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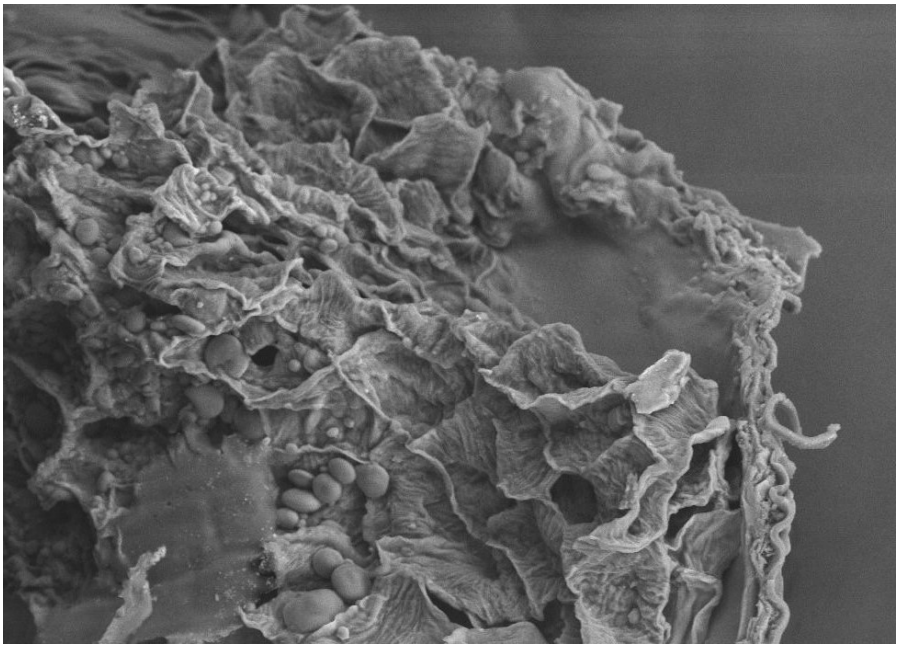
Modification of wheat bran arabinoxylan extracts for functional properties in breadmaking

Solja Pietiäinen



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Abstract

Wheat bran is a milling side stream rich in dietary fiber and bioactive compounds that is predominantly used for animal feed. Increasing the food use of wheat bran as a fiber ingredient could improve the nutritional quality of baked products and sustainability of cereal processing.

In this thesis, the potential of modified wheat bran arabinoxylan extracts as fiber ingredients was explored by investigating the relationship between extract properties and functionality in breadmaking. Ferulic acid content and molar mass of extracts were reduced, and the effect of these modified extracts on dough and bread quality was analyzed. An extraction process combining extreme temperatures with a decolorization process was tested to increase extraction yield while improving extract properties.

Reduction of arabinoxylan molar mass improved both dough and bread quality in terms of dough extensibility and specific volume of bread. This effect was connected to viscosity and water-holding capacity of the extracts. Hydrolyzed and feruloylated extracts produced bread and dough quality closest to control, supporting the potential for feruloylation and molar mass reduction to be used in combination to enhance bread quality and shelf life. Yields from subcritical water extraction comparable to alkali extraction were achieved through increased treatment temperature while improving the color of the extracts through ultrafiltration and active charcoal treatment.

The findings of this thesis demonstrate the potential of modified wheat bran arabinoxylan extracts as functional fiber ingredients, and therefore help to facilitate the use of wheat bran in production of health-promoting ingredients for bread.

Keywords: wheat bran; arabinoxylan; breadmaking; feruloylation; fiber

Modifiering av vetekli-arabinoxylanextrakt för funktionella egenskaper i bröd

Sammanfattning

Vetekli är en sidoström från malning rik på kostfiber och bioaktiva föreningar som mest används för djurfoder. Att öka användningen av vetekli som fiberingrediens skulle kunna förbättra näringskvaliteten hos bakade produkter och hållbarheten i spannmålsvärdekedjan.

Denna avhandling undersökte potentialen hos modifierade vetekli-arabinoxylanextrakt vid brödtillverkning genom att undersöka sambandet mellan bearbetningen av vetekli, och egenskaper och brödtillverkningsfunktionalitet av modifierade vetekli-arabinoxylanextrakt. Ferulinsyrainnehållet och molmassan av extrakt reducerades, och effekten av dessa modifierade extrakt på deg- och brödkvalitet analyserades. En extraktionsprocess som kombinerar extrema temperaturer med en avfärgningsprocess testades för att öka extraktionsutbytet och förbättra extraktegenskaperna.

Minskning av molmassan förbättrade både deg- och brödkvaliteten, och denna effekt var kopplad till extraktegenskaper i form av viskositet och vattenhållande förmåga. Hydrolyserade och feruloylerade extrakt gav bröd- och degkvaliteten närmast kontroll, vilket stöder potentialen för feruloylering och molarmassareduktion som kan användas i kombination för att förbättra brödkvaliteten och hållbarheten. Utbyten från subkritisk vattenextraktion jämförbar med alkaliextraktion uppnåddes med ökad behandlingsintensitet samtidigt som färgen på extrakten förbättrades genom ultrafiltrering och behandling med aktivt kol.

Resultaten av denna avhandling visar potentialen hos modifierade vetekliarabinoxylanextrakt som funktionella fiberingredienser, och underlättar därför användningen av vetekli vid produktion av hälsofrämjande ingredienser till bröd.

Nyckelord: vetekli; arabinoxylan; bakning; ferylsyra; fiber

Dedication

To Vellamo,
my dearest little bread lover

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Pietiäinen, S., Moldin, A., Ström, A., Malmberg, C., & Langton, M. (2022). Effect of physicochemical properties, pre-processing, and extraction on the functionality of wheat bran arabinoxylans in breadmaking – A review. *Food Chemistry*. 383, 132584. https://doi.org/10.1016/j.foodchem.2022.132584

II. Pietiäinen, S., Jimenez-Quero, A., Moldin, A., Strom, A., Katina, K., and Langton, M. (2024). Feruloylation and hydrolysis of arabinoxylan extracted from wheat bran: Effect on bread quality and shelf-life. *Journal of Cereal Science.* 117, 103920. https://doi.org/10.1016/j.jcs.2024.103920

III. Pietiäinen S, Lee Y, Jimenez-Quero A, Katina K, Maina NH, Hansson H, Moldin A, Langton M. (2024). Feruloylation and Hydrolysis of Arabinoxylan Extracted from Wheat Bran: Effect on Dough Rheology and Microstructure. *Foods*. 13, 2309. https://doi.org/10.3390/foods13152309

IV. Pietiäinen S, Hansson H, Jimenez-Quero A, Ström. A, Katina K, Moldin A, Langton M. Improving color profile of wheat bran arabinoxylan extracts after subcritical water extraction with ultrafiltration and active charcoal treatment. Manuscript.

All published papers are published open access.

The contribution of Solja Pietiäinen to the papers included in this thesis was as follows:

I. Designed the literature study together with co-authors, wrote the original manuscript draft and had the main responsibility of the manuscript revision.

II. Designed the study together with the co-authors, conducted modification of arabinoxylan extracts, test baking, all measurements of bread quality, and measurements on extract composition and properties (excluding monosaccharide composition, ferulic acid content and molar mass distribution), analyzed the data, wrote the original manuscript draft and had the main responsibility of the manuscript revision.

III. Designed the study together with the co-authors, conducted modification of arabinoxylan extracts, measurements of extract composition and properties (excluding molar mass distribution), and all measurement of dough quality, analyzed the data, wrote the original manuscript draft and had the main responsibility of the manuscript revision.

IV. Designed the study together with the co-authors, conducted extractions and measurements of extract composition and properties (excluding molar mass distribution), analyzed the data and wrote the original manuscript.

The following paper was published during the timeframe of the doctoral project but is not part of this thesis:

V. Zhang D, Rudjito RC, Pietiäinen S, Chang SC, Idström A, Evenäs L, Vilaplana F, Jiménez-Quero A. (2023). Arabinoxylan supplemented bread: From extraction of fibers to effect of baking, digestion, and fermentation. *Food Chemistry*. 413, 135660. https://doi.org/10.1016/j.foodchem.2023.135660

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Abbreviations

AACC	American Association of Cereal Chemists
AE	Alkali extraction
ANOVA	Analysis of Variance
AX	Arabinoxylan
A/X ratio	Arabinose to Xylose Ratio
Đ	Dispersity index
DDT	Dough Development Time
DST	Dough Stability Time
FA	Ferulic Acid
FAX	Feruloylated Arabinoxylan
G’	Storage modulus
G’’	Loss modulus
H-FAX	Hydrolyzed and feruloylated arabinoxylan
H-UAX	Hydrolyzed and low feruloylated arabinoxylan
HPAEC-PAD	High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection
HPLC	High-Performance Liquid Chromatography
M_n	Number average molar mass
M_w	Weight average molar mass
Px	Pixel
PLS	Partial least squares
SEC	Size exclusion chromatography
SWE	Subcritical Water Extraction
TPA	Texture Profile Analysis
UAX	Low-feruloylated Arabinoxylan
UPLC-PDA	Ultra Performance Liquid Chromatography with Photodiode Array Detection
WEAX	Water Extractable Arabinoxylan
WHC	Water Holding Capacity
WUAX	Water Unextractable Arabinoxylan

1. Introduction

Wheat (*T. aestivum*) is one of the most cultivated crops worldwide with a global annual total harvest of around 770 million tons (FAO, 2019). A large part of this wheat is milled through a process where the endosperm rich in starch and gluten is separated from the other grain parts. The purest refined wheat flour is highly valued due to its superior sensory and technological properties in food applications. Wheat bran, the outer layers of the wheat grain separated from the endosperm in milling, makes up around 15 % of the wheat grain weight (Prückler et al., 2014). This enormous side stream is predominantly used for animal feed, despite being rich in many nutrients such as dietary fiber, minerals, and bioactive compounds (Katileviciute et al., 2019). Thus, it could be used as a health-promoting ingredient to increase the nutritional quality of food products.

Globally, all 195 countries evaluated in the Global Burden of Disease study (GBD, 2017 Diet Collaborators, 2019) failed to reach the recommended daily fiber intake. In Sweden, only 30 % of people reach the recommended fiber intake (Livsmedelsverket, 2010). Therefore, there is a clear need to find new and innovative ways to incorporate more fiber into our diets to both improve public health and prevent diet-related diseases. In a world with increasing uncertainty regarding food security due to climate change, political conflicts, and growing population, using the crops we produce in the most efficient way possible is becoming increasingly important. Utilizing wheat bran for food ingredients would increase the efficiency and sustainability of cereal processing by directing more edible material directly to human consumption instead of animal feed.

Bread is a staple food in most parts of the world and therefore increasing the use of wheat bran in bakery applications could improve both the nutritional quality of bakery products and sustainability of cereal processing.

However, wheat bran is known to negatively impact bread quality in terms of volume, texture, and sensory properties, which is why it is separated from the endosperm in the first place (Khalid et al., 2017). During recent decades, wheat bran and its extracts have been extensively studied in breadmaking, and the negative effect of wheat bran addition has been closely related to the fiber in bran (Khalid et al., 2017). Arabinoxylan is the main fiber component in wheat bran, and understanding the role of arabinoxylan properties and their modifications in bread applications could therefore help us increase the use of wheat bran in baking.

The aim of this work was to determine the relationship between the properties of modified wheat bran arabinoxylan extracts and their functionality in breadmaking. This work was expected to provide new insights on how the processing of wheat bran can be used to improve the breadmaking properties of wheat bran fiber extracts to facilitate the use of wheat bran in the production of functional bread ingredients.

2. Wheat bran arabinoxylan in breadmaking

This chapter will provide an overview of wheat bran arabinoxylans and their functionality in breadmaking by discussing the findings from Paper I combined with findings from additional studies relevant to work in Papers II-IV. Based on the literature review in Paper I, there is an extensive number of studies on modified wheat bran extracts in breadmaking from the past decades, but comparing results from these studies is often challenging due to differences in raw materials, extraction methods, and baking process. This overview is framed to focus on wheat bran arabinoxylan and modified wheat bran arabinoxylan extracts and their functionality in breadmaking. In terms of arabinoxylan extraction, only extraction methods relevant to the papers included in this thesis are discussed.

2.1 Wheat bran arabinoxylan

2.1.1 Wheat bran arabinoxylan structure

Wheat bran contains around 30 % of arabinoxylan, which is a structural component found in bran cell walls (Maes & Delcour, 2001). Arabinoxylan is composed of a β-(1→4)-linked β-D-xylopyranose backbone substituted by α-D-arabinofuranosyl units (Figure 1), and xylose units can be either unsubstituted, mono-substituted in C2, mono-substituted in C3, or di-substituted in both C2 and C3 positions (Darvill et al., 1980). Although the main part of wheat bran comes from the pericarp tissue, wheat bran is a composite containing other grain tissues, including aleurone layer, epidermis, and testa (Antoine et al., 2003; Ordaz-Ortiz & Saulnier, 2005). As the structure of arabinoxylan varies between these tissues, the milling

process and quantity of these different grain parts affect the structure of arabinoxylan found in wheat bran (Antoine et al., 2003).

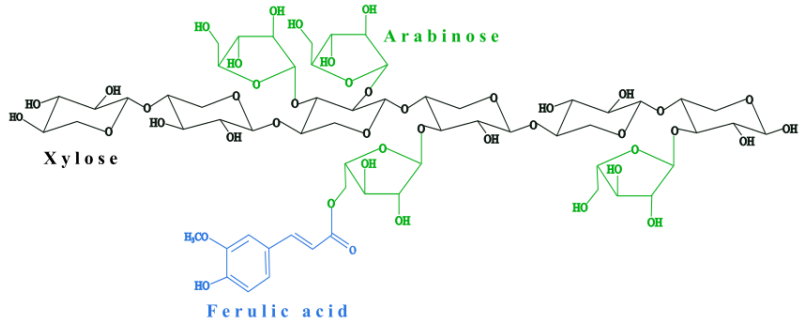

Figure 1. Schematic diagram of the structure of arabinoxylan, showing the xylose backbone (black), arabinose substitutions (green), and ferulic acid (blue) (Paper I).

The arabinose substitution of xylose backbone plays an important role in the physicochemical properties of arabinoxylan as the distribution of arabinose side chains determines arabinoxylan conformation and affect the arabinoxylan properties such as solubility, thermal degradation, and interactions with other molecules (Pavlovich-Abril et al., 2016, Yan et al., 2019). The substitution pattern of the xylose backbone of arabinoxylan varies, and arabinoxylan chains can be divided into both highly and less branched regions (Courtin & Delcour 2002; Heikkinen et al., 2013). Arabinose to xylose (A/X) ratio varies in different bran layers, with the ratio increasing from 0.31 in the inner aleurone layer to 1.0-1.1 towards the outer bran layers (Antoine et al., 2004; Zhang et al., 2014).

In wheat bran, the arabinose side chain can be further esterified with ferulic acid (FA) which has antioxidant, anti-inflammatory, antiviral, antiallergic, antimicrobial, antithrombotic, and anticarcinogenic activity (Kumar & Pruthi, 2014). Therefore, feruloylation could offer additional health benefits from an arabinoxylan addition besides increasing the fiber content (Koegelenberg & Chimphango, 2017; Pihlajaniemi et al., 2020). FA has been suggested to cross-link polysaccharide chains via peroxidase-meditated oxidative coupling, forming ferulate dimers, trimers, and even tetramers (Li et al., 2020; Mnich et al., 2020). FA can also form covalent ether-linkages with lignin and there are indications that it can even link arabinoxylan to proteins by forming a dehydroferulic acid-tyrosine cross-

link (Piber & Koehler, 2005). FA can be removed from arabinoxylan with saponification, wherein aqueous alkali cleaves the ester-linkages between FA and arabinoxylan (Juhnevica-Radenkova et al., 2021).

2.2 Arabinoxylan properties

2.2.1 Extractability

Interactions between arabinoxylan and different cell wall components form a robust and almost non-wettable, strong phenolic copolymer, leading to around 95 % of wheat bran arabinoxylan being water unextractable (Escarnot et al., 2011). Water unextractable arabinoxylan (WUAX) has been reported to have a higher molar mass, FA content, and A/X ratio compared to water extractable arabinoxylan (WEAX) (Maes & Delcour, 2002). This leads to more covalent and non-covalent interactions between WUAX and other cell wall components, tightly binding WUAX into the cell wall matrix (Zhou et al., 2010). WEAX is located on the cell surface and lacks these interactions, making it more easily extractable (Escarnot et al., 2011).

Due to the poor extractability of most wheat bran arabinoxylan, several processes have been tested and developed to increase the arabinoxylan extractability from wheat bran. Based on findings from Paper I, subcritical water extraction (SWE) offers a promising alternative to alkaline extraction (AE) for extracting feruloylated arabinoxylan with potential bioactivity. A detailed comparison on the effects of different extraction methods and process parameters on arabinoxylan yields and properties can be found in Paper I.

2.2.2 Molar mass

Molar mass is a crucial property of wheat bran arabinoxylan that affects its physicochemical properties including solubility and viscosity (Hou et al., 2020; Kale et al., 2010). Molar mass of arabinoxylan varies between different grain parts and can be influenced via processing and extraction (Wang et al., 2014). Based on a comparison of different extraction methods in Paper I, WEAX typically has a lower molar mass compared to WUAX. Enzymatic hydrolysis can be used to produce lower molar mass arabinoxylan or even arabinoxylan oligosaccharides with specific functionality (Paper I).

2.2.3 Solubility & Viscosity

Solubility and viscosity are important properties of wheat bran arabinoxylan because they influence arabinoxylan functionality in breadmaking (Koegelenberg & Chimphango, 2017; Xiao et al., 2021). The solubility is strongly affected by arabinose side groups, with a low degree of substitution leading to aggregation and lower solubility as more flexible unsubstituted regions align with each other more easily (Izydorczyk & Biliaderis, 1995; Li et al., 2017; Pitkänen et al., 2009). This interaction between polymer chains can affect viscosity and cause precipitation, which can in turn cause issues in dough mixing and gluten development (Heikkinen et al., 2013; Koegelenberg & Chimphango, 2017; Xiao et al., 2021). Covalent cross-linking by FA further decreases solubility even in highly branched arabinoxylan as FA-mediated cross-linking increases these chain-chain interactions (Li et al., 2017; Saulnier et al., 2007; Xiao et al., 2022). Higher molar mass has been linked to lower solubility and higher viscosity, as larger molecules have more intermolecular interactions leading to an increased resistance to flow (Buksa et al., 2016).

2.2.4 Water-holding capacity

Water-holding capacity, often expressed as the amount of water retained by fiber during centrifugation, is crucial to the functionality of arabinoxylan in bread as it determines the distribution of water between different bread components (Saulnier et al., 2007). The relatively high water-holding capacity of arabinoxylan has been associated with the degree of substitution and capacity to form crosslinks via FA, as the entanglement of arabinoxylan chains increases the water-holding capacity (Izydorczyk & Biliaderis, 1992).

2.2.5 Health effects

Arabinoxylan is the main dietary fiber component in wheat and therefore one of the main contributors regarding the health benefits connected to the consumption of wheat bran. When used in food products, wheat bran arabinoxylan can be used to reach nutritional claims related to high fiber content in food products, and wheat bran fiber has a health claim on reduction in intestinal transit time and for an increase in faecal bulk (EFSA, 2019).

In addition to these allowed claims, arabinoxylan has shown potential in many studies to affect glucose tolerance, insulin response, cholesterol metabolism, oxidative stress and prebiotic activity (Broekaert et al., 2011;

Chen et al., 2019; Fadel et al., 2018; Scazzina et al., 2013; Zhang et al., 2014). The health effects of arabinoxylan have been linked to its structure and properties, especially in terms of molar mass, degree of substitution, and feruloylation. Lower molar mass arabinoxylan has been shown to have a higher prebiotic potential and the potential to improve glucose tolerance, while higher molar mass with higher viscosity has been suggested to be linked to cholesterol lowering ability (Bhattacharya et al., 2020; Boll et al., 2016).

FA exhibits activity against oxidation and inflammatory processes, and meditates prebiotic responses of arabinoxylan (Zhang et al., 2021). Feruloylated wheat bran arabinoxylan has been shown to have an antioxidant capacity even after baking and *in vitro* fermentation, thus incorporating feruloylated arabinoxylan could offer additional health benefits in bread besides increasing the fiber content (Koegelenberg & Chimphango, 2017; Snelders et al., 2014; Zhang et al., 2021; Zhang et al. 2023). However, there are currently no allowed health claims for feruloylated wheat bran arabinoxylan in the European Union.

