Journal of Biomed Research

Zhu et al., J Biomed Res.
2022;3(1):30-36.

Mini Review

Utilizing wheat arabinoxylans as a potent functional biomaterial for fabrication of hydrogels: A mini review

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Received date: August 11, 2022
Accepted date: August 22, 2022

Citation: Zhu Q, Wang P, Zhang M. Utilizing wheat arabinoxylans as a potent functional biomaterial for fabrication of hydrogels: A mini review. J Biomed Res. 2022;3(1):30-36.

Abstract

Arabinoxylans (AXs) are the major structural cell wall component of various cereal brans. As an important dietary fiber source, AXs contain ferulic acids, proteins and other functional ester compounds. These functional components offer AXs well-established health benefits, such as prebiotic effect, antioxidant property, antitumor activity and immunomodulatory property. In addition, the presence of ferulic acids could trigger the covalent cross-linking of AX molecules under oxidative conditions, and therefore AX could be used as a promising functional biomaterial for fabrication of hydrogels. The formed covalent gels are resistant to harsh digestive condition, which could be developed as colon-targeted delivery system for food and biomedical application. This article aims to summarize the extraction methods of wheat arabinoxylans, discuss the covalent gelling mechanism of AX, and review the potential application of AX in food and biomedical industries.

Keywords: Wheat arabinoxylans, Extraction methods, Gelling mechanism, Colon-target delivery system

Introduction

Wheat bran is an abundant industrial by-product of wheat milling operation, about 10~25% bran is inevitably produced during the processing of wheat products. It has been reported that the annual output of wheat bran could reach 3000 tons in China, and it has exceeded 200 million tons from a global perspective [1]. Most wheat brans (about 85%) are used for winemaking, animal feed and papermaking, and the utilization of deep process and high-efficiency industries are rare [2]. As shown in **Figure 1**, wheat bran is composed of outer and inner pericarp, testa, hyaline layer and aleurone layer. The inner and outer pericarps are composed of xylans, lignin and cellulose [3]. High contents of dietary fiber (around 50%) and varied phytochemicals (e.g. vitamins, phenolic acids, minerals) in the bran and aleurone make wheat bran have high health benefits [4]. In addition, wheat bran is abundant, low-cost natural source, and therefore wheat bran has a great potential for the development of novel healthy food products.

Wheat bran arabinoxylan is an important dietary fiber, accounting for more than 80% of wheat non-starch polysaccharides. It has been reported that AX can be selectively degraded in the intestinal tract to produce arabinoxylan oligosaccharides (AXOS), short-chain fatty acids (SCFA), and other health-promoting bioactive factors, which is beneficial for promoting the growth of intestinal microorganisms [4,5]. The presence of functional groups makes AXs possess health-promoting benefits, such as enhancing body's immune system [6], regulating the lipid and glucose metabolism and antitumor effects [7]. The viscosity of AX can affect their fat-binding capacity and easily improve the postprandial blood glucose response [8-9]. The presence of ferulic acid on the side chain of AXs make them a natural antioxidant and excellent immune system enhancer [10]. Overall, there is a huge application potential of AXs in food, cosmetic, and medical industries.

In addition to bioactive functions, arabinoxylans have some unique physicochemical properties, such as rheological properties, water holding capacity and oxidative cross-linking (gelling) properties.

