viscosity of dough (Pavlovich-Abril et al., 2016). This can be explained by the higher tendency of low-substituted AX to aggregate and its reduced ability to retain water, allowing for cross-linking with gluten starch complexes in the dough. A tendency for aggregation of AX with low substitution of arabinofuranosyl has been observed previously, and even low molar mass samples tend to aggregate in water (Pitkänen et al., 2009). Lu et al. (2020) found that wheat bran AX viscosity in solution increased with the sodium hydroxide concentration used during extraction, but decreased with higher extraction temperatures and longer extraction times. The monosaccharide composition of different AX is likely to affect the viscosity of its dispersions (Izydorczyk & Biliaderis, 1992; Kale et al., 2015; Yan et al., 2019), but exactly how remains to be resolved.

#### 2.2.4. Emulsion properties

While AX has been shown to have marginal ability to adsorb onto droplet interfaces, much of the emulsifying ability of AX has been attributed to phenolic groups or proteins attached to AX, rather than the AX itself (S. Li, Chen, Cheng, Yang, Cai, He, Du, Liu, Liu, Zeng, & Li,

2021; Lv, Chen, Yin, & Liu, 2019). Protein-AX conjugates have been shown to stabilize emulsions better than wheat bran AX or protein alone, making them potential novel emulsifiers (Lv et al., 2019). Kaur, Singh, Yadav, Bhinder, and Singh (2021) compared the emulsifying ability of alkali-extracted and water-extracted wheat bran AX, and found that alkali extraction resulted in good emulsion activity in terms of initial droplet size and ability of the emulsion to maintain droplet size during three days of accelerated storage. They suggested that this may be related to some extent to the higher molar mass of alkali-extracted AX, but to a greater extent to the higher amounts of protein residues in alkali-extracted AX, as proteins tend to adsorb better to the interfacial layer.

Yan et al. (2019) found that the extraction temperature applied during alkali extraction also affects the emulsifying properties of wheat bran AX, with higher extraction temperature of 85 °C improving the emulsifying properties compared to 25 °C. This might be due to the higher molar mass and degree of substitution achieved with higher extraction temperatures. However, wheat bran AX did not perform well compared with AX from other cereal sources in that study, probably due to its purity and structure, as the best emulsifying ability was observed for high molar mass and highly branched corn bran AX with larger amounts of protein and lipid residues (Yan et al., 2019).

#### 2.2.5. Health effects

The health effects of wheat bran AX have not been widely investigated, but wheat endosperm AX is reported to have several health-promoting effects (Ciudad-Mulero et al., 2020; Jefferson & Adolphus, 2019). Studies have found that wheat endosperm AX has the potential to lower postprandial glucose and insulin response, affect cholesterol metabolism, protect from oxidative stress, and reduce the risk of coronary heart disease (Chen et al., 2019; Fadel et al., 2018b; Scazzina, Siebenhandl-ehn, & Pellegrini, 2013; Z. Zhang et al., 2014). Wheat endosperm AX also has an approved health claim from the European Food Safety Authority (EFSA Panel on Dietetic Products. Nutrition and Allergies (NDA). (2011) (NDA), 2011) for reducing post-prandial glycemic response when 8 g of wheat endosperm fiber with at least 60% AX content is used per 100 g of carbohydrates (EFSA Panel on Dietetic Products. Nutrition and Allergies (NDA). (2011) (NDA), 2011). However, this claim does not consider AX from other sources.

Health effects of AX are linked to its structure, especially to molar mass and degree of substitution. The cholesterol-lowering effect of nonstarch polysaccharides is suggested to be related to their viscosity and molar mass (Shelat et al., 2010). More intact structure deriving from high molar mass and low degree of substitution makes polysaccharides less accessible for human enzymes, influencing their effect on human metabolism (Scazzina et al., 2013). Low molar mass AX oligosaccharides have been shown to have the potential to improve glucose tolerance and prebiotic potential (Bhattacharya et al., 2020; Boll et al., 2016). Wheat endosperm AX stimulates probiotic bacteria, presumably by crossfeeding of lactobacilli and bifidobacteria with degradation products from carbohydrate-degrading bacteria (Bhattacharya et al., 2020). Chen et al. (2019) showed that a low degree of substitution increases the antioxidant activity of AX from whole grain wheat, probably due to an increase in specific functional groups of xylan, including ferulate residues. All these findings indicate that wheat bran AX may have some health-promoting effects.

#### 3. Arabinoxylan extraction process from wheat bran

As the complex cell wall matrix makes economic and sustainable extraction of AX challenging, several processes have been developed to increase AX extraction efficiency from wheat bran. These processes usually combine different pretreatments, extraction methods, and AX purification steps to obtain higher yields and AX purity. A general outline of the AX extraction process is presented in Fig. 2.

Most of the studies included in this review were performed at

# WHEAT BRAN PRETREATMENT

#### Chemical/enzymatic

Destarching
Delignification
Protein removal
Defatting

#### Mechanical/electrical

Milling Extrusion Ultrasound Microwave



#### **AX EXTRACTION**

Water Subcritical water Alkali Enzymatic



#### **AX PURIFICATION**

Fig. 2. Flow chart of arabinoxylan (AX) extraction process from wheat bran.

laboratory scale, but several studies have concluded that upscaling of AX extraction from wheat bran to pilot scale is possible (Hollmann & Lindhauer, 2005; Jacquemin et al., 2015; Rudjito et al., 2019). While adding process steps may increase the AX yield and improve its purity,

the production costs increase with every added step. Wheat bran AX is still not produced commercially, and increasing the value of this currently low-cost raw material requires an efficient process that results in premium food ingredients with proven beneficial technological and nutritional functionality (Misailidis et al., 2009).

#### 3.1. Wheat bran pretreatment for arabinoxylan extraction

Pretreatments combined with intense extraction methods are usually needed to increase the susceptibility of wheat bran to extraction and reach higher extraction yields (Hell et al., 2015; Rudjito et al., 2019). Several pretreatments have been used to increase AX yield and modify AX properties (Table 1. In general, removal of other wheat bran components before AX extraction has been observed to increase AX yield, as co-extraction of these components interferes with AX extraction (Koegelenberg & Chimphango, 2017; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). Hell et al. (2015) compared different mechanical, chemical, and enzymatic pretreatments of bran in terms of total mass loss, residual sugar content, and AX location, and found that pretreatment efficiency depended on which wheat bran layer was used, indicating that different parts of the bran are amenable to varying extraction conditions during processing. This partly explains the reported differences in the sugar content of samples (Hell et al., 2015), as AX composition varies in different bran layers. For example, Hell et al. (2015) found that peroxidase treatment was more effective for the outer bran layers, but relatively ineffective for deeper layers.

