

Communication

Feruloylated Arabinoxylans from Maize Distiller's Dried Grains with Solubles: Effect of Feruloyl Esterase on their Macromolecular Characteristics, Gelling, and Antioxidant Properties

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Received: 14 October 2019; Accepted: 12 November 2019; Published: 16 November 2019



Abstract: Distiller's dried grains with solubles (DDGS) are co-products of the maize ethanol industry. DDGS contains feruloylated arabinoxylans (AXs), which can present gelling, antioxidant, and health-promoting effects. However, AXs presenting high ferulic acid (FA) content can exhibit delayed fermentation by the colonic microbiota. Therefore, partial deferuloylation of AXs from DDGS while preserving the polysaccharide gelling and antioxidant properties could add value and favor the sustainable development of bioethanol plants. The aim of this work was to partially deferuloylated AXs from DDGS using feruloyl esterase and to evaluate the polysaccharide macromolecular characteristics, gelling, and antioxidant properties. The AXs presented FA and FA dimer contents of 3.27 and 0.30 $\mu\text{g}/\text{mg}$ polysaccharide, respectively, which decreased to 1.26 and 0.20 $\mu\text{g}/\text{mg}$ polysaccharide, respectively, in feruloyl esterase-treated AXs (FAXs). The molecular weight and intrinsic viscosity of FAXs were slightly less than those of AXs. The Fourier transform infrared spectroscopy data of AXs and FAXs were similar, confirming that the enzyme did not modify the polysaccharide molecular identity. FAX gels (2% w/v) exhibited a decrease in elasticity by 43% in relation to that of AXs gels. The antioxidant capacity of FAXs was reduced by 32% and 43% (DPPH and ABTS method, respectively), compared with that of AXs. The FAX gelling and antioxidant properties were -comparable to those reported for other AXs in the literature. Feruloyl esterase may offer an interesting approach for the design of functional FAXs as value-added products recovered from DDGS.

Keywords: cereal co-products; polysaccharides; ferulic acid; gels; antioxidants

1. Introduction

Maize is the largest source for bioethanol as a transportation fuel in areas such as the United States, the European Union, and China [1]. One of the significant co-products of the maize-based bioethanol generation is the distiller's dried grains with solubles (DDGS) fraction, which is usually utilized as a feed ingredient in animal production. DDGS have been previously investigated as a potential source of arabinoxylans (AXs) [2,3]. AXs are formed by a linear chain of β -1,4 xyloses, which may or may not be substituted by α -1,3 and α -1,2 arabinose units. Consequently, there may be three different forms of substitution: unsubstituted, mono-substituted, and di-substituted. The arabinose to xylose ratio (A/X) or degree of substitution refers to the number of arabinose units attached to the xylose backbone [4]. AXs can contain ferulic acid (FA) in their structure in such a way that some arabinose residues are ester-linked on O-5 to FA (Figure 1) [5]. The FA in AXs allows these polysaccharides to gel in the presence of specific oxidizing agents. AXs undergoing oxidative gelation was first reported by Durham [6] and Fausch et al. [7], demonstrating that the FA bonded with AXs is involved in this

oxidative gelation. The gelling mechanism of AXs may implicate chemical (FeCl_3) or enzymatic (laccase and peroxidase/ H_2O_2) oxidizing agents, which induce the formation of phenoxy free radicals [8,9]. The coupling of these phenoxy free radicals results in the formation of dimers and trimers of FA, which allows the chains of polysaccharides to cross-link, forming an aqueous gel [9,10]. Laccase produce the oxidative cross-linking of phenolic compounds employing molecular oxygen as the final electron acceptor [11]. The AX gels are neutral, colorless, and odorless. Due to their covalent nature, these gels have interesting characteristics, such as high water absorption capacity and pH, temperature, and ionic stability. These characteristics and their meso- and macroporous structure make AX gels highly suitable for the formulation of delivery systems of drugs in the colon [12]. AX gels present some advantages when compared to other polysaccharide gels. AX gels are quickly formed; their cross-links are much stronger and are stable when subjected to heat and pH changes and do not exhibit syneresis after prolonged storage [4]. The functional properties of AXs are related to their structural characteristics, such as their arabinose to xylose ratio (A/X), molecular weight (M_w) and FA content. In this regard, several studies have focused on investigating the relative influence of each structural characteristic on AX properties by varying the AX source. In particular, the FA content has been considered to be of significant importance in AX gelling and in their antioxidant capability [13,14]. AXs extracted from DDGS can present health-promoting effects [2], which could add value and favor the sustainable development of bioethanol plants. Nevertheless, it has been reported that highly feruloylated AXs and FA-cross-linked AXs present a reduced rate of fermentation by colonic microbiota [15,16]. Therefore, partial deferuloylation of AXs extracted from DDGS while preserving the polysaccharide gelling and antioxidant properties would allow for the efficient utilization of this co-product. In this regard, partially deferuloylated AXs presenting sufficient gelling capabilities would be attractive, for example, for the preparation of efficient colon-targeted delivery systems exhibiting antioxidant characteristics.

