



Maize processing waste water arabinoxylans: Gelling capability and cross-linking content

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ABSTRACT

Water-soluble feruloylated arabinoxylans were extracted from maize processing waste water (nejayote) generated from tortilla-making industries. Nejayote arabinoxylans (NAX) presented a ferulic acid content of 0.23 µg/mg, an arabinose to xylose ratio (A/X) of 0.65, an intrinsic viscosity, $[\eta]$, of 183 ml/g and a molecular weight (Mw) of 60 kDa. Laccase-induced gels were obtained from 4% (w/v) NAX solution while, at lower concentrations, no gelation was observed. Laccase covalent cross-linking of NAX led to the formation of diferulic (di-FA) and triferulic (tri-FA) acid structures. 4% (w/v) NAX gel presented di-FA and tri-FA contents of 0.02 and 0.01 µg/mg NAX, respectively, and a G' value of 2 Pa. Recuperation of this gum from a low-value maize by-product could represent a commercial advantage over other gums commonly used in the food industry.

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1. Introduction

Maize nixtamalisation is important in Mexico as half of the total volume of consumed food is maize. Nixtamalisation consists of cooking maize grains in a lime solution; after soaking for 2–8 h, the supernatant (called nejayote) is discarded. The remaining material is then ground to obtain nixtamal (dough or masa), used to prepare a variety of products, tortilla being the most popular one (Billeb de Sinibaldi & Brezan, 2001). The nixtamalisation process degrades and solubilises maize cell wall components, thus facilitating pericarp removal. As a matter of fact, the nejayote, in general, contains more than 60% of non-starch polysaccharides (Arámula-Villa, Barrón-Ávila, González-Hernández, Moreno-Martínez, & Luna-Bárceñas, 2001). Alkali-soluble non-cellulosic cell wall polysaccharides, present in maize pericarp (mainly arabinoxylans), show interesting functional properties as thickeners, stabilisers, emulsifiers and film and gel formers. Due to the fact that nejayote obtained from nixtamalisation is a highly alkaline waste water, with high chemical and biological oxygen demand, it is considered an environmental pollutant. A typical maize nixtamalisation facility, processing 50 kg of maize every day, uses over 75 l of water per day and generates nearly the equivalent amount of alkaline waste water on a daily basis. Thus, alternatives for nejayote residues utilisation in Mexico are needed.

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Arabinoxylans are non-starch polysaccharides, from the cell walls of cereal endosperm (Fincher & Stone, 1974), constituted of a linear $-(1\rightarrow4)$ -xylopyranose backbone and *-L*-arabinofuranose residues as side chains on O3 and O2 and O3 (Izydorczyk & Biliaderis, 1995). Arabinoxylans can present some of the arabinose residues ester-linked on (O)-5 to ferulic acid (FA) (3-methoxy, 4 hydroxy cinnamic acid) (Smith & Hartley, 1983) (Fig. 1). Dehydrodimers of ferulic acid (di-FA structures) may serve to cross-link cell wall polymers and contribute to the mesh-like network of the cell wall (Bunzel, Ralph, Bruening, & Steinhart, 2006; Iiyama, Lam, & Stone, 1994). Arabinoxylans can gel by covalent cross-linking, involving FA oxidation by some chemical or enzymatic (laccase/O₂ and peroxidase/H₂O₂ system) free radical-generating agents (Figueroa-Espinoza & Rouau, 1998; Geissman & Neukom, 1973; Hosney & Faubion, 1981). Five main di-FA (5-5', 8-5' benzo, 8-O-4', 8-5' and 8-8' di-FA) are identified in gelled arabinoxylans, the 8-5' and 8-O-4' forms being generally preponderant (Carvajal-Millán, Guigliarelli, Belle, Rouau, & Micard, 2005a; Figueroa-Espinoza & Rouau, 1998; Schooneveld-Bergmans, Dignum, Grabber, Beldman, & Voragen, 1999; Vansteenkiste, Babot, Rouau, & Micard, 2004) (Fig. 2). The involvement of a trimer of ferulic acid (4-O-8', 5'-5'-dehydrotriferulic acid) in laccase cross-linked wheat or maize bran arabinoxylans has been reported (Carvajal-Millán et al., 2005a; Carvajal-Millán et al., 2007). In addition to covalent cross-links (di-FA, tri-FA), the involvement of physical interactions between arabinoxylan chains was suggested to contribute to the arabinoxylan gelation and gel properties (Carvajal-Millán et al., 2007;