#### 3.1.1. Chemical and enzymatic pretreatments

3.1.1.1. Destarching. Most recent studies on AX extraction from wheat bran have included a destarching step in their AX extraction process, as destarching can help to increase both the yield and purity of AX (Rudjito et al., 2019; Ruthes et al., 2020). While destarching can lead to lower total extracted solids, due to removal of easily extractable material, Rudjito et al. (2019) showed that it improves AX yield and increases the purity of AX to 70% of total carbohydrates. They also found destarching

Table 1
Reported effect (increase/decrease/no effect) of different wheat bran pretreatments on total solid yield, arabinoxylan (AX) yield, AX purity, ferulic acid (FA) content, starch content, and AX molar mass.

| Pretreatment       | Total solid yield   | AX yield  | AX purity  | A/X   | FA content  | Starch content  | Molar mass                             |
|--------------------|---|---|--|---|---|---|--|
| Destarching        | Decreases ( Rudjito et al., 2019) Increases ( Mathew et al., 2017)          | Increases (Rudjito et al.,<br>2019; Yilmaz-Turan,<br>Jiménez-Quero,<br>Moriana, et al., 2020) | Increases (Rudjito<br>et al., 2019; Yilmaz-<br>Turan, Jiménez-<br>Quero, Moriana,<br>et al., 2020)<br>Decreases (Mathew<br>et al., 2017) | No effect (Yilmaz-<br>Turan, Jiménez-<br>Quero, Moriana,<br>et al., 2020) | Increases (Yilmaz-<br>Turan, Jiménez-<br>Quero, Moriana,<br>et al., 2020) | Decreases (Rudjito<br>et al., 2019; Yilmaz-<br>Turan, Jiménez-<br>Quero, Menzel,<br>et al., 2020) |  |
| Protein<br>removal | Increases (<br>Yilmaz-Turan,<br>Jiménez-Quero,<br>Moriana, et al.,<br>2020) | Decreases (Mathew<br>et al., 2017; Yilmaz-<br>Turan, Jiménez-Quero,<br>Moriana, et al., 2020) | ct dis, 2017)  |   |   | Increases (Yilmaz-<br>Turan, Jiménez-<br>Quero, Moriana,<br>et al., 2020)                         |  |
| Defatting          | Increases ( Rudjito et al., 2019)   | No effect (Rudjito et al., 2019)  | No effect (Rudjito et al., 2019)   |   |   |   |  |
| Milling            | ·   | Increases (Demuth et al., 2020)   |  | Decreases (Demuth et al., 2020)   | Increases (Rosicka-<br>kKaczmarek et al.,<br>2018)                        | Increases (Caprez et al., 1986)   | Decreases (<br>Demuth<br>et al., 2020) |
| Extrusion          |   | Increases (Fadel,<br>Ashworth, et al., 2018;<br>Roye et al., 2020;<br>Zeitoun et al., 2010)   |  |   |   |   |  |
| Ultrasound         |   | Increases (Ebringerová<br>& Hromádková, 2002;<br>Jiang et al., 2019; J.<br>Wang et al., 2014) |  | Increases<br>(Hromádková &<br>Ebringerova, 1999;<br>Liu et al., 2020)     | Increases<br>(Hromádková &<br>Ebringerova, 1999)                          |   | Decreases (<br>Liu et al.,<br>2020)    |
| Microwave          |   | Increases (Jiang et al., 2019)  |  | ый ст ал, 2020)   |   |   |  |

alone to be more beneficial than combining it with a defatting step, due to enzymatic activity caused by physical changes occurring during the defatting process. Mathew, Karlsson, and Adlercreutz (2017) found that a destarching step alone without protein removal improved total yield, but decreased the AX content in the extract.

3.1.1.2. Protein removal. As proteins make up a large part of wheat bran, their co-extraction during the process might interfere with AX extraction (Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). Prior protein fractionation would also enable their use in other applications, such as human food or animal feed, and therefore increase the usage of wheat bran even further (Arte et al., 2015). Yilmaz-Turan, Jiménez-Quero, Moriana, et al. (2020) found that protein removal by fermentation and alkali extraction increased carbohydrate yield, but decreased wheat bran AX yield. However, on combining protein removal with a destarching step they were able to significantly increase the AX content of the extract from 52% to 63% compared with the control. The protein removal step also increased the A/X ratio of extracts, which Yilmaz-Turan, Jiménez-Quero, Moriana, et al. (2020) attributed to the specificity of the protein extraction step towards the aleurone layer, which has a lower A/X ratio. Mathew et al. (2017) were able to extract wheat bran AX with high purity (71%) in a process that included an enzymatic protein removal step, although the protein removal step alone decreased AX yield compared with the control.

3.1.1.3. Delignification. As lignin associates with other cell wall components and decreases the water extractability of AX, sodium hypoclorite, chlorine, and hydrogen peroxide are commonly used for delignification of wheat bran before isolation of AX (Bataillon, Mathaly, Nunes Cardinali, & Duchiron, 1998; Börjesson, Larsson, Westman, & Ström, 2018; Maes & Delcour, 2001). Delignification solutions react with lignin to form water-soluble oxidation products, facilitating AX extraction (Maes & Delcour, 2001). Delignification efficiency depends on pH and chemical composition of the extractant liquid. Maes and Delcour (2001) found the optimum hydrogen peroxide concentration for non-starch polysaccharide extraction from wheat bran to be 2%, as any further increase in concentration did not affect extraction yield. Delignification has been observed to improve AX purity when extracting AX from barley husks (Glasser, Kaar, Jain, & Sealey, 2000). However, Bataillon et al. (1998) found that using 40% sodium chlorite in AX extraction for destarched wheat bran did not affect AX purity. If lignin is not removed, it can cause discoloration of bread when AX is added as a bakery ingredient (Koegelenberg & Chimphango, 2017). Unfortunately, most literature regarding delignification in AX extraction is relatively old and no newer research has been reported on effect of delignification step on wheat bran AX extractability.

3.1.1.4. Defatting. Wheat bran contains also small amounts of lipids, and some studies have included a lipid removal step before AX isolation to increase the AX extraction yield (Anderson & Simsek, 2019). Defatting can affect extract composition compared with untreated bran, although the results in yield of the extracted material using subcritical water extraction showed a slight improvement (Rudjito et al., 2019). However, these authors concluded that it is not an essential process step for AX isolation from wheat bran, because it did not improve AX purity or AX extraction yield. They attributed the higher total yield to the defatting process removing other components present in wheat bran.

#### 3.1.2. Mechanical and electrical pretreatments

3.1.2.1. Milling. Reducing the particle size of wheat bran by milling has long been known to affect both bran composition and AX extracted from bran (Caprez, Arrigoni, Amadò, & Neukom, 1986; Demuth, Betschart, & Nyström, 2020; Rosicka-kaczmarek, Komisarczyk, & Nebesny, 2018). Caprez et al. (1986) compared the chemical composition of unmilled

coarse bran and milled bran (<0.4 mm) and found milled bran to have lower levels of both insoluble and soluble fiber, but over 10% higher starch content. They attributed these differences partly to bran parts being lost during the milling process. Demuth et al. (2020) found that milling the bran to <0.5 mm particle size increased the yield of waterextractable AX and decreased the A/X ratio significantly compared with unmilled wheat bran. Milling also reduced the molar mass average of WEAX significantly, from 403 kDa to 134 kDa. These results indicate that the high-energy input from milling is able to destroy the cell wall structure and break bonds inside AX molecules, making AX more watersoluble. Decrease in A/X-ratio compared to untreated sample also indicates that milling increases AX extraction from inner parts of wheat kernel that have a lower amount of arabinose substitution (Antoine et al., 2004). According to Rosicka-kaczmarek et al. (2018), bran particle size affects the antioxidant potential of extracts, with particle size > 315 µm resulting in the highest antioxidant potential.