2.3 Wheat bran arabinoxylan in breadmaking

Arabinoxylan has been extensively researched as a functional ingredient in baking due to its impact on several important baking factors, including gluten development, water-holding and binding, starch retrogradation, and rheology (Paper I). The effects of arabinoxylan properties and concentration on dough and bread properties from Paper I are summarized in Table 1. Based on Paper I, the observed positive effects were mostly obtained using WEAX or more soluble arabinoxylan with a low molar mass and high arabinose content. This suggests that arabinoxylan properties are crucial to determine the effect of arabinoxylan addition to bread quality. Since wheat bran predominantly contains WUAX with poor functionality in baking, it is important to understand how structure and properties are connected to arabinoxylan functionality to increase the usability of WUAX in baking applications.

Table 1. *Effect of arabinoxylan (AX) structure and properties (A/X ratio, molar mass (HMM, high molar mass; LMM, low molar mass), ferulic acid (FA) content of AX, AX solubility, AX water-holding capacity (WHC) and AX addition level on dough and bread properties (dough properties, specific volume and crumb structure, crumb texture, and health effects) (Adapted form Paper I)*

	A/X ratio	Molar mass of AX	FA content of AX	AX solubility	AX water holding capacity	AX addition level to bread
Dough properties	Low ratio increases dough viscosity (Pavlovich-Abril et al., 2016; Wang et al., 2020) and causes uneven mixing (Koegelenberg & Chimphango, 2017); no effect (Kaur et al., 2019).	LMM improves dough processing (Guo et al., 2018).	Increase in FA improves dough extensibility (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 (Li et al., 2012).	1 % causes uneven structure, 3 % changes structure completely (Espinosa-Ramírez et al., 2020).
Volume and structure		LMM increases volume (Buksa et al., 2016; P. Wang et al., 2019) and results in good crumb formation (Buksa & Krystyjan, 2019); no effect (Koegelenberg & Chimphango, 2017).	Increase in FA decreases volume (Koh & Ng, 2009).	WEAX improves surface smoothness (P. Wang et al., 2019); WUAX had no effect (Ma et al., 2018).	Increase in water-holding increases volume (Buksa et al., 2016).	10 % significantly decreases volume and causes coarser structure, 2 and 5 % have no effect (Zhang et al., 2019); 2 % improves structure (Wang et al., 2019); 1-3 % increases, over 6 % decreases (Buksa et al., 2016).
Texture		LMM decreases hardness of fresh bread (Buksa et al., 2016; Wang et al., 2019), HMM prevents long-term starch retrogradation (Wang 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 (Wang et al., 2019).
Health effects	Low ratio increases antioxidant activity and decreases availability to gut bacteria (Chen et al., 2019; Scazzina et al., 2013).	HMM promotes bowel health (Scazzina et al., 2013), LMM 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 lower glycemic response, insoluble promotes bowel health (Scazzina et al., 2013).		

Although the effect of arabinoxylan addition on bread quality is linked to arabinoxylan properties, the variation in bread with arabinoxylan incorporation cannot be fully explained with differences in arabinoxylan structure (Skendi et al., 2011; Wang et al., 2019). Bread is a complex system, and the impact of arabinoxylan is influenced by the inclusion level, the quality of wheat flour, other ingredients, and the baking process (Paper I). These factors render results highly system specific and comparisons between different studies complicated, as even minor changes in ingredients and baking process can significantly affect outcomes.

2.3.1 Arabinoxylan interactions with gluten

Gluten network formation during dough mixing and hydration is a key determinant of bread properties, and arabinoxylan both directly and indirectly interferes with gluten network development via chemical and physical mechanisms (Kaur et al., 2019; Wang et al., 2020). The chemical mechanisms, including non-covalent interactions such as hydrogen bonding and hydrophobic interactions, occurs between hydroxyl groups in arabinoxylan and gluten's amide groups, as suggested by Zhou et al. (2021) in their review about fiber-protein interactions. This cross-linking between gluten and arabinoxylan can increase the extensibility of dough, and resultingly strengthen the gluten network (Wang et al., 2020). Arabinoxylan's ability to have chemical interactions with gluten is directly linked to its structure, and arabinoxylan with a low FA content and low level of substitution has been shown to decrease dough strength, likely due to less chemical interactions between arabinoxylan and gluten (Wang et al., 2020).

The physical mechanisms, such as competition for water, are particularly important for WUAX with a high water-holding capacity. The high water-holding capacity of wheat bran arabinoxylan causes water migration from the gluten network to arabinoxylan and consequently less available water for gluten development, leading to disruption of the gluten network formation and a lowered baking quality (Wang et al., 2020; Li et al., 2012). Wheat bran arabinoxylan also causes partial agglomeration and irregular distribution of gluten proteins (Kaur et al., 2019; Frederix et al., 2004). Incorporation of arabinoxylan with FA increases the amount of free water within a dough system compared to arabinoxylan without FA (Wang et al. 2020). Liu et al. (2023) suggested FA moiety in arabinoxylan to improve the viscoelasticity of gluten due to moderate polymerization of

gluten and cross-linking between gluten and arabinoxylan. This indicates that feruloylation of arabinoxylan could also potentially improve dough network formation during baking.

2.3.2 Arabinoxylan interactions with starch

Starch is another fundamental dough component, and its unique gelatinization and retrogradation behavior significantly impacts bread properties (Hou et al., 2020). Water-soluble arabinoxylan has been suggested to form complexes with soluble starch extracts or proteins on starch granule surfaces (Rosicka-Kaczmarek et al., 2017; Wang et al., 2019). Arabinoxylan can also decrease the swelling power and solubility of starch, likely by inhibiting granule swelling and starch leaching (Hou et al., 2020). Low molar mass arabinoxylan has more interactions with starch compared to high molar mass arabinoxylan, which has been suggested to lead to more inhibition of amylose leaching and amylose–lipid complex formation and therefore the prevention of starch gelatinization (Hou et al., 2020; Wang et al., 2019). Although starch swelling is an important part of dough formation, limited starch swelling by low molar mass arabinoxylan has also been suggested to lead to good bread crumb formation (Buksa & Krystyjan, 2019).

2.3.3 Dough rheology

The mechanical properties of dough, particularly dough rheology, govern the quality of bread and therefore provide valuable insights when predicting bread quality (Osella et al., 2008; Xu et al., 2019). As discussed earlier, arabinoxylan can have high viscosity and water-holding capacity, which affects the formation of the gluten network and starch swelling. Due to these factors, arabinoxylan addition has a large impact on dough properties, and arabinoxylan incorporation has been shown to cause uneven dough matrix, affect bubble interface in dough, and cause changes in dough microstructure (Espinosa-Ramírez et al., 2020, Frederix et al., 2004; Koegelenberg & Chimphango, 2017; Xiao et al., 2021). Arabinoxylan addition increases dough stiffness and promotes the formation of a more compact protein network in dough, which has been suggested to be caused by the high water-holding capacity of arabinoxylan initiating immobilization of water during dough resting (Guo et al., 2018; Hemdane et al., 2017; Li et al., 2012; Saeed et al., 2016). Competition for water with gluten also prolongs dough

development time and reduces dough stability, leading to weaker doughs (Biliaderis et al., 1995; Wang et al. 2020; Xiao et al., 2021).

Arabinoxylan has been shown to increase the elastic and viscous moduli of bread dough and increase dough firmness (Li et al., 2024; Molina et al., 2021; Wang et al., 2020). While Pavlovich et al. (2016) observed lower A/X ratios to increase dough viscosity and induce aggregation, Kaur et al. (2019) concluded that the A/X ratio did not influence dough rheology. Reduction of molar mass by enzymatic hydrolysis has been shown to decrease dough viscosity, indicating that the effect of arabinoxylan addition to dough rheology can be influenced with a reduction of molar mass (Meeus et al., 2021). Further, Snelders et al. (2014) showed that high FA content can increase dough extensibility and decrease resistance to extension. Addition of free FA has also been reported to improve the rheological properties of gluten with incorporated WEAX due to its competition for the interaction sites with gluten (Wang et al., 2002). However, Koh & Ng (2009) observed that the addition of free FA can weaken gluten strength due to reduced gluten-gluten interactions. These conflicting results indicate that the effect of FA could be system specific and related to both the amount of added FA and the baking process.

2.3.4 Dough microstructure

As arabinoxylan influences the viscoelastic properties of dough and interferes with gluten formation, arabinoxylan addition also affects the microstructure of dough. Arabinoxylan has been shown to form a fibrous network that is dispersed between the gluten strands in dough, leading to a weaker gluten matrix (Sun et al., 2022; Zhang et al., 2014). This disruption results in less compact gluten structure affecting both dough elasticity and extensibility. The high water-holding ability of arabinoxylan also leads to localized hydration zones within the dough, which prevents full gluten formation (Sun et al., 2022). Enzymatic hydrolysis of arabinoxylan has been shown to improve the gluten network structure by reducing disruptions of gluten network and feruloylation by increasing cross-linking of gluten (Sun et al., 2022).

2.3.5 Bread volume and structure

Volume is one of the most industrially important bread quality parameters related to the ability of the bread matrix to retain fermentation gas (Courtin

& Delcour, 2002; Zhang et al., 2019). Bread volume is directly linked to crumb structure, and they are both influenced by water retention and gas cell formation. The effect of arabinoxylan addition to bread volume and structure depends on arabinoxylan structure, properties, and addition level (Paper I). Soluble WEAX has been reported to increase the viscosity of aqueous dough phase, strengthening gluten-starch networks, and improving bread volume (Adams et al., 2017; Courtin & Delcour, 2002). Arabinoxylan 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 et al., 2021). Dough with incorporated arabinoxylan can also bind more water due to the improved water-holding capacity from arabinoxylan which increases bread weight (Ayala-Soto et al., 2017). However, if the gluten network is disrupted by arabinoxylan addition, it will no longer retain gas as efficiently, which will be directly reflected in the bread volume (Courtin & Delcour, 2002).

The positive effects of arabinoxylan addition to bread volume and structure have been reported for addition levels varying between 0.8 and 5 % (Koegelenberg & Chimphango, 2017; Wang et al., 2019; Zhang et al., 2019), but higher levels lead to excessive dough viscosity, reduced gas retention, and lower bread volume (Damen et al., 2012). Decrease in bread volume has been reported for arabinoxylan addition levels as low as 2-3 % (Bieneik et al., 2024; Kucerova et al., 2013), and addition levels above 10 % severely compromise the normal gluten network formation (Damen et al., 2012; Zhang et al., 2019). Molar mass reduction of arabinoxylan has been shown to improve wheat bread volume, while high FA has been observed to have either no effect or decrease bread volume (Bieniek et al., 2024; Koh & Ng, 2009; Schooneveld-Bergmans et al., 1999; Snelders et al., 2014).

2.3.6 Bread texture

Bread texture is a key attribute to consumers perception of bread quality (Gellynck et al., 2006), and arabinoxylan addition has been observed in many studies to impact the softness of bread crumb (Pihlajaniemi et al., 2020; Wang et al., 2019; Zhang et al., 2019). These changes are most likely due to the previously discussed structure and property dependent effect of arabinoxylan on the dough network. Similar to bread volume, WEAX can, in low addition levels, strengthen the gluten network and improve bread softness (Wang et al., 2019). Water binding by arabinoxylan can also

promote crumb softness by increasing the amount of water within the dough (Zhang et al., 2019). If arabinoxylan addition disrupts the gluten network formation, it can lead to an undeveloped gluten network, low gas retention, and a denser and harder bread structure (Kucerova et al., 2013). The effect of arabinoxylan on crumb texture is connected to the addition level, and numerous studies have demonstrated that lower arabinoxylan additions of 5 % and below are able to increase crumb softness (Pihlajaniemi et al., 2020; Wang et al., 2019; Zhang et al., 2019), whereas higher addition levels ranging from 3 to 10 % increase crumb hardness (Kucerova et al. 2013; Zhang et al., 2019). These differences in results between studies on optimal arabinoxylan addition level for crumb softness might be due to differences in arabinoxylan structure and properties, as reduction of molar mass has been shown to lessen the inhibiting effect of arabinoxylan on gluten network formation and result in softer bread crumb (Bieniek et al., 2024).

2.3.7 Bread staling

Staling is a complex phenomenon wherein bread gradually loses its freshness during storage. It leads to the hardening of breadcrumb and therefore bread texture is closely related to the perceived freshness and high quality by consumers (Fadda et al., 2014). Starch retrogradation, where amylose and amylopectin that has leached out of granules due to gelatinization and swelling during baking reorganizes back into more crystalline structure during storage, is a main contributor to bread staling. However, because staling also involves water migration and redistribution, and gluten transformation, cross-linking between partially solubilized starch and gluten proteins has been suggested to cause bread hardening during staling (Choi et al., 2008).

Some studies have found that the addition of arabinoxylan increases the crumb softness during storage. Low molar mass arabinoxylan has been shown to prevent recrystallization of amylose and therefore retard short-term retrogradation, while high molar mass arabinoxylan, on the other hand, has been observed to have a greater impact on long-term retrogradation by suppressing recrystallization of amylopectin (Biliaderis et al., 1995; Wang et al., 2019). This could be partly due to interactions between arabinoxylan and starch but also related to the altered amount of available water in bread system, as the amount of available water in the bread matrix has a huge impact on starch recrystallization (Izydorczyk & Biliaderis, 2006; Liu et al.,

2020). However, it has also been noted that arabinoxylan can increase starch retrogradation by increasing the water content of bread which leads to increased starch mobility (Courtin & Delcour 2002; Izydorczyk & Biliaderis, 2006).

3. Aims

The overall aim of this thesis was to determine the relationship between the processing of wheat bran, properties of wheat bran arabinoxylan extracts, and the functionality of modified wheat bran arabinoxylan extracts in breadmaking. This work was expected to provide new insights into how arabinoxylan processing can improve its breadmaking properties, and therefore promote the use of wheat bran as functional bread ingredients.

The specific objectives of this work were:

- Identify potential processes for wheat bran arabinoxylan modification for breadmaking based on existing literature (Paper I).

- Modify wheat bran arabinoxylan extracts by both reducing ferulic acid content with saponification and molar mass with enzymatic hydrolysis to evaluate their combined effect on bread quality and shelf-life (Paper II).

- Investigate the relationship between the composition and properties of modified arabinoxylan extracts, and dough rheology and microstructure to further explore the role of feruloylation and molar mass in breadmaking (Paper III).

- Explore high intensity subcritical water extraction to reach arabinoxylan yields comparable to alkali extraction while improving extract color with ultrafiltration and active charcoal treatment (Paper IV).

4. Methods

This chapter provides an overview of the methods used to first modify and characterize, then incorporate arabinoxylan extracts in dough and observe the impact of arabinoxylan addition to the dough and bread properties. All the methodology is described in detail in the respective papers (Paper II-IV).

4.1 Production of modified arabinoxylan extracts

Feruloylated arabinoxylan (FAX) obtained from a pilot scale SWE process described by Zhang et al. (2023) was used as a starting material to produce modified arabinoxylan extracts for Papers II and III. Briefly, a low FA arabinoxylan extract (UAX) was prepared from an original FAX extract by removing FA with mild alkaline saponification. Hydrolyzed extracts with (H-FAX) and without (H-UAX) FA were produced from unhydrolyzed extracts by enzymatic hydrolysis. The enzymatic hydrolysis process for feruloylated wheat bran arabinoxylan has been previously optimized by Ruthes et al. (2017) and was not part of this work. The preparation of extracts was repeated separately for Papers II and III. A more detailed description of the production of modified arabinoxylan extracts can be found in Papers II and III.

4.2 Characterization of arabinoxylan extracts

4.2.1 Chemical compositions

To evaluate the effect of hydrolysis and FA removal on arabinoxylan extract structure, chemical composition of FAX, UAX, H-FAX, and H-UAX was characterized in Papers II and III, and monosaccharide composition of

arabinoxylan extracts in Paper IV. The monosaccharide composition of arabinoxylan extracts was quantified following a two-step sulfuric acid hydrolysis using high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Klason lignin content was determined as the acid-insoluble residue after hydrolysis. Arabinoxylan content was calculated based on the total content of arabinose and xylose from the monosaccharide analysis. The protein content of extracts was determined by either the Kjeldahl or Dumas combustion method. Ash content was determined using the AACC total ash method. Total starch and β-glucan content of arabinoxylan extracts was determined using resistant starch and mixed linkage β-glucan assay kits based on colorimetric methods. For Paper II, FA content was quantified with high performance liquid chromatography (HPLC) after a one-step alkali extraction. For Paper III, extraction was completed separately for ethanol-soluble free, conjugated, and ethanol-insoluble esterified bound FA and all three extracts were quantified using ultra performance liquid chromatography with photodiode array detector (UPLC-PDA).

4.2.2 Extract properties

To evaluate the effect of extraction, saponification, and enzymatic hydrolysis on arabinoxylan extract properties, molar mass of arabinoxylan extracts was measured in Papers II-IV by size exclusion chromatography (SEC). Additionally, rheological properties and water holding capacity were measured for FAX, UAX, H-FAX, and H-UAX in Paper III.

4.3 Dough quality

4.3.1 Water absorption, dough development time, and dough stability time

For dough with incorporated arabinoxylan extracts, 5 % of arabinoxylan was added by replacing flour based on arabinoxylan content of extracts in Papers II and III. The arabinoxylan extracts were pre-dispersed in water using heat and mixing before dough preparation. In Paper II, farinograph measurements were done for bread doughs including all bread ingredients, and in Paper III for model dough containing only flour, water and arabinoxylan extracts. Water absorption, dough development time (DDT), and dough stability time

(DST) were determined. Optimal water absorption and DDT obtained from farinograph results were used to produce doughs for both deformation measurements and microscopy.

4.3.2 Dough rheology

To provide information on the effect of arabinoxylan extract addition on dough rheology in Paper III, large and small deformation measurements were taken for model dough samples. All doughs were prepared with a farinograph using the optimal water absorption and DDT for the flour/extract mixtures. The model doughs contained only wheat flour, water, and arabinoxylan extracts. Extensibility and resistance to extension were determined with uniaxial extension measurements at large deformations using a texture analyzer equipped with a Kieffer dough/gluten extensibility rig. Storage and loss modulus were determined with small deformation rheological measurements using an oscillation test.

4.3.3 Dough microstructure

To visually examine the effect of arabinoxylan extracts on gluten network formation in Paper III, dough microstructure was examined from doughs prepared the same way as for rheological measurements (see 4*.3.2 Dough rheology*). Dough samples were first fixated, dehydrated, and then embedded. Embedded samples were then sectioned, stained, and examined with a light microscope (Paper III).

4.4 Bread quality

4.4.1 Breadmaking process

To evaluate the effect of different arabinoxylan extracts on bread quality in Paper II, wheat breads were baked with FAX, UAX, H-FAX, and H-UAX according to the straight-dough procedure (AACC, 2000). Breads were prepared in a farinograph with 0 (Control), 1, 2, and 5 % of arabinoxylan by replacing flour based on arabinoxylan content of extracts. The calculated fiber content for control bread, and bread with 1 %, 2 %, and 5 % added arabinoxylan was 1.7 %, 2.5 %, 3.3 %, and 5.7 %, respectively. The other ingredients used in the bread recipe can be found in Paper II. Farinograph water absorption and DDT were determined for each dough and final doughs

for bread-baking were produced using optimal water absorption and DDT based on farinograph measurements.

4.4.2 Specific volume and crumb structure

Specific volume was determined for all breads after three hours of cooling (Paper II). Crumb structure was first evaluated visually followed by an image analysis from slices cut from the middle of the bread loaf. Slices were scanned and images were processed and analyzed to generate an estimate of mean cell size in number of pixels (px).

4.4.3 Bread shelf life

In Paper II, crumb texture was measured at 0, 7, and 14 days after baking using texture profile analysis (TPA). As potential prevention of crumb staling by arabinoxylan was expected to be related to the prevention of moisture migration due to arabinoxylans high water binding ability, moisture content was determined at 0, 7, and 14 days after baking from both crumb and crust samples (Paper II).

4.5 Improvement of arabinoxylan extraction process for bread applications

To develop an extraction process to produce an arabinoxylan ingredient for breadmaking while also minimizing coloring and maximizing arabinoxylan yield, two extraction processes combining subcritical water extraction, enzymatic hydrolysis, ultrafiltration, and decolorization were developed and compared to a more conventional alkali extraction (Paper IV). In the first process design (SE), subcritical water extraction and enzymatic hydrolysis were completed separately by subjecting wheat bran to subcritical water extractions and then taking the extraction residues further to enzymatic hydrolysis (Figure 2B). In the second extraction process, subcritical water extraction and enzymatic hydrolysis were completed in the same pressure cell to improve the scalability of the extraction process (CES) (Figure 2C). These processes were compared to conventional alkali extraction (AE) (Figure 2A). A more detailed description of extraction processes can be found from Paper IV.

