Chapter 4

# Gelation of Arabinoxylans from Maize Wastewater — Effect of Alkaline Hydrolysis Conditions on the Gel Rheology and Microstructure

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**Abstract**

The purpose of this research was to extract arabinoxylans (AX) from maize wastewater generated under different maize nixtamalization conditions and to investigate the polysaccharide gelling capability, as well as the rheological and microstructural characteristics of the gels formed. The nixtamalization conditions were 1.5 hours of cooking and 24 hours of alkaline hydrolysis (AX1) or 30 minutes cooking and 4 hours of alkaline hydrolysis (AX2). AX1 and AX2 presented yield values of 0.9% and 0.5% (w/v), respectively. Both AX samples presented similar molecular identity (Fourier Transform Infra-Red) and molecular weight distribution but different ferulic acid (FA) content. AX1 and AX2 presented gelling capability under laccase exposure. The kinetics of gelation of both AX samples was rheologically monitored by small amplitude oscillatory shear. The gelation profiles followed a characteristic kinetics with an initial increase in the storage modulus (G') and loss modulus (G") followed by a plateau region for both gels. AX1 presented higher G' than AX2. In scanning electron microscopy (SEM) images, both gels present an irregular honeycomb microstructure. The lower FA content in AX2 form gels presenting minor elasticity values and a more fragmented microstructure. These results indicate that nixtamalization process conditions can modify the characteristics of AX gels.

**Keywords:** Ferulated arabinoxylans, nejayote, gelling, maize wastewater

## 1. Introduction

Nixtamalization is a process widely used in Mexico, the southern United States, Central America, Asia, and parts of Europe. This process consists of cooking maize grains in a lime solution, after soaking for 2–15 hours, the supernatant called maize wastewater or commonly known as "nejayote" is discarded [1, 2]. The remaining material is then ground to obtain nixtamal (dough or masa), used to prepare a variety of food products such as tortillas and related products [3]. A typical maize nixtamalization facility, processing 50 kg of maize every day, uses over 75 L of water per day and generates nearly the equivalent amount of alkaline wastewater on a daily basis [4]. Nejayote is considered an environmental pollutant because it is an alkaline wastewater, with high chemical and biological oxygen demand. The estimated monthly volume of nejayote generated in Mexico is about 1.2 $m^3$ [1, 5, 6]. Thus, alternatives for maize wastewater utilization are needed.

Nejayote is rich in maize bran residues as during the nixtamalization process this tissue is removed from the maize kernel. Non-starch polysaccharides are major constituents of maize bran, 30% of which are ferulated arabinoxylans (AX) [2, 7, 8]. Therefore, nixtamalization degrades and solubilizes maize cell wall components, mainly AX, which can be recovered in maize wastewater [4]. Nixtamalization conditions such as cooking temperature, lime concentration, and soaking period could affect the structural and functional characteristics of AX but, to our knowledge, the effect of maize nixtamalization conditions on AX properties has not been investigated.

AX are the main non-starchy polysaccharides of cereal grains, constituted of a linear backbone of β-(1→4)-linked D-xylopyranosyl units to which $\alpha$-L-arabinofuranosyl substituents are attached through O-2 and/or O-3. Some of the arabinose residues are ester linked on (O)-5 to FA (3-methoxy, 4 hydroxy cinnamic acid) [9, 10]. AX can form covalent gels by oxidation of FA resulting in the formation of dimers (di-FA) and trimers (tri-FA) of FA as covalent cross-linking structures [11]. AX gels are stabilized by covalent linkages, which make them stable to temperature, pH or ionic strength changes; these characteristics would allow their passage through the gastrointestinal tract being further fermented by colonic microflora [12, 13]. In addition, AX gels could have potential application as a microencapsulation system for colon-specific delivery due to their porous structure (mesh size from 48 to 400 nm), high water absorption capacity (up to 100 g of water for gram of polymer), and dietary fiber nature [9, 10, 14].

The molecular features of AX depend on the source and the process extraction [10]. This characteristics as chemical structure, molecular weight and FA content affect their gelling ability and therefore functional properties of gels [11]. The purpose of this research was to extract AX from maize wastewater generated under two different maize nixtamalization conditions and investigate the polysaccharide gelling capability as well as the rheological and microstructural characteristics of the gels formed.

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## 2. Experimental

### 2.1. Materials

Nejayote was provided by two tortilla-making in Northern Mexico and used to extract AX. Laccase, which is copper-containing oxidase enzyme (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) extracted from *Trametes versicolor* and the other chemical products were purchased from Sigma Co. (St. Louis, MO, USA).