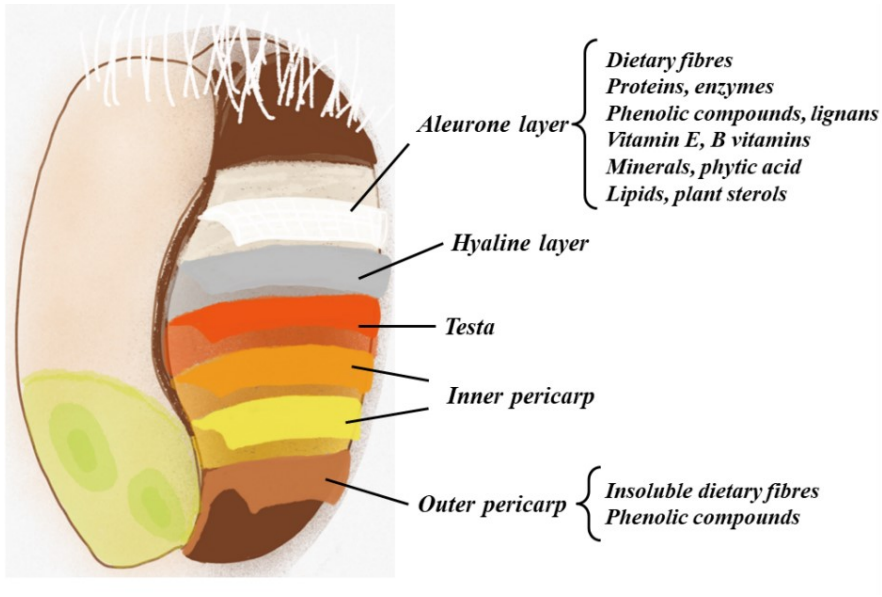

Figure 1: Wheat grain, showing consists of wheat bran. Adapted from Brouns et al. (2012).

It is worth noting that the extraction methods of AXs have a significant effect on their fine structure and subsequent functional properties, and suitable extraction methods are necessary for obtaining arabinoxylan with desired properties. This article aims to summarize the extraction methods of wheat bran arabinoxylan and compare their structural difference. More importantly, the gelling property of AXs has been discussed to provide the theoretic basis for broadening their application in varied fields.

Isolation of Wheat Arabinoxylans

Arabinoxylans have a complex structure, consisting of a linear skeleton of β-(1→4)-linked D-xylopyranosyl (Xylp) units, through the O-3 of xylose residues and/or O-2,3 position is connected with α-L-arabinofuranosyl substituent (Araf). Due to the diverse forms of xylose residues at O-2 and O-3 positions, they may be unsubstituted (uX), monosubstituted (mX) or disubstituted (dX) [11], with a small amount of α-D-glucuronic acid or 4-O-methyl-glucuronic acid, galactose pyranose, and xylose pyranose present on the side chain of AXs. Generally, arabinoxylans are divided into water-extractable arabinoxylan (WEAX) and water unextractable arabinoxylan (WUAX). WEAX accounts for only a minority in wheat bran, which is about 6% of the total AX content [12]. WUAX could interact with protein, lignin and other components via hydrogen bonds and covalent ether bonds, making it insoluble in water and more difficult to extract from wheat bran [2,13].

The extraction method could directly affect the monosaccharide composition, molecular weight and substitution degree of AXs. As shown in **Table 1**, the molecular weight of wheat bran arabinoxylans ranges from 10 kDa to 10,000 kDa by using different extraction methods under different conditions [2,14-18]. The substitution degree of AX is expressed by the ratio of arabinose to xylose (Ara/Xyl). The A/X ratios of wheat bran arabinoxylan are approximately in the range of 0.4-0.9 [2,14-18]. In addition, the ethanol concentration also affects the monosaccharide composition of AX. According to previous study, wheat brans underwent 20%-80% ethanol fractionation treatment could obtain AX fractions with Ara/Xyl ratios ranging from 0.31 to 0.85 [19].

Extraction process of AXs includes raw material pretreatment, extraction, separation, and purification and final freeze-drying treatment. After milling and sieving process, wheat brans are treated with heat-stable α-amylase to remove the starch. The current extraction methods are roughly divided into chemical treatment, enzyme treatment and physical treatment. The most common extraction method is the alkaline method, which has better selectivity and higher extraction yield. Zhou et al. found that the peroxide-assisted alkaline extraction was 50% more effective than enzymatic treatment for AX extraction [20]. Alkaline treatment could improve the solubilization of hemicellulose, but a stronger alkaline solution treatment would destroy some functional groups of AX. For example, hydroxycinnamic acid and acetic acid could partially or completely disappear after alkaline treatment, and the esterification degree of the fraction is lower [21,22]. The physical extraction technologies, including extrusion, ball milling, steam, ultrasonic treatment, subcritical water extraction, microwave treatment [19,23-25], could be used for AX extraction. Mechanical processes are efficient, while the energy input required for the equipment is high and the reaction conditions are strict. Hell et al. have compared the difference between several extraction methods including the alkaline extraction method, ball milling method and extrusion method [23]. It has been reported that hydrogen peroxide has the highest solubilization effect on AX, increasing by 105%. Although the ball milling method results in low yield, it has high specificity to AX and the extraction process is green. As for the extrusion method, it has high selectivity but low extraction rate. Overall, the ball milling tends to be the best method. Recently, subcritical water extraction (SWE) had drawn researchers' much attention, due to its green process and high extraction efficiency [24]. It has been stated that AX extracted by SWE method owns higher molecular weight and more ferulic acid content, which is beneficial for enhancing the cross-linking ability of AX molecules [2]. The enzymatic method is more environmentally friendly than the