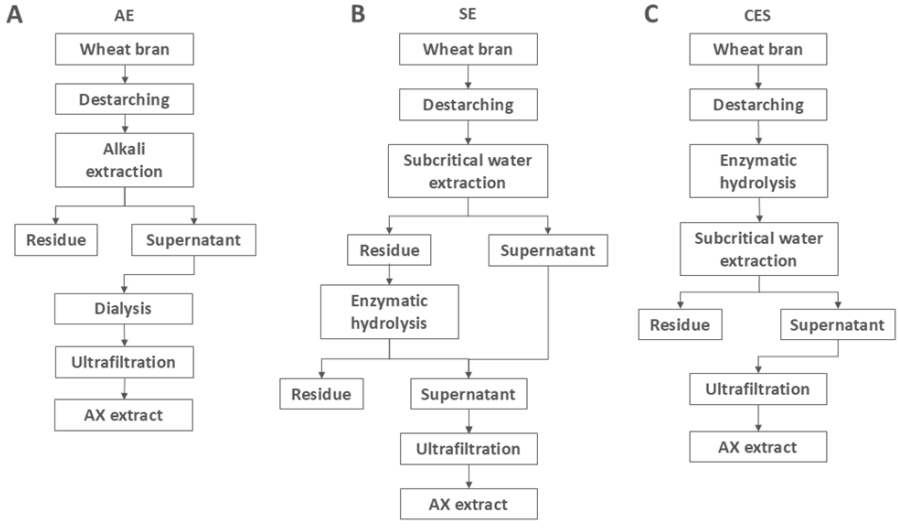

Figure 2. Flow charts for extraction processes used for arabinoxylan extraction. A = Alkali extraction (AE), B = Subcritical water extraction + enzymatic hydrolysis (SE), and C = combination of enzymatic hydrolysis and subcritical water extraction (CES). (Paper IV).

4.6 Statistical analysis

All measurements were conducted at least in duplicate, with results reported as mean values ± standard deviation. Type-III ANOVA was used in Papers II-IV to identify differences at a 95 % confidence level, determined by Tukey's pairwise comparisons. A multivariate analysis was performed with partial least squares regression analysis (PLS) to investigate relationships between extracts and bread properties in Paper II. Extract properties were set as predicting X-variables, and bread and dough properties were set as dependent Y-variables. In Paper III, the relationship between extract and dough properties was examined using 2-tailed Pearson correlation with linear relationships evaluated through regression analysis.

5. Results and discussion

5.1 Effect of hydrolysis and feruloylation on arabinoxylan extracts

5.1.1 Composition of modified arabinoxylan extracts

To understand the effect of reduction of FA and molar mass on the arabinoxylan extracts, the chemical composition was determined in Papers II and III (Table 2). Total carbohydrate content varied between 66.5 and 71.1 g/100 g in Paper II and 56.2 and 75.9 g/100 g in Paper III. The relative arabinoxylan content (% of total carbohydrates) of the extracts was higher in Paper II, varying between 78.5 and 81.6 %, compared to Paper III where relative arabinoxylan content was between 70.7 and 73.0 %. A/X ratios in Paper II were relatively low, varying between 0.1 – 0.2, and were slightly higher in Paper III (0.3 – 0.4).

Table 2. *Carbohydrate, arabinoxylan (AX), starch, mixed linkage β-glucan, Klason lignin, protein, and ash content of the extracts in Papers II and III. Mean value ± SD. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX=Hydrolyzed low-feruloylated arabinoxylan extract*

	FAX		**UAX**		**H-FAX**		**H-UAX**	
	Paper II	**Paper III**	**Paper II**	**Paper III**	**Paper II**	**Paper III**	**Paper II**	**Paper III**
Total carbohydrates (g/100g)[a]	66.4 ±11.8	75.9 ±6.6	71.1 ±2.1	69.4 ±2.6	66.5 ±0.8	56.2 ±13.3	70.4 ±0.6	67.5 ±0.8
AX (%)[b]	79.0 ±2.0	70.7 ±2.1	80.6 ±0.1	73.0 ±0.2	78.5 ±0.2	71.1 ±0.0	81.6 ±0.0	72.0 ±0.1
A/X[c]	0.1	0.4 ±0.1	0.3	0.3 ±0.0	0.2	0.3 ±0.0	0.2	0.3 ±0.0
Starch (g/100g)	4.8 ±0.9	0.8 ±0.0	6.3 ±0.2	0.7 ±0.0	5.6 ±0.0	1.0 ±0.0	7.4 ±0.1	1.1 ±0.1
Mixed linkage β-glucan (g/100g)	6.3 ±0.0	5.6 ±0.6	6.5 ±0.1	6.0 ±1.3	5.6 ±0.1	5.3 ±0.22	4.2 ±0.0	5.9 ±0.1
Protein (g/100g)	2.2 ±0.4	2.7 ±0.0	2.8 ±0.2	1.7 ±0.0	2.9 ±0.4	2.6 ±0.0	1.7 ±0.3	1.7 ±0.0
Kalson lignin (g/100g)	-	3.3 ±1.0	-	2.0 ±1.0	-	2.6 ±1.7	-	3.4 ±1.1
Ash (g/100g)	-	4.7 ±0.0	-	7.1 ±0.0	-	4.4 ±0.0	-	7.2 ±0.0

[a]Total carbohydrate content was calculated based on total content of arabinose, rhamnose, galactose, glucose, xylose, and mannose. [b]AX content was calculated based on the total content of arabinose and xylose and expressed as % of the total carbohydrate content. [c]Ratio between arabinose and xylose

When comparing the amounts of other components, the extracts had a relatively similar amount of protein (1.7 – 2.8 g/100 g) and β-glucan (4.2 – 6.5 g/100 g). The amount of starch was higher in Paper II (4.8 – 7.4) compared to Paper III (<1.1 %) which is likely due to different amounts of starch in the original FAX extracts. Reduction of FA content by saponification increased ash content in Paper III, which was suggested to be related to the removal of other components during the alkali treatment leading to concentration of ash as reported previously by Rasool et al. (1995). However, no clear increase in total carbohydrate content was detected in extracts after FA reduction in Paper III. As samples were only washed with

ethanol and not dialyzed after alkaline treatment, a more probable explanation for increased ash content is the formation of carboxylate salts in the saponification process.

Based on the chemical composition measurements, there were certain differences in the composition of both the original FAX extract and modified arabinoxylan extracts between the two studies. This is not necessarily surprising as the FAX extracts for the different studies were produced separately on a pilot scale from different wheat bran batches. Wheat bran is a side stream with highly varying quality, and there might have been considerable variation in the wheat bran composition between these extractions. These differences in the extract composition need to be kept in mind when comparing results from these two studies throughout the rest of the results & discussion chapter.

FA content of the arabinoxylan extracts in Papers II and III is presented in Figure 3. In Paper II, where the total amount of FA was measured after a single step phenolic extraction, hydrolysis increased the amount of FA from 4.7 mg/g for FAX to 10.8 mg/g for H-FAX. It was then suggested to be due to enzymatic hydrolysis concentrating FA in the remaining arabinoxylan extract as enzymes might be more active on unsubstituted non-feruloylated regions of arabinoxylan. However, a more detailed analysis of FA was completed in Paper III, where phenolic extraction was conducted separately for ethanol-soluble free (representing unbound free FA), ethanol soluble conjugated (representing FA bound to shorter arabinoxylan chains), and ethanol-insoluble esterified (representing FA bound to arabinoxylan) ferulic acids. A more detailed description of differences between these FA groups can be found in Paper III. In Paper III, enzymatic hydrolysis slightly decreased the total amount of FA from 11.2 mg/g in FAX to 8.8 mg/g in H-FAX.

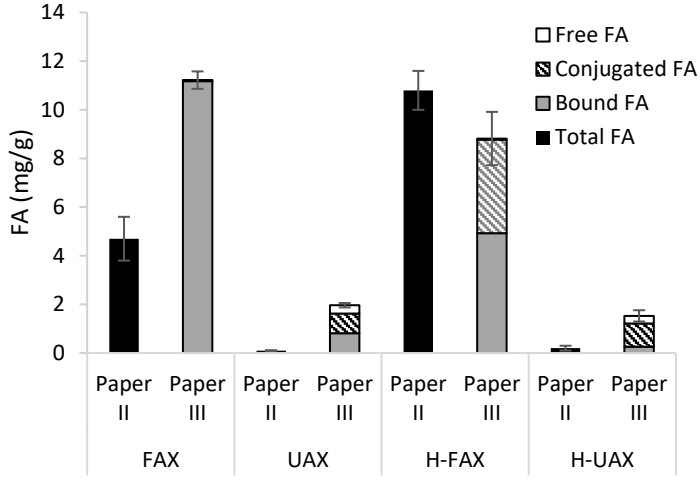

Figure 3. Ferulic acid (FA) content (mg/g) of extracts in Papers II and III. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX=Hydrolyzed low-feruloylated arabinoxylan extract.

Differences in the results between the two studies could be explained by differences in phenolic extraction, as Alara et al. (2021) concluded in their review on extraction of phenolic compounds that a single step extraction can change the recovery of phenolic components from plant samples. Based on the results from Paper III, hydrolysis seems to turn a large part of the bound FA into conjugated FA. This could be explained by the partial hydrolysis producing shorter feruloylated arabinoxylans with increased ethanol solubility in the phenolic extraction. It appears that the single step extraction is unable to liberate all bound FA and is therefore more effective towards free and conjugated FA, leading to this method underestimating the total amount of FA. In Peper III, higher levels of residual FA in extracts with reduced FA content compared to Paper II were suggested to be caused by differences in ethanol washing step. However, based on the above discussion, the 3-step extraction may simply show more residual FA in these samples as this process seems to be more efficient in extracting FA.

5.1.2 Properties of modified arabinoxylan extracts

To connect the differences observed in composition of the modified arabinoxylan extracts to their properties and to understand how these

modifications can be used to alter bread and dough quality, the extract properties were studied in term of molar mass, solution viscosity, and WHC. Molar mass was measured for modified arabinoxylan extracts in Papers II and III, and viscosity and WHC in Paper III (Table 3). The weight average molar mass (M_w) in Paper II was 698 kg mol^{-1} for FAX and 714 kg mol^{-1} for UAX, and in Paper III 485 kg mol^{-1} and 464 kg mol^{-1}, respectively. Hydrolysis decreased M_w 5 to 7 times for both FAX and UAX in Paper III. In Paper II, there were differences in molar mass reduction between extracts UAX and FAX, as the M_w was reduced only 3.6 times for UAX but 24 times for FAX. The enzyme used in the hydrolysis was an endo-1,4-β-xylanase, that hydrolyzes the linear β-1,4-xylan backbone, and its activity has been shown to be restricted by arabinose substitution (Rudjito et al., 2023). In Paper II, FAX had a lower A/X ratio (0.1) compared to UAX (0.3), and it is possible that the higher arabinose substitution in UAX has restricted the enzyme activity leading to less efficient hydrolysis.

Table 3. *Extract properties in terms of molar mass (number-average molar mass (M_n), weight-average molar mass (M_w), and dispersity index (Đ)) in Papers II and III, and solution viscosity (mPa.s) and water-holding capacity (WHC) in Paper III. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract.*

	FAX		**UAX**		**H-FAX**		**H-UAX**	
	Paper II	**Paper III**	**Paper II**	**Paper III**	**Paper II**	**Paper III**	**Paper II**	**Paper III**
M_w (kg mol^{-1})	698	485	714	464	29	67	199	95
M_n (kg mol^{-1})	317	126	200	218	24	37	59	17
Đ	2.2	3.8	3.6	2.1	1.2	2.6	3.4	3.9
Viscosity (mPa.s)	-	100 ±18	-	149 ±44	-	39 ±1.0	-	30 ±8.9
WHC[a]	-	3.1 ±0.1	-	3.3 ±0.2	-	0.7 ±0.2	-	0.9 ±0.1

[a]g H2O/g dry sample

In addition to directly affecting the enzyme activity, differences in A/X ratio of extracts in Papers II and III might have influenced results of molar mass measurements by altering the arabinoxylan solubility. Low substituted arabinoxylan has a high tendency to aggregate which affects the molar mass measurement, as the results represent the mass of these aggregates rather than arabinoxylan itself. The M_w for unhydrolyzed fractions in Papers II and III were higher than previously reported values for SWE extracts (Paper I), which could indicate that the observed values partly represent the mass of arabinoxylan aggregates. The formation of aggregates might also restrict enzyme activity as aggregated arabinoxylan can be less available for enzymatic hydrolysis. The extracts in Paper III had higher A/X ratios compared to extracts in Paper II, making them potentially more available for hydrolysis due to their higher solubility. If solubility is very poor, it can also lead to the formation of aggregates that are large enough to be filtered away

during the preparation of samples, leading to the results only reflecting the arabinoxylan population with higher solubility. Without more detailed knowledge about enzyme activity and formations of aggregates, it is difficult to conclude whether these values represent the true molar mass of the arabinoxylan and what has caused the observed differences in the hydrolysis efficiency.

Extract properties in terms of molar mass, viscosity, and WHC expectedly correlated with each other, supporting the vast existing evidence that higher molar mass increases both WHC and viscosity of arabinoxylan solutions. Molar mass reduction through enzymatic hydrolysis had a significant effect on all extract properties, which is in accordance with numerous previously published studies (Biliaderis et al., 1995; Bieniek et al., 2024, Zhang et al., 2019). Reduction of FA content through alkaline saponification had little to no effect on extract properties.

5.2 Effect of arabinoxylan structure and properties on dough quality

5.2.1 Water absorption, dough development time, and dough stability

As extract modification was observed to alter the properties of the arabinoxylan extracts, the next step was to evaluate how these changes will be reflected in dough quality. Farinograph measurements of dough water absorption and dough development time were investigated in Papers II and III, and dough stability time in Paper III. The results for doughs with 5 % arabinoxylan addition are presented in Figure 4 as the change in each property compared to control dough to make comparison between the studies easier. For water absorption, unhydrolyzed fractions FAX and UAX showed the highest increase. For UAX, the increase was 34.7 and 36.6 % in Papers II and III, respectively, and for FAX 36.2 and 28.7 % in Papers II and III, respectively. In both studies, hydrolysis decreased the water absorption compared to unhydrolyzed extracts, H-FAX increasing it 7.0 and 5.7 % in Papers II and III, respectively, and H-UAX 25.0 % and 3.8 % in Papers II and III, respectively. This is most likely due to the lowered WHC of the hydrolyzed extracts related to their lower molar mass, as discussed in section *5.1.2 Properties of modified arabinoxylan extracts*. Reduction of FA content

through alkaline saponification had little to no effect on dough water absorption.

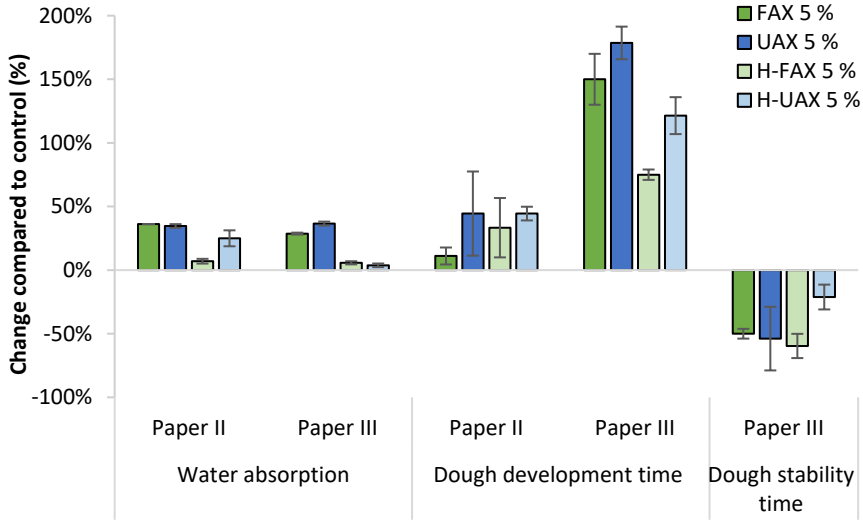

Figure 4. Change in water absorption, dough development time, and dough stability for doughs with 5 % arabinoxylan addition compared to control dough in Paper II and III. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract.

Addition of arabinoxylan extracts also increased the DDT compared to control in Paper III, and this effect was more severe for unhydrolyzed extracts. This indicates that the arabinoxylan addition delayed the formation of the gluten network, as previously described by Liu et al. (2023). In both studies, the feruloylated extracts also seem to have a smaller impact on DDT, suggesting that FA might play a minor role in improving gluten formation. In Paper II, the dough farinograph measurements were taken using the bread dough used for baking, whereas in Paper III the farinograph measurements were taken for mixtures of flour and fiber extracts only, which can explain why DDT increased considerably more and showed larger differences between samples in Paper III compared to Paper II.

Arabinoxylan extract addition decreased DST, indicating that doughs with arabinoxylan extracts couldn't maintain maximum consistency under mixing as well as control dough. As already indicated by WHC, farinograph water absorption correlated strongly with the extract properties molar mass,

viscosity, and WHC. Also, DDT correlated with all the above-mentioned properties (Paper III), supporting the existing evidence that water holding by arabinoxylan delays the formation of gluten during dough mixing resulting in weaker doughs (Biliaderis et al., 1995; Wang et al., 2020; Xiao et al., 2021).

5.2.2 Dough extensibility and resistance to extension

To better evaluate the effect of the addition of modified arabinoxylan extracts on large deformations that dough undergoes during processing, dough extensibility and resistance to extension were measured in Paper III (Figure 5). Arabinoxylan extract addition was found to decrease dough extensibility compared to control, showing that an incorporation of arabinoxylan weakens the dough network as it less capable of stretching before breaking. Dough extensibility is also an indicator of bread volume, as it reflects the doughs' ability to expand during fermentation and baking, and a stronger and elastic gluten network has been shown to slow down gas diffusion from dough during baking (Biliaderis et al., 1995; Janssen et al., 2020; Wang et al., 2019). Arabinoxylan extract addition also increased resistance to extension, demonstrating that these doughs were stiffer because they resist deformation more. As large deformation measurements mostly reflect the protein-protein interactions in dough (Amemiya et al. 1992), these results indicate that the addition of arabinoxylan extracts disrupts the gluten network. This was also supported by farinograph results on DDT and DST (Figure 4).

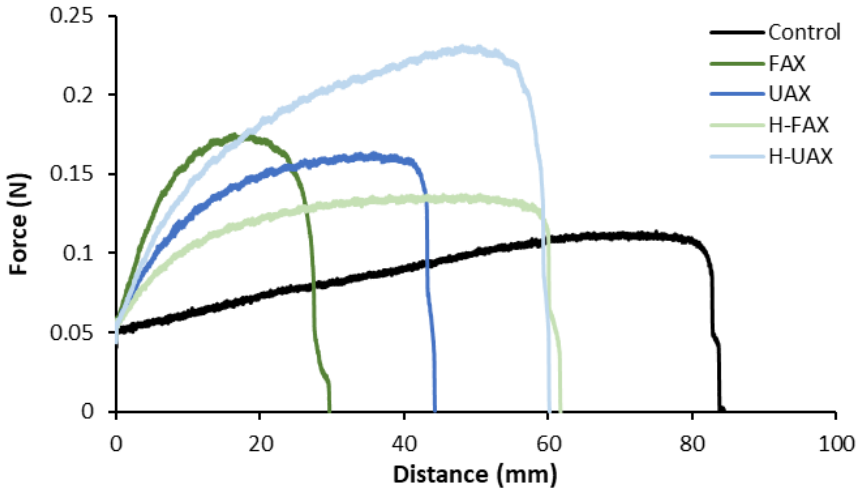

Figure 5. Extension curves with measured force (N) as the function of distance (mm) for control doughs and doughs prepared with 5 % arabinoxylan addition in Paper III. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract.

Hydrolysis of arabinoxylan extracts improved dough extensibility, which suggests that unhydrolyzed extracts disturb the dough formation more than hydrolyzed extracts. As extensibility also negatively correlated with molar mass, viscosity and WHC, the results for dough extensibility further strengthen the evidence that decreased viscosity and water-holding by hydrolyzed arabinoxylan extracts disturb the gluten network formation less compared to unhydrolyzed fractions. Although H-FAX was closest to control in both resistance to extension and extensibility, no clear effect from feruloylation could be detected as FAX showed the most extreme reduction of dough extensibility. Arabinoxylan with a low FA content and low level of substitution has been shown to decrease dough strength which is likely due to less chemical interactions between arabinoxylan and gluten (Wang et al., 2020). H-UAX had the highest resistance to extension, but a similar extensibility compared to H-FAX, indicating that dough with incorporated H-UAX was extremely firm but still relatively extensible compared to unhydrolyzed extracts.