### 2.2. Methods

#### *2.2.1. Arabinoxylans extraction*

AX from nejayote were extracted as previously described [15]. Maize wastewater generated under two commercial nixtamalization conditions were used: 1.5 hours of cooking and 24 hours of alkaline hydrolysis (AX1) or 0.5 hours of cooking and 4 hours of alkaline hydrolysis (AX2) (Scheme 1).

#### *2.2.2. Fourier Transform Infra-Red (FT-IR) spectroscopy*

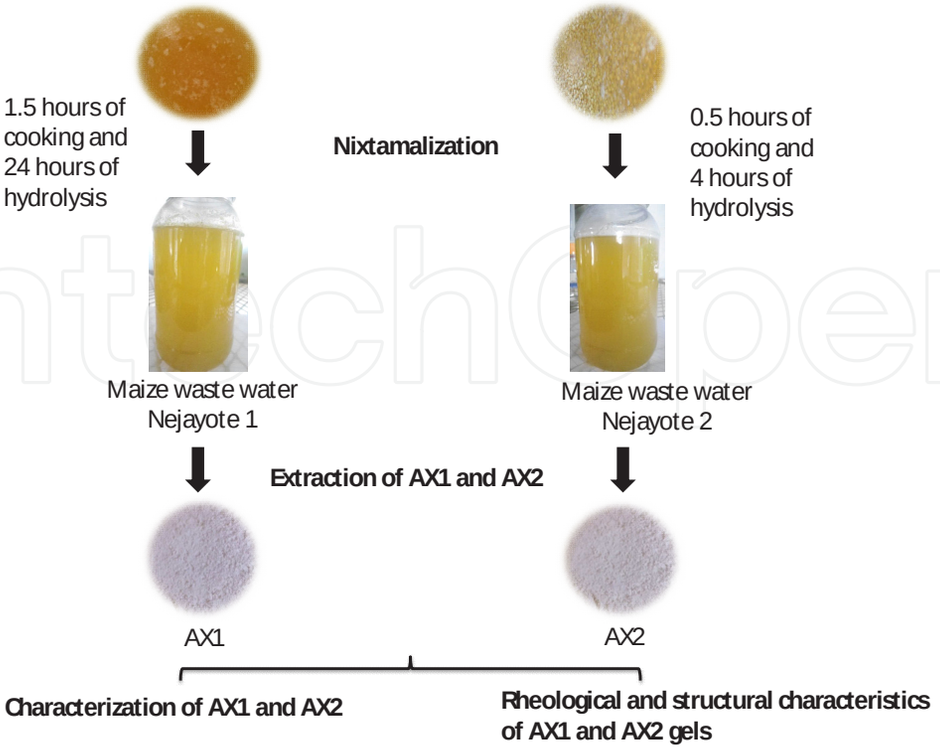
FT-IR spectra of dry AX1 and AX2 were recorded on a Nicolet FT-IR spectrometer (Nicolet Instrument Corp. Madison, WI, US). The samples were pressed into KBr pellets (2 mg/200 mg KBr). A blank KBr disk was used as background. The spectra were measured in absorbance mode from 400–4000 $cm^{-1}$ resolution [16].

#### *2.2.3. Molecular weight distribution*

Molecular weight distribution of AX1 and AX2 was determined by Size Exclusion-High Performance Liquid Chromatography (SE-HPLC) at 38°C using a TSKgel (Polymer Laboratories, Shropshire, UK) G500 PMWX column (7.8 x 300 mm). 20 μL of AX1 and AX2 solutions (0.5% w/v in 0.1 M $LiNO_3$) filtered through 0.2 μm (Whatman) were injected, and Water 2414 Refractive Index Detector was used for detection. Isocratic elution was performed at 0.6 mL/min with 0.1 M $LiNO_3$ filtered through 0.2 μm. Molecular weights were estimated after universal calibration with pullulans (polysaccharide extracted from the fermentation medium of the *Aureobasidium pullulans* consisting of maltotriose units) as standards (P50 to P800) [17].

#### *2.2.4. Ferulic acid content*

FA content in AX1 and AX2 were quantified by Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) after de-esterification step as previously described [4, 18]. An Alltima C18 column (250 × 4.6 mm) (Alltech Associates, Inc., Deerfield, IL, USA) and a photodiode array detector Waters 996 (Millipore Co., Milford, MA, USA) were used. Detection was followed by UV absorbance at 320 nm. The measurements were performed in triplicate.