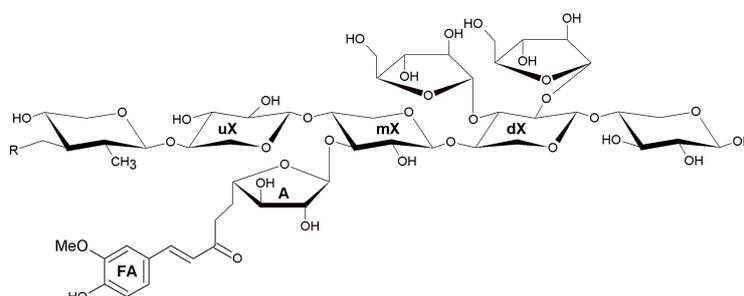


Figure 1. Basic structure of feruloylated arabinoxylans. Arabinose (A); ferulic acid (FA); unsubstituted xylose (uX); monosubstituted xylose (mX); disubstituted xylose (dX).

Previous studies have reported the chemical modification of the FA content in AXs [12,17]. However, compared with chemical modification, enzymatic modification of the FA content in polysaccharides has the advantages of high specificity, high efficiency, and few side effects [18]. The use of feruloyl esterase to reduce the FA content in AXs extracted from ragi and wheat flours has been previously studied [19]. Those authors only reported a decrease in the relative viscosity of feruloyl esterase-treated AXs under peroxidase exposure. In this context, to the best of our knowledge, the modification of the FA content in AXs from DDGS using a feruloyl esterase has not been reported elsewhere. This work aimed to investigate the partial deferuloylation of FA in AXs from DDGS and to evaluate its effect on polysaccharide gelling and their antioxidant properties. This strategy could represent an alternative for the design of functional AXs as value-added products for the food and biomedical industry as well as an opportunity in sustainable food waste utilization.

2. Materials and Methods

2.1. Materials

AXs were extracted from DDGS given by a feed supply manufacturer in the Northwest in Mexico. All chemical products and laccase (E.C. 1.10.3.2) from *Trametes versicolor* were bought from Sigma Chemical Co. (St Louis, MO, USA). Feruloyl esterase enzyme (E.C. 3.1.1.73) was a kind gift from Biocatalysts Limited (Cardiff, UK).

2.2. Methods

2.2.1. Extraction of Feruloylated Arabinoxylans (AXs)

AXs were extracted as previously reported [3]. In brief, DDGS (500 g) was treated with 2500 mL of absolute ethanol to remove the lipophilic components (100 rpm, 25 °C, 12 h). The DDGS/ethanol mixture was filtered (2.7 µm), and the recovered DDGS was then boiled in 3500 mL of water for 30 min and again filtered (2.7 µm). Afterward, 2500 mL of 0.5 N NaOH was mixed with the DDGS at 100 rpm, 25 °C for 30 min in darkness, and then acidified to pH 4 with a 6 N HCl solution. The mix was centrifuged at 12096 g, 20 °C for 15 min, and the supernatant was precipitated with 65% (v/v) ethanol at 4 °C for 12 h. The precipitate was dried by solvent exchange with 80% v/v ethanol, absolute ethanol, and acetone to obtain AXs.

2.2.2. Partial Deferuloylation of AXs by Feruloyl Esterase

Feruloyl esterase was selected because it cleaves the ester linkages of the carboxylic group in phenolic acids (ferulic and p-coumaric acid) and the C5-hydroxyl of the α-L-arabinosyl side chains of xylans (Figure 2).

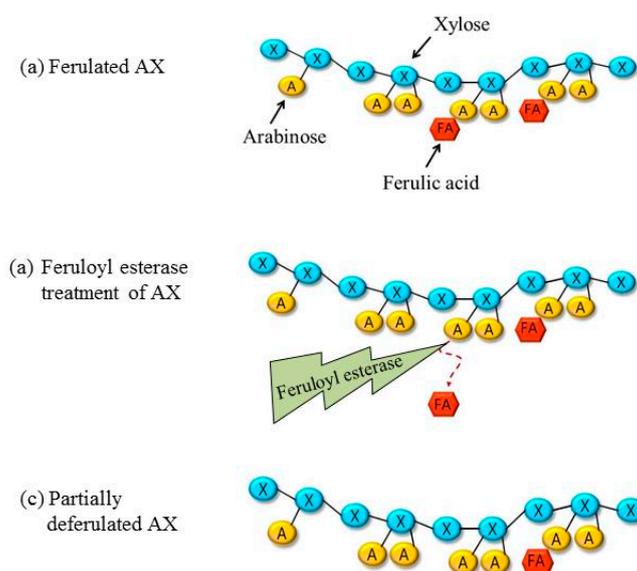


Figure 2. Schematic representation of partial AX deferuloylation by feruloyl esterase treatment.