5.2.3 Dough storage and loss modulus

Although the large deformation extensional measurements discussed earlier are important to predict the breadmaking quality of flour, they provide limited fundamental rheological information (Amemiya et al., 1992). Therefore, small deformation rheological measurements were taken in Paper III to provide information on the effect of modification of arabinoxylan extracts to viscoelastic properties of dough. All doughs exhibited elastic behavior as the storage modulus (G') was higher than the loss modulus (G'') (Figure 6). G' represents the elastic properties of the dough and this increases as the dough becomes stiffer (Mani et al., 1992). Hydrolyzed extracts H-UAX and H-FAX decreased G', indicating that reduction of molar mass by hydrolysis decreased dough stiffness. Even though WUAX has been previously shown to increase the elastic and viscous moduli of bread dough (Molina et al., 2021; Li et al., 2024; Wang et al., 2020), FAX was shown to increase G' only on lower frequency ranges. For G'', which is related to doughs flow properties, hydrolyzed extracts showed lower values compared to control on the lower frequency ranges used.

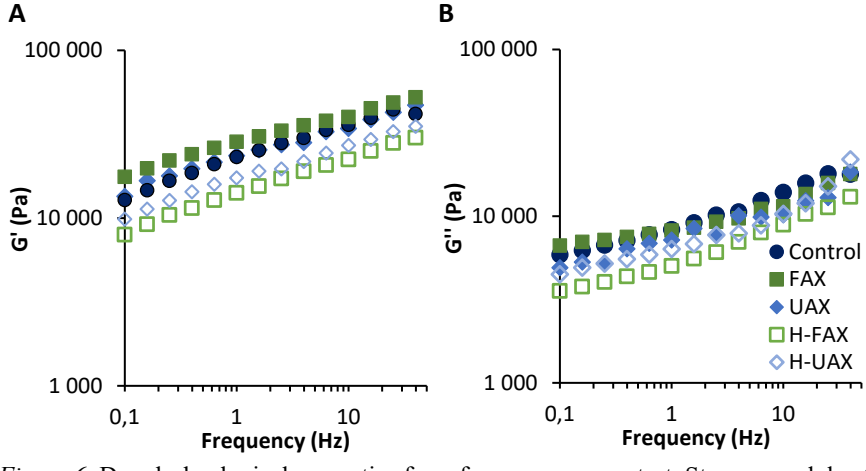

Figure 6. Dough rheological properties from frequency sweep test. Storage modulus G' (A) and loss modulus G'' (B) as a function of frequency at 25 °C for doughs with and without arabinoxylan extract incorporation in Paper III. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract.

These results for dough storage and loss modulus were mostly in line with large deformation measurements (Figure 5), where hydrolyzed extracts were

also observed to decrease dough firmness and showed higher extensibility compared to unhydrolyzed extracts. However, on small deformations the hydrolyzed fractions showed lower G' and G'' compared to control, whereas in large deformations the control clearly stood out by having the highest extensibility and lowest resistance to extension. The protein-protein interactions, that govern the dough behavior on large deformations, are partly masked by starch-starch and starch-protein interactions with the small frequency ranges used in small deformation measurements (Amemiya et al., 1992). Reduction of molar mass has been reported to increase arabinoxylan interactions with starch, leading to better bread quality (Hou et al., 2020; Wang et al., 2019). Moreover, limited starch swelling by low molar mass arabinoxylan has been observed to improve breadcrumb formation (Buksa & Krystyjan, 2019), which could also be related to the lower dough viscosity. This suggests that the extracts could have affected the starch-starch and starch-protein interactions in the dough, leading to changes in dough rheology.

Even though H-FAX incorporation produced dough with the lowest G' and G'', FAX addition did not clearly affect dough rheology compared to control. This suggests that there is no clear effect from feruloylation, possibly related to low FA content in extracts. Indeed, Snelders et al. (2017) have previously observed FA levels similar to FAX and H-FAX (0.1 – 1.7 %) to be too low to cause any effects on dough rheology.

5.2.4 Dough microstructure

As fiber addition was observed to affect farinograph results and dough rheology, dough microstructure was visualized in Paper III to observe if these results were linked to the effect of modified arabinoxylan extract on the gluten network. For control dough, the micrographs showed a continuous gluten network that occupied the entire space between the starch granules (Figure 7). Incorporation of arabinoxylan extracts caused disruptions on this gluten network, shown to decrease in stained protein area and more unstained background. This indicates that incorporating fiber into the dough disrupts the formation of a normal gluten network, which is in accordance with previous studies that have reported arabinoxylan to form networks between gluten strands, leading to disrupted gluten matrix (Sun et al., 2022; Zhang et al., 2014).

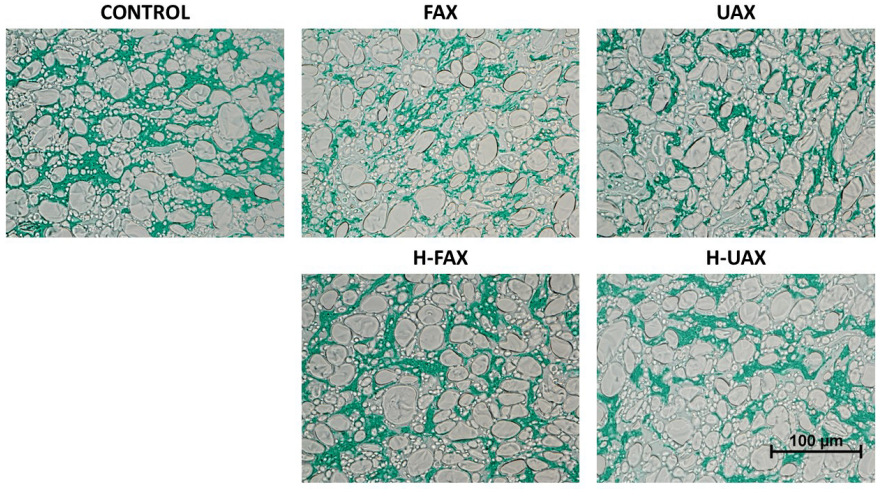

Figure 7. Dough microstructure for control dough and doughs with 5 % arabinoxylan addition in Paper III. Magnification x40. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract.

Unhydrolyzed extracts UAX and FAX disrupted the gluten network more than H-FAX and H-UAX, suggesting that they were hindering the gluten formation more than hydrolyzed extracts. For FAX and UAX, there were also other visible non-protein and non-starch particles, which may have been residual bran particles. These particles could also cause physical hindrance to dough formation, as suggested by Molina et al. (2021). It has also been reported that WUAX disrupts the gluten network microstructure while enzymatically hydrolyzed arabinoxylan improves it (Sun et al., 2022). The results from the visualization of dough microstructure supported the results from the farinograph and large deformation measurements, where hydrolyzed extracts were observed to have a less severe effect on dough formation during mixing and disturb the protein-protein interactions in dough less than unhydrolyzed fractions. Although FA has been reported to improve dough microstructure (Wang et al., 2020), no clear effect from extract feruloylation on microstructure was observed.

5.3 Effect of arabinoxylan structure and properties on bread quality

5.3.1 Specific volume and crumb structure

To understand how changes in arabinoxylan structure and properties affect bread properties, the effect of modified arabinoxylan extracts on bread was evaluated in Paper II. Fiber incorporation decreased specific volume, and this effect was related to both fiber extract and addition level (Figure 8). At 5 % fiber addition, all other extracts significantly decreased specific volume compared to control except H-FAX. Both hydrolyzed extracts H-UAX and H-FAX had a less severe effect on the specific volume compared to unhydrolyzed extracts FAX and UAX. Also, feruloylated extracts FAX and H-FAX resulted in higher volumes at 5 % extract addition level compared to their non-feruloylated counterparts, even though this increase was not statistically significant. The results for dough rheology and microstructure, where the hydrolyzed extracts were observed to produce more extensible doughs and disrupt the gluten network less compared to unhydrolyzed extracts, reflected the observed lower specific volume for unhydrolyzed extracts (Figure 5 and 7). Based on image analysis where crumb structure was measured, the changes in crumb structure were in line with the results for specific volume, as larger average cell size could be expected to result in larger bread volume. Results for the image analysis can be found in Paper II.

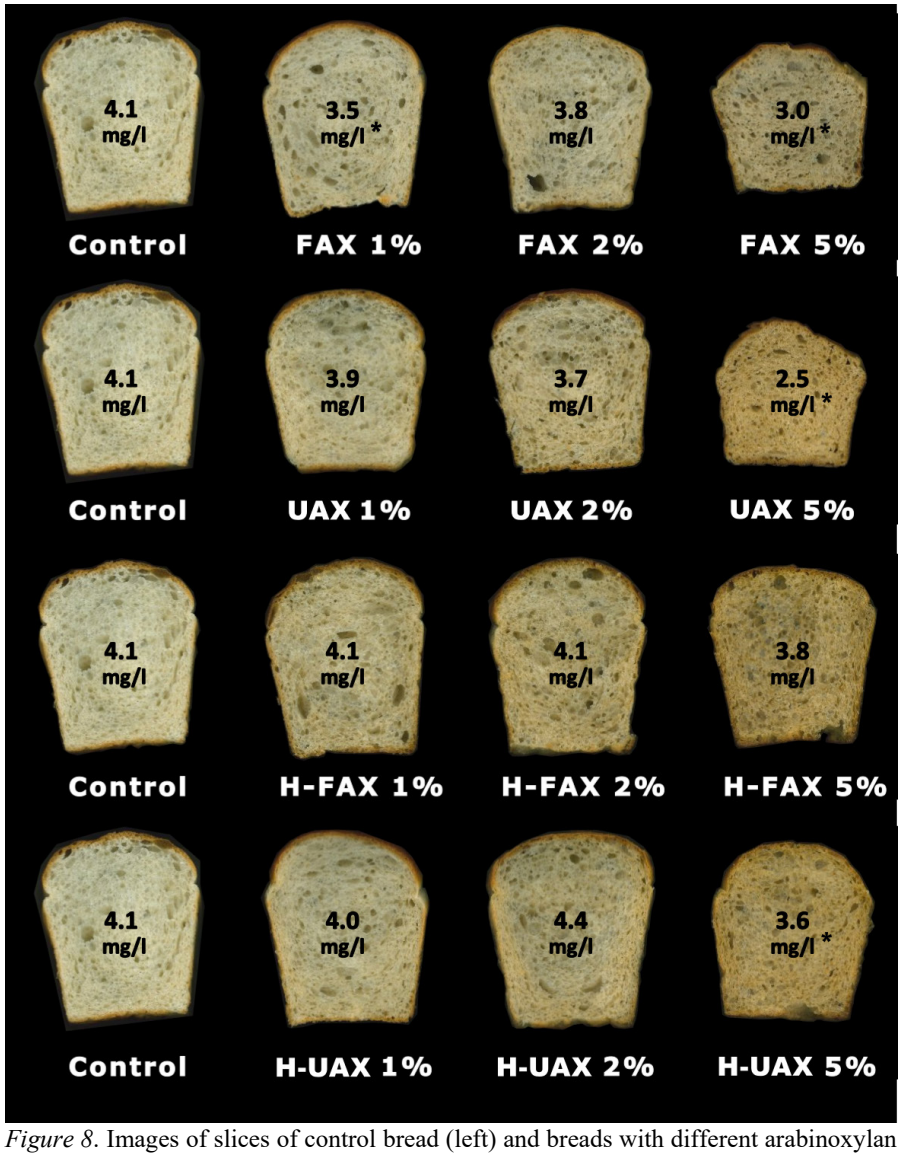

Figure 8. Images of slices of control bread (left) and breads with different arabinoxylan extracts at different addition levels (1%, 2%, 5%) and their specific volume (mg/l). FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract. *$p<0.05$ compared with control.

5.3.2 Crumb texture and shelf life

To evaluate the effect of modified arabinoxylan extracts on bread texture and moisture loss during storage, breadcrumb texture and shelf life in terms of staling rate (representing change in hardness (N) during storage) and moisture loss (representing change in moisture content (%) during storage) were studied in Paper II. Only FAX 5 % was able to prevent the moisture loss of breadcrumbs during a 14-day storage period (Figure 9A). When looking at the texture of fresh breadcrumbs, an increase in fiber addition level increased the crumb hardness (Paper II). Although both unhydrolyzed extracts UAX and FAX increased crumb hardness of fresh bread compared to control (Paper II), the staling rate of bread was affected only by addition of UAX 5 % (Figure 9B), indicating that these breads were hardening more than control.

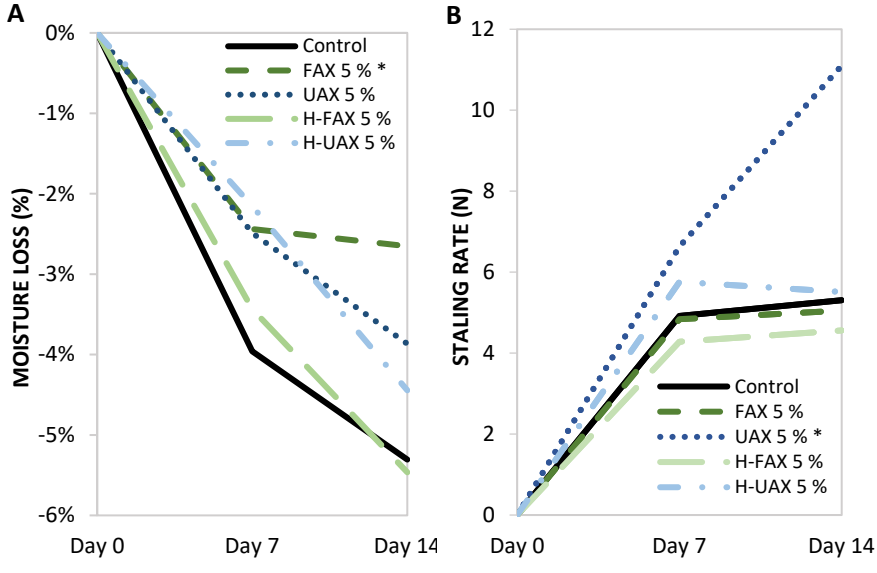

Figure 9. Changes during the 14-day storage period in (A) crumb moisture content (%) and (B) staling rate (change in hardness (N)) of breadcrumbs in breads with 5 % arabinoxylan addition in Paper II. FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract; H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract. *$p<0.05$ compared with control.

Although it was hypothesized that staling is related to the amount of water in the bread matrix, bread hardening did not correlate with the water-holding capacity of the extracts or bread crumb moisture loss. UAX extracts, that

increased the staling rate, had the highest water-holding capacity and longest dough development time and resulted in the most disrupted gluten network and lowest specific volume. This suggests that the observed hardening of breadcrumbs might be connected to changes in the gluten network and its elasticity, as UAX extract seems to have had the most severe effect on gluten formation. As changes in starch are known to play an important role in staling, it is possible that high water absorption and high water content in bread has increased the amount of gelatinized starch, leading to faster retrogradation (Choi et al., 2008). However, staling is a complex process involving several possible contributors, such as starch retrogradation, and it is impossible to make deeper conclusions on the effect of arabinoxylan extracts on staling based solely on their effect on crumb hardness.

Interestingly, the feruloylated extracts FAX and H-FAX had a slightly lower staling rate compared to low-feruloylated extracts UAX and H-UAX, despite this effect not being statistically significant. This is in line with the previously presented results for dough and bread properties, as feruloylated extracts and especially H-FAX showed lower DDT, and a higher specific volume compared to their non-feruloylated counterparts. This was also visible in large deformation measurements and microstructure of the doughs, where H-FAX was closest to the control indicating the least disturbed gluten network.

5.3.3 Relationship between extract modification, dough quality, and bread quality

As both Paper II and III demonstrated that modification of arabinoxylan extracts affects bread properties, a correlation coefficients and regression analysis was conducted to model the relationships between extract properties and dough quality parameters (Table 4). Extract composition in terms of monosaccharide, FA, β-glucan, or lignin content did not correlate with either extract or dough properties, which indicates that differences in extract composition aren't directly linked with extract properties in dough. This suggests that differences in results in dough properties might be more connected to the properties of the arabinoxylan in extracts in terms of molar mass, viscosity, and WHC rather than differences in their composition, as extract properties correlated strongly with each other and dough properties. Molar mass, viscosity, and WHC correlated with extensibility, elastic modulus, farinograph water absorption, and dough development time. This

supports the existing evidence that lower water-holding by hydrolyzed arabinoxylan with lower molar mass is connected to lower viscosity. This in turn decreases the water absorbance of dough, delaying dough formation less, and results in stronger doughs with a higher extensibility compared to unhydrolyzed extracts.

Table 4. *Pearson's correlation coefficient matrix between extract and dough properties in Paper III. Total= extract total monosaccharide content, A/X=extract A/X ratio, AX=extract AX content, FA=extract ferulic acid content, β-glucan= β-glucan content, Lignin=lignin content, M_w=weight average molar mass, Viscosity=extract viscosity, WHC=extract water-holding capacity, RE=dough resistance to extension, E=dough extensibility, G'=storage modulus, G''=loss modulus, WA=farinograph water absorption, DDT=dough development time, DST=dough stability time*

	Total	**AX**	**A/X**	**FA**	**β-glucan**	**Lignin**	**M_w**	**Viscosity**	**WHC**	**RE**	**E**	**G'**	**G''**	**WA**	**DDT**
AX	**1.00*** **														
A/X	-0.69	-0.71													
FA	-0.4	-0.42	**0.74***												
β-glucan	-0.11	-0.12	0.05	-0.33											
Lignin	0.39	0.36	0.06	0.2	0.22										
M_w	0.23	0.24	0.18	0.14	0.19	-0.14									
Viscosity	0.28	0.3	-0.01	-0.1	0.41	-0.23	**0.88****								
WHC	0.22	0.23	0.13	0.1	0.15	-0.22	**0.99*****	**0.90*****							
RE	0.46	0.45	-0.12	-0.43	0.18	0.15	0.18	0.01	0.12						
E	-0.18	-0.17	-0.41	-0.43	0.02	-0.17	**-0.85****	**-0.61***	**-0.81****	-0.21					
G'	0.41	0.41	0.15	0.25	0.36	0.18	**0.87****	**0.70***	**0.81****	0.32	**-0.79****				
G''	0.23	0.22	0.28	0.34	0.44	0.43	0.52	0.47	0.41	0.16	-0.48	**0.81****			
WA	0.25	0.26	0.03	-0.03	-0.37	-0.29	**0.97*****	**0.95*****	**0.97*****	0.23	**-0.72***	**0.74***	0.18		
DDT	0.08	0.09	0.1	-0.36	0.02	-0.33	**0.80***	**0.81***	**0.82***	0.58	-0.63	0.59	0.09	**0.79***	
DST	0.29	0.28	-0.29	-0.54	0.51	0.45	-0.32	-0.35	-0.31	0.67	0.13	-0.06	0.05	-0.43	0.03

*p-values below 0.05(*), 0.01(**) and 0.001(***) based on regression analysis

To model the relationships between properties of modified arabinoxylan extracts and bread quality, a multivariate analysis was completed for extract and bread properties in Paper II. Based on the PLS model, arabinoxylan content, β-glucan content, and molar mass were the most important extract properties according to variance importance to protection (Figure 10). In ANOVA on the cross-validated residuals of Y-variables, water absorption, specific volume, and crumb moisture content showed significantly different values ($p<0.05$) (Paper II). Samples separated most with 5 % fiber addition, indicating that lower fiber addition levels may not have as significant of a role on bread properties, and could be used in baking without modifying arabinoxylan properties. This minor effect of 1-2 % arabinoxylan extract addition could also be seen in the specific volume of bread (Figure 8), where only FAX decreased the specific volume on these lower addition levels. High water absorption was associated with lower bread quality, which is in line with results from Paper III (Table 4), as it positively correlated with hardness and DDT, and negatively correlated with bread volume. This further supports the evidence that lower water absorption by hydrolyzed extracts improves bread quality and indicates that dough rheological properties are a good indicator of bread quality in a fiber enriched dough matrix.