**Scheme 1.** Nixtamalization conditions and arabinoxylans extraction (AX1, AX2).

*2.2.5. Gel preparation*

AX1 and AX2 solutions at 10% (w/v) were prepared in 0.1 M sodium acetate buffer pH 5.5. Laccase (1.675 nkat per mg polysaccharide) was used as a cross-linking agent. AX1 and AX2 gels were allowed to develop for 4 hours at 25°C. The measurements were performed in duplicate.

*2.2.6. Rheological test*

Small amplitude oscillatory shear was used to follow the gelation process of AX1 and AX2 solutions at 10% (w/v). Solutions were mixed with laccase (1.675 nkat per mg AX) and immediately placed on the parallel plate geometry (4.0 cm in diameter) of a strain controlled rheometer (Discovery HR-3 rheometer; TA Instruments, New Castle, DE, US). Exposed edges were covered with silicone oil to prevent evaporation. The dynamic rheological parameters used to evaluate the gel network were the storage modulus (G'), loss modulus (G''), crossover point (G'>G''), and tan delta (tan δ, G''/G'). AX1 and AX2 gelation were monitored at 0.25 Hz and 5% strain. At the end of the network formation a frequency sweep (0.01–10 Hz) was carried out. Rheological measurements were performed in duplicate [4].

*2.2.7. Microstructure*

AX1 and AX2 gels at 10% (w/v) were frozen in liquid nitrogen and lyophilized at −37°C/0.133 mbar overnight in a Freezone 6 freeze drier (Labconco, Kansas, MO). The microstructure of

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the freeze-dried gels was studied by scanning electron microscopy (SEM) (JEOL 5410LV, JEOL, Peabody, MA, USA) at low voltage (20 kV) and Secondary Electron Imaging (SEI) mode [19].

*2.2.8. Statistical analysis*

FA content was made in triplicates and the coefficients of variation were lower than 5%. Small deformation measurements were made in duplicates and the coefficients of variation were lower than 5%. All results are expressed as mean values.

## 3. Results and discussion

### 3.1. Extraction and characterization of AX1 and AX2

AX1 and AX2 presented yield values of 0.9 and 0.5 % (w/v), respectively. This is consistent with a previous report where poorer AX yield of extraction were registered at lower times of alkaline hydrolysis [20]. Nevertheless, the AX yields found in the present study were smaller than those previously reported [21], which could be related to the maize varieties used in each investigation. The yield found in this study is similar to reported by other sources of AX as wheat flour (0.5 %) [16]. In spite of low yield values, recuperation of AX from wastewater could be an advantage for future industrial applications of this polysaccharide. It could also provide an alternative use this highly alkaline waste generated in large quantities.

The structural features of AX1 and AX2 were analyzed by FT-IR spectroscopy (Figure 1). The spectra were similar for both AX samples indicating a similar chemical structure characteristic of AX from maize and other sources [16, 17]. The region of 1200–850 $cm^{-1}$ is typical of AX [17, 22, 23]. The maximum absorption band (~1035 $cm^{-1}$) could be assigned to C-OH bending with signals at 1,070 and 898 $cm^{-1}$ that were related to the antisymmetric C-O-C stretching mode of the glycosidic bond and β(1-4) linkages between the xylose units [16, 17, 24]. Phenolic compounds and proteins have specific absorption bands in the 1500 – 1,700 $cm^{-1}$ [23]. The region from 3500–1800 $cm^{-1}$ is the fingerprint region of polysaccharides, with two bands (3,400 $cm^{-1}$ corresponding to stretching of the OH groups and 2,900 $cm^{-1}$ corresponding to the $CH_2$ groups) [16, 25]. These results suggest that nixtamalization conditions used in the present study do not affect the structural features of AX. However, in this work the effect of two alkaline treatments was also investigated on the FA content and physicochemical characteristics of AX1 and AX2.

The gel permeation chromatography profile of AX1 and AX2 are presented in Figure 2. Molecular weight distribution profiles were similar for both AX showing a major peak at molecular weight region of ~250 kDa (high molecular weight region), a similar behavior has been previously reported for maize AX [17].