Table 1: Extraction of wheat bran arabinoxylan with varied structural characteristics.

Source	Pretreatment	Extraction condition	Separation and purification condition	Yield (%)	Total AX (%)	Mw (kDa)	A/X ratio	Reference
Water unextractable solids of wheat bran	Destarch	1 M NaOH (1:10, w/v)	3 volumes of ethanol precipitation	54.0	≈69	76	0.69	Li et al., 2020 [2]
Wheat bran	Destarch	0.5 M NaOH, 150 rpm, 4 h (1:6, w/v)	3 volumes of ethanol precipitation	3.7-5.6	68-72	1250-1490/3.57-3.83	0.46-0.54	Wang et al., 2019 [14]
Water unextractable solids of wheat bran	Destarch	0.5 M $Ba(OH)_2$, 16 h (1:10, w/v)	3 volumes of ethanol precipitation,	48.0	0.36	70	0.78	Li et al., 2020 [2]
Wheat bran	Destarch	Endoxylanase, 55°C/24h hydrothermal treatment	4 volumes of ethanol precipitation	19.5	62.5	12/160	0.52	Aguedo et al., 2014 [15]
Wheat bran	Destarch	$RuCl_3$/Al-MCM-48/180°C	——	——	78.0	9	0.53	Sánchez-Bastardo et al., 2017 [16]
Wheat bran	Destarch	Endoxylanase, 55°C/24 h vacuum filtration	membranes ultrafiltration	4.2-4.3	77.8-78.3	5/12.5	0.68-0.73	Aguedo et al., 2014 [15]
Wheat bran	Destarch, deprotein	Sunzymes, 180 W ultrasound bath	——	14.3	0.62	——	——	Wang et al., 2014 [17]
Wheat bran	——	Electrostatic separation	50μm/4000Pa air jet sieving	8.0	0.43	——	——	Wang et al.2015 [18]
Water unextractable solids of wheat bran	Destarch	160°C, Subcritical water extraction	3 volumes of ethanol precipitation	——	≈66.0	39	0.52	Li et al., 2020 [2]

chemical method, while the enzymatic reaction requires a specific and strict environment. In addition, the high price of enzymes requires a constant search for alternatives [23]. Since each method has its pros and cons, in future, more efforts should be focused on to explore novel extraction technique, in order to enhance the extraction efficiency, improve the quality of extracted arabinoxylan, reduce the cost and guarantee the green extraction process.

Gelation Mechanism of Wheat Arabinoxylans

AX with ferulic acid can form gel under the action of oxidizing agents such as laccase, peroxidases, hydrogen peroxide. In the early stage of AX gelation, ferulic acids are oxidized and gradually disappeared, transforming into ferulic acid dimers (di-FA), and ferulic acid trimers (tri-FA). The oxidant could attack the hydrogen atoms of the hydroxyl group on the ferulic acid ring to obtain a resonance-stable (C-4, C-5, C-8) phenoxy group, and then the unpaired electron coupling of free radicals (phenoxy/alkoxy) forms the covalent bond to connect two adjacent arabinoxylan chains, contributing to the gelation of AX molecules [26,27]. As shown in **Figure 2**, five forms of ferulic acid dimers including 5-5', 8-5' benzo, 8-8', 8-O-4', 8-5' could be generated during AX gelation [28]. In addition, during laccase coupling reaction, the conformation of AX converts into a more ordered and closer-packing structure with higher degree of crystallinity as well as an increased molecular weight. During the process of O_2/laccase-induced cross-linking of wheat AX gelation, 8-5' accounts for 80%, and tri-FA in the form of 4-O-8' and 5'-5"-dehydroferric acids are also present [26]. Han et al. reported that after 2 h of gelation, around 70%-96% esterified FA were oxidized [29]. The eFAs were transformed into 8-5' Benzofuran dimers, 8-O-4' and 8-5' diFA. It was worth noting that the covalent cross-linking process of AX mainly depended on the AX structure, especially the position and contents of FA [30]. A high proportion of 5-5' di-FA in the AX gel could lower the effective elasticity and further affected the gel performance [31]. Zhang et al. found that the increase in contents of decarboxylated 8-5' di-FA favored the formation of AX gels [32]. Overall, there is insufficient research investigating the influence of formed di-FA and tri-FA on the gel performance of AX gel, which will be an important research subject on elucidating the gelling mechanism of AX in future studies.