Feruloyl esterase activity was measured by reverse-phase liquid chromatography using ethyl ferulate as a substrate. An assay mix (1 mL) containing the enzyme extract (3×10^{-3} mg/mL), ethyl ferulate (5 mM) and MOPS (100 mM, pH 6) was incubated in darkness with gentle stirring at 40 °C for 60 min. The reaction was stopped by adding glacial acetic acid (200 µL). The FA released in solution was recovered by adding ethyl acetate (3 mL, 2×), centrifuged (3820 g, 25 °C, 5 min), and the supernatant was evaporated at 40 °C under nitrogen in the dark [20]. The recovered FA was suspended in a methanol:water:acetic acid (40:59:1) solution, filtered (0.45 µm), and injected onto the

HPLC system (Waters Co., Milford, MA, USA) at 25 °C. An analytical Supelcosil LC-18-BD column (Supelco Inc., Bellefont, USA) was used with an isocratic solution of methanol:water:acetic acid (40:59:1) at 0.6 mL/min. FA was detected by UV absorbance at 280 nm in a photodiode array detector (PDA) (Waters Co., Milford, MA, USA). The assay was carried out in triplicate. One unit (U) of activity was defined as the amount of enzyme releasing one μmol of FA per minute at pH 6 and 40 °C. For AX deferuloylation, a 2% (w/v) AX solution was prepared in MOPS (100 mM, pH 6.0) and stirred at 4 °C for 12 h. Feruloyl esterase was added to the AX solution (721 μU enzyme/mg polysaccharide) at 40 °C in the dark over 24 h with gentle stirring. The enzymatic reaction was stopped by adding glacial acetic acid (1:5 v/v). The reaction mixture was precipitated in 75% (v/v) ethanol at 4 °C for 12 h. The precipitate was filtered and dried by solvent exchange with 80% (v/v) ethanol, absolute ethanol, and acetone to obtain FAXs [12].

2.2.3. FA, Dimers (di-FA) and Trimer of FA (tri-FA) Content

After the saponification of the AXs and FAXs, the FA, dimers and trimer of FA content was quantified by reverse-phase liquid chromatography as mentioned before [9]. Two milliliters of 2 N NaOH was added to 50 mg of AXs in a nitrogen-enriched atmosphere. The samples were incubated under agitation at 50 rpm and 35 °C for 2 h in darkness (KS 3000 ic control, IKA, Wilmington, NC, USA). Subsequently, 100 μL of 3,4,5-trimethoxycinnamic acid (TMCA) and 5 mL of 4 N hydrochloric acid were added to both samples. The pH was adjusted to 2. Phenolics were extracted twice with 5 mL of diethyl ether and then evaporated to dryness at 40 °C under nitrogen gas (Dri-Block DB-3A, Techne, UK). The extract was recovered in 1 mL of methanol:water (50:50), filtered (0.45 μm), and quantified using a chromatographic system (Waters Co, Milford, MA, USA). A Supelcosil LC-18-BD column (Supelco, Inc., Bellefonte, PA, USA) and a PDA (Waters Co, Milford, MA, USA) were used. Detection was achieved by measuring UV absorbance at 320 nm.

2.2.4. Macromolecular Characteristics

The samples (5 mg) were mixed in 1 mL of 50 mM $\text{NaNO}_3/0.02\%$ NaN_3 at 80 °C for 2 h with constant stirring. The biopolymer solutions were centrifuged at 15,000 $\times g$ and 20 °C for 10 min. Once the supernatants were filtered (0.21 μm), the samples were analyzed by size-exclusion high-performance liquid chromatography (SE-HPLC) with a multi-angle laser light scattering (MALLS) DAWN/HELEOS-II detector, an Optilab T-rex refractometer, and a ViscoStar-II viscometer (Wyatt Technology Corp., Santa Barbara, CA, USA). The SE-HPLC system consisted of two columns, Shodex OH-pak SBH-Q-804 and 805 columns (Shodex Showa Denco K.K., Tokyo, Japan), and a chromatographic system (Agilent Technologies, Inc., Santa Clara, CA, USA). The elution of AXs and FAXs was performed with 50 mM $\text{NaNO}_3/0.02\%$ NaN_3 at 0.6 mL/min and 25 °C. Weight average molecular weight (M_w), intrinsic viscosity ($[\eta]$), and polydispersity index ($PDI = M_w/M_n$) were determined by Astra 6.1 software (Wyatt Technology Corp., Santa Barbara, USA). The dn/dc value of 0.146 mL/g previously reported for AXs was used [21].

2.2.5. Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectrums of AXs and FAXs powder were recorded on a Nicolet iS50 FT-IR spectrophotometer (Nicolet Instrument Corp., Madison, WI, USA). The AXs and FAXs were placed on a smart iTX module, and the spectrums were recorded in the range of 4000–500 cm^{-1} with 4 cm^{-1} resolution and 32 scans [3]. OMNIC 9.3.32 software was used for data processing.