FA content in AX1 and AX2 was $0.012 \pm 2.7 \times 10^{-5}$ and $0.008 \pm 1.4 \times 10^{-4}$ (μg/mg polysaccharide), respectively. These values are lower than those reported for other maize wastewater AX (0.23 μg/mg polysaccharide) [4]. Heating temperature, lime concentration, hydrolysis time, and exposure to light could have affected the amount of FA present in AX. In a previous report found that the best conditions of alkaline hydrolysis for FA extraction (from brewer's spent

grain) are low NaOH concentration (2.0%), temperature of 120°C, and a short reaction time (90 minutes) [26]. In the present work, maize grains cooking in a lime solution was performed during 90 minutes and 30 minutes for AX1 and AX2, respectively; but after heating, long soaking periods were used (24 hours and 4 hours for AX1 and AX2, respectively), which could explain the lower FA content in the polysaccharide. This is congruent with a previous study where the FA content in AX was dependent of the time of alkaline hydrolysis [20].

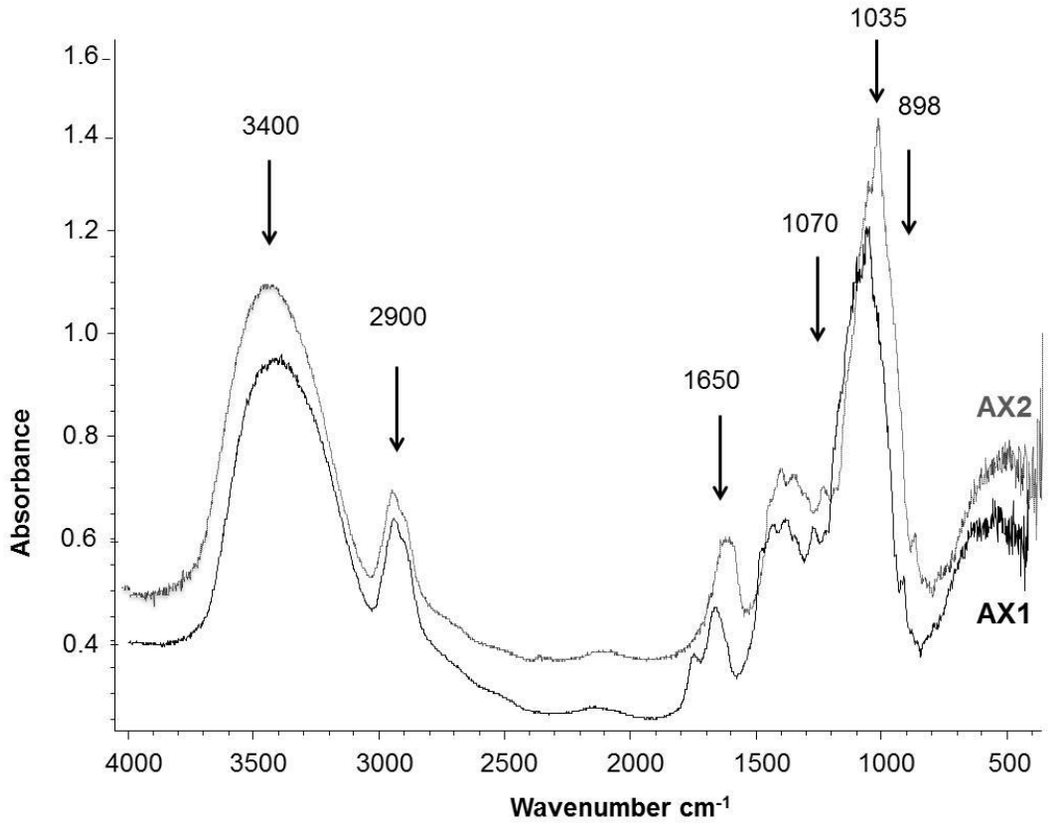


**Figure 1.** FT-IR of AX1 and AX2. The arrows indicate the characteristic absorption bands.

### 3.2. Rheological and structural characteristics of AX gels

AX1 and AX2 solutions at 10% (w/v) presented gelling capability under laccase exposure (Figure 3). The kinetics of gelation of these solutions was rheologically monitored by small amplitude oscillatory shear. Figure 4 shows the development of storage modulus (G'), loss (G") modulus, and tan δ (G"/G') versus time of 10% (w/v) AX1 and AX2 solutions undergoing oxidative gelation by laccase.