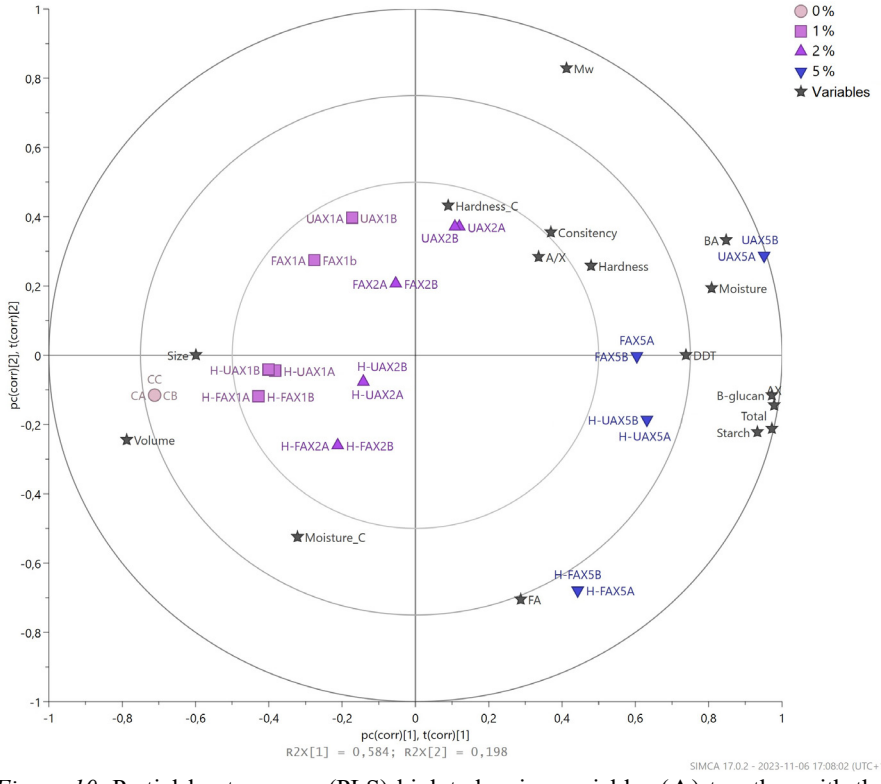

Figure 10. Partial least squares (PLS) biplot showing variables (★) together with the different bread samples, colored based on arabinoxylan (AX) addition level (0% = ●; 1% = ■; 2% = ▲; 5% = ▼ (Paper II). FAX = Feruloylated arabinoxylan extract; UAX = low-feruloylated arabinoxylan extract; H-FAX = Hydrolyzed feruloylated arabinoxylan extract, H-UAX = Hydrolyzed low-feruloylated arabinoxylan extract, Total = total carbohydrate content, A/X = A/X-ratio, Mw = molecular weight average of AX, FA = ferulic acid content, B-glucan = β-glucan content, Starch = starch content, BA = water absorption, DDT = dough development time, Consistency = final dough consistency, Volume = specific volume of bread, Size = average cell size of breadcrumb, Moisture = moisture content of fresh breadcrumb, Moisture_C = moisture loss, Hardness = hardness of fresh breadcrumb, Hardness_C = staling rate.

In Paper II it was concluded that other extract components might further increase water absorption and therefore affect bread quality as β-glucan was identified as one of the most important extract properties according to the variable importance in projection. However, this effect was not observed in Paper III, where extract composition was not found to correlate with any

dough quality parameters investigated (Table 4). For the bread with 5 % of H-FAX, that was closest to control in dough extensibility and bread volume, high FA content and low molar mass were the main contributors to bread properties. Although feruloylated arabinoxylan slightly improved the bread volume, no consistent evidence on the positive effect of FA on bread quality was seen. This might be due to the low FA content in extracts, as discussed earlier in section 5.2.3 (*Dough storage and loss modulus*). H-FAX had also the lowest M_w from all extracts (Table 3), which might contribute to the observed results.

5.4 Improvement of arabinoxylan extraction process for bread applications

5.4.1 Extraction yield

In Papers II and III, reduction of arabinoxylan molar mass improved properties of bread with added arabinoxylan even with low degree arabinose substitution. These findings suggest that harsher subcritical water extraction conditions, previously avoided in arabinoxylan extraction to preserve molecular structures, could be used to increase arabinoxylan yield as degradation of arabinoxylan is not an issue for arabinoxylan functionality in breadmaking. Therefore, two processes combining subcritical water extraction with high treatment temperatures and enzymatic hydrolysis were tested to increase arabinoxylan yield compared to alkali extraction (Paper IV). A more detailed description of these processes can be found in Paper IV.

The total solid and arabinoxylan yields of the 3 different extraction processes are presented in Figure 11. SE extraction, where subcritical water extraction and enzymatic hydrolysis were conducted separately, increased total solid yield to 23.1 % with 200 °C 15 min and to 23.4 % with 210 °C 10 min compared to alkali extraction, that reached a total solid yield of 13.6 %. Increasing treatment intensity further to 20 minutes in 210 °C decreased total solid yield to 11.2 %. Most SE treatment combinations also increased arabinoxylan yield compared to alkali extraction, although the increase was statistically significant only for SE 200 °C 15 min reaching 31 % arabinoxylan yield. Even though SE 210 °C 10 min was comparable to SE 200 °C 15 min in total solid yield, it had a lower arabinoxylan yield,

illustrating that increasing the temperature from 200 to 210 °C decreases arabinoxylan extraction and increases coextraction of other compounds, as reported by Ruthes et al. (2017). Increasing treatment time to 20 min with 210 °C further decreased arabinoxylan yield to only 4.7 %.

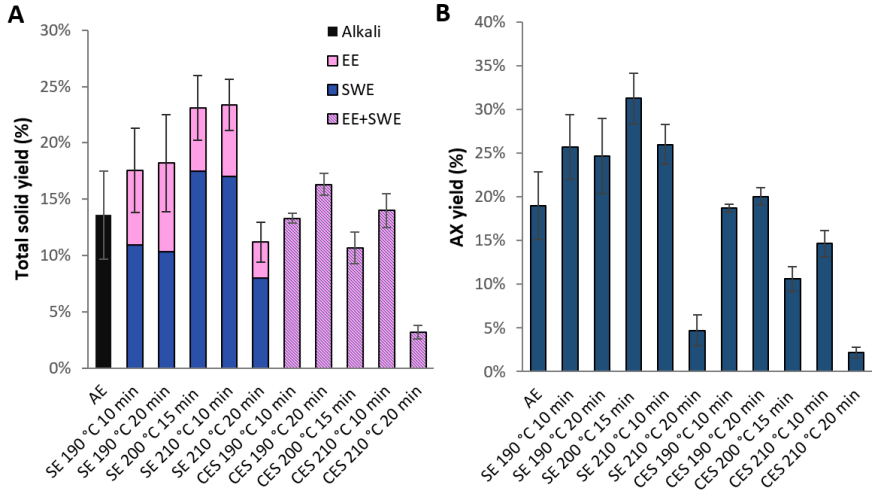

Figure 11. Total solid yield (% of destarched bran dry weight) (A) and arabinoxylan (AX) yield (% of AX content in destarched wheat bran) (B) of alkali extract (AE), extracts from subcritical water extraction + enzymatic hydrolysis (SE), and extracts from combination of enzymatic hydrolysis and subcritical water extraction (CES) with different time and temperature combinations. Error bars represent standard deviation for total yield and total AX yield. Alkali = alkali extraction, SWE = subcritical water extraction, EE = enzymatic hydrolysis.

CES extracts, where subcritical water and enzymatic hydrolysis were conducted in the same extraction cell to improve scalability of process, did not increase extraction yield compared to AE and showed lower arabinoxylan yields compared to SE extracts. This could be due to the lower molar mass distribution of these extracts, and the larger number of small oligomers created in the extraction being filtered out during ultrafiltration. However, even the less effective but scalable CES process was able to reach arabinoxylan yields comparable to alkali extraction. This indicates that both SE and CES processes could potentially be used to replace the alkali extraction process to extract arabinoxylan with lower A/X ratios and molar mass suitable for bread applications.

5.4.2 Extract decolorization

As high temperatures used during SWE cause extract darkening, purification and decolorization of SWE extracts was tested to improve extract usability in food applications. Extracts were first purified with ultrafiltration and then treated with activated charcoal to reduce coloring. The combination of treatment time and temperature had a clear effect on the color of extracts before purification and decolorization, 200 °C 15 min extract being the darkest, followed by 210 °C 10 min extract, as seen from their L-values representing color lightness that decreased compared to other extracts (Figure 12A). These process parameter combinations were also the ones that gave the highest extraction yields, indicating that these most efficient conditions also increase the extraction of the compounds causing the coloring of these extracts. From all the extracts, 190 °C 10 min extract had the lightest color. Extracts treated with 190 °C 20 min, 200 °C 15 min, and 210 °C 10 min were significantly higher in redness, represented by higher a-values (Figure 12B). Increase in treatment temperature to 210 °C also increased extract yellowness (Figure 12C). Ultrafiltration did decrease extract darkness and increase yellowness and redness. Active charcoal treatment decreased the a-values, indicating decreased redness and increased greenness of extracts compared to ultrafiltrated samples but otherwise did not have a significant effect on extract color profile. The results suggest that optimization of process parameters, ultrafiltration, and activated charcoal treatment can be used to improve the color profile of extracts from SWE.

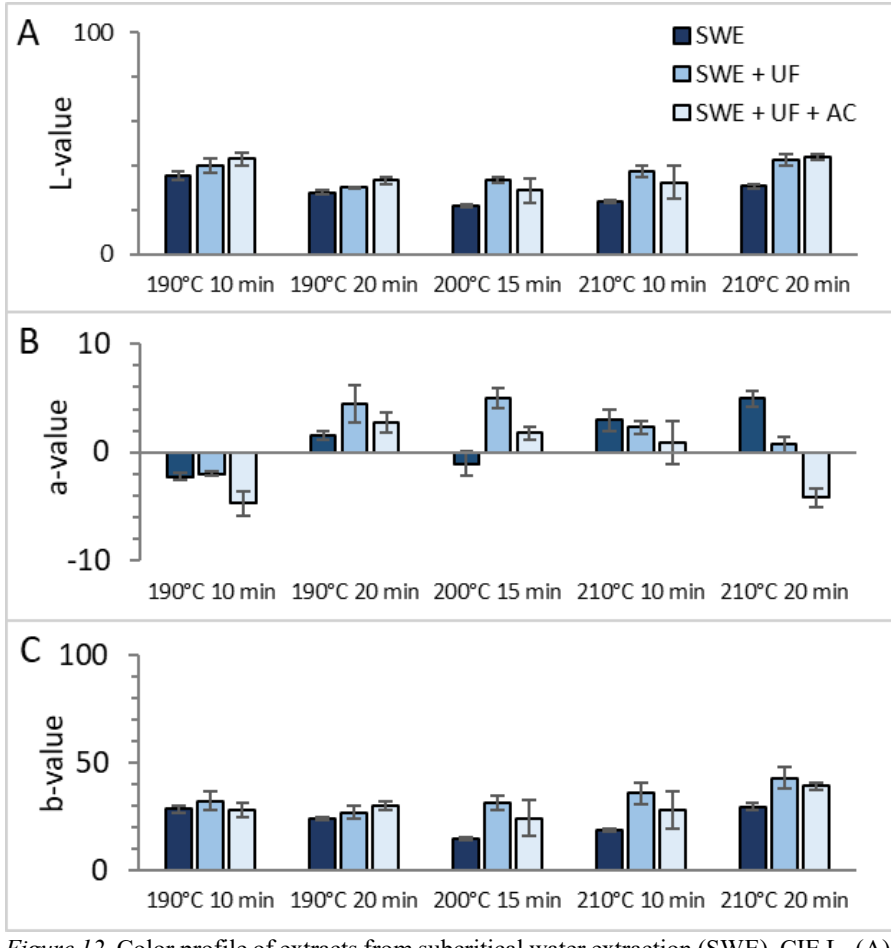

Figure 12. Color profile of extracts from subcritical water extraction (SWE). CIE L- (A), a- (B) and b- (C) values for extracts after extraction (SWE), ultrafiltration (UF), and active charcoal treatment (AC).

6. Conclusions

In this thesis, the relationship between the processing of wheat bran, properties of wheat bran arabinoxylan extracts, and the functionality of modified wheat bran arabinoxylan extracts in breadmaking was explored. This thesis provided a comprehensive understanding of how feruloylation and hydrolysis of wheat bran arabinoxylan extracts can be used to enhance dough and bread quality, and therefore facilitate the use of wheat bran in production of value-adding ingredients for bread products.

Reduction of ferulic acid content and molar mass with saponification, hydrolysis, and subcritical water extraction were identified as promising processes to reduce the negative impact of wheat bran arabinoxylan in breadmaking through a comprehensive review of the existing literature.

Modification of wheat bran arabinoxylan extract by reduction of molar mass and ferulic acid was found to influence bread quality. Reduction of molar mass with hydrolysis improved specific volume and decreased the hardness of fresh bread compared to unhydrolyzed extracts. Hydrolyzed and feruloylated extracts produced the bread closest to control, supporting the potential for feruloylation and molar mass reduction to be used in combination to enhance bread quality and shelf life.

The findings strengthened the evidence that dough properties and microstructure are related to the properties of arabinoxylan extracts, as the hydrolyzed extracts with lower molar mass, viscosity, and water-holding capacity were observed to produce more extensible doughs and disrupt the gluten network less compared to unhydrolyzed extracts. Hydrolyzed and feruloylated extract produced dough closest to control, suggesting that modifications of arabinoxylan extracts can be used to tailor arabinoxylan properties for specific applications.

This thesis work also advanced the subcritical water extraction of wheat arabinoxylan to achieve yields comparable to alkali extraction, while simultaneously improving the color of the extracts through ultrafiltration and active charcoal treatment. This suggests that these improvements could facilitate the use of increased treatment intensity in subcritical water extraction, and therefore improve the extraction efficiency while improving extract properties for bread applications.

7. Future perspectives

Despite this thesis work demonstrating the potential of modified wheat bran arabinoxylan extracts in breadmaking, future research and work is still needed to facilitate the use of wheat bran in production of fiber ingredients for baking. As this work suggests the effect of arabinoxylan on gluten network formation to be the main contributor on the effect of arabinoxylan addition to dough, future research should focus on further understanding the molecular mechanisms by which arabinoxylan extracts interact with dough components, particularly gluten. Indeed, the mechanism regarding how arabinoxylan affects bread staling by influencing gluten and starch interactions offers interesting possibilities for future research.

In this work, desirable properties for arabinoxylan extracts were identified and this knowledge was used to increase the extraction yield of arabinoxylan extraction process. Future work should be directed towards optimizing the arabinoxylan extraction further and exploring the possibilities of upscaling the ingredient production on pilot and industrial scale to develop an industrially viable process to produce arabinoxylan in a cost-efficient way.

To justify investments required for setting up industrial production of an arabinoxylan ingredient, arabinoxylan must show market potential by offering added value compared to competing ingredients. Although this thesis has demonstrated how modification of arabinoxylan extracts can improve their functionality in bread, future work should focus on further optimizing the structure of these extracts to reach the technological functionality of existing fiber ingredients. Applications beyond bread should also be further explored to increase the potential market by investigating functionality of wheat bran arabinoxylan in other food products.

One of the societal motivations for the need of a wheat bran arabinoxylan ingredient in this work was the possibility to increase sustainability and efficiency of cereal processing by redirecting a side stream to food ingredient production. Calculations of the actual sustainability and efficiency of the process are needed, as more detailed knowledge on these impacts could help in motivating the industry to start directing wheat bran to the production of these ingredients to increase the circularity and efficiency of their production.

The findings of this thesis suggest that there is potential for using modified arabinoxylan extracts to increase fiber content of bread. However, there are currently no health claims related to wheat bran arabinoxylan and the health-related added value from arabinoxylan ingredient is solely based on the increased fiber content. Exploring the health benefits of specific arabinoxylan extracts with defined structural properties could provide valuable insights for both developing new food ingredients with tailored health effects and potentially help reaching for a health claim for wheat bran arabinoxylan.

Although the focus of this thesis was on improving the quality of bread with high fiber content, these products will never be able to improve the public health by increasing fiber intake if consumers are not interested in eating fiber-enriched products. On a societal level, there is a great need to increase the consumer awareness of the importance of dietary fiber and future research should be directed to support this goal. The increased knowledge could then hopefully translate into an increased consumer interest towards fiber-enriched products leading to improved public health and help in directing resources for the development of next generation fiber ingredients.

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Popular science summary

Wheat is the world's largest crop in terms of value and a large part of wheat is milled into refined flour through a process where the inner grain parti rich in starch and gluten is separated from the other grain parts. Milling produces an enormous side stream of wheat bran that is predominantly used for animal feed despite it containing many nutrients such as dietary fiber, minerals, and bioactive compounds. Therefore, increasing the use of wheat bran in products such as bread could improve their nutritional quality and sustainability of cereal processing. However, wheat bran has a negative impact on bread quality, which is mainly due to the fiber in wheat bran. Arabinoxylan is the main fiber component in wheat bran, and understanding its properties in bread applications could facilitate the use of wheat bran as a fiber ingredient in baking.

This work investigated how processing can be used to improve the breadmaking properties of wheat bran arabinoxylan. Wheat bran arabinoxylan extract was treated to reduce its molecular size, because this is known to improve its properties in baking. A bioactive compound called ferulic acid, that is naturally present in wheat bran, was then removed. Arabinoxylan with ferulic acid could potentially add additional health benefits to bread, but its effect on baking needed to be investigated. The structure and properties of these extracts were analyzed, and they were then used in dough and bread. In this way, it was possible to connect the arabinoxylan structure and properties to differences observed in dough and bread quality. After understanding the optimal arabinoxylan properties for improving dough and bread quality, an extraction process using water with pressure and extreme temperatures, combined with a decolorization process, was tested to increase extraction yield while improving extract properties for bread applications.

Based on the results, arabinoxylan extracts with a larger molecular size had both a higher viscosity and water-holding capacity, and these are usually indicators of poor properties in baking. These extracts also produced bread with the smallest volume and hardest texture. Contrastingly, reducing the molecular size resulted in volumes comparable to bread baked without extracts. Extracts with more ferulic acid and smaller molecular size resulted in the best bread and dough quality, supporting the potential for ferulic acid and reduction of molecular size to be used in combination to improve bread quality.

The findings of this thesis demonstrate that there is potential for using these modified extracts as functional ingredients to increase fiber content of bread while also improving bread quality. Overall, this thesis provides a comprehensive understanding of how modifying wheat bran arabinoxylan properties can enhance the functionality of wheat bran fiber in breadmaking and therefore facilitate the use of wheat bran in production of value-adding ingredients for bread products.

Populärvetenskaplig sammanfattning

Vete är världens största skörd i värde och en stor del av vetet mals till raffinerat mjöl i en process där den stärkelse- och glutenrika inre delen separeras från de andra spannmålsdelarna. Malning producerar en enorm sidoström av vetekli som mest används till djurfoder även om det innehåller många näringsämnen som kostfiber, mineraler och bioaktiva komponenter. Därför kan en ökad användning av vetekli i produkter som bröd förbättra deras näringskvalitet och hållbarhet för spannmålsbearbetning. Vetekli har dock en negativ påverkan på brödkvaliteten, vilket främst beror på fibern i vetekli. Arabinoxylan är den huvudsakliga fiberkomponenten i vetekli, och att förstå dess egenskaper i bröd kan underlätta användningen av vetekli som fiberingrediens i bakning.

Detta arbete undersökte hur bearbetning kan användas för att förbättra brödframställningsegenskaperna hos veteklifiber. Veteklifiber behandlades för att minska dess storlek, eftersom detta är känt för att förbättra dess egenskaper vid bakning. Sedan togs bort en bioaktiv komponent som kallas ferulsyra som finns naturligt i vetekli. Fiber med ferulsyra skulle potentiellt kunna lägga till ytterligare hälsofördelar till bröd, men dess effekt på bakning behövde undersökas. Strukturen och egenskaperna hos dessa fibrer analyserades, och de användes sedan i deg och bröd. På så sätt kunde fiberstrukturen och egenskaperna kopplas till skillnader i deg och brödkvalitet. Efter att ha förstått de optimala fiberegenskaperna för att förbättra deg- och brödkvalitet, testades en extraktionsprocess med vatten med tryck och extrema temperaturer kombinerat med en avfärgningsprocess för att öka extraktionsutbytet och förbättra extraktegenskaperna för brödtillämpningar.

Baserat på resultaten hade fibrer med större storlek högre viskositet och vattenhållande förmåga, vilket indikerar dåliga egenskaper vid bakning.

Dessa fibrer gav också bröd med den minsta volymen och hårdaste konsistensen. Däremot resulterade en minskning av fiberstorleken i volymer jämförbara med bröd bakat utan fiber. Fibrer med mer producerad ferulsyra och mindre storlek resulterade i den bästa bröd- och degkvaliteten, vilket stöder potentialen för ferulsyra och storleksminskning att användas i kombination för att förbättra brödkvaliteten.

Resultaten av denna avhandling visar att det finns potential att använda dessa modifierade fibrer som funktionella ingredienser för att öka fiberinnehållet i bröd och samtidigt förbättra brödkvaliteten. Sammantaget ger denna avhandling en omfattande förståelse för hur modifierande veteklifiberegenskaper kan användas för att förbättra funktionaliteten hos veteklifiber i brödtillverkning och därför underlätta användningen av vetekli vid produktion av värdeskapande ingredienser för brödprodukter.