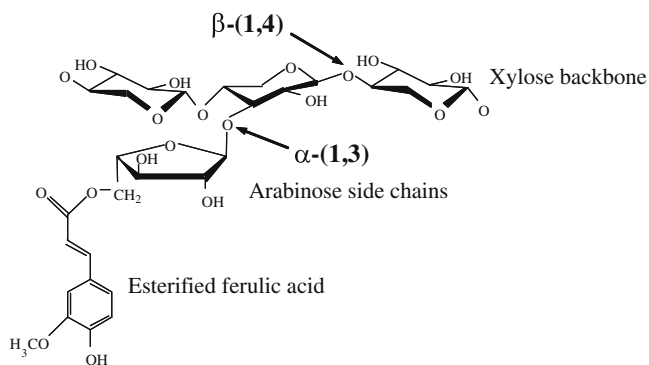


Fig. 1. Chemical structure of arabinoxylans.

Vansteenkiste et al., 2004). Arabinoxylan gels have an interesting technological potential as they are mostly stabilised by covalent linkages, which make them stable upon heating and they exhibit no syneresis after long times of storage (Izydorczyk & Biliaderis, 1995; Vansteenkiste et al., 2004).

The purpose of this research was to recover arabinoxylans from nejayote and to characterise their physicochemical and gelling properties as a new source of maize arabinoxylans for the food industry.

2. Materials and methods

2.1. Materials

Nejayote was kindly provided by a local tortilla-making industry in northern Mexico. Laccase (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) was obtained from *Trametes versicolor*. All chemical products were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Nejayote arabinoxylans (NAX) extraction

NAX were extracted as described by Carvajal-Millan, Rascón-Chu, and Márquez-Escalante (2005b).

2.3. Neutral sugars

Neutral sugars content in NAX was determined after hydrolysis with 2 N trifluoroacetic acid at 120 °C for 2 h. The reaction was stopped on ice and the extracts were evaporated under air at 40 °C, and rinsed twice with 200 μ l of water. The evaporated extract was solubilised in 500 μ l of water. Sorbitol was used as internal standard. Samples were filtered (0.45 μ m, Whatman) and analysed by high performance liquid chromatography (HPLC) using a Supelcogel Pb column (300 \times 7.8 mm; Supelco, Inc., Bellefont, PA) eluted with water (filtered 0.2 μ m, Whatman) at 0.6 ml/min