The gelation profiles followed a characteristic kinetics with an initial increase in G' and G", followed by a plateau region for both gels. This behavior reflects an initial formation of covalent linkages between FA of adjacent AX molecules producing a three-dimensional network [19]. At the end of gelation, G' and G" were 78 and 13 Pa for AX1, respectively, while for AX2 they were 32 Pa and 8 Pa for G' and G", respectively (Table 1). Similar kinetics of gelation have been

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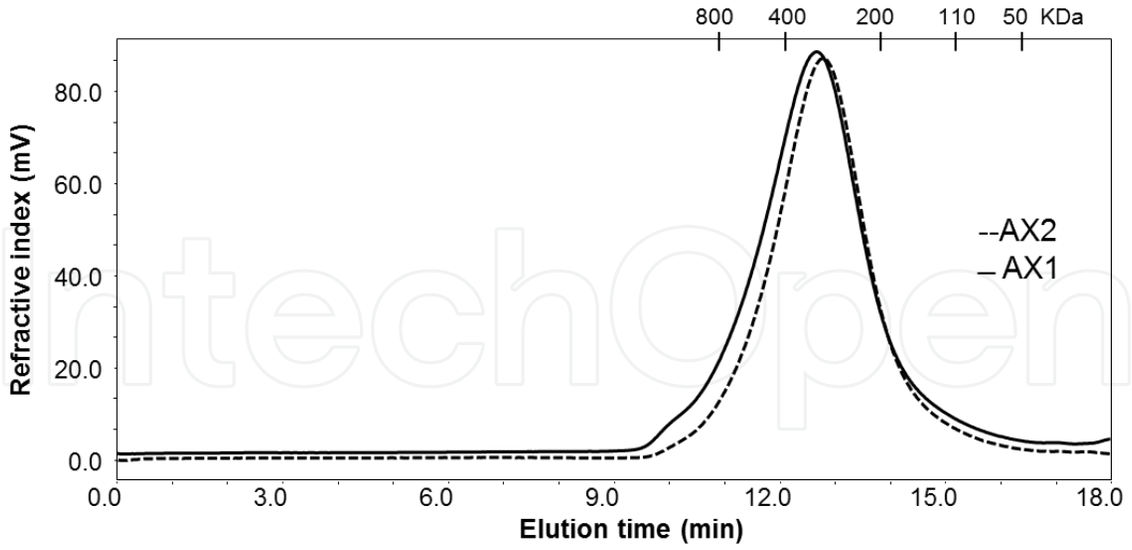


**Figure 2.** Elution profiles of AX1 and AX2. Pullulan molecular weight markers (kDa) used as calibration scales are shown at the top.

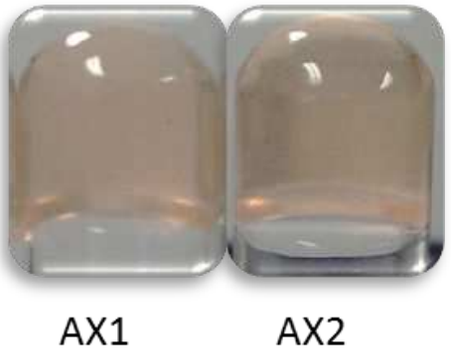


**Figure 3.** AX1 and AX2 gels at 10% (w/v).

previously reported for maize bran AX gels [17, 27, 28]. AX1 gel presented higher elasticity value in comparison to AX2 gel, which can be attributed to its higher FA content.

Gelation time (tg) at crossover point (G' > G") was 26 min and 40 min for AX1 and AX2, respectively. The tg value indicates the sol/gel transition point and at this point G' = G". The lower FA content in AX2 compared with AX1 could have affected the cross-linking of AX chains and retard the gel formation. The tan δ (G"/G') values decreased during the AX1 and AX2 gelation indicating the formation of a more elastic covalent system (Figure 4) [17]. The tan δ calculated at the end of the test, were 0.16 for AX1 and 0.24 for AX2, indicating than AX1 gel is more elastic than AX2 gel [29].

Niño-Medina et al. [21] reported nejayote AX gels (4% and 8%, w/v) with smaller G' values (2 and 4 Pa, respectively) and a higher crossover point (150 min) than those found in the present work. On the other hand, Ayala-Soto et al. [30] reported nejayote AX gels (4 % w/v) that showed a fluid-like behavior with G' of 5.9 Pa. Such differences might have its origin in the structural

and/or conformational characteristics of these macromolecules [21, 31]. Possible differences in structure such as arabinose and FA distribution throughout the AX molecule could explain the variance in the rheological characteristics of the gels formed.