3.1.2.2. Extrusion. Extrusion is a mechanical treatment that has been found to increase AX extractability when combined with alkali extraction. Zeitoun, Pontalier, Marechal, and Rigal (2010) were able to increase the amount of purified extract from destarched wheat bran by 24% on using twin-screw extrusion with alkaline extraction, though they did not state the exact extruder settings required for these results. Fadel et al. (2018a) used twin-screw extrusion at screw speeds of 80 and 160 rpm to increase WEAX extractability from wheat endosperm from 9% to 15%, attributing the effect mostly to increased solubility of low molar mass fractions. They also recorded an increase in yield with increased extrusion temperature, maximum temperature being 140 °C. Zeitoun et al. (2010) and Jacquemin et al. (2015) concluded that the main advantages of the twin-screw extrusion assisted method are the shorter residence time and lower water consumption. Similarly to twinscrew extrusion, Roye et al. (2020) showed that double-pass extrusion of wheat bran increases extraction of WEAX and it also removes more ferulic acid compared with single-pass extrusion. For double pass extrusion, they used a screw speed at 310 rpm, moisture content of 27 % and maximum temperature of 120 °C.

3.1.2.3. Ultrasonication. Ultrasonication is a mechanical treatment in which sound energy above the human hearing range (>20 kHz) is applied to samples. The mechanical energy caused by pressure variation and cavitation during ultrasound treatment facilitates the release of both more available extractives and less extractable cell wall components with shorter treatment times and lower temperatures (Hromádková, Košť álová, & Ebringerová, 2008). Ultrasound used in combination with alkali extraction has been shown to increase antioxidant activity and extraction yield of hemicelluloses from corn and wheat bran, and to reduce the extraction time compared with alkali extraction alone (Ebringerová & Hromádková, 2002; Hromádková, Kováčiková, & Ebringerová, 1999). Ultrasound treatment has been shown to either decrease or increase the A/X ratio, which might be explained by combining different auxiliary treatments with ultrasound in the extraction process (Hromádková et al., 1999; Liu et al., 2020). Jiang et al. (2019) found that a combination of ultrasonication, microwave treatment, and alkali extraction was an efficient method for extracting bioactive AX from corn bran. They reached their maximum AX yield of 28 % with ultrasonic power 500 W, sodium hydroxide concentration 0.30 mol/L and ultrasonic-microwave synergetic time 25 min. J. Wang et al. (2014) optimized ultrasound-assisted enzymatic extraction of wheat bran AX, resulting in 12.9% AX yield. They used an endo-1,4-bxylanase (EC3.2.1.8) from Bacillus subtilis, a raw material concentration of 50 g/l, enzyme dose 4.5 g/l, extraction temperature 50 °C, extraction time 70 min, and ultrasonic power 180 W. These studies show the potential of ultrasound-assisted extraction processes when applied in extraction and modification of AXs with high yields from cereal byproducts (Liu et al., 2020; Z. Zhang et al., 2014).

3.1.2.4. Microwave treatment. Although microwave treatment has not been studied in terms of wheat bran pretreatment, microwave-assisted extraction has been observed to enhance AX extraction yield from other cereal sources. In contrast to the mechanical sound waves used in ultrasonic treatment, microwave treatment uses electromagnetic waves in frequencies between 300 MHz and 300 GHz. Electromagnetic waves cause dielectric heating due to molecular dipole rotation of mostly water, but also some fats and sugars (Rose & Inglett, 2010). As microwaves mostly cause heating, increased AX yields from microwaveassisted extraction are most likely due to increased temperature during treatment. However, microwave treatment has been shown to be a useful method for more efficient and controlled heating, decreasing extraction times when used for AX extraction from barley husks (Roos, Persson, Krawczyk, Zacchi, & Stålbrand, 2009). Roos et al. (2009) found that increasing microwave treatment times from 2 to 15 min and temperature from 120 to 200 °C decreased molar mass of AX but increased AX yield. Changing pH from acidic (pH 3.7) or neutral (pH 6.5) to alkali conditions increased molar mass but decreased yield up to pH 8. According to the literature review by Zhang et al. (2014), the yield of water-extractable hemicelluloses can be increased with microwave treatment, but the effect is dependent on temperature and time. Rose and Inglett (2010) were able to reach the maximum increase in total solid yield of corn bran extract using 180  $^{\circ}$ C for 5 to 10 min. 180  $^{\circ}$ C for 10 min was also the optimal treatment combination for maximizing release of AX oligosaccharides. Yoshida, Tsubaki, Teramoto, and Azuma (2010) obtained a carbohydrate yield of 59% from corn pericarp by combining microwave treatment with hydrothermal water extraction, reaching maximal yield with heating temperature of 176.5 °C, come-up time 2 min, heating time 16 min and solid to liquid ratio 1:20.

#### 3.2. Arabinoxylan extraction

In AX extraction, several methods can be used to separate AX from the cell wall matrix. As AX is covalently and non-covalently bound to the wheat bran cell wall matrix, harsher chemical methods have generally been used in the past for extraction. Several alternative extraction methods using water, enzymes, and physical treatments have also been developed over the years. The choice of extraction method affects the extraction efficiency, but also the AX properties. Lack of uniformity or of standard methodology for AX extraction makes comparing results from different sources challenging, due to great variation in the extraction processes used in different studies, which can greatly reflect on yield, purity and molecular features of the solubilized AX fractions. Different extraction methods are compared in terms of yield and AX properties in Table 2.

#### 3.2.1. Water extraction

Although AX is mostly bound to a non-wettable cell wall matrix, water extraction remains one of the most common methods of wheat bran AX extraction, due to its convenience and low cost. However, water extraction yields are low compared with those in many other treatments, because the gentle conditions of water extraction are not sufficient to disrupt the cross-linking in cell walls (Izydorczyk & Biliaderis, 2006; Skendi, Biliaderis, Izydorczyk, Zervou, & Zoumpoulakis, 2011). Water extraction of AX happens often in a long process containing several steps where other bran components are first removed in aqueous conditions and then concentrated fiber is precipitated (Li et al., 2020; Pavlovich-Abril et al., 2016; Wang et al., 2019). Process conditions during these steps can vary greatly, temperatures between 20 and 95 °C and pH between 2.2 and 7.5.

WEAX from wheat bran is reported to have lower molar mass and lower A/X ratio, indicating a lower degree of substitution compared with alkali-extracted and subcritical water-extracted AX (C. Li et al., 2020). C. Li et al. (2020) also found that WEAX from wheat bran contains only a few ferulic acid dehydrodimers, indicating negligible covalent cross-links in the WEAX. This lack of cross-linking in WEAX

explains its solubility to water. The water extract in that study also contained a considerable amount of glucan (42%), suggesting a need for a further purification step in combination with water extraction (C. Li et al., 2020).