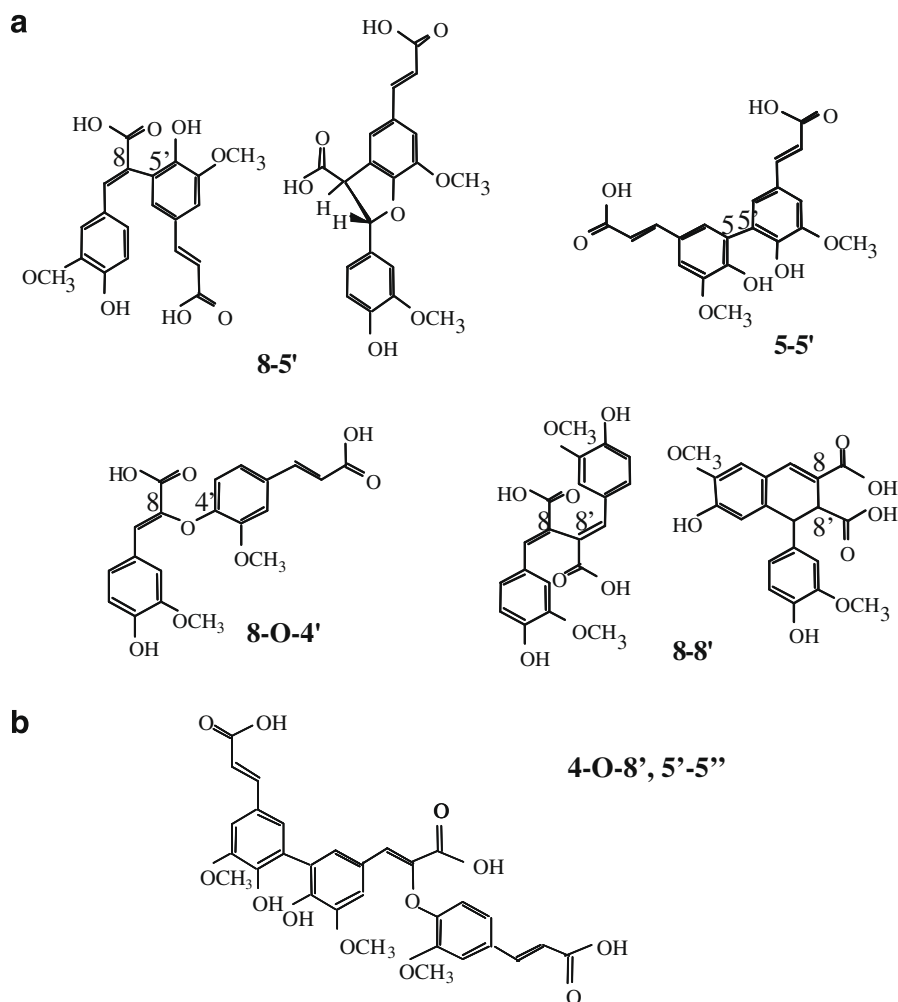


Fig. 2. Dehydromers of ferulic acid (di-FA) (a) and dehydrotrimer of ferulic acid (tri-FA) (b) chemical structures.

and 80 °C. A refractive index detector Star 9040 (Varian, St. Helens, Australia) was used.

2.4. Proteins

Protein content in NAX was determined according to the Bradford method (Bradford, 1976).

2.5. Ash

Ash content was determined according to the AOAC methods (Association of Official Analytical Chemists, 2002).

2.6. Viscosity determinations

Specific viscosity (η_{sp}) of NAX solutions was measured with an AVS 400 capillary viscosimeter (Schott Geräte, Hofheim, Germany), equipped with an Oswald capillary tube (flow water time 75.15 s). The η_{sp} was related to the NAX concentration (η_{sp}/C) to obtain their reduced viscosity, η_{red} (ml/g), according to Rao (1993). The intrinsic viscosity $[\eta]$ was determined (Kraemer, 1938; Mead & Fous, 1942).

2.7. NAX gel preparation

A NAX solution (4% w/v) was prepared in 0.05 M citrate phosphate buffer, pH 5. Laccase (1.68 nkat per mg NAX) was added to NAX solution. Gels were allowed to form for 2 h at 25 °C.

2.8. Ferulic acid (FA), dimers of ferulic acid (di-FA) and trimer of ferulic acid (tri-FA) in NAX and NAX gels

FA, di-FA and tri-FA contents in NAX and NAX gels were quantified by high performance liquid chromatography (HPLC) after a deesterification step, as described by Vansteenkiste et al. (2004). Tri-FA levels were quantified as described by Rouau et al. (2003). 100 mg of sample were allowed to react with 1 ml of 2 N NaOH for 2 h in the dark at 35 °C under argon. After adding 3,4,5-trimethoxy-trans-cinnamic acid (TMCA, internal standard, 10 µg), the pH was adjusted to 2.0 ± 0.2 with 4 N HCl. Phenolics were extracted twice with diethyl ether, and evaporated at 30 °C under argon. The dried extracts were solubilised in 0.50 ml of methanol/water/acetic acid (40/59/01), filtered (0.45 µm) and injected (20 µl) into the HPLC, using a Supelcosil LC-18-DB (250 × 4.6 mm) (Supelco, Inc., Bellefont, PA) column. Detection was by UV absorbance at 280 nm. Isocratic elution was performed, using methanol/water/acetic acid (40/59/01) at 0.6 ml/min at 35 °C. A Varian 9012 photodiode array detector (Varian, St. Helens, Australia) was used to record the ferulic acid spectra. A Star Chromatography Workstation system control version 5.50 was used.

2.9. Rheological measurements

Rheological tests were performed by small amplitude oscillatory shear (AR-1500ex, TA Instruments, USA), as reported by Vansteenkiste et al. (2004). NAX gelation was studied for 4 h at 25 °C. Measurements were carried out in the linear domain at 0.25 Hz and 5% strain at 25 °C.

2.10. Statistical analysis

Chemical determinations were done in duplicates and the coefficients of variation were lower than 5%. Rheological measurements were done in duplicate and the coefficients of variation were lower than 8%. All results are expressed as mean values.

Table 1

Composition of nejayote arabinoxylan (NAX) gum.

Arabinose ^a	32.0 + 0.80
Xylose ^a	49.0 + 1.90
Glucose ^a	5.10 + 0.40
Galactose ^a	3.70 + 0.20
Mannose ^a	0.60 + 0.04
Protein ^a	4.50 + 0.20
Ash ^a	5.10 + 0.21
Ferulic acid ^b	0.23 + 0.01
Diferulic acids ^b	0.58 + 0.01
Triferulic acid ^b	0.30 + 0.01

All results are obtained from duplicates.

^a Results are expressed in g/100 g NAX matter

^b Phenolics are expressed in µg/mg NAX.