In fact, the gelling process of AX is a complex system, and it depends on several factors including the molecular weight, concentration, ferulic acids, A/X ratios and so on [2,14,27,33-35]. As shown in **Table 2**, AX with different structural properties exhibit varied gelling properties. Generally, arabinoxylans with higher molecular weight (Mw), ferulic acids and purity are easier to

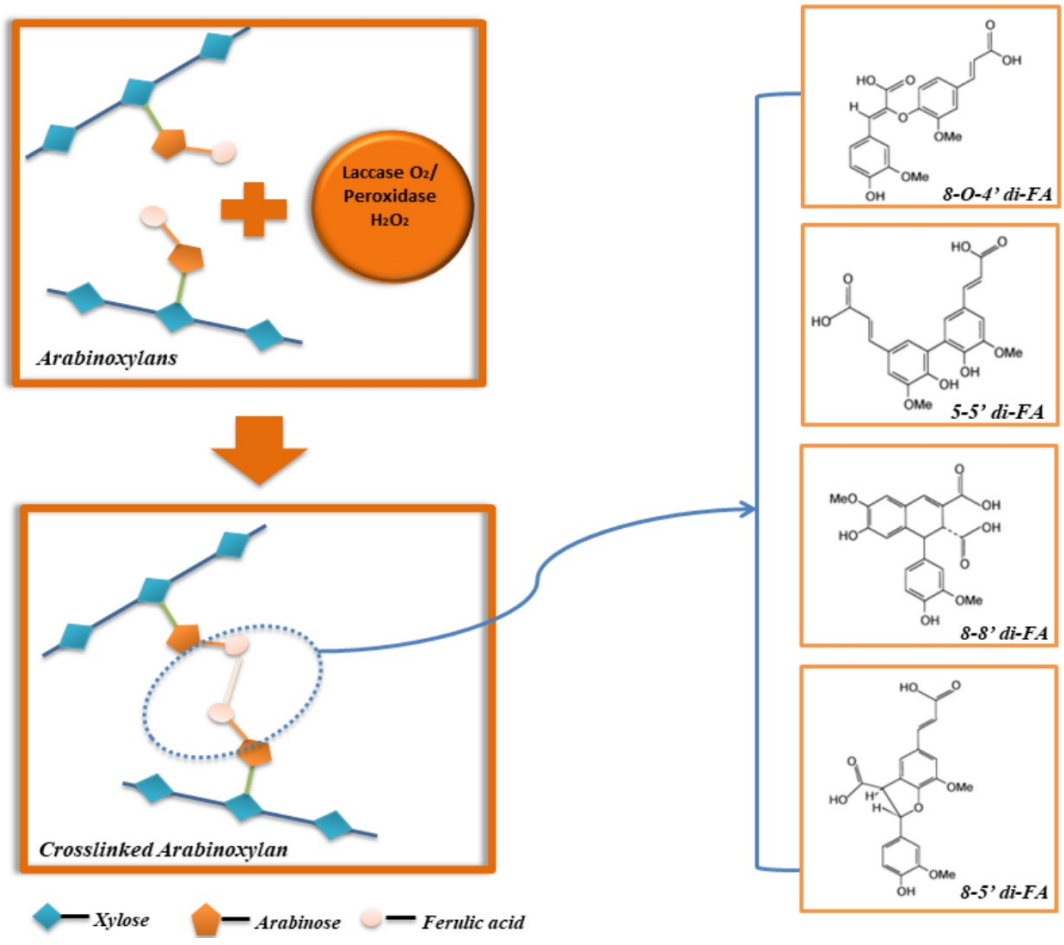

Figure 2: The transformation of FA into various di-FA during covalent crosslinking of AX.