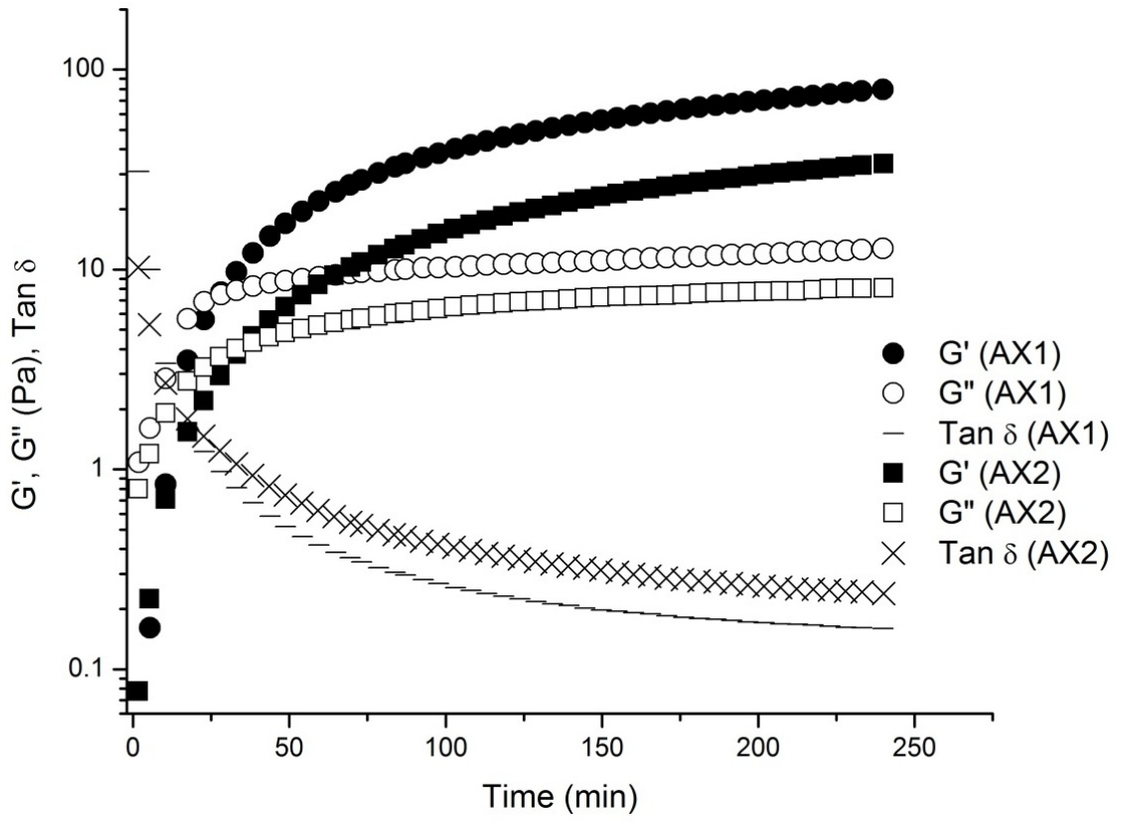


**Figure 4.** Monitoring the storage (G') and loss modulus (G'') of AX1 and AX2 solutions (10% w/v) during gelation by laccase at 0.25 Hz and 25°C.

| AX | Hydrolysis time (h) | Ferulic acid, FA (μg/mg AX) | Gelation time, tg (min) | G' (Pa) | G'' (Pa) | Tan delta (δ, G''/G') |
|---|---|---|---|---|---|---|
| AX1 | 24 | $0.012 \pm 2.7 \times 10^{-5}$ | 26 | 78 | 13 | 0.16 |
| AX2 | 4 | $0.008 \pm 1.4 \times 10^{-4}$ | 40 | 32 | 8 | 0.24 |

**Table 1.** FA content in AX1 and AX2 and rheological characteristics of the gels formed at 10% (w/v).

The mechanical spectra of AX1 and AX2 after gelation (Figure 5) exhibited a solid-like behavior with G'>G''. The mechanical spectra of AX gels with a linear G' independent of frequency and G'' much smaller than G' and dependent of frequency have been previously reported [11, 16, 17, 27]. AX1 and AX2 gels G' increase at frequency values, this may indicate the presence of physical interactions in the polymer network in addition to the covalent bonds induced by laccase.