2.2.6. Gelation

AXs and FAXs 2% (w/v) gels were formed at 25 °C using laccase as a cross-linking agent (1.675 nkat/mg polysaccharide) as described previously [16,22]. The gelation kinetics of the AXs and FAXs were monitored employed small-amplitude oscillatory shear analysis. Rheological measurements were carried out using a rheometer (Discovery HR-2, TA Instruments, New Castle, DE, USA) at 0.25 Hz

and 5% strain for 1 h. The mechanical spectrum of the gels was collected by a frequency sweep from 0.1 to 10 Hz at 5% strain. All measurements were carried out at 25 °C.

2.2.7. Antioxidant Capacity

The antioxidant capacity of the AXs and FAXs was measured by 2,2'-azinobis-(ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assays. The ABTS method was performed as previously reported. [23]. To prepared the ABTS radical cation (ABTS^{•+}), 0.034g of ABTS (concentration final of 7 mM) was mix with 10 mL of 2.45 mM potassium persulfate, and keep at room temperature for approximately 16 h. The ABTS^{•+} was mixed with ethanol:water (50:50 v/v) until the absorbance was 0.7 (\pm 0.02). One milligram of each sample (AXs and FAXs) were mixed with 6 mL of the ABTS^{•+} reagent for 2 min and then centrifuged at 9200 g for 2 min. The absorbance of the supernatant was measured at 734 nm at 7, 15, and 30 min. The DPPH method was carried out, as described elsewhere [24]. The DPPH reagent (1.8 mg) was dissolved in 50 mL methanol:water (60:40). The reaction medium was made as follows: 400 μ L sample (2 mg/mL), 250 μ L methanol, and 750 μ L DPPH (final concentration of 45 μ M). The reaction medium was mixed and kept in the dark for 35 min. The absorbance at 515 nm of the medium was measured at 40 and 60 min. The antioxidant capacity was expressed as mmol of Trolox equivalent antioxidant capacity (TEAC) per kg of the sample using a dose-response curve of 0–20 μ M for both the ABTS and DPPH methods. The analyses were carried out in triplicate.

2.2.8. Statistical Analysis

Chemical determinations were performed by triplicate with coefficients of variation lower than 7%. Measurements of small deformation were made in triplicate with coefficients of variation lower than 9%. All results are expressed as the mean values.

3. Results

3.1. Extraction and Characterization of AXs

AXs were extracted from 500 g of DDGS to yield 4.7% (w AXs/w DDGS) on a dry matter basis, which is higher than the value previously reported using 30 min of alkaline hydrolysis (2.5% w AXs/w DDGS) [3]. Extracted AXs consisted of white-colored powder with fine and granulated parts (Figure 3). AXs from cereal bran are water unextractable (WUAXs), as they present high molecular weights and a high FA content; however, after alkaline treatment under controlled conditions as those used in the present study, WUAXs from maize bran can be extracted in water. It is important to mention that AXs present in cereal endosperm are water extractable (WEAXs); however, the extraction of WEAXs is more expensive than that of the AXs used in the present study because the use of enzymes is required and extraction yields are lower (~0.5% w WEAXs/w cereal endosperm), and the cereal endosperm is usually destined for human consumption, for example, in the production of wheat flour [9,21]. In contrast, in the present investigation, AXs extraction did not involve enzymes, the yield was higher, and the source was a maize co-product (DDGS from maize bioethanol plants).

The composition of AXs is shown in Table 1. The arabinose + xylose content in the AXs extracted from DDGS was 70.5% dry basis (db), which is higher than the value reported by other authors for AXs recovered from DDGS (58.1% and 64.0%) [9,25]. The arabinose to xylose ratio (A/X) was 0.7, indicating a moderately branched structure in the range of that previously reported for AXs from DDGS (1.1 and 0.51) [9,25]. Small levels of glucose, galactose, and mannose were also quantified. The FA content (3.27 μ g/mg AXs) was lower than the value previously reported using 30 min of alkaline hydrolysis (7.53 μ g/mg AXs) [3], which could be attributed to an extensive chemical de-esterification of FA during 1 h of alkaline hydrolysis used in the present work. However, the FA content reported in AXs extracted from DDGS is higher than that reported for AXs isolated from other maize co-products, such as maize wastewater generated during nixtamalization (0.012–0.23 μ g/mg AXs) [26]. These

differences may be related to the partial de-esterification of FA attached to AX chains occurring during the cooking of maize grains under alkaline conditions. During maize bioethanol production, no chemical treatment is involved, allowing for better preservation of FA in the AX molecules. The di-FA and tri-FA contents in the AXs were 0.27 and 0.01 $\mu\text{g}/\text{mg}$ AXs, respectively. The presence of di-FA and tri-FA in AXs from different cereals has been previously reported and are related to the possible presence of cross-linked AXs chains [3]. The di-FA structures present in AXs were 8-5', 5'-5' and 8-O-4' at 68, 31, and 1%, respectively, while the 8-8' di-FA was not detected. The high proportion of 8-5' and 5'-5' di-FA structures in maize AXs has been indicated in earlier investigations [3,22]. In cell walls, the 8-5' dimer has been reported to be the most abundant di-FA due to the relatively high reactivity of its radical structures [12].