Table 2: Changes in the structure and composition of AX during laccase triggered gel formation.

Source	Phenolic acid profile		AX content (%)	Oxidation system	G′/G″ (Pa) /tanδ	Mw (kDa)	A/X ratio	Reference
	FA content (μg/mg)	Diferulic acids (di-FA)						
Wheat bran AX	2.17	8-O-4'/8-5'	6	Laccase	9800-10000/2200-5000/≤0.8	——	——	Khalighi et al., 2019 [26]
Wheat bran AX	6.22-7.39	——	5	Laccase	77-184/64-96/0.52-0.83	1250-1490 /3.57-3.83	0.46-0.54	Wang et al., 2019 [14]
Wheat bran AX	14.2	8-O-4'/5-5', 8-5'	2	Laccase	100-200//0.01-0.02	39	0.52	Li et al., 2020 [2]
Wheat bran AX	0.006-0.009	——	5-6	Laccase	——	66-77	0.76-0.83	Berlanga-Reyes et al., 2011 [32]
Wheat bran AX	0.008	——	5	Laccase	177/20/0.11	74	0.8	Berlanga-Reyes et al., 2014 [33]
Wheat endosperm AX	0.526	——	2	Laccase	31//0.160	504	0.66	Morales-Ortega et al., 2014 [34]

form hydrogels. Berlanga-Reyes et al. found that prolonged alkaline extraction time could reduce the FA contents and A/X ratios, and thereby hampering the cross-linking ability of extracted AX and reducing the prepared gels' hardness [32]. More recent studies by Wang et al. [14] and Han et al. [29] also confirmed that AX with higher FA contents and Mw showed a higher oxidative cross-linking property under the action of laccase, and the obtained gels had denser network structure and higher viscoelastic modulus.

Application of Wheat Arabinoxylans Gels

AX, as a dietary fiber, are resistant to digestive enzyme and can be degraded and fermented by microorganism in the colon [36]. Therefore, covalent AX hydrogels could be developed for novel colon-specific delivery system for hydrophilic drugs and bioactive compounds (**Figure 3**). It has been reported that the structure feature of AX gels could significantly affect their degradation property in the colon. AX gel with a higher cross-linking density and more compact structure could slow down the degradation of three-dimensional gel structure when they are fermented in the intestine by *B. longum* and *B. adolescentis* [37]. In comparison to AX-free culture substrate, the AX gel samples produce significantly higher short chain fatty acids (SCFAs) during fermentation. It has been stated that during the degradation of AX gels, partial AX chains could be converted to SCFAs such as acetic acids, propionic and butyric acids by the *Bifidobacterium* mix. Therefore, AX gels can be well developed for designing controlled delivery system activated by the colon microbiota. Carvajal-Millan et al. have fabricated AX gels as oral delivery system for insulin, to develop a painless therapy for diabetics [38]. It has been reported that the proteolysis of insulin encapsulated in AX gels is reduced to 17% in comparison to pure insulin solution, and the AX gels allow the release of insulin in the colon. The water-extractable arabinoxylan (WEAX) hydrogels are prepared via laccase treatment to encapsulate the protein drug model bovine serum albumin (BSA) [30]. The diffusion coefficient of BSA from AX gels tends to decrease with the increase in cross-linking degree and AX concentrations, and therefore the release property of protein drug could be controlled by regulating the structure of AX hydrogels. In a more recent study, arabinoxylan was used as biomaterial to prepare pH-responsive microgel for BSA delivery [39]. The obtained results showed that only 14.53% BSA was released in simulated gastric fluids, while 66.64% BSA was released in intestinal solutions at pH7.4, indicating that AX microgels had superior controlled release properties of BSA. In addition, small molecules caffeine could be encapsulated in AX gels, and the gels were stable in the stomach acid condition [40]. Overall, these studies have showed the great potential for development of AX gel as delivery system for hydrophilic drugs, which would broaden its practical application in biomedical field.