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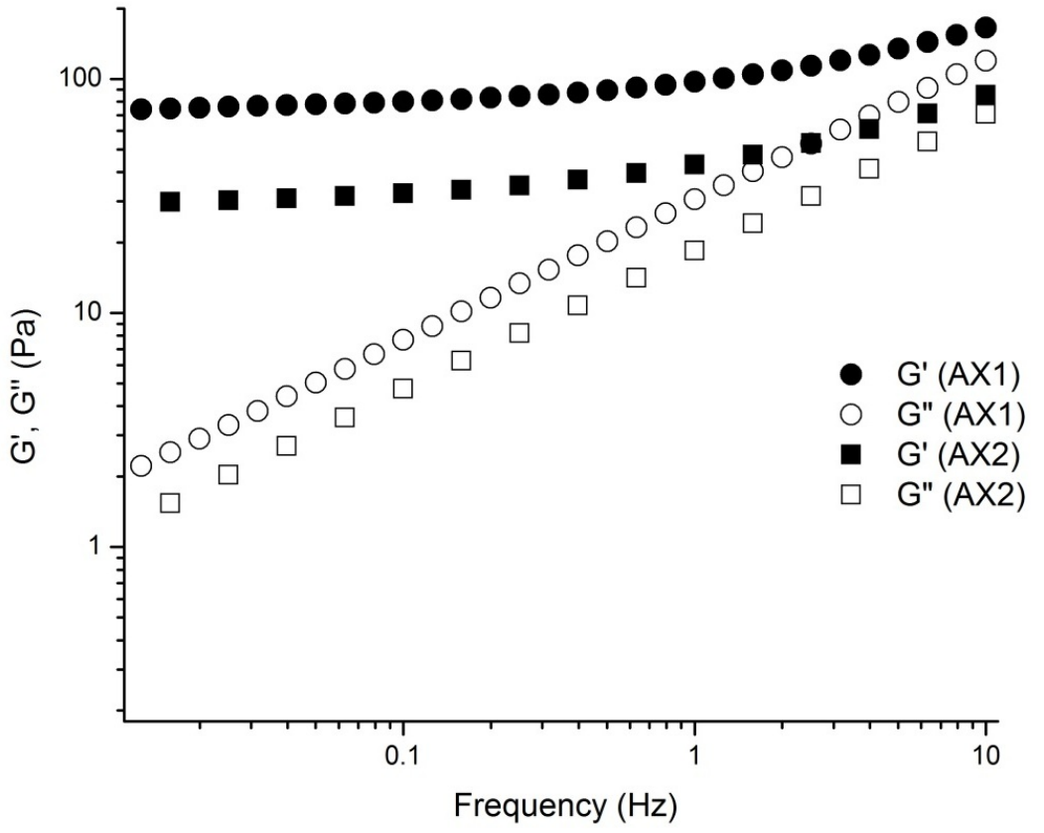


**Figure 5.** Mechanical spectra of AX1 and AX2 gels at 4 h. Rheological measurements made at 25 °C and 5% strain.

The images from SEM of the lyophilized gels AX1 and AX2 are shown in Figure 6. Both gels present many connections and resemble an imperfect honeycomb. In general, the microstructural characteristics of AX1 and AX2 are similar to those previously reported for lyophilized wheat and maize bran AX gels [16, 17, 22]. Nevertheless, AX2 gel appears to have a more fragmented morphology with a rougher and heterogeneous surface (Figure 6c, d). These microstructural dissimilarities between AX1 and AX2 gels could explain the differences in G' values of the gels, since a more compact and defined microstructure could give stronger gels.

The average inner diameter of the AX1 and AX2 cells were approximately 30 μm and 50 μm, respectively. Higher cell dimensions were reported in lyophilized AX gels (>200 μm) [17, 19], and this difference could be related to the method used to freeze the gels before lyophilization. In the present study AX gels were frozen by immersion in liquid nitrogen (fast congelation), while previous studies [17, 19] reported AX gel congelation at -20°C for several hours (slow congelation).

An important aspect of achieving a high-quality frozen material, particularly with high water content such as gels, is the freezing rate. Fast congelation results in a better-preserved structure (i.e., finer ice crystals). The microstructural characteristics of AX1 and AX2 gels were similar to those reported in AX gels frozen by fast congelation [32, 33] under similar conditions to those used in the present study. In those previous studies [32, 33], AX gels can be also compared with an irregular honeycomb structure in which pore diameters ranged from 12 μm to 50 μm.