#### 3.2.2. Subcritical water extraction

Water extraction can be combined with mechanical treatments, such as hydrothermal treatment, to increase the extraction yield of AX. Subcritical water extraction (SWE) is a hydrothermal treatment that utilizes pressure to keep water in the liquid state at elevated temperatures (100–374 °C) (C. Li et al., 2020). The harsh conditions in the commonly used alkali extraction process remove functional groups such as acetyl, uronic acid, and phenolic substitutions (C. Li et al., 2020); Ruthes et al., 2020). SWE offers an alternative method to obtain feruloylated AX from wheat bran with potential bioactivity and other additional functionality from ferulic acid (C. Li et al., 2020; Rudjito et al., 2019; Ruthes et al., 2020).

In SWE, higher extraction times and temperatures increase the extraction yield and purity of AX (Rudjito et al., 2019; Ruthes, Martínez-Abad, Tan, Bulone, & Vilaplana, 2017; Ruthes et al., 2020; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). Wheat bran SWE extracts change from glucan-rich to containing higher amounts of ferulovlated AX with extraction time, improving the purity AX (Ruthes et al., 2020; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). According to Ruthes et al. (2017), Ruthes et al. (2020), wheat bran AX purity is at its highest after 15–30 min and extraction yield is at its highest at 160  $^{\circ}\text{C}$ and pH 7. C. Li et al. (2020) operated SWE at different temperatures  $(120-180 \,^{\circ}\text{C})$ , initial pHs (4-10) and treatment durations  $(10-120 \, \text{min})$ . They obtained highest extraction yields using high temperatures (180  $^{\circ}$ C), low pH (pH 4) and long treatment times (120 min). However, this also increased the co-extraction of other components. Increasing temperature results in faster extraction, but it also causes degradation and depolymerization of wheat bran AX and reduces the molar mass (Fadel et al., 2018b; C. Li et al., 2020; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020). The use of high temperatures in SWE also decreases the A/X ratio, suggesting that the side-chains are more vulnerable to hydrolysis than the xylan backbone (C. Li et al., 2020). Comparing alkali and subcritical water extraction, Yilmaz-Turan, Jiménez-Quero, Moriana, et al. (2020) found SWE-extracted wheat bran AX to have significantly lower A/X ratio and attributed this to the susceptibility of the arabinose moieties to hydrolysis at high temperatures during SWE. They also suggested combining SWE with protein isolation to improve extraction yields, as other bran components solubilized during SWE might interfere with AX extraction.

#### 3.2.3. Alkali extraction

In alkaline extraction, hydroxyl ions disrupt covalent and hydrogen bonds and cause repulsion between molecules by changing the charge of uronic acids (Fadel et al., 2018b; Hollmann & Lindhauer, 2005). Alkaline conditions also hydrolyze ester linkages between AX and ferulic acid (Ruthes et al., 2020). This liberates AX efficiently from the complex bran cell wall matrix, making alkali extraction one of the most widely used isolation methods for AX extraction from wheat bran. Compared with other extraction methods, alkali extraction is able to achieve the highest AX extraction yields, molar mass (<717 kDa), and purity, because it lowers the starch and  $\beta$ -glucan content of AX extracts (Anderson & Simsek, 2019; Lu et al., 2020; Ruthes et al., 2017; Yilmaz-Turan, Jiménez-Quero, Menzel, et al., 2020). Alkali treatment does not hydrolyze arabinose moieties, which also leads to higher A/X ratios in AX extracts (Pihlajaniemi et al., 2020; Yilmaz-Turan, Jiménez-Quero, Menzel, et al., 2020).

Several different alkali solutions have been used for extraction to increase AX yields, including sodium, potassium, and calcium hydroxide, hydrogen peroxide, and barium ions (Bergmans, Beldman, Gruppen, & Voragen, 1996; Maes & Delcour, 2001; Ruthes et al., 2017; Z. Zhang et al., 2014). According to Ruthes et al. (2017), monovalent hydroxides

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Table 2
Comparison of steps and parameters used in water extraction, subcritical water extraction (SWE), alkali extraction, and enzymatic extraction of arabinoxylan (AX), and their effect on AX yield, AX extract purity % of dry weight, DW), A/X ratio, molecular weight (MW) and ferulic acid (FA) content of extracts.

|   | Extraction |  |                           |   |                                |                        |  |                   |                                   |              |             |                                  |
|---|------------|--|---------------------------|---|--------------------------------|------------------------|--|-------------------|-----------------------------------|--------------|-------------|----------------------------------|
| Reference   | Туре       | Pretreatments  | Solid:<br>liquid<br>ratio | Details   | Extraction<br>temperature (°C) | Extraction<br>duration | Purification method  | AX<br>yield       | AX extract<br>purity (% of<br>DW) | A/X<br>ratio | MW<br>(kDa) | FA content (mg g <sup>-1</sup> ) |
| (Demuth et al., 2020)                                       | Water      | Destarching, protein removal, β-glucan removal       | 13:90                     |   | 60                             | 30 min                 | Ethanol precipitation  | 9.3 <sup>b</sup>  | 42                                | 0.48         | 403         | 3.0                              |
| (Kaur et al., 2021)   | Water      | Destarching  |                           |   | 95                             | 60 min                 | Ethanol precipitation  | 4.5 <sup>b</sup>  |                                   |              |             | 3.5-4.9                          |
| (P. Wang et al., 2019)                                      | Water      | Destarching,<br>amyloglycosidase, protein<br>removal | 1:10                      |   | 65                             | 90 min                 | Ethanol precipitation, 20–70%                                  |                   | 83                                | 0.85         | 513         |                                  |
| (Rudjito et al., 2019)                                      | SWE        | Destarching  | 1:17                      | Sequential extraction                             | 160                            | 60 min                 |  | 46.7 <sup>a</sup> |                                   | 0.69         |             | 3.3                              |
| (Ruthes et al., 2017)                                       | SWE        | Destarching  |                           |   | 160                            | 15 min                 |  | 31.2 <sup>a</sup> | 72                                | 0.49         | 193         | 5.6                              |
| (Ruthes et al., 2020)                                       | SWE        | Destarching  |                           |   | 160                            |                        |  | 27.5 <sup>a</sup> | 68                                |              | 337         | 7.1                              |
| (Yilmaz-Turan, Jiménez-<br>Quero, Moriana, et al.,<br>2020) | SWE        | Destarching  | 1:10                      |   | 160                            | 30 min                 | Ethanol precipitation, 95%                                     | 7.5 <sup>a</sup>  | 76                                | 0.2          | 126         | 10.3                             |
| (Aguedo et al., 2014)                                       | Alkali     | Destarching  | 1:20                      | 0.44 M NaOH                                       | 80                             | 15 h                   | Ultrafiltration, 100 kDa                                       | 20.8 <sup>a</sup> |                                   | 0.94         | 670         | < 0.001                          |
| (Anderson & Simsek, 2019)                                   | Alkali     | Defatting, destarching, protein removal              | 1:18                      | 3% NaOH   | 50                             |                        | Ethanol precipitation, 95 %                                    |                   | 73                                | 0.51         | 717         |                                  |
| (Bhattacharya et al., 2020)                                 | Alkali     | Destarching, protein removal                         |                           | 0.5 M NaOH  | 80                             | 16 h                   | Ultrafiltration, 30 kDa  |                   | 778                               | 1.2          |             | < 0.001                          |
| (Börjesson et al., 2018)                                    | Alkali     | Acid hydrolysis,<br>delignification                  | 1:20                      | 1 M NaOH  | 25                             | 16 h                   | Destarching,<br>delignification, pre-<br>hydrolysis            | 11 <sup>b</sup>   |                                   | 1            |             |                                  |
| (Chen et al., 2019)   | Alkali     | Destarching, protein removal                         | 5:100                     | 0.16 mol/l  | 80                             | 90 min                 | Ethanol precipitation  | 19.8ª             |                                   | 1.14         | 210         |                                  |
| (Deralia, Maire, Lund,<br>Larsson, & Str, 2021)             | Alkali     | Acid hydrolysis,<br>delignification                  | 1:12                      | 1 M NaOH,   |                                | 16 h                   | Destarching, protein<br>removal, ethanol<br>precipitation 95 % | 13 <sup>a</sup>   | 72                                | 0.77         | 163         | n.d.                             |
| (Guo et al., 2018)  | Alkali     | Destarching, protein removal                         | 1:20                      | 0.26 M NaBH4                                      | 65                             | 3 h                    | Proof-man 20 10  | 6.9 <sup>a</sup>  | 79                                | 0.76         | 491         | 0.7                              |
| (Ruthes et al., 2017)                                       | Alkali     | Destarching, protein removal                         | 1:8                       | 0.5 M NaOH  | 80                             | 16 h                   | Ethanol precipitation, 95%                                     | 27.6 <sup>b</sup> | 73                                | 0.74         | 458         | 0.2                              |
| (Yan et al., 2019)  | Alkali     | Destarching, defatting,<br>water extraction          |                           | 0.625 M sodium<br>hydroxide                       | 85                             | 2.5 h                  | Ethanol precipitation, 100 %                                   | 6.3ª              | 85                                |              |             | 2.9                              |
| (Yilmaz-Turan, Jiménez-<br>Quero, Menzel, et al.,<br>2020)  | Alkali     | Destarching  | 1:8                       | 0.5 M   | 80                             | 16 h                   |  | 31.1 <sup>a</sup> | 73                                | 0.7          | 263         | n.d.                             |
| (Aguedo et al., 2014)                                       | Enzymatic  | Destarching  | 1:11                      | Endo-xylanase<br>from <i>Bacillus</i><br>subtilis | 55                             | 2 h                    | Ultrafiltration, 100 kDa                                       | 4.2 <sup>a</sup>  |                                   | 0.73         | 12.5        |                                  |
| (Zhou et al., 2010)   | Enzymatic  | Destarching, protein removal                         | 1:12                      | 150U xylanase                                     | 60                             | 2 h                    | Ethanol precipitation, 65%                                     | 12.4 <sup>b</sup> |                                   | 0.56         |             | 0.4                              |