In recent years, arabinoxylan based gels have been widely investigated and developed to encapsulate the functional nutrients which are unstable and easily degraded during storage, processing and human digestion process. Morales-Ortega et al. have successfully developed AX gels as delivery system for the probiotics *B. longum*, and it has been reported that the presence of probiotics could affect the crosslinking of AX and results in the a slight reduction of AX gel elasticity [34]. Carvajal-Millan's research team has fabricated a series of AX based gel particles for probiotic entrapment using electrospray technique [41-42]. The simulated gastrointestinal digestion results show that the prepared AX gel particles are not degraded in the stomach and small intestinal, which is beneficial for colon targeted delivery of probiotics. Previous study by Hernández-Espinoza et al. has reported that arabinoxylan gel could encapsulate lycopene, and the AX concentrations are the dominant factor modulating lycopene release. The increase in AX concentration could decrease the diffusion coefficient of lycopene [43].

Except for development of delivery system, the utilization of AX-based gel system as biomaterial have drawn researchers' much attention more recently. Proteins and AX are used as substrates to fabricate interpenetrating polymer network (IPN) gel, due to more compact gel structure and strengthened mechanical properties [44,45]. Khan et al. have employed the hydrothermal method to fabricate AX-graphene oxide based functional composite hydrogels for bone tissue engineering [46]. Graphene oxide could increase AX gels' mechanical property. In addition, the fabricated composite gel has antibacterial activity and favored for cell viability and proliferation, which could be used for wound healing.

Conclusion and Future Trend

Wheat bran arabinoxylan and its gel formulations are of high nutritive values, which is beneficial for development of both functional foods and biomedical products. The extraction method and the subsequent structural composition of obtained AX significantly affect their gel forming capacity, and therefore the structure-function relationship should be investigated more clearly to illustrate the potential gelling mechanism. More importantly, more eco-friendly extraction technique should be explored and established to improve the extraction efficiency, and to avoid the loss of functional components (e.g. ferulic acids, proteins, etc) from the commonly-used alkaline extraction method. Due to the fact that AX could be degraded by the colonic bacterial enzymes, AX based gels have huge

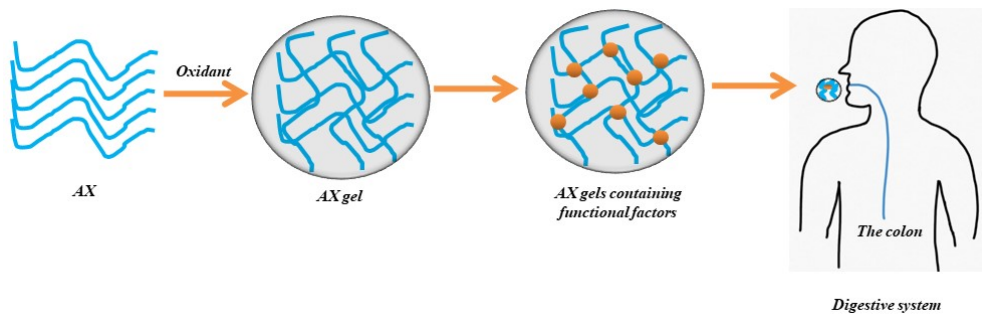

Figure 3: AX gel as a nutrient delivery vehicle.

potential to be designed as an ideal carrier for colon-specific delivery of protein/peptide drugs and bioactive compounds. However, pure AX gels have poor mechanical property and uncontrolled porous gel structure, which may significantly affect their delivery performance. In addition, the poor mechanical property of AX gel also could limit its application in tissue engineering. In future, fabrication of AX based interpenetrating polymer network gel system or addition of micro/nano-polymer as reinforcing materials can provide a simple and valid strategy to strengthen the composite gel structure.

Author Contribution Statement

QZ and MZ conceived, proofread and wrote the manuscript. PW reviewed and revised the manuscript.

Acknowledgments

This work was financially supported by Natural Science Foundation of Tianjin (20JCQNJC00080), Tianjin "131" Innovative Talent Team Project (201926).

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