Figure 3. AXs extracted from DDGS.

Table 1. Composition of AXs extracted from DDGS.

Arabinose ^a	28.81 \pm 1.30
Xylose ^a	41.70 \pm 1.95
Glucose ^a	3.36 \pm 0.31
Galactose ^a	4.20 \pm 0.06
Mannose ^a	0.96 \pm 0.07
Protein ^a	3.20 \pm 0.05
Ferulic acid ^b	3.27 \pm 0.46
Diferulic acid ^b	0.30 \pm 0.02
Triferulic acid ^b	0.010 \pm 0.001

^a Values expressed in g/100 g AXs dry matter. ^b Phenolics are expressed in $\mu\text{g}/\text{mg}$ AXs dry matter. All results were obtained from triplicate analyses.

3.2. Partial Deferuloylation of AXs by Feruloyl Esterase

The initial FA content in the AXs (3.27 $\mu\text{g}/\text{mg}$ polysaccharide) decreased to 1.26 $\mu\text{g}/\text{mg}$ polysaccharide when the sample was treated with feruloyl esterase, indicating that 61.5% of the FA esterified to the AX chains was removed. In the present study, the AXs and FAXs presented an average FA residue content of 5 and 2 FA molecules per 1000 xylose units, respectively. Previous investigations have reported 6-2 FA molecules per 1000 xylose residues in AXs from wheat, rye, triticale, and barley [4]. By using chemical de-esterification (lime treatment), a range of wheat flour AX samples with FA contents varying from 2.3 to 0.1 $\mu\text{g}/\text{mg}$ AXs was prepared, which corresponded to FA residue contents from 2.5 to 0.1 molecules per 1000 xyloses, respectively [12]. Those authors found that in AXs the minimal FA content to gelling is 0.5 FA residues per 1000 xyloses. However, in the present study, it was not possible to decrease the FA content in AXs beyond 1.26 $\mu\text{g}/\text{mg}$ polysaccharide (2 FA molecules per 1000 xylose units) even at longer feruloyl esterase incubation times, which could be attributed to the high arabinose substitution degree ($A/X = 0.7$) in relation to the value reported in the study using chemical de-esterification ($A/X = 0.6$) [12]. A high amount of branched arabinose molecules from the xylose backbone may make feruloyl esterase access to de-esterification sites difficult. The di-FA content in the FAXs was 0.20 $\mu\text{g}/\text{mg}$ polysaccharide, suggesting that some polysaccharide chains might be cross-linked [3,21]. The comparative percentages of each di-FA in FAXs were 58%, 36%, and 3% for 8-5', 5'-5' and 8-O-4', respectively.

The macromolecular characteristics of FAXs were not significantly affected by feruloyl esterase treatment (Table 2). The M_w and $[\eta]$ values in the FAXs slightly decreased in comparison to those of the AXs, probably related to the small decrease in the di-FA content from 0.30 to 0.20 $\mu\text{g}/\text{mg}$ polysaccharide, which suggests a low amount of cross-linked AX chains in the sample.

Table 2. Macromolecular characteristics of AXs and FAXs.

Sample	Molecular weight M_w (kDa)	Polydispersity index $PDI = (M_w/M_n)$	Intrinsic viscosity $[\eta]$ (mL/g)
AXs	270	1.2	100
FAXs	250	1.2	95

Coefficients of variation < 7%.

The SE-HPLC chromatograms of the AXs and FAXs are presented in Figure 4. The M_w values found for AXs and FAXs in the present work were lower than those previously published for other AXs from DDGS in the literature (5900–4463 kDa) [9], possibly due to the mobile phase used by these authors (water). It has been reported that the chromatogram shape and the repeatability of retention time are considerably enhanced when the water is changed by sodium nitrate as the mobile phase [27]. The AXs and FAXs exhibited an index of polydispersity in the range reported for other AXs isolated from DDGS (1.0–1.8) (1.3–1.7). The elution profile of the FAXs was almost to the same as that of the AXs, showing that enzymatic treatment did not depolymerize the polysaccharide backbone (Figure 4). The M_w , I , and $[\eta]$ values of AXs and FAXs were in the range reported for other maize-derived AXs in the literature [3,9,22,28].