<sup>&</sup>lt;sup>a</sup> Total solid yield % of bran dry weight. <sup>b</sup>AX yield % of bran dry weight.

|                  | A/X ratio   | Molar mass of AX   | Ferulic acid content of AX  | AX solubility   | AX water holding capacity   | AX addition level to bread  |
|------------------|---|--|---|---|---|---|
| Dough properties | Low ratio increases<br>dough viscosity (<br>Pavlovich-Abril et al.,<br>2016; M. Wang et al.,<br>2020) and causes uneven<br>mixing (Koegelenberg &<br>Chimphango, 2017); no<br>effect (Kaur et al., 2019). | LMW improves dough processing (Guo et al., 2018).  | Increase in FA improves dough extensibility (P. Wang et al., 2019). | WUAX destroys bubble interface in dough (Xiao et al., 2021).  | Increased water holding increases dough viscosity ( Kaur et al., 2019) and causes inferior baking quality (J. Li et al., 2012). | 1 % causes uneven structure,<br>3 % changes structure<br>completely (Espinosa-<br>Ramírez et al., 2020).  |
| Specific volume  |   | LMW increases volume ( Buksa et al., 2016; P; Wang et al., 2019); no effect (Koegelenberg & Chimphango, 2017).   | Increase in FA decreases volume (Koh & Ng, 2009).                   |   | Increase in water holding increases volume (Buksa et al., 2016).  | 10 % significantly decreases,<br>2 and 5 % have no effect (<br>Zhang et al., 2019); 1–3 %<br>increases, over 6 % decreases<br>(Buksa et al., 2016). |
| Crumb texture    |   | LMW decreases hardness<br>of fresh bread (Buksa<br>et al., 2016; P; Wang<br>et al., 2019), HMW<br>prevents long-term starch<br>retrogradation (P. Wang<br>et al., 2019). |   |   | Increase in water holding decreases hardness (Buksa et al., 2016).  | 10 % significantly increases, 2 and 5 % no effect (Zhang et al., 2019); 1 % decreases hardness (P. Wang et al., 2019).                              |
| Crumb structure  |   | LMW result in good<br>crumb formation (Buksa<br>& Krystyjan, 2019).  |   | WEAX improves surface<br>smoothness (P. Wang et al.,<br>2019); WUAX had no effect (<br>Ma et al., 2018).    |   | 10 % causes coarser structure,<br>2 and 5 % no effect (Zhang<br>et al., 2019); 2 % improves<br>structure (P. Wang et al.,<br>2019).                 |
| Health effects   | Low ratio increases antioxidant activity and decreases availability to human gut bacteria (Chen et al., 2019; Scazzina et al., 2013).   | HMW promotes bowel health (Scazzina et al., 2013), LMW has prebiotic activity and improves glucose tolerance (Bhattacharya et al., 2020; Boll et al., 2016).             | FA increases antioxidant activity (Chen et al., 2019).              | Soluble fibers lowers<br>glycemic response, insoluble<br>promotes bowel health (<br>Scazzina et al., 2013). |   |   |

give higher yields but lower AX extract purity compared with divalent hydroxides. Increasing the concentration of the alkali agent increases the A/X ratio and decreases the ferulic acid content of extracts (Kale, Hamaker, & Campanella, 2013; Pihlajaniemi et al., 2020). Similarly to SWE treatment, increasing the treatment temperature from 25 °C to 85 °C during alkali extraction decreases the molar mass of wheat bran AX, but increases the A/X ratio and extraction yield (Yan et al., 2019).

As AX differs in different bran layers, differences in A/X ratio may indicate that alkali extraction is able to solubilize AX from outer bran layers with higher arabinose content (Pihlajaniemi et al., 2020; Ruthes et al., 2017). Pihlajaniemi et al. (2020) suggested that, based on increased A/X ratio, alkali treatment may be able to improve extraction of the outer pericarp AX from wheat bran. However, reported A/X ratios in products from alkali extraction vary greatly between different publications.