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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; WUAX, water-unextractable 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 β-(1 → 4)-linked β-D-xylopyranose backbone (Fig. 1). Xylose in the backbone can be unsubstituted, mono-substituted in *C*2, mono-substituted in C3, or di-substituted in both *C*2 and C3 by α-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 alkali-extracted 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 cross-linking 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

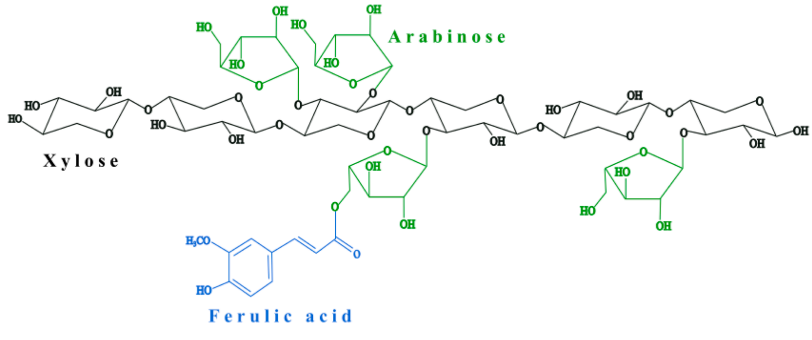

Fig. 1. Schematic diagram of the structure of arabinoxylan, showing the xylose backbone (black), arabinose substitutions (green), and ferulic acid (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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 non-starch 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 cross-feeding 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

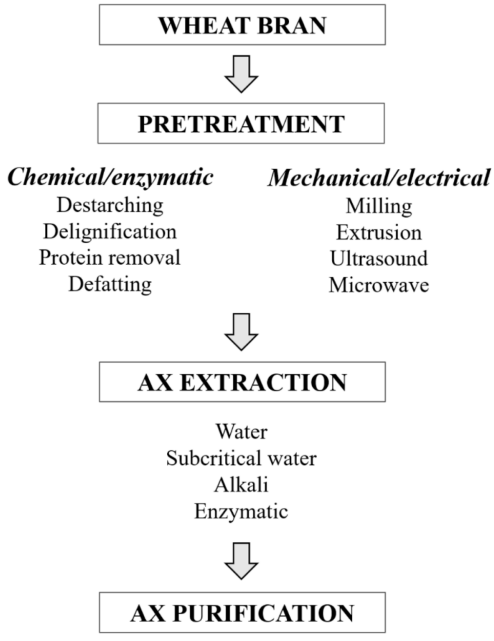

Fig. 2. Flow chart of arabinoxylan (AX) extraction process from wheat bran.

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., 2019; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)	Increases (Rudjito et al., 2019; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020) Decreases (Mathew et al., 2017)	No effect (Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)	Increases (Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)	Decreases (Rudjito et al., 2019; Yilmaz-Turan, Jiménez-Quero, Menzel, et al., 2020)	
Protein removal	Increases (Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)	Decreases (Mathew et al., 2017; Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)				Increases (Yilmaz-Turan, Jiménez-Quero, Moriana, 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-kKaczmarek et al., 2018)	Increases (Caprez et al., 1986)	Decreases (Demuth et al., 2020)
Extrusion		Increases (Fadel, Ashworth, et al., 2018; Roye et al., 2020; Zeitoun et al., 2010)					
Ultrasound		Increases (Ebringerová & Hromádková, 2002; Jiang et al., 2019; J. Wang et al., 2014)		Increases (Hromádková & Ebringerova, 1999; Liu et al., 2020)	Increases (Hromádková & Ebringerova, 1999)		Decreases (Liu et al., 2020)
Microwave		Increases (Jiang et al., 2019)					

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 water-extractable 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 water-soluble. 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 twin-screw 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, Košťálová, & 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-*b*-xylanase (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 by-products (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 microwave-assisted 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 °C for 5 to 10 min. 180 °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 feruloylated 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 °C and pH 7. C. Li et al. (2020) operated SWE at different temperatures (120–180 °C), initial pHs (4–10) and treatment durations (10–120 min). They obtained highest extraction yields using high temperatures (180 °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 β-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

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	Type	Pretreatments	Solid: liquid ratio	Details	Extraction temperature (°C)	Extraction duration	Purification method	AX yield	AX extract purity (% of DW)	A/X ratio	MW (kDa)	FA content (mg g^{-1})
(Demuth et al., 2020)	Water	Destarching, protein removal, β-glucan removal	13:90		60	30 min	Ethanol precipitation	9.3[b]	42	0.48	403	3.0
(Kaur et al., 2021)	Water	Destarching			95	60 min	Ethanol precipitation	4.5[b]				3.5–4.9
(P. Wang et al., 2019)	Water	Destarching, amyloglycosidase, protein 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[a]		0.69		3.3
(Ruthes et al., 2017)	SWE	Destarching			160	15 min		31.2[a]	72	0.49	193	5.6
(Ruthes et al., 2020)	SWE	Destarching			160			27.5[a]	68		337	7.1
(Yilmaz-Turan, Jiménez-Quero, Moriana, et al., 2020)	SWE	Destarching	1:10		160	30 min	Ethanol precipitation, 95%	7.5[a]	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[a]		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, delignification	1:20	1 M NaOH	25	16 h	Destarching, delignification, pre-hydrolysis	11[b]		1		
(Chen et al., 2019)	Alkali	Destarching, protein removal	5:100	0.16 mol/l	80	90 min	Ethanol precipitation	19.8[a]		1.14	210	
(Deralia, Maire, Lund, Larsson, & Str, 2021)	Alkali	Acid hydrolysis, delignification	1:12	1 M NaOH,		16 h	Destarching, protein removal, ethanol precipitation 95 %	13[a]	72	0.77	163	n.d.
(Guo et al., 2018)	Alkali	Destarching, protein removal	1:20	0.26 M NaBH4	65	3 h		6.9[a]	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[b]	73	0.74	458	0.2
(Yan et al., 2019)	Alkali	Destarching, defatting, water extraction		0.625 M sodium hydroxide	85	2.5 h	Ethanol precipitation, 100 %	6.3[a]	85			2.9
(Yilmaz-Turan, Jiménez-Quero, Menzel, et al., 2020)	Alkali	Destarching	1:8	0.5 M	80	16 h		31.1[a]	73	0.7	263	n.d.
(Aguedo et al., 2014)	Enzymatic	Destarching	1:11	Endo-xylanase from *Bacillus subtilis*	55	2 h	Ultrafiltration, 100 kDa	4.2[a]		0.73	12.5	
(Zhou et al., 2010)	Enzymatic	Destarching, protein removal	1:12	150U xylanase	60	2 h	Ethanol precipitation, 65%	12.4[b]		0.56		0.4

[a] Total solid yield % of bran dry weight. [b]AX yield % of bran dry weight.

Table 3
Effect of arabinoxylan (AX) properties (A/X ratio, molar mass of AX (HMW, high molecular weight; LMW, low molecular weight), ferulic acid (FA) content of AX, AX solubility and AX water holding capacity) and AX addition level to bread on dough and bread properties (specific volume, crumb texture, crumb structure, health effects).

	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 dough viscosity (Pavlovich-Abril et al., 2016; M. Wang et al., 2020) and causes uneven mixing (Koegelenberg & Chimphango, 2017); no 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, 3 % changes structure completely (Espinosa-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, 2 and 5 % have no effect (Zhang et al., 2019); 1–3 % increases, over 6 % decreases (Buksa et al., 2016).
Crumb texture		LMW decreases hardness of fresh bread (Buksa et al., 2016; P; Wang et al., 2019), HMW prevents long-term starch retrogradation (P. Wang 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 crumb formation (Buksa & Krystyjan, 2019).		WEAX improves surface smoothness (P. Wang et al., 2019); WUAX had no effect (Ma et al., 2018).		10 % causes coarser structure, 2 and 5 % no effect (Zhang et al., 2019); 2 % improves structure (P. Wang et al., 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 glycemic response, insoluble promotes bowel health (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 β-(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). Water-holding 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 gluten-free 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 crystallization-related 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 7-day 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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Feruloylation and hydrolysis of arabinoxylan extracted from wheat bran: Effect on bread quality and shelf-life

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ABSTRACT

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

1. Introduction

Wheat bran, a low-value side-stream currently used mostly for animal feed, has an estimated annual global production volume of 150 million tons (Prückler et al., 2014). Wheat bran contains many nutritionally interesting components, such as dietary fiber and bioactive compounds, that could be used in food applications, increasing the value of this by-product (Katileviciute et al., 2019). One such compound is arabinoxylan (AX), an abundant dietary fiber component in wheat bran composed of a β-(1 → 4)-linked β-D-xylopyranose backbone substituted with arabinofuranosyl (Darvill et al., 1980). Arabinose can be further linked to ferulic acid (FA) via an ester bond (Izydorczyk and Biliaderis, 1995). Although increased dietary fiber intake would reduce the risk of various nutrition-related diseases, AX is not widely utilized by the baking industry, partly due to its reported negative effect on bread quality (Pietiäinen et al., 2022). New solutions are therefore needed to enable the high fiber addition levels required for nutrition benefits.

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

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

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

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

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

2. Materials and methods

2.1. Materials

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

2.2. Hydrolyzation of arabinoxylan

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

2.3. Characterization of fractions

2.3.1. Monosaccharide composition

The monosaccharide composition of AX fractions was determined using a method developed by Sluiter et al. (2008) with modifications. First, 3 mL 72% H_2SO_4 (Sigma-Aldrich, Stockholm, Sweden) were added to 200 mg of sample, which was kept under vacuum for 15 min and incubated for 1 h at 30 °C with stirring every 20 min. Next, 84 g of water were added and the samples were autoclaved at 125 °C for 1 h. The samples were then vacuum-filtered and diluted to a total volume of 100 mL. Fucose was added as an internal standard and the samples were filtered (0.45 μm). Monosaccharide composition was analyzed using HPAEC-PAD (ICS 3000 Dionex, Thermo Scientific, Sunnyvale, USA) equipped with an AEC column (CarboPac PA 1 analytical 4 × 250 nm, Thermo Scientific, Sunnyvale, USA). All samples were analyzed in duplicate.

2.3.2. Total starch content

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

2.3.3. β-glucan content

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

2.3.4. Protein content

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

2.3.5. Ferulic acid content

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

2.3.6. Molar mass distribution

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

2.4. Breadmaking

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

2.5. Water absorption and dough development time

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

2.6. Bread measurements

Bread parameters were measured on the day of baking (visual

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

2.6.1. Specific volume

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

2.6.2. Crumb texture by texture profile analysis

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

2.6.3. Crumb structure by image analysis

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

2.6.4. Moisture content of crumb and crust

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

2.7. Experimental design and statistical analysis of data

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

3. Results and discussion

3.1. Characterization of fractions

3.1.1. Chemical composition

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

3.1.2. Molar mass distribution

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

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

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

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

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

[a] Total carbohydrate content was calculated based on total content of arabinose, rhamnose, galactose, glucose, xylose, and mannose.
[b] AX content was calculated based on the total content of arabinose and xylose.
[c] Ratio between arabinose and xylose.

Table 2
Molar mass number-average molecular weight (M_n), weight-average molecular weight (M_w), and dispersity index (Đ) of the different fractions analyzed. F = feruloylated; H = hydrolyzed.

	AX	FAX	H-AX	H-FAX
M_n (kg mol^{-1})	200	317	59	24
M_w (kg mol^{-1})	714	698	199	29
Đ	3.6	2.2	3.4	1.2

Table 3
Water absorption (% of flour weight), dough development time (DDT, min), final dough consistency (BU), cell average size (pixel (px)), and specific volume of control bread and breads with different arabinoxylan (AX) fractions and addition levels (1, 2, 5 %). Mean value ± SD. F = feruloylated; H = hydrolyzed.

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

[a] $p < 0.05$ compared with control. .

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

3.3. Visual evaluation of bread

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

3.4. Crumb structure

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

3.5. Specific volume

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

3.6. Bread shelf-life

3.6.1. Moisture content

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

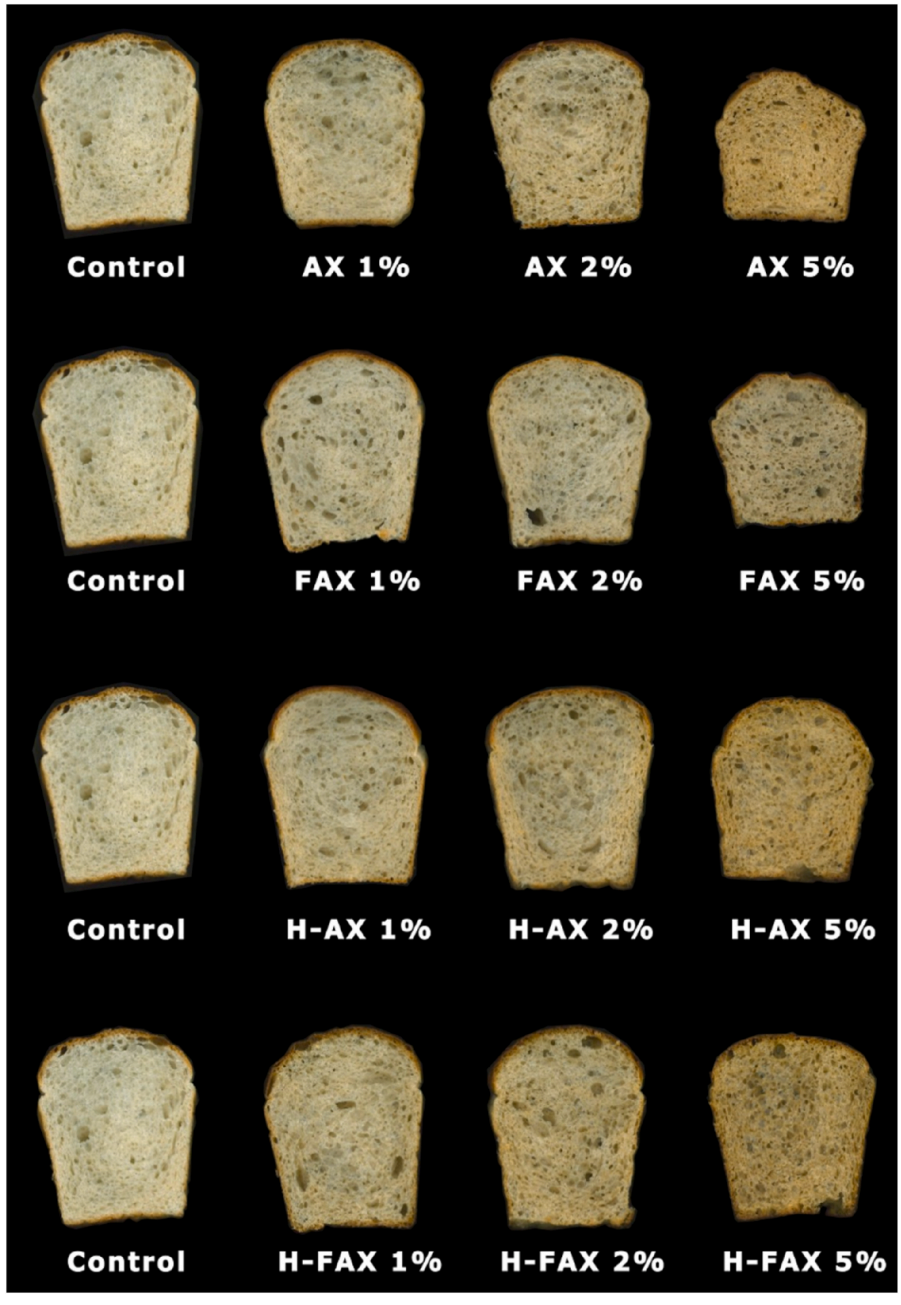

Fig. 1. Images of slices of control bread (left) and breads with different arabinoxylan (AX) fractions at different addition levels (1%, 2%, 5%). F = feruloylated; H = hydrolyzed.

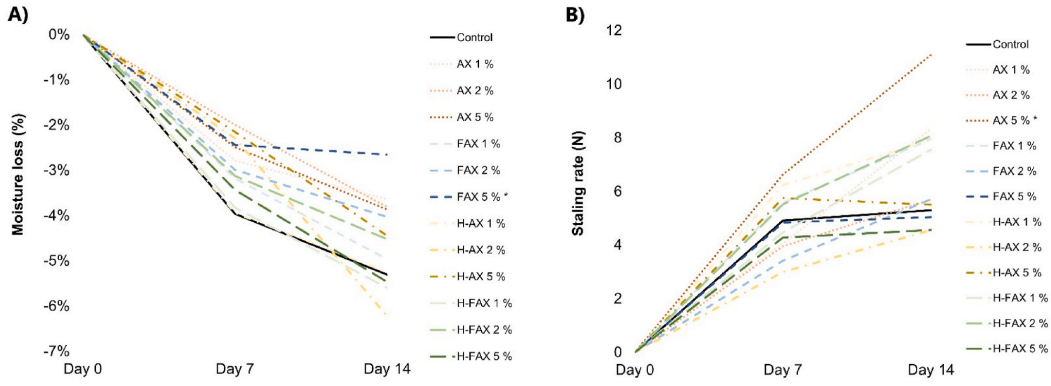

Fig. 2. Changes during the 14-day storage period in (A) crumb moisture content (%) (n = 3) and (B) staling rate of bread crumb (n = 2) in breads made with different arabinoxylan (AX) fractions at different addition levels (1%, 2%, 5%). F = feruloylated; H = hydrolyzed. *p < 0.05 compared with control.

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

3.6.2. Crumb texture

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

3.7. Impact of fraction composition on dough and bread properties

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

Water absorption by optimal wheat bran AX levels has been observed to improve bread quality in some studies (Biliaderis et al., 1995; Buksa et al., 2016). However, based on multivariate analysis, increased water absorption was associated with negative impact on bread quality in the present study (Table 3). Water absorption was positively correlated with the carbohydrate content of fractions, crumb moisture content, crumb hardness, and DDT, and these variables were in turn negatively correlated with specific volume and cell average size. This indicates that excess amounts of water and increased dough viscosity may prevent formation of proper crumb cell structure and hence decrease specific volume and produce harder bread crumb. Molar mass was negatively correlated with specific volume, suggesting that higher molar mass of AX might further increase the amount of excess water in dough and therefore decrease volume. However, differences in water absorption might not be only related to AX structure, as impurities in AX extracts possibly contributed to the baking properties of the fractions (see section 3.2). This theory was supported by the results of multivariate analysis, where β-glucan content was one of the most important fraction properties according to variable importance in projection. This, combined with the high water-holding capacity of β-glucan (Lazaridou and Biliaderis, 2007), indicates that the extreme increase in water absorption might be due to impurities in the AX extracts used in this study.

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

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

4. Conclusions

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

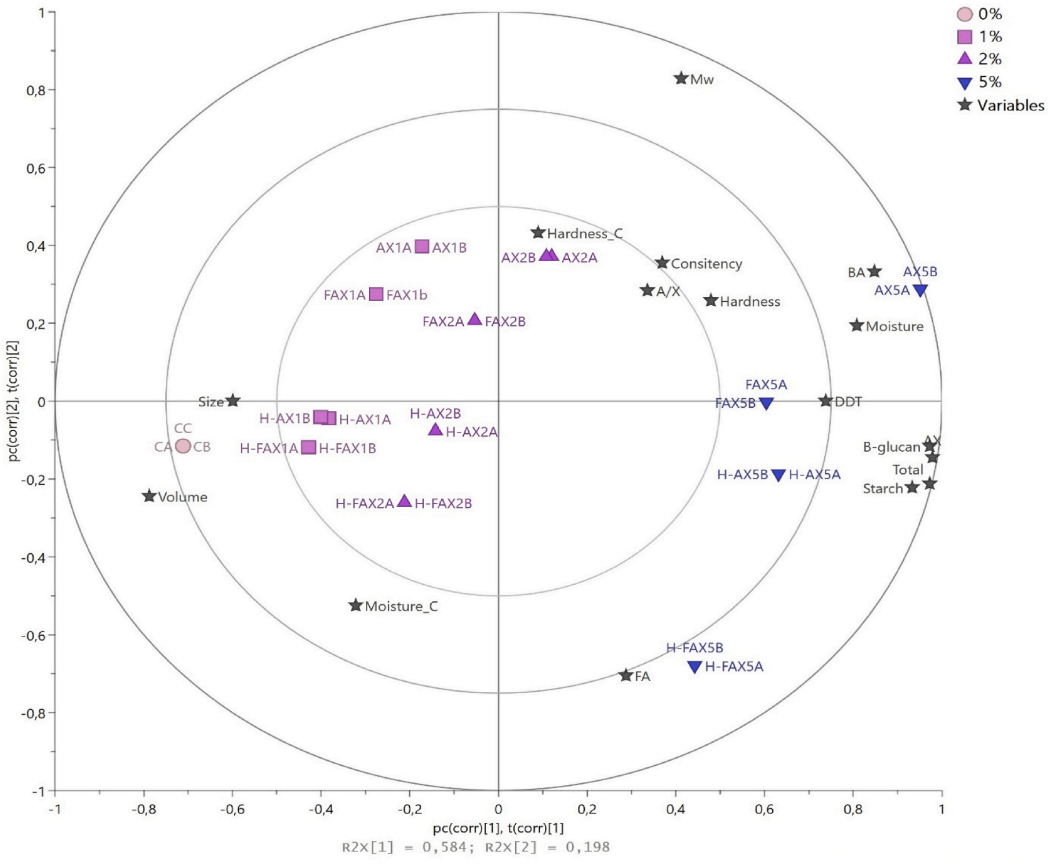

Fig. 3. Partial least squares (PLS) biplot showing variables (★) together with the different samples analyzed, colored based on arabinoxylan (AX) addition level (0% = ●; 1% = ■; 2% = ▲; 5% = ▼, F = feruloylated, H = hydrolyzed). Total = total carbohydrate content, A/X = A/X-ratio, Mw = molecular weight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

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

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Availability of data and material

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

CRediT authorship contribution statement

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

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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

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Article

Feruloylation and Hydrolysis of Arabinoxylan Extracted from Wheat Bran: Effect on Dough Rheology and Microstructure

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Abstract: Feruloylated arabinoxylan (AX) is a potential health-promoting fiber ingredient that can enhance nutritional properties of bread but is also known to affect dough rheology. To determine the role of feruloylation and hydrolysis of wheat bran AX on dough quality and microstructure, hydrolyzed and unhydrolyzed AX fractions with low and high ferulic acid content were produced, and their chemical composition and properties were evaluated. These fractions were then incorporated into wheat dough, and farinograph measurements, large and small deformation measurements and dough microstructure were assessed. AX was found to greatly affect both fraction properties and dough quality, and this effect was modulated by hydrolysis of AX. These results demonstrated how especially unhydrolyzed fiber fractions produced stiff doughs with poor extensibility due to weak gluten network, while hydrolyzed fractions maintained a dough quality closer to control. This suggests that hydrolysis can further improve the baking properties of feruloylated wheat bran AX. However, no clear effects from AX feruloylation on dough properties or microstructure could be detected. Based on this study, feruloylation does not appear to affect dough rheology or microstructure, and feruloylated wheat bran arabinoxylan can be used as a bakery ingredient to potentially enhance the nutritional quality of bread.