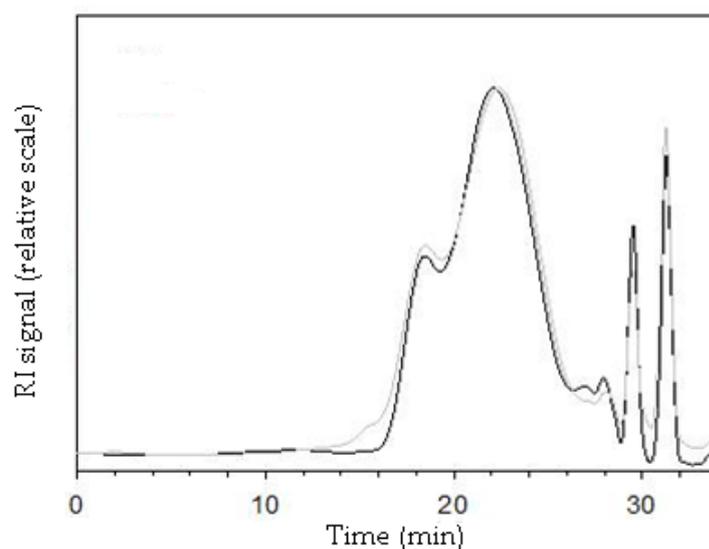


Figure 4. SE-HPLC chromatograms of AXs (—) and FAXs (---). RI: refractive index.

The FT-IR spectra ($4000\text{--}400\text{ cm}^{-1}$) of the AXs and FAXs were very similar, confirming that feruloyl esterase releases FA without altering the molecular identity of this polysaccharide (Figure 5a). The $1200\text{--}900\text{ cm}^{-1}$ interval (fingerprint region) displays the typical spectra for xylan-type polysaccharides (Figure 5b). This region of the spectrum is constituted by absorption bands of C-O and C-C stretching and C-OH bending vibrations of the polymer backbone [29]. Both signals at 1164 cm^{-1} (denoted as the shoulder) and 1120 cm^{-1} (not defined as the shoulder) are assigned to C-O and C-C stretching vibrations. A ring vibration produces the shoulder at 1073 cm^{-1} , and the dominant band at 1038 cm^{-1} is due to COH side groups [30]. The band at 990 cm^{-1} is not perceptible, suggesting a high arabinose side substitution (O-3 position) on the xylan backbone. The band at 905 cm^{-1} is related to the glycosidic C1-H1 deformation mode with ring vibration contribution and OH bending vibration,

which confirms the β -1, 4 glycosidic bond between the xylopyranose units of the main xylan chains [31]. A chain of simple xylopyranose units should not have visible absorption bands at 1800–1500 cm^{-1} (Figure 5c). Consequently, the bands in this region could be attributed to the vibration of a diverse type of substituent in the main chain or the xylan side chains [31]. In this regard, a band at 1645 cm^{-1} that mostly corresponds to polypeptide carbonyl stretching (amide I) [29] was also identified, confirming the presence of residual protein in the samples [3]. This band overlaps with the secondary absorption bands of FA (1690, 1620, and 1600 cm^{-1}) [31]. The primary absorption band of FA is an assignment at 1517 cm^{-1} , in our case, at 1519 cm^{-1} [30]. However, in the present study, this band is not clear due to overlap with the band at 1540 cm^{-1} , which could be related to amide II [29]. As Figure 5c shows, the band at 1645 cm^{-1} is more defined, and consequently the band at 1540 cm^{-1} is relatively more intensive in the AX spectrum than in the FAX spectrum, which could be associated with the higher FA content in AXs in relation to FAXs. The broadband with a maximal signal at 3346 cm^{-1} is related to the vibration of hydroxyls involved in both intra- and intermolecular hydrogen bonds (Figure 5a) [31]. The bands at 2928 and 2875 cm^{-1} correspond to asymmetric and symmetric stretch vibrations of CH_2 groups, respectively (Figure 5a). These vibrations could be explained by the presence of such groups in xylopyranose rings of the backbone xylan [32].