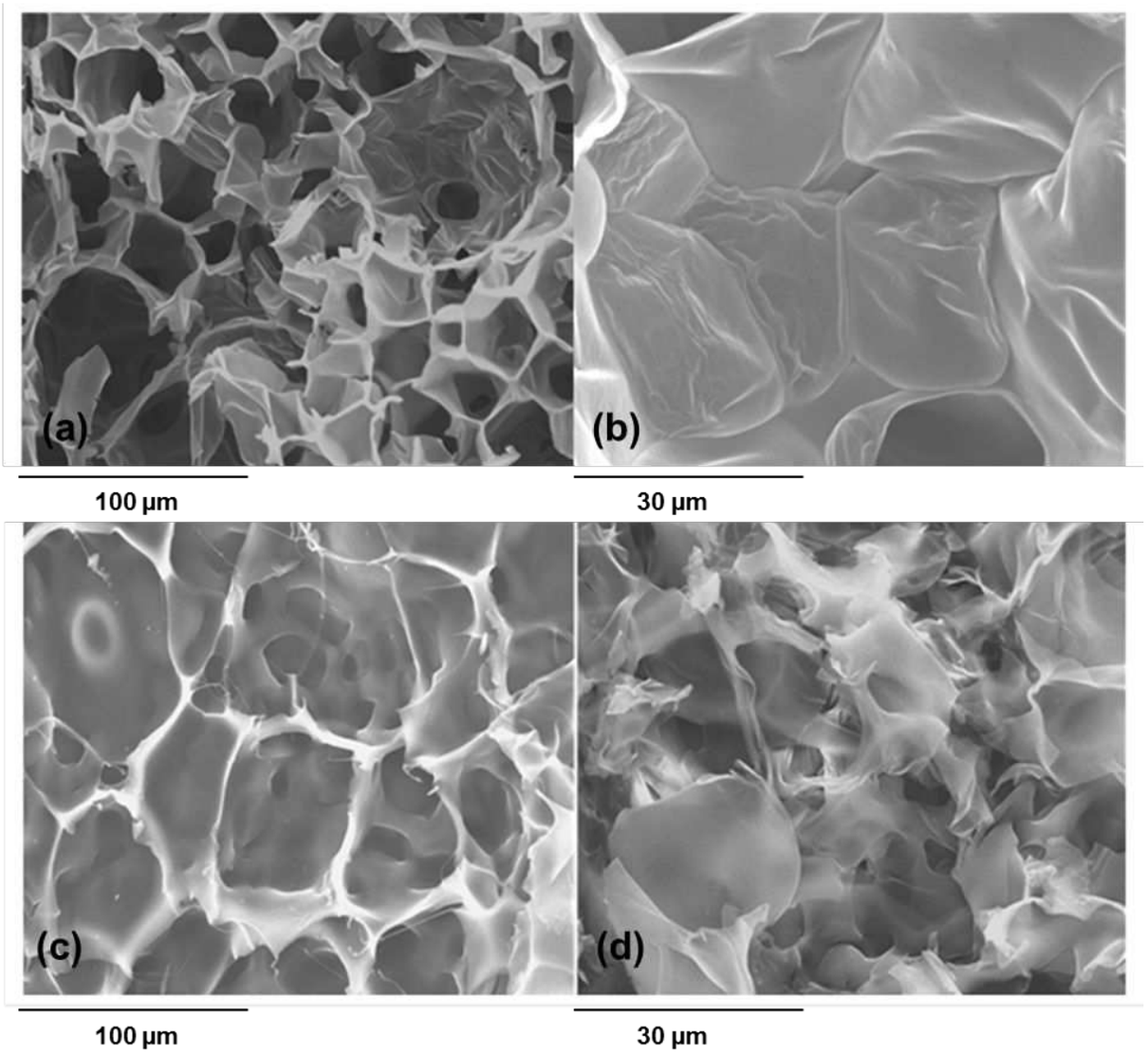


**Figure 6.** The SEM of lyophilized AX1 (a, b) and AX2 (c, d) gels. a and c at 500x magnification; b and d at 2000x magnification.

The entrapment of biomolecules or microorganisms in high cell dimension (>200µm) AX gels has been previously reported [14, 19, 31], but reducing cell dimensions of AX gels could increase the possibility of carried out smaller compounds or cells. Therefore, AX1 and AX2 gels microstructural characteristics could be of interest for the development of designed delivery systems, which could allow alternative uses for maize wastewater.

## 4. Conclusion

AX1 and AX2 presented similar molecular identity (FT-IR) and molecular weight distribution, but different FA content. Both AX1 and AX2 presented gelling capability under laccase exposure. The lower FA content in AX2 form gels presenting minor elasticity values and a

more fragmented microstructure. These results indicate that nixtamalization process conditions can modify the characteristics of AX gels. Environmental concerns have triggered research on alternative nixtamalization processes rendering residues with less environment impact. AX recovering from this kind of less pollutant maize wastewater could be an interesting research subject in order to explore the structural and functional properties of this hydrocolloid.

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