Keywords: rheology; arabinoxylan; hydrolysis; ferulic acid; dough

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1. Introduction

Wheat bran is a low-value by-product form wheat processing that has an estimated annual global production volume of 150 million tons [1]. It is currently utilized mostly as animal feed even though it contains many health-promoting components, such as dietary fiber, that could be used as food ingredients to increase the value of wheat bran side stream [2]. Arabinoxylan (AX) is an abundant dietary fiber component in wheat bran composed of a β-(1→4)-linked β-D-xylopyranose backbone substituted with an arabinofuranosyl group [3]. Arabinose can be further linked to ferulic acid (FA) via an ester bond [4], and feruloylated AX has been shown to have antioxidant properties even after baking and fermentation [5,6]. This suggests that feruloylated AX could offer further health benefits when added to bread, as food antioxidants and their anti-inflammatory properties have been linked to the prevention of cardiovascular diseases and cancer [7]. Despite the availability of wheat bran and the well-known health benefits of increased dietary fiber intake, wheat bran AX is currently not utilized as a bread ingredient partly due to its

negative effect on bread quality [8]. Like many fibers, also AX can have a detrimental effect on bread quality, often leading to decreased volume and denser bread crumb especially with fiber addition levels of 5% or above [8]. New approaches are therefore required to enable the use of feruloylated wheat bran AX as a bread ingredient.

We have recently demonstrated how hydrolysis and feruloylation of wheat bran AX can improve its properties as bread ingredient, as feruloylated and hydrolyzed arabinoxylan produced the bread closest to control in volume and crumb structure [9]. This indicates that modifying AX structure and properties in terms of feruloylation and hydrolysis can help improve the quality of bread with incorporated fiber. However, the effect feruloylation and hydrolysis of AX on bread quality remains unclear as contrary results exist. The mechanical properties of the dough govern the quality of bread [10] and understanding of dough quality can therefore provide valuable insights into the bread-baking quality of feruloylated AX. Some studies suggest that FA can strengthen gluten network via covalent cross-linking [11–14] when others have observed addition of FA to reduce the amount of disulfide bonds and glutenin macropolymer content [15] and increase dough firmness with lower concentrations [16]. Wang et al. [17] observed an improvement in dough extensibility with external ferulic acid, 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. However, many of the previously mentioned studies used free FA rather than feruloylated AX. Previous research has demonstrated that reducing molar mass through hydrolysis is crucial for AX functionality in dough [18,19], but to our knowledge its connection to feruloylation and their combined effect on AX dough rheology and microstructure has not been studied previously. More knowledge on the connection between AX feruloylation, hydrolysis, and dough properties are therefore needed to understand the effect of AX structure on dough quality and hence facilitate use of feruloylated AX as a bread ingredient.

The aim of this study was to determine the effect of partial hydrolysis and feruloylation of wheat bran AX on wheat dough rheology and microstructure. Unhydrolyzed and hydrolyzed AX with high and low levels of FA were incorporated in dough. Dough water absorption, development time and stability were determined using a farinograph and dough extensibility and rheological characteristics were measured in large and small deformations. Microstructure of doughs was observed using light microscopy. This work provides new insights into how feruloylation and hydrolysis of AX affect dough properties and microstructure and therefore enable the use of wheat bran AX as a functional bread ingredient to enhance the technological and nutritional quality of bread with incorporated fiber.

2. Materials and Methods

2.1. Materials

Feruloylated wheat bran arabinoxylan (FAX) was supplied by Lantmännen (Stockholm, Sweden). The process to produce this fraction in pilot scale has been previously detailed by Zhang et al. [5]. Wheat flour (Special Vetemjöl, Lantmännen, Sweden) was bought from local supermarket. All chemicals and reagents were purchased from Sigma-Aldrich (St. Luis, MO, USA) and were analytical grade, unless otherwise stated.

2.2. Preparation of Arabinoxylan Fractions

2.2.1. Removal of Ferulic Acid

To produce a fraction without ferulic acid (AX), original FAX fraction was subjected to mild saponification using a process from Zhang et al. [5] with modification. FAX was mixed with 0.5 M NaOH and sample was stirred 4 h 20 °C. After saponification, pH was adjusted to pH 7 with 0.5 M acetic acid. AX was precipitated with ethanol (99.6%, 1:4 v/v) by stirring for 2 h followed by cooling overnight (4 °C). Arabinoxylan was filtered through a porous metal plate (40 µm) washed 3 times with 75% (v/v) ethanol and freeze dried.

2.2.2. Hydrolysis of Arabinoxylan

To produce hydrolyzed fractions (H-FAX and H-AX), FAX and AX were enzymatically hydrolyzed using a process previously described and optimized by Ruthes et al. [20]. First the fraction were first solubilized in water by using a solid:liquid ratio of 1:10, adjusting pH to pH 5 using 0.5 M acetic acid and then heating to 60 °C under constant mixing. Then Pentopan Mono BG, a 1,4-β-xylanase (2500 FXU-W/g, Novozymes, Lyngby, Denmark) was added at 20 U/g AX. After incubation for 24 h in 37 °C, the enzyme was inactivated by heating to 100 °C for 5 min and fractions were then freeze dried.

2.3. Characterization of Fraction Composition

2.3.1. Monosaccharide Composition and Klason Lignin Content

The monosaccharide composition was determined from AX fractions as previously described by Lu et al. [21] with modifications. 1 mL 72% H_2SO_4 was added to 70 mg of sample and kept under vacuum for 1.5 h in 20 °C. After incubation, 29 mL of water was added, and the samples were autoclaved at 125 °C for 1 h. The samples were then vacuum filtered with 10 mL of hot water and diluted. For reference, a standard solution containing glucose, arabinose, xylose, rhamnose and mannose was prepared the same way as samples. Monosaccharide composition was analyzed using HPAEC with a pulsed amperometry detector (ICS 3000 Dionex, Thermo Scientific, Sunnyvale, CA, USA) equipped with an AEC column (CarboPac PA1 guard 4 × 50 mm and CarboPac PA 1 analytical 4 × 250 mm, Thermo Scientific, Sunnyvale, CA, USA). Klason lignin content was determined as the acid-insoluble residue after hydrolysis.

2.3.2. Basic Nutrient Composition

The protein content of fractions was measured by their total N content using the Dumas combustion method in triplicate with a factor of 6.25 applied to calculate the protein content. Ash content was assessed in triplicate following the AACC total ash method [22] The total starch content of the AX fractions was determined using a resistant starch assay kit (Resistant Starch Assay Kit (Rapid), Megazyme Ltd., Wicklow, Ireland), with the total starch content calculated as the sum of resistant and digestible starch. The β-glucan content was measured in triplicate with a β-glucan assay kit (Mixed Linkage Assay Kit, Megazyme Ltd., Wicklow, Ireland).

2.3.3. Ferulic Acid Content

The ethanol-soluble free and conjugated ferulic acid, as well as the ethanol-insoluble esterified bound ferulic acid content of fractions, were extracted and quantified as previously described by Li et al. [23] with modifications. All samples were prepared in triplicates. 100 mg of sample was extracted with 80% ethanol, sonicated for 10 min, and the supernatant was collected after centrifugation. This process was repeated three times, and the combined supernatants were evaporated under nitrogen (extract A). For conjugated ferulic acid, dried extract A was hydrolyzed with 800 µL 2 M NaOH (Merk KGaA, Darmstadt, Germany) and incubated for 16 h after oxygen removal. The pH was then adjusted to pH 2 using 12 M HCl, and the conjugated ferulic acids were extracted with ethyl acetate three times (extract B). For the bound ferulic acid, the residual pellet obtained after extraction with 80% ethanol, was hydrolyzed with 2 M NaOH (Merk KGaA, Darmstadt, Germany) followed by 16 h incubation at 20 °C. After centrifugation, the collected supernatant was adjusted to pH 2 with 12 M HCl followed by the addition of approximately 30 mg of NaCl (≥99.5%, Merk KGaA, Darmstadt, Germany), and then extracted with ethyl acetate three times (extract C). All ferulic acid extracts were evaporated with nitrogen, re-dissolved in 10% methanol and centrifuged (14,000 × *g*, 15 min, 20 °C) using Amicon filter (0.5 mL, 10 K, Merck Millipore Ltd., Co., Cork, Ireland) before analysis. Extract A was used as is for free ferulic acid, extract B for conjugated ferulic acid and extract C for bound ferulic acid.

The ferulic acid standard was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) and the stock solution was prepared in methanol (1 mg/mL). The stock

solution was diluted with Milli-Q water and utilized for preparation of the calibration solution. Ferulic acid content was quantified using an ACQUITY ultra high-performance liquid chromatography system with a photodiode array detector (UPLC-PDA, Waters, Milford, MA, USA) at a wavelength of 320 nm. Separation was conducted using an ACQUITY UPLC HSS T3 column (1.8 µm, 2.1 × 150 mm, Waters) connected with a VanGuaurd precolumn (2.1 × 5 mm, Waters), at 40 °C. Mobile phases A and B were 0.5% formic acid in Milli-Q water and Acetonitrile, respectively. The flow rate was 0.5 mL/min, and the linear gradient was as follows: 0 min, 90% A; 10 min, 80% A; 14 min, 10% A, 15 min, 10% A; 16 min, 90% A with a re-equilibrum time of 4 min. The data was processed by Empower 3 (Waters) and Excel (Microsoft).

2.4. Characterization of Fraction Properties

2.4.1. Molar Mass Distribution

Molar mass distribution was analyzed using size exclusion chromatography (SEC) (SECurity 1260, Polymer Standard Services, Mainz, Germany), following the method from Ruthes et al. [20].

2.4.2. Water Holding Capacity of Fractions

Water holding capacity (WHC) of fractions was determined in triplicate according to method described previously by Hemdane et al. [24] with modifications. 0.5 g of sample was weighted into a tube with 5 mL of water, and left to room temperature for 60 min after mixing. Samples were then centrifuged (10,000× *g* 10 min at 20 °C). The supernatants were separated and pellets were turned upside down for 15 min to remove excess water. WHC was expressed as g of water retained by 1 g of dry matter.

2.4.3. Rheological Properties of Fractions

Rheological properties of fractions were determined from AX solutions that were prepared by mixing fractions with water (4% *w*/*v*) and stirring continuously at 60 °C for 2 h. Rheological properties were measured using a Discovery HR-3 rheometer (TA Instruments, New Castel, DE, USA) with a 40 mm cone plate. Flow curves were obtained at a shear rate range from 0.01 to 1000 1/s.

2.5. Dough Quality

2.5.1. Water Absorption, Dough Development Time (DDT) and Dough Stability

Water absorption, dough development time (DDT) and dough stability were measured for wheat flour and mixtures of flour and 5% of arabinoxylan fractions with a farinograph (Brabender GmhH, Duisburg, Germany) following AACC method 54-21.01 [25]. 5% addition level was chosen based on our previous study on the effect of feruloylated and hydrolyzed AX fractions on bread quality [9], as lower addition levels did not result in clear differences in bread quality. Fractions were added by replacing flour with arabinoxylan based on fractions' AX content. The fractions were premixed with water and heated to 80 °C under constant stirring, and then cooling to room temperature before dough preparation. Water absorption was defined as the amount of water required to reach 500 BU and expressed as percentage of flour weight. DDT was defined as the time from water addition to the dough reaching peak consistency.

2.5.2. Large Deformation Rheological Measurements

Uniaxial extension measurements at large deformations were performed using a texture analyzer (TA-XT Plus, Stable Micro System, Surrey, UK) equipped with a Kieffer dough/gluten extensibility rig (Stable Micro System, Surrey, UK). Control dough was prepared using only flour and water. Doughs containing AX fractions were prepared by adding 5% of fraction by replacing flour based on fractions' AX content. The fractions were dispersed in water prior to dough preparation by stirring while heating to 80 °C and cooling to room temperature. Doughs were then mixed in farinograph using optimal

water absorption and DDT obtained from farinograph results. After mixing in farinograph, doughs were gently rolled into balls and placed relaxing in incubator 30 °C for 20 min. After relaxing, a piece of dough was cut from the center of the dough ball and rolled gently into a sausage shape and placed into an oiled molder and compressed into dough strips. The molder containing the dough strips was placed back into incubator for 40 min 30 °C. After relaxing, a dough strip was removed from the molder and clamped between the plates of the Kieffer rig prior to each test. The samples were tested using 2.0 mm/s test speed and 75.0 mm distance. 6 dough strips were tested from each dough.

2.5.3. Small Deformation Rheological Measurements

Small deformation rheological measurements were performed by oscillation test using a Haake MARS 40 rheometer (ThermoScientific, Sunnyvale, CA, USA) with parallel-plate geometry (35 mm). The doughs were prepared the same way as for dough extensibility test (Section 2.5.2). Dough piece was placed between the plates and pressed to 1 mm gap. Then excess dough was removed and 200/50 cS fluid (Dow Corning Corporate, Midland, MI, USA) was applied to sample edges to prevent sample drying. After 2 min resting time, an oscillation test was done using 0.01% strain based on the linear viscoelastic region of the samples at 25 °C in the frequency range of 0.05–50 Hz. The rheological characteristics were expressed as the storage (G′) and loss modulus (G″).

2.5.4. Dough Microstructure

Dough samples (5 × 5 × 5 mm) were cut from doughs prepared for small deformation rheological tests (Section 2.5.3) and fixated in glutaldrehyde (2.5%) for 24 h. Samples were then dehydrated with a series of ethanol in increasing concentrations and infiltrated and hardened using Technovit 7100 (KULZER, Hanau, Germany). Hardened samples were sectioned into 5 µm sections with a ultramicrotome (Leica Microsystems GmbH, Leica EM UC6, Wetzlar, Germany), stained with light green and examined with a microscope (Nikon, Ecplise Ni-U, Tokio, Japan) equipped with a 40× (0.75 NA) apochromatic objective. Images were captured with Nikon Digital Sight DS-Fi2 camera (Nikon, Japan).

2.6. Experimental Design and Statistical Analysis of Data

All measurements were conducted at least in duplicate, with results reported as mean values ± standard deviation. For dough and fraction properties, type-III ANOVA was used to identify differences at a 95% confidence level, determined by Tukey's pairwise comparisons. The relationship between fraction and dough properties was examined using 2-tailed Pearson correlation with linear relationships evaluated through regression analysis. All data analyses were performed using R (version R 4.3.0, The R Foundation for Statistical Computing, Vienna, Austria), unless otherwise stated.

3. Results & Discussion

3.1. Fraction Composition

Chemical composition of fractions is presented in Table 1. The fraction modification with hydrolysis or saponification did not affect relative AX content ($p > 0.05$), and AX content was 70.7–73.0% of total carbohydrates for all fractions. However, the amount of total carbohydrates did vary between fractions, with FAX having the highest AX content of 75.9%, followed by AX, H-AX and H-FAX with AX contents of 69.4, 67.5 and 56.2%, respectively. H-FAX had a relatively low amount of carbohydrates compared to other fractions. The results of monosaccharide composition are known to be closely related to the hydrolysis process [26], indicating that sample material might have affected the acid hydrolysis of H-FAX. This difference in AX content should be taken into account when considering results for dough quality. The fractions were added to doughs based on their AX content, so doughs with H-FAX also contained higher levels of other fraction components compared to other fractions. The A/X ratio decreased from 0.36 for FAX to 0.26–0.27 for H-FAX, AX and H-AX, indicating that processing of fractions decreased the

amount of arabinose substitution. The ash content was 4.7 and 4.4 for FAX and H-FAX, and 7.1 and 7.3 for AX and H-AX, respectively, indicating that ash content was increased by alkali treatment, as reported previously by Rasool et al. [27]. Glucose, starch, protein and Klason lignin contents were similar between fractions.

Table 1. Carbohydrate, arabinoxylan (AX), starch, mixed linkage β-glucan, Klason lignin, protein, and ash content of the fractions (dwb). Mean value ± SD. F = feruloylated; H = hydrolyzed.

	FAX	H-FAX	AX	H-AX
Total carbohydrates (g/100 g) [1]	75.9 ± 6.6 [a]	56.2 ± 13.3 [b]	69.4 ± 2.6 [b]	67.5 ± 0.8 [b]
AX % [2]	70.7 ± 2.1 [a]	71.1 ± 0.0 [a]	73.0 ± 0.2 [a]	72.0 ± 0.1 [a]
Glc % [3]	23.1 ± 2.8 [a]	26.4 ± 0.1 [a]	24.5 ± 0.2 [a]	25.6 ± 0.1 [a]
Gal % [3]	3.0 ± 0.4 [a]	2.5 ± 0.2 [a]	2.5 ± 0.0 [a]	2.5 ± 0.0 [a]
A/X [4]	0.4 ± 0.1 [a]	0.3 ± 0.0 [a]	0.3 ± 0.0 [a]	0.3 ± 0.0 [a]
Mixed linkage β-glucan (g/100 g)	5.6 ± 0.6 [a]	5.3 ± 0.2 [a]	6.0 ± 1.3 [a]	5.9 ± 0.1 [a]
Starch (g/100 g)	0.8 ± 0.0 [a]	1.0 ± 0.0 [a]	0.7 ± 0.0 [a]	1.1 ± 0.1 [a]
Klason lignin (g/100 g)	3.3 ± 1.0 [a]	2.6 ± 1.7 [a]	2.0 ± 1.0 [a]	3.4 ± 1.1 [a]
Protein (g/100 g)	2.7 ± 0.0 [a]	2.6 ± 0.0 [a]	1.7 ± 0.0 [b]	1.7 ± 0.0 [b]
Ash (g/100 g)	4.7 ± 0.0 [a]	4.4 ± 0.0 [a]	7.1 ± 0.0 [b]	7.2 ± 0.0 [b]

[1] Total carbohydrate content was calculated based on total content of arabinose, rhamnose, galactose, glucose, xylose, and mannose. [2] AX content was calculated based on the % of arabinose and xylose of total carbohydrate content. [3] % of total carbohydrate content. Glc = glucose, Gal = galactose. [4] Ratio between arabinose and xylose. Different letters indicate significant differences ($p < 0.05$).

FA content of fractions is presented in Figure 1. FAX had the highest amount of FA, 11.2 mg/g, of which almost all were bound FA. Hydrolysis reduced the amount of FA slightly to 8.8 mg/g for H-FAX. For H-FAX, 44% of FA was conjugated, indicating that hydrolysis has converted a portion of the insoluble FA bound to AX into ethanol-soluble conjugated FA. This is likely due to enzyme activity, which hydrolyzes xylose backbone into smaller AX fragments with attached FA. This process can increase ethanol solubility of FA, and therefore increases the proportion of conjugated FA. Fractions with low FA content still contained traces of FA, which indicates that pilot scale saponification was not able to remove FA as efficiently as previously [9]. However, the FA extraction was more detailed and contained 3 extraction steps instead of one, which might also affect the amount of FA detected in samples. Saponification reduced the total FA content to 2.0 and 1.5 mg/g for AX and H-AX, respectively. Like H-FAX, also H-AX contained less bound and more conjugated FA compared to unhydrolyzed AX. In this study, the focus was only on the differences in total amount of FA and the differences in the type of FA were not considered. However, bound and free FA have been shown to differ in their antioxidant capacity [15], indicating that there could even be differences in the functionality between conjugated and bound FA in breadmaking.