#### 3.2.4. Enzymatic extraction

Enzymatic extraction is an alternative method for AX isolation where wheat bran AX solubility is increased usually by enzymatic degradation of the xylan backbone (Z. Zhang et al., 2014). In enzymatic extraction, endo-β-(1, 4)-xylanases from glycoside hydrolase (GH) families 10 and 11 are often used to hydrolyze  $\beta$ -(1–4)-linkages in the xylose backbone, partially solubilizing WUAX and depolymerizing WEAX (Courtin & Delcour, 2001; Mathew et al., 2017; Santala, Lehtinen, Nordlund, Suortti, & Poutanen, 2011). Xylanases from the GH11 family are characterized by high substrate specificity and high catalytic efficiency, and preferentially cleave unsubstituted areas of the AX backbone chain, whereas xylanases from the GH10 family exhibit broader catalytic versatility and lower substrate specificity and have the ability to hydrolyze xylose linkages closer to the side-chain residues (Bender et al., 2017). AX is more effectively solubilized from destarched wheat bran by GH11 xylanases (41-49% of total AX) than by GH10 xylanases (18-26% of total AX) (Z. Zhang et al., 2014).

Compared with alkaline extraction, enzymatic extraction results in considerably lower extraction yields, possibly caused by endogenous enzyme inhibitors in bran and the partially crystalline structure of lignocellulose (Z. Zhang et al., 2014). Due to lignification and the strong cell wall matrix, the outer layers of bran are resistant to enzymatic treatment, with the aleurone layer being the most accessible for enzymes (Hell et al., 2015; Vangsøe, Sørensen, & Bach Knudsen, 2019). Enzymatic treatment also affects the molecular structure by decreasing the molar mass 10-fold compared with alkali treatment, but preserves ferulic acid and antioxidant functionality well (Ruthes et al., 2017; Zhou et al., 2010). Moreover, enzymatic treatment has been shown to increase AX extraction yield when combined with other methods such as alkali treatment (Beaugrand et al., 2004)

#### 3.3. Purification of arabinoxylan

Alcohol precipitation is a step that can be used to purify AX and increase AX yield from extracts (Liu et al., 2020; Mathew et al., 2017). Ethanol reduces the solubility of polysaccharides and enhances their precipitation, but also influences AX properties (P. Wang et al., 2019). Increasing the ethanol concentration results in AX fractions with increased molar mass, amount of di-substituted xylose, and A/X ratio, but has a negative effect on ferulic acid content (Dervilly-Pinel et al., 2001; Peng, Nie, Li, Huang, & Li, 2019; P; Wang et al., 2019). J. Li and Du (2019) found wheat beer AX solubility to be highest at 50–67% ethanol concentration. Peng et al. (2019) came to the same conclusion, suggesting use of ethanol concentrations of 50% and 65% to maximize the AX molar mass from corn stems. Higher ethanol concentrations in purification might lead to co-aggregation of AX with other polysaccharides and minerals, thus decreasing AX purity (Jie Li & Du, 2019; Mathew et al., 2017).

A purification step is crucial to AX functionality as a bread ingredient, because impurities in AX concentrates or isolates are often

associated with lower bread quality (L. Zhang, van Boven, Mulder, Grandia, Chen, Boom, & Schutyser, 2019). However, materials and solvents make up a major part of the production costs for AX and ethanol purification of wheat bran AX is currently not viable on an industrial scale as high concentrations of ethanol are needed, in equal ratios to AX (Misailidis et al., 2009). One of the most commonly used alternative purification methods for AX is ultrafiltration (Aguedo, Fougnies, Dermience, & Richel, 2014; Jacquemin et al., 2015; Thuvander & Jönsson, 2019). In addition to concentrating AX, ultrafiltration removes small molecules and leaves AX in aqueous solution (Jacquemin et al., 2015). However, ultrafiltration results in lower purity compared with alcohol precipitation and can still leave the wheat bran AX extract dark (Jacquemin et al., 2015). In order to evaluate the industrial feasibility of AX production for ingredient purposes, the level of AX purity that is actually necessary for baking applications needs to be determined.

#### 4. AX in breadmaking

Arabinoxylan from different sources has been widely studied as a potential functional ingredient in baking applications because it affects several important baking factors, including water-holding and binding, starch retrogradation, and rheology (Biliaderis, Izydorczyk, & Rattan, 1995; Liu et al., 2020). The effect on bread quality is generally related to AX properties, but not all differences in bread can be fully explained by differences in AX structure (Skendi et al., 2011; P; Wang et al., 2019). Effect of AX properties and addition level on dough and bread properties is summarized in Table 3. Bread is a complex food system and the effect of AX is further affected by the inclusion level of AX, the quality of wheat flour, the other ingredients used, and the baking process itself (Izydorczyk & Biliaderis, 1992; Kale et al., 2010; L. Zhang et al., 2019). This makes comparisons among different studies challenging, as even minor changes in ingredients and baking process are known to greatly affect the results.

The reported optimal AX addition level varies greatly among different publications, mostly due to the varying degree of AX purification and its molecular characteristics (Biliaderis et al., 1995; Izydorczyk & Biliaderis, 2006) but addition levels above 5% generally start having a clear negative effect on bread quality (L. Zhang et al., 2019). However, L. Zhang et al. (2019) were able to increase wheat bran AX addition level from 5 to 10 % and still obtain comparable quality to control bread, by adjusting the recipe based on dough water absorption. According to Buksa, Nowotna, and Ziobro (2016), the maximum rye AX addition level to rye bread is dependent on rye AX structure and levels of other ingredients. Those authors were able to double the rye AX level from 3% to 6% by changing from highly cross-linked rye AX to hydrolyzed, more soluble rye AX, and by increasing the protein content of the dough. These findings indicate that high-quality bread can be achieved with increased amounts of added AX, if the recipe and baking process are adjusted for fiber addition.

#### 4.1. Water-holding capacity

Wheat bran AX is very efficient in binding water, with a water-holding capacity of about five- to 10-fold higher than protein and starch (M. Wang et al., 2020). In terms of unfractionated wheat bran, water binding by the wheat bran has been suggested to be the most influential factor affecting bread quality (Hemdane et al., 2015). With optimal wheat bran AX addition levels, water absorption by AX can increase dough yield and improve bread quality by increasing loaf volume and moisture content and decreasing crumb hardness (Biliaderis et al., 1995; Buksa et al., 2016; Kaur et al., 2019). According to Kaur et al. (2019), increased water absorption from wheat bran AX improves the viscoelastic properties of dough. In their study on rye AX, Buksa et al. (2016) found fiber addition of 1 to 3 % to improve specific volume of bread. Higher addition levels of AX (6–12 %) did in turn lead to excessive water absorption, decreased viscosity of the dough during baking,

and insufficient gas retention (Buksa et al., 2016). However, adjusting the amount of added water in bread recipe to compensate for increased water absorption from AX seems to facilitate the use higher AX addition levels while maintaining bread quality (L. Zhang et al. 2019). Waterholding capacity depends on the solubility of wheat bran AX, and WUAX has significantly higher water-holding capacity compared with WEAX (L. Zhang et al., 2019). This leads to a greater amount of water in bread fortified with WUAX.