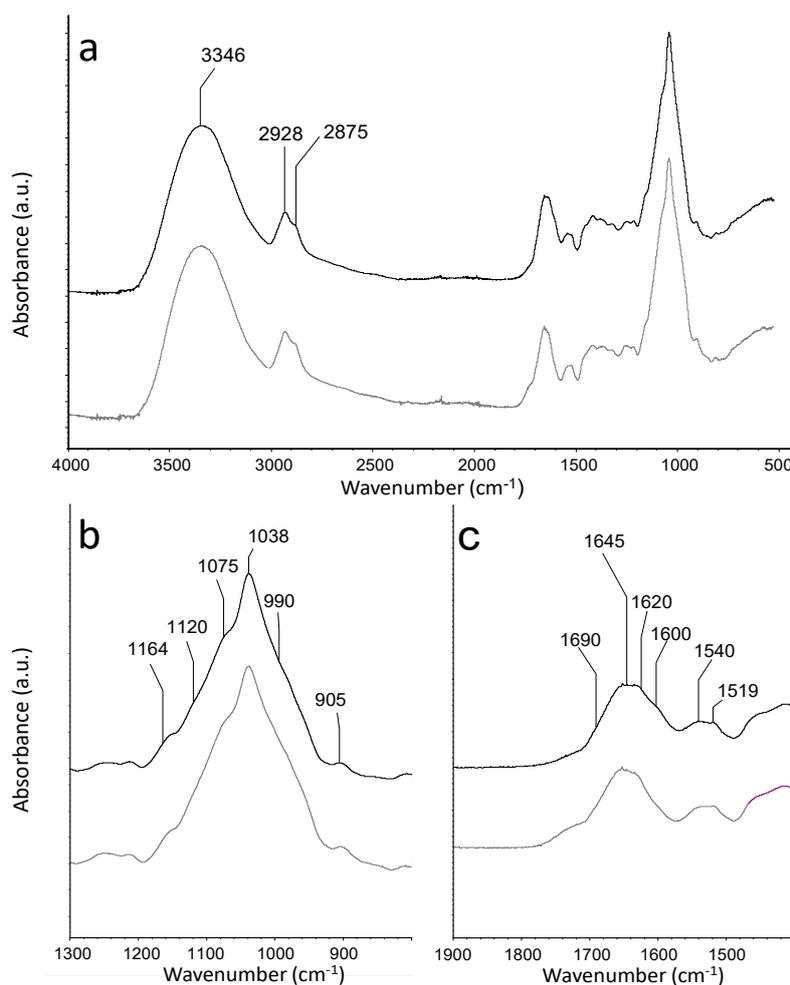


Figure 5. FT-IR spectra of AXs (—) and FAXs (—) at 4000–500 cm^{-1} (a), 1200–900 cm^{-1} (b), and 1800–1500 cm^{-1} (c).

3.3. AX and FAX Gelling and Antioxidant Properties

The kinetics of the gelation of AXs and FAXs was rheologically investigated by small-amplitude oscillatory shear analysis. Similar gelation kinetics were observed for both samples with a lag time

(~5 min) followed by a rapid G' rise until nearly constant values (plateau region) (Figure 6a). The final G' values for the AXs and FAXs gels (at 3600 sec) were 195 Pa and 112 Pa, respectively. The gelation time ($G' = G''$) was lower in the AXs gel (13 min) than in the FAX gel (16 min). The G' value decreased, and the gelation time was higher for the FAXs gel than for the AXs gel, which is related to the partial removal of FA in the molecule after feruloyl esterase treatment. These results were similar to those reported for gels obtained from wheat endosperm AXs, which were partially de-esterified by a chemical process [12], confirming the fundamental role of FA in the elastic properties of AXs gels. The mechanical spectra of the AXs and FAXs gels after 1 h of gelation are presented in Figure 6b. The AXs and FAXs gels exhibited typical gel behavior, with G' being independent of frequency and higher than G'' [33]. Lower G' values were observed for FAXs gels than for AXs gels under the frequency range used in the present study. Nevertheless, the G'' values were almost superposed for both samples, indicating that the viscous contribution to the gel structure was similar. From the G' and G'' values in the AXs and FAXs gels at 1 Hz the frequency used during polysaccharide gelation, $\tan \delta$ (G''/G') values were calculated. The $\tan \delta$ values increased from 0.004 to 0.005 when the AX initial FA content was reduced from 3.27 to 1.26 $\mu\text{g}/\text{mg}$ AXs. This change in the $\tan \delta$ value means an increase in the flexibility of the polymer chain of the gel [34]. These mechanical spectra are similar to others reported for wheat- and maize-derived AX gels [3,12,20]. These results demonstrate that even when the FAX gel exhibited a reduction in its G' value in relation to that of the AXs gel, the gel rheological characteristics are similar to those reported for other AXs gels in the literature [3,12,20].

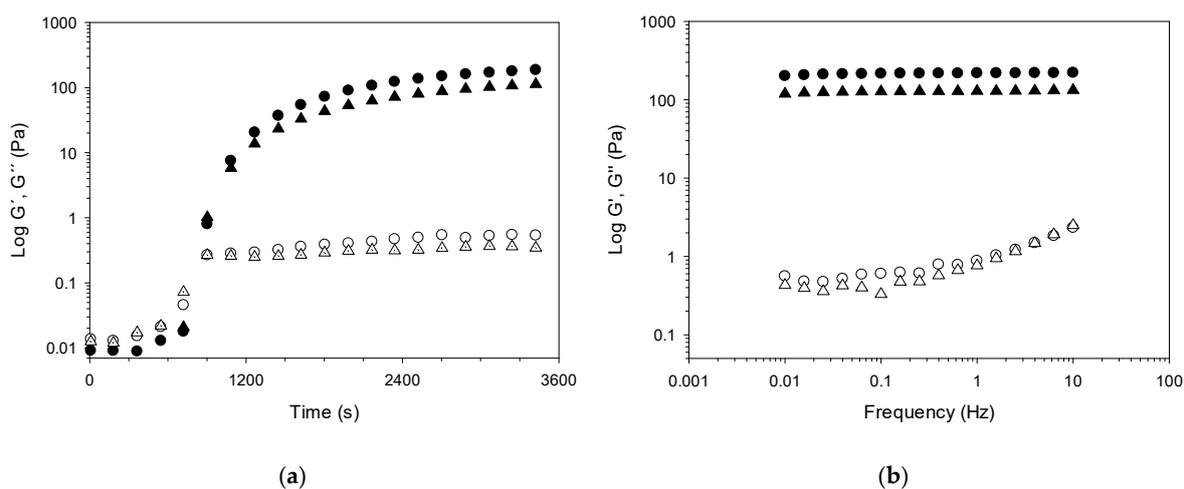


Figure 6. (a) Monitoring the storage modulus (G') and loss modulus (G'') of 2% (w/v) AX and FAX solutions during gelation by laccase (25 °C, 1 Hz, and 5% strain). (b) Mechanical spectra of AX and FAX gels induced by laccase (25 °C and 5% strain). AX gel (circle), FAX gel (triangle), G' (filled symbol), and G'' (empty symbol).