3.2. Fraction Properties

Fraction properties in terms of molar mass, WHC and solution viscosity are presented in Table 2. Molar mass was expectedly reduced by hydrolysis, and weight average molar mass reduced from 485 to 67 for FAX and 464 to 95 kg mol^{-1} for AX. Molar mass correlated strongly with both viscosity and WHC of fractions (Appendix A), indicating that hydrolysis decreases both solution viscosity and WHC. The correlation between molar mass and both WHC and viscosity has been widely reported [12,28,29], and the strong tendency of high molar mass AX to absorb water has been shown to play a key role in its functionality in dough. Buksa et al. [29] reported that relative viscosities were positively correlated with the molar mass of arabinoxylan fractions.

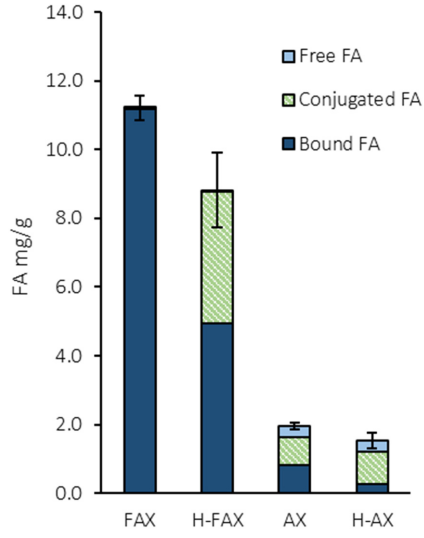

Figure 1. Ferulic acid content of fractions (dwb). Different letters on each bar indicate significant difference ($p < 0.05$). F = feruloylated; H = hydrolyzed.

Table 2. Fraction properties in terms of molar mass (number-average molecular weight (Mn), weight-average molecular weight (Mw), and dispersity index (Đ), solution viscosity (mPa.s) and water holding capacity (WHC). Different letters indicate significant difference ($p < 0.05$). F = feruloylated; H = hydrolyzed.

	FAX	H-FAX	AX	H-AX
Mw (kg mol^{-1})	485	67	464	95
Mn (kg mol^{-1})	126	37	218	17
Đ	3.8	2.6	2.1	3.9
Viscosity (mPa.s) [1]	100 ± 18 [a]	39 ± 1.0 [b]	149 ± 44 [a]	30 ± 8.9 [b]
WHC [2]	3.1 ± 0.1 [a]	0.7 ± 0.2 [b]	3.3 ± 0.2 [a]	0.9 ± 0.1 [b]

[1] At 1/s. [2] g H_2O/g dry sample.

3.3. Dough Properties

3.3.1. Water Absorption, Dough Development Time (DDT) and Dough Stability

Farinograph results for control dough and doughs prepared with 5% fiber fractions are presented in Table 3. Fiber addition increased water absorption and DDT, and decreased dough stability with all fractions ($p < 0.05$). Hydrolysis decreased water absorption of FAX and AX from 78.5 and 83.3 to 64.5 and 63.3%, respectively ($p < 0.05$). Similar to WHC, also farinograph water absorption correlated strongly ($p < 0.001$) with molar mass and viscosity of fractions (Appendix A). This effect has been shown previosuly by Biliaderis et al. [19].

Table 3. Water absorption (% of flour weight), dough development time (DDT, min) and dough stability (min) of sample doughs. Mean value ± SD. F = feruloylated; H = hydrolyzed.

	Control	FAX	H-FAX	AX	H-AX
Water absorption (%)	61.0 ± 0.7	78.5 ± 0.6 ***	64.5 ± 0.8 *	83.3 ± 1.3 ***	63.3 ± 0.9
Development time (min)	2.8 ± 0.8	7.0 ± 1.4 *	4.9 ± 0.2	7.8 ± 1.0 *	6.2 ± 0.2
Dough stability (min)	5.2 ± 0.2	2.6 ± 0.1 **	2.1 ± 0.2 **	2.4 ± 0.6 **	4.1 ± 0.4

p-values below 0.05 (*), 0.01 (**) and 0.001 (***) indicate statistically significant differences at the 95%, 99% and 99.9% conficende level compared to control, respectively.

DDT of FAX and AX was also decreased from 7.0 and 7.8 to 4.9 and 6.2, respectively, and DDT was also found to correlate with molar mass, viscosity and WHC of fraction (Appendix A). Notably, H-FAX had the DDT closest to control despite the high content of other fraction components in H-FAX doughs due to lower AX content in fraction, as discussed in Section 3.1. DDT represent the time required to develop a dough with optimal consistency, and the increased DDT has been linked to the higher water absorption, leading to competition for water between arabinoxylan, starch and gluten, and therefore delaying gluten network formation [30]. H-AX was the only fraction that did not reduce dough stability time (DST) ($p > 0.05$).

3.3.2. Large Deformation Rheological Measurements

Large deformation extensional measurements, while limited in providing fundamental rheological information, are crucial for assessing the strength of wheat flour due to the large deformations encountered during dough processing, such as mixing, sheeting, and baking [31]. Extension curves for control dough and doughs prepared with 5% fiber fractions are presented in Figure 2, where maximum force (N) represents dough resistance to extension and distance to break (mm) represents doughs extensibility. Fiber addition decreased dough extensibility and increased resistance to extension compared to control, indicating that fiber addition increases dough firmness and produces weaker doughs. Hydrolyzed fractions H-FAX and H-AX showed higher extensibility compared to their unhydrolyzed counterparts FAX and AX. As dough extensibility is usually related with dough strength [17], these results show that fiber addition weakened the doughs, and this effect was more severe for unhydrolyzed fractions. Fiber incorporation has been previously shown to decrease extensibility of dough due to breakage of the starch-gluten matrix, restricting the retention of gas in the gluten network and preventing gluten agglomeration [20,32]. As extensibility was also found to correlate negatively with molar mass, viscosity and WHC of fractions (Appendix A), these results further support the existing evidence that the higher WHC, water absorption and viscosity of unhydrolyzed fractions disrupt the gluten network to a larger extent compared to their hydrolyzed counterparts.

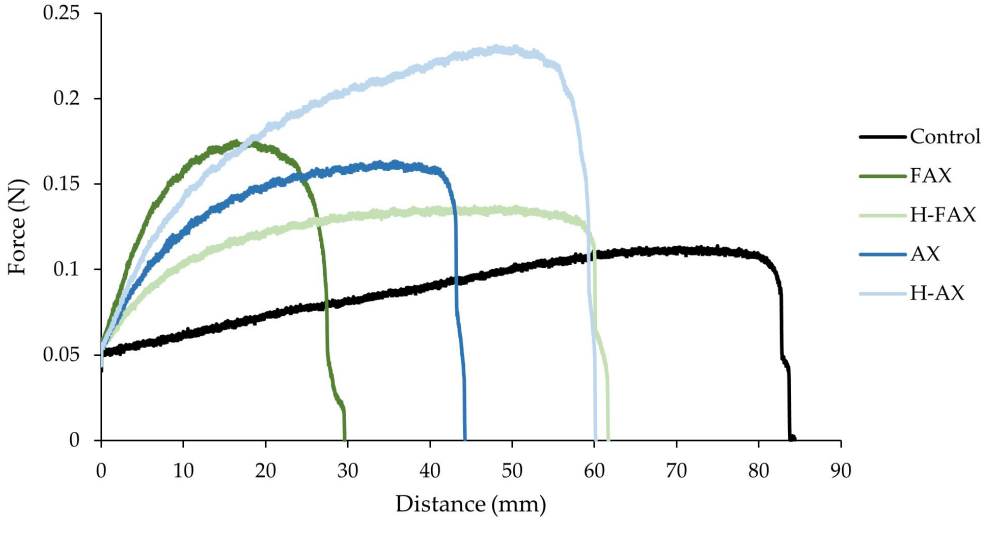

Figure 2. Extension curves with measured force (N) as a function of distance (mm) for control dough and doughs prepared with 5% fiber fractions. F = feruloylated; H = hydrolyzed.

H-AX showed the highest resistance to extension but was still able to maintain a similar extensibility compared to control while increasing resistance to extension, doughs prepared with H-AX being firm but relatively strong compared to other fractions. These results were in line with the farinograph results (Section 3.3.1), where H-AX improved the dough stability compared to other fractions. The other hydrolyzed fraction H-FAX was closest to control in terms of resistance to extension and extensibility. This was in line with results from previously published baking trails using similar fractions [9], where H-FAX produced a bread comparable to control at the 5% addition level. This indicates that hydrolyzed fractions disrupt the starch-gluten matrix less compared to their unhydrolyzed counterparts. Hydrolyzed AX fractions might also promote the connectivity of the gluten network by AX-starch-gluten interactions as suggested previously by Li et al. [33]. Even though Wang et al. [17] observed wheat bran AX with low ferulic acid content directly, no difference between feruloylated and unferuloylated fractions was observed in this study.

3.3.3. Small Deformation Rheological Measurements

An oscillation test was performed to provide more fundamental information about the effect of arabinoxylan incorporation on the viscoelastic properties of wheat dough. Figure 3 shows the frequency sweep (G′ and G″) curves of control dough and doughs enriched with AX fractions. Dough is a complex system showing both elastic and adhesive behavior [17]. We found the storage modulus (G′) for all doughs tested to be higher than the loss modulus (G″), showing a predominant elastic behavior. Addition of unhydrolyzed fractions increased G′ compared to control, while hydrolyzed fractions H-FAX and H-AX decreased G′ compared to control. G′ was also correlated with molar mass, viscosity and WHC of fractions (Appendix A). G′ describes the materials ability to store deformation energy in an elastic manner, with higher G′ indicating higher mechanical rigidity. These results indicate that unhydrolyzed AX increases the dough stiffness, while hydrolyzed AX increases dough softness. The addition of AX and bran particles in general has been previously observed to increase the storage modulus of dough [33], which has been linked the high WHC of AX leading to water immobilization during dough resting and hence a stiffer dough [24]. The loss modulus (G″) is associated with dough flow properties, such as extensibility and adhesiveness [17], and higher extensibility is linked to increased dough strength. For G″, sample doughs followed a similar pattern compared to G′, unhydrolyzed fractions having the highest values and hydrolyzed fractions the lowest, but the loss modulus was not correlated to fraction properties.

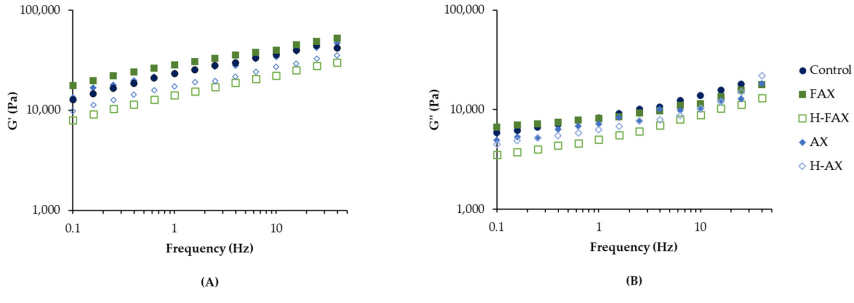

Figure 3. Dough rheological properties from frequency sweep test. Storage modulus G′ (**A**) and loss modulus G″ (**B**) as a function of frequency at 25 °C for doughs with and without fiber incorporation.

Small deformation measurements did not directly correlate with the large deformation measurements. This was somewhat expected as in small deformation measurements within the frequency range used in this study (0.05–50 Hz), it has been suggested that protein-protein interactions crucial for dough properties are partly masked by starch-starch and starch-protein interaction [31]. It is therefore likely that the large deformation measurements

represent more the protein-protein interactions and the small deformation measurements starch-starch and starch-protein interaction. No clear effect from AX feruloylation on small-scale deformation could be detected between fractions. Even though some authors have seen FA to increase dough softness and therefore decrease the negative effect of fiber addition on dough, Snelders et al. [16] observed that a FA content of 0.1–1.7% was not high enough to see any positive effects from addition of AX oligosaccharides. Therefore, minimal effect could be expected with the low FA amounts in the AX fractions used in this study.

3.3.4. Dough Microstructure

To visualize changes in dough as an effect of fiber incorporation, doughs were visualized using light microscopy, and gluten was stained green to confirm the expected disturbance of gluten network. Captured images from dough samples are presented in Figure 4. For the control dough with no added fiber, the gluten network occupied almost all areas between starch granules and left almost no unstained background area. For unhydrolyzed fractions FAX and AX, the gluten network seemed more disrupted compared to control, and large portion of areas between starch granules were left unstained. Also, longer non-protein and non-starch particles, that were expected to be residual bran particles from AX fractions, were visible in FAX and AX doughs. The presence of these particles might indicate that residual bran particles in unhydrolyzed AX fractions might also cause physical hindrance to dough formation, as previously reported by Molina et al. [34]. In doughs with hydrolyzed fractions H-FAX and H-AX, the amount of unstained background was less than for hydrolyzed fractions, supporting the explanation that hydrolyzed fractions have less effect on the gluten network formation than unhydrolyzed fractions. Previously Zhu et al. [30] have reported AX incorporation in dough to increase the amount of unstained background in light microscopy. This is supported by Frederix et al. [32], who have shown that supernatant viscosity of AX enriched batters affects gluten agglomeration and the impact was more severe for high molar mass AX than low molar mass AX. Döring et al. [35] observed the incorporation of 5% AX in dough to inhibit protein network formation and cause protein agglomeration. They suggested that increased water content leads to dilution of dough and therefore proteins with less stretching ability, also previosuly described by Jekle and Becker [36].

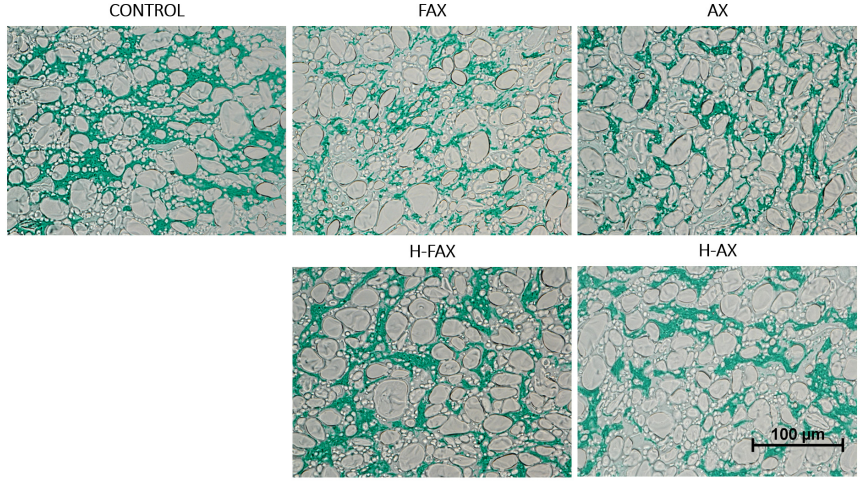

Figure 4. Dough microstructure for control dough and doughs with fiber fractions. Protein stained light green. Magnification 40×. Pixels: 975 × 731.

4. Conclusions

Inclusion of wheat bran AX was observed to greatly affect the rheology of wheat dough. This effect appeared to be modulated by hydrolysis of AX, which, in turn, correlated with changes in fraction properties. Hydrolysis of AX fractions decreased the molar mass, solution viscosity and water holding capacity of the AX fractions. Additionally, it resulted in reduced water absorption and dough development time, while showing higher extensibility compared to their unhydrolyzed counterparts FAX and AX. Based on large deformation measurements, fiber addition decreased dough extensibility and increased resistance to extension compared to control, with a more profound effect observed for unhydrolyzed fractions. Addition of unhydrolyzed fractions increased and hydrolyzed fractions decreased G' compared to control, suggesting that unhydrolyzed AX increases the dough stiffness on small deformations. Results from both large and small deformation measurements demonstrate how especially unhydrolyzed fiber fractions produced firm and stiff doughs with poor extensibility, attributed to fiber disturbing formation of gluten network. Notably, fraction properties such as molar mass, viscosity and water holding capacity strongly correlated with dough extensibility and storage modulus, indicating their role in increasing viscosity and water holding by high molar mass AX. Light microscopy indicated that hydrolyzed fractions might disturb the gluten network less compared to unhydrolyzed fractions FAX and AX. While H-FAX produced doughs closest to control in terms of water absorption, dough development time and extensibility, no clear effects from AX feruloylation on dough properties or microstructure could be detected. Based on this study, feruloylation does not affect dough quality and feruloylated wheat bran arabinoxylan can be utilized as a bakery ingredient to potentially improve the quality of bread with incorporated fiber. However, differences in fraction composition make drawing firm conclusions difficult and further work is needed to evaluate the importance of other fraction compounds to the observed results.

Author Contributions: S.P.: Conceptualization, Methodology, Investigation, Validation, Formal analysis, Visualization, Project administration, Writing—Original draft. Y.L.: Methodology, Investigation, Writing—review & editing. A.J.-Q.: Investigation, Writing—review & editing. H.H.: Methodology, Writing—review & editing. K.K.: Conceptualization, Supervision, Resources, Writing—review & editing. N.H.M.: Supervision, Resources, Writing—review & editing. A.M.: Conceptualization, Supervision, Writing—review & editing. M.L.: Resources, Funding acquisition, Conceptualization, Supervision, Writing—review & editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: Author Solja Pietiäinen and Annelie Moldin were employed by the company Lantmännen. The contribution of the author Solja Pietiäinen in the paper is Conceptualization, Methodology, Investigation, Validation, Formal analysis, Visualization, Project administration, Writing—Original draft. The contribution of the author Annelie Moldin is Conceptualization, Supervision, Writing—review & editing. The authors declare that this study received funding from Lantmännen. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A

Table A1. Pearson's correlation coefficient matrix between fraction and dough properties. Total= fraction total monosaccharide content, A/X = fraction A/X ratio, AX = fraction AX content, FA = fraction ferulic acid content, Mw = weight average molar mass of fractions, Viscosity = Fraction viscosity, WHC = fraction water holding capacity, RE = dough resistance to extension, E = dough extensibility, WA = farinograph water absorption, DDT = dough development time, DST = dough stability time.

	Total	AX	A/X	FA	B-Glucan	Lignin	Mw	Viscosity	WHC	RE	E	G′	G″	WA	DDT
AX	1.00 ***														
A/X	−0.69	−0.71													
FA	−0.40	−0.42	0.74 *												
B-glucan	−0.11	−0.12	0.05	−0.33											
Lignin	0.39	0.36	0.06	0.20	0.22										
Mw	0.23	0.24	0.18	0.14	0.19	−0.14									
Viscosity	0.28	0.30	−0.01	−0.10	0.41	−0.23	0.88 **								
WHC	0.22	0.23	0.13	0.10	0.15	−0.22	0.99 ***	0.90 ***							
RE	0.46	0.45	−0.12	−0.43	0.18	0.15	0.18	0.01	0.12						
E	−0.18	−0.17	−0.41	−0.43	0.02	−0.17	−0.85 **	−0.61 *	−0.81 **	−0.21					
G′	0.41	0.41	0.15	0.25	0.36	0.18	0.87 **	0.70 *	0.81 **	0.32	−0.79				
G″	0.23	0.22	0.28	0.34	0.44	0.43	0.52	0.47	0.41	0.16	−0.48	0.81 **			
WA	0.25	0.26	0.03	−0.03	−0.37	−0.29	0.97 ***	0.95 ***	0.97 ***	0.23	−0.72 *	0.74 *	0.18		
DDT	0.08	0.09	0.10	−0.36	0.02	−0.33	0.80 *	0.81 *	0.82 *	0.58	−0.63	0.59	0.09	0.79 *	
DST	0.29	0.28	−0.29	−0.54	0.51	0.45	−0.32	−0.35	−0.31	0.67	0.13	−0.06	0.05	−0.43	0.03

p-values below 0.05 (*), 0.01 (**) and 0.001 (***) indicate statistically significant differences at the 95%, 99% and 99.9% confidence level, respectively.

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Wheat bran is a milling side stream that could be used as an ingredient to improve the nutritional quality of bread and sustainability of cereal processing. The aim of this thesis was to investigate the relationship between the properties of modified wheat bran arabinoxylan extracts and their functionality in breadmaking. The results showed the potential of modified arabinoxylan extracts as functional fibre ingredients, and therefore help to facilitate the use of wheat bran in production of health-promoting bread ingredients.

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