#### 4.2. Dough properties

Dough properties, especially rheological properties, are useful for evaluating baking performance and predicting the quality of bread (Xu, Wang, & Li, 2019). Addition of wheat bran AX interferes with gluten development, prolonging dough development time and reducing stability (Xiao et al., 2021). Wheat bran WUAX is known to affect pore distribution and destroy the bubble interface in dough (Xiao et al., 2021). For AX extracted from maize, even addition of 1% AX to glutenfree bread dough has been observed to cause an uneven dough matrix, while addition of 3% AX changes the dough microstructure completely (Espinosa-Ramírez, Garzon, Serna-Saldivar, & Rosell, 2020). The negative effects of AX on dough properties seem to be related especially to AX solubility as the insoluble AX aggregates have been shown to cause uneven dough mixing and destroy bubble interface in dough (Koegelenberg & Chimphango, 2017; Xiao et al., 2021). While Kaur et al. (2019) concluded that the A/X ratio of wheat bran AX does not have any marked influence on the rheological properties of wheat flour dough, studies by Pavlovich-Abril et al. (2016) and M. Wang et al. (2020) have shown that lower A/X ratio induces aggregation of molecules. Differences in molar mass distribution of wheat bran AX have been suggested to have an impact on dough strength and explain differences between studies (Kaur et al., 2019; Pavlovich-Abril et al., 2016). According to Guo, Yang, and Zhu (2018), low molecular weight wheat bran AX improves the processing properties of dough compared with high molecular weight AX, due to increased interactions between water, starch, and gluten.

#### 4.3. Arabinoxylan interactions with gluten

The formation of a gluten network during dough mixing and hydration is one of the main determinants of bread properties (M. Wang et al., 2020). Wheat bran AX affects dough formation, and hence bread quality, by interfering with gluten network formation both indirectly and directly (Kaur et al., 2019; M. Wang et al., 2020). According to Zhou et al. (2021), the physical mechanisms such as competition for water are as crucial to gluten-AX interactions as the chemical mechanisms linked to AX and gluten structure. In their review on dietary fiber-protein interactions, Zhou et al. (2021) suggested that interaction between soluble AX and gluten happens mostly non-covalently via hydrogen bonding and hydrophobic interactions due to hydroxyl groups in polysaccharides can interact non-covalently with amide groups in gluten proteins. For insoluble fibers, the interactions are dominated by degree of swelling and hydration.

M. Wang et al. (2020) observed that dough extensibility improved with external ferulic acid addition, suggesting that wheat bran AX with low ferulic acid content directly decreases the extensibility of dough due to less cross-linking between gluten and AX. Wheat bran AX has high water-binding capacity, so it also indirectly disrupts gluten network formation by leaving less water for the gluten network development (M. Wang et al., 2020). Increased water absorption by AX leads to water migration from the gluten network to AX, which can result in inferior baking quality (J. Li et al., 2012). Wheat bran AX addition to dough also leads to partial agglomeration and irregular distribution of proteins (Kaur et al., 2019).

Interactions of AX with gluten proteins influence the texture and loaf volume of bread, because a stronger and more elastic gluten network

due to AX and gluten interactions can slow down gas diffusion from dough during baking (Biliaderis et al., 1995; Janssen, Wouters, Meeus, Moldenaers, Vermant, & Delcour, 2020; P; Wang et al., 2019). P. Wang et al. (2019) found that WEAX from wheat bran can reduce heat-induced polymerization of gluten, resulting in larger loaf volume and softer texture in steamed bread. They observed this effect to be stronger for AX with lower molar mass and higher degree of substitution.

#### 4.4. Arabinoxylan interactions with starch

Starch is a crucial component in baked products and its distinct gelatinization and retrogradation behavior have a great impact on bread properties (Hou et al., 2020). In particular, WEAX from both wheat endosperm and wheat bran has been shown to form complexes with soluble starch fractions or proteins present on the surface of starch granules (Rosicka-Kaczmarek, Tkaczyk, Makowski, Komisarczyk, & Nebesny, 2017; P. Wang et al., 2019). WEAX decreases the swelling power and solubility of starch, possibly by inhibiting starch granule swelling with limited leaching of starch (Hou et al., 2020). Both Hou et al. (2020) and P. Wang et al. (2019) have shown that low molar mass AX (~60 kDa) inhibits starch gelatinization more than high molar mass AX (360-500 kDa). This is possibly caused by inhibition of amylose leaching and amylose-lipid complex formation due to stronger interactions between starch and low molar mass AX (Hou et al., 2020). However, limited starch swelling caused by hydrolyzed rye AX with low molar mass has also been observed to result in good bread crumb formation (Buksa & Krystyjan, 2019).

There are also indications that AX interactions with starch could retard starch retrogradation and hence increase bread storability (Biliaderis et al., 1995; Hou et al., 2020; P; Wang et al., 2019). High and low molar mass AX fractions both retard starch retrogradation, but AX with higher molar mass and a higher degree of substitution has been found more to be efficient in preventing long-term retrogradation due to preferential binding to amylopectin (Hou et al., 2020; P; Wang et al., 2019). P. Wang et al. (2019) found that even though low molar mass AX retarded short-term retrogradation by preventing recrystallization of amylose, high molar mass AX had a more significant effect on long-term retrogradation by suppressing recrystallization of amylopectin. Increased molar mass of wheat bran AX causes a starch crystallizationrelated enthalpy reduction, which might be related to interactions between AX and starch or to a decreased amount of available water in the bread system (Liu et al., 2020). However, starch recrystallization, as probed by calorimetry, is greatly influenced by the water contents of the composite starch-gluten-fiber matrix, particularly the water concentration in the starch component which is modulated by the presence of soluble fiber such as AX (Izydorczyk & Biliaderis, 2006).

#### 4.5. Specific volume

Specific volume is an important bread quality parameter that is affected by crumb structure, moisture content, and dough gas retention (L. Zhang et al., 2019). The effect of AX on loaf volume seems to be heavily dependent on fiber amount and AX properties. Several studies have demonstrated that AX from wheat bran can increase or maintain the specific volume of different types of bread with optimized AX addition levels (Koegelenberg & Chimphango, 2017; Ma, Lee, & Baik, 2018; P. Wang et al., 2019; L. Zhang et al., 2019). However, the optimal addition level for maximizing specific volume varies greatly between different studies. L. Zhang et al. (2019) observed an increase in volume at all wheat bran AX addition levels up to 5%, while P. Wang et al. (2019) found increased loaf volume of steamed bread only with 1% wheat bran WEAX addition and observed the effect to be higher with AX purified with higher ethanol concentration. Koegelenberg and Chimphango (2017) found wheat bread to maintain its volume compared with a control with an addition level of 0.8% wheat bran AX with 2.5% flour removal. Higher wheat bran AX addition levels are reported to

compromise bread quality and addition levels of 10–18% have a significant negative effect on bread volume (Damen et al., 2012; L. Zhang et al., 2019).

The initial increase in specific volume with wheat bran AX addition has been attributed to increased strength and elasticity of the gluten-starch network. AX has also been suggested to interact with proteins adsorbed at air-water interfaces and increase the stability of dough gas bubbles, improving gas retention (Janssen, Wouters, Chatzigiannakis, Delcour, & Vermant, 2021). When the AX amount increases further, AX disturbs the formation of a stable gluten network by increasing viscosity, binding water, and lowering gas retention (Damen et al., 2012). Incorporation of ferulic acid has been observed to reduce specific volume, which indicates that feruloylated AX might have a similar effect (Koh & Ng, 2009). Ferulic acid is known to decrease AX solubility, which might explain the observed reduction in specific volume (Schooneveld-Bergmans et al., 1999).