The TEAC values of AXs and FAXs are shown in Table 3, as determined by the procedures using DPPH and ABTS solutions. Higher antioxidant activity values were observed when using ABTS than when using the DPPH method. It has been previously reported that ABTS is an unstable radical cation that reacts with antioxidants to generate compounds with antioxidant activity, thus leading to the overestimation of the antioxidant activity in a sample. In contrast, the relatively stable radical DPPH can be easily reduced by extracting hydrogen from hydrogen donors [35]. The results show that the partial deferuloylation of AXs reduced their antioxidant activity by 36% and 42% using the DPPH and ABTS methods, respectively. This reduction in antioxidant activity in FAXs may be attributed to the partial release of FA esterified to the polysaccharide. A previous study reported that AXs with a high amount of FA residues show high antioxidant activity [36]. The effect of Mw on the antioxidant activity of AXs is not clear. It has been suggested that a decrease in AX Mw could improve their antioxidant activity due to their higher solution mobility and, thus, their enhanced radical-scavenging activity [36].

Another study showed that in AXs, a higher *M_w* gave stronger antioxidant activity [37]. However, in the present study, feruloyl esterase treatment reduced the *M_w* of the AXs by only 7% (Table 2). Thus, the polysaccharides still had a relatively high *M_w*; in this regard, the *M_w* the AXs did not seem to impact their antioxidant activity. The antioxidant capacity values obtained for the AXs and FAXs are in the range reported for other AXs from DDGS (12–68 mmol TEAC/kg) [17,38] and higher than those reported in AXs from other maize co-products (6–16 mmol TEAC/kg) [14,39]. The results suggested that AXs and FAXs have great potential as free-radical scavengers in the food, pharmaceutical, medical, and cosmetic industries, among others.

Table 3. Antioxidant capacity of AXs and FAXs.

Sample	DPPH (mmol TEAC/kg)	ABTS (mmol TEAC/kg)
AXs	28 ± 2	67 ± 6
FAXs	18 ± 3 *	39 ± 3 *

All values are the mean ± standard deviation of three repetitions. * Value in each column is significantly different at $p \leq 0.001$.

4. Conclusions

AXs from DDGS can be partially deferuloylated by feruloyl esterase treatment, allowing for a reduction of 61.5% in FA content without affecting the polysaccharide molecular identity. The molecular weight and intrinsic viscosity of feruloyl esterase-treated AXs (FAXs) are slightly reduced, probably due to the decrease in di-FA content, which could moderate the presence of cross-linked polysaccharide chains. FAXs present gelling and antioxidant properties similar to those reported for other maize-derived AXs in the literature. FAX gels (2% w/v) exhibit a storage modulus value 43% lower than that of AXs gels at the same polysaccharide concentration. The antioxidant capacity of the FAXs is diminished by 32% and 43% using the DPPH and ABTS methods, respectively, in relation to that of AXs. These results confirm the fundamental part of FA in the gelling capability and antioxidant activity of this polysaccharide. Feruloyl esterase treatment could offer an exciting opportunity for the reliable design of AXs as value-added products for the food and biomedical industries as well as an opportunity in sustainable maize waste utilization. FAXs would be attractive as designed encapsulating and radical scavenging agents, for example, for the preparation of efficient colon-targeted delivery systems without the reduced rate of fermentation by the colonic microbiota.

Author Contributions: Conceptualization, J.A.M.-E. and E.C.-M.; Methodology, J.A.M.-E. and E.C.-M.; Validation, J.A.M.-E. and E.C.-M.; Formal Analysis, J.A.M.-E. and E.C.-M.; Investigation, J.A.M.-E. and E.C.-M.; Writing—Original Draft Preparation, J.A.M.-E.; Writing—Review and Editing, E.C.-M.; Visualization, J.A.M.-E. and E.C.-M.; Supervision, E.C.-M.; Project Administration, E.C.-M.; Funding Acquisition, E.C.-M.

Funding: This research was funded by ‘Fund to support research on the Sonora-Arizona region 2019’, Mexico (Grant 20614to E. Carvajal-Millan).

Acknowledgments: The authors are pleased to acknowledge Itzel Aguirre-Franco, Alma C. Campa-Mada, and Karla G. Martínez-Robinson (CIAD) for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

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