#### 4.6. Crumb structure

The crumb structure of bread is affected by water retention and gas cell formation, and can therefore be influenced by AX addition. High addition levels of wheat bran AX (10%) increase porosity and give a more irregular cell structure, probably due to high water retention by AX, interfering with gluten network formation and causing a weaker gluten network (L. Zhang et al., 2019). Lower addition levels of 2% and below can improve crumb structure by increasing porosity and result in more homogenous cell structure, contributing to higher volume and softer texture (P. Wang et al., 2019). WEAX has been shown to improve surface smoothness, probably due to the influence of wheat bran AX on the gluten network, but WUAX has not been shown to have a significant effect on crumb structure (Ma et al., 2018; P; Wang et al., 2019).

#### 4.7. Texture

Crumb hardness, i.e., the force required to compress crumb, has been observed in many studies to be affected by wheat bran AX addition (Pihlajaniemi et al., 2020; P. Wang et al., 2019; L. Zhang et al., 2019). Changes in hardness are most likely caused by interactions between AX and the gluten network. Wheat bran AX can also increase the amount of water in bread, which softens the composite structure (L. Zhang et al., 2019). Similarly to specific volume, the effect of AX addition on crumb structure seems to be dependent on the AX addition level, which might to some extent explain differences in results from different studies. P. Wang et al. (2019) were able to reduce the firmness of Chinese steamed bread by 44% with addition of 1% WEAX from wheat bran. They also observed bread firmness to be influenced by the ethanol concentration used during AX isolation, with AX purified using 60% ethanol producing the softest bread. Pihlajaniemi et al. (2020) found that addition of wheat bran AX syrup resulted in a significantly softer bread. L. Zhang et al. (2019) found that wheat bran AX supplementation up to 5% did not have a negative impact on texture, but that bread with 10% AX addition was significantly harder. A study by Espinosa-Ramírez et al. (2020) on use of maize bran AX in breadmaking of pan bread found no significant effects on hardness, but a negative effect on springiness, cohesiveness, and resilience compared with control bread. Bread with high molar mass AX from wheat endosperm has also been found to be softer during a 7day storage period compared with breads with low molar mass AX (Biliaderis et al., 1995).

#### 4.8. Nutritional quality

The main nutritional advantage of adding wheat bran to bread is the increased dietary fiber content in the bread. Wheat bread fortified with AX from wheat endosperm has been shown to have as beneficial an effect on glycemic control in rats as whole grain rye bread, indicating that AX-fortified products might be able to achieve nutritional quality

comparable to that of whole grain products (Hartvigsen, Jeppesen, Lærke, Njabe, Knudsen, & Hermansen, 2013). The inclusion threshold for labeling a product as having "high fiber content" is over 6 g of dietary fiber per 100 g of product. L. Zhang et al. (2019) achieved this level by adding 10% wheat bran AX to wheat bread, but they also needed to adjust the bread recipe and baking process to reach acceptable bread quality attributes with this high AX addition rate.

In vitro digestion experiments of both AX-starch mixture and AX-fortified bread have shown that the higher molar mass of wheat bran AX gives a better inhibitory effect against starch digestibility, leading to a higher percentage of resistant starch (Liu et al., 2020). This indicates that AX with different properties could be used to change the glycemic index value of fortified bread. Ferulic acid linked to AX also displays antioxidant activities in food, hence offering additional health benefits besides increasing the fiber content (Koegelenberg & Chimphango, 2017).

While processing can be used to alter AX functionality, some health effects can be lost when AX is added to bread (Arcila, Weier, & Rose, 2015). The baking process itself causes changes in AX, as mixing, fermentation, baking, and xylanases present in flour promote aggregation of WEAX and solubilization of WUAX (Nishitsuji, Whitney, Nakamura, Hayakawa, & Simsek, 2020). This might affect, besides the quality attributes of the baked product, the nutritional functionality of AX and is an issue that still needs to be investigated.

#### 4.9. Sensory quality

Sensory quality plays a key role in consumer acceptance of bread and fiber addition has been observed in many studies to significantly affect sensory quality. Addition of wheat bran AX has been shown to improve both the softness and texture of bread, and hence increase overall acceptability with optimal fiber addition levels (P. Wang et al., 2019). However, while AX can be used to increase the dietary fiber content and improve the texture of bread, fiber incorporation into bread is also known to have negative effects on other sensory attributes of bread (Grigor, Brennan, Hutchings, & Rowlands, 2016; Hemdane et al., 2015). In particular, the high addition rates needed to label products "high in fiber" can cause a significant decrease in the overall acceptability of bread (L. Zhang et al., 2019). Consumers may be interested in health-promoting bread, but the bread still needs to maintain an acceptable quality even with added health benefits.

Pihlajaniemi et al. (2020) found that the effect of adding high AX wheat bran syrup on wheat bread sensory quality was dependent on the syrup extraction method, with water-extracted syrup resulting in comparable quality to control bread but alkali-extracted syrup causing off-flavors. Pure AX in itself is colorless, but the purity of fiber fractions is rarely 100% and they usually affect color, creating a darker bread (L. Zhang et al., 2019). As the molecular characteristics and the physicochemical properties of AX as well as the processing conditions can modify the AX functionality in baking applications, selecting AX with suitable physicochemical properties might help to produce bread with better quality (Foschia, Peressini, Sensidoni, & Brennan, 2013).

#### 5. Conclusions

Preprocessing treatment, extraction method, and processing parameters affect AX properties and functionality, indicating that the AX extraction process can be adjusted to produce AX with targeted functionality for baking applications. However, differences in the raw materials and methods used in different studies make it difficult to draw firm conclusions, and more research is needed on the complex interactions between processing, molecular structure, and functionality of this polysaccharide from the cell walls of wheat bran.

Wheat bran AX has potential as a functional bakery ingredient that can be used to improve bread volume, crumb structure, texture, and nutritional value, but this functionality is dependent on AX properties

especially in terms of solubility. Most positive results were obtained using WEAX or more soluble AX with lower molar mass and higher arabinose content, indicating that these properties seem to be in a key role defining the effect of AX addition on bread quality. Tailoring the extraction process to produce more soluble AX with suitable physicochemical properties might enable incorporation of fiber in larger amounts while maintaining acceptable bread quality. Nevertheless, less soluble high molar mass AX might have additional health benefits compared to soluble AX and for example improve prevention of starch retrogradation during storage. Better understanding of required fiber functionality is needed to determine the optimal AX properties for bakery applications.

The AX addition level, interactions between AX and other components, and the baking process also affect the quality of AX-fortified bread. Even though most reviewed studies found low addition levels of AX to improve several bread properties, high fiber addition levels needed for reaching the current requirements for health claims seem to result in a bread with compromised quality. Optimizing baking process and bread recipe, especially in terms of amount of added water and protein, could improve bread quality and help increasing the amount of added fiber in fortified bread even further. Overall, this review of the literature indicated that use of AX with tailored properties together with properly optimized baking process could help increasing the amount of added fiber in bread while maintaining or even improving bread quality.

#### **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.

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