Table 2

FA, di-FA and tri-FA contents in 4% (w/v) laccase-induced NAX gels.

FA (µg/mg NAX)	0.060 + 0.001
di-FA (µg/mg NAX)	0.020 + 0.001
tri-FA (µg/mg NAX)	0.010 + 0.001

All results are obtained from duplicates.

3. Results and discussion

3.1. NAX extraction and characterisation

Composition of NAX is presented in Table 1. Pure arabinoxylans represented 81%, dry basis (db), of the recovered NAX gum. This value was estimated from the sum of xylose + arabinose. The ratio arabinose to xylose was high (A/X = 0.65), indicating a moderately branched structure, similar to that reported by Singh, Doner, Johnston, Hicks, and Eckhoff (2000) in maize bran arabinoxylans. A higher A/X ratio (0.85) was recently reported by Carvajal-Millán et al. (2007) in a maize bran gum. Residues of glucose, galactose, mannose, proteins and ash were also detected in this NAX gum. The FA content (0.23 µg/mg NAX) was lower than that reported by Carvajal-Millán et al. (2007) in maize bran arabinoxylans (0.34 µg/mg). Di-FA and tri-FA were also detected in NAX (0.58 and 0.30 µg/mg NAX, respectively), suggesting that some arabinoxylan chains might be inter- and/or intra-cross-linked. The di-FA and tri-FA contents in NAX were higher than the FA content. Carvajal-Millán et al. (2007) and Lapierre, Pollet, Ralet, and Saulnier (2001) reported similar results for a maize bran arabinoxylan extracted under controlled alkaline conditions. The relative percentages of each di-FA structure were: 15, 20 and 65%, for the 8-5' (mainly in the benzofuran form), 8-O-4' and 5-5' structures, respectively. Similar relative percentages were recently reported by Carvajal-Millán et al. (2007) in maize bran arabinoxylans. The intrinsic viscosity ($[\eta]$) value and viscosimetric molecular weight (Mw) of NAX were 183 ml/g and 60 kDa. These values are lower than that reported for maize bran arabinoxylans (Carvajal-Millán et al., 2007). This low Mw allowed the formation of NAX solutions at 4% (w/v), which is higher than that reported in the literature for other arabinoxylan cereal sources (Carvajal-Millán, Guigliarelli, Belle, Rouau, & Micard, 2005a) (see Table 2).

3.2. NAX gelation and cross-linking content

Treatment of NAX with laccase as oxidising agent results in the development of a three-dimensional network. Fig. 3 shows the evolution of storage (G') and loss (G'') moduli of laccase-treated NAX solution (4.0%, w/v) as a function of time. NAX solution exhibited gradual increase in the G' value throughout the entire reaction time. The rheological measurements showed that the crossover

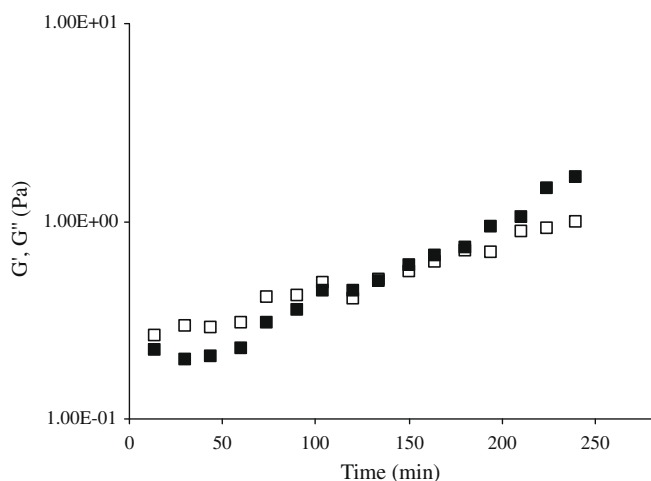


Fig. 3. Monitoring of the storage (G' , ■) and loss (G'' , □) moduli of NAX solution (4% w/v) during gelation by laccase. Rheological test at 0.25 Hz, 5% strain and 25 °C.

point of the G' and G'' curves ($G' > G''$) was at 150 min. The $\tan \delta$ (G''/G') value, calculated at the end of the test, was 0.5, indicating the presence of an elastic covalent system (Ross-Murphy, 1984). This behaviour is similar to that previously reported for arabinoxylan gels cross-linked by laccase or peroxidase/ H_2O_2 systems (Carvajal-Millán et al., 2005a; Izydorczyk, Biliaderis, & Bushuk, 1990a; Vansteenkiste et al., 2004). Nevertheless, the G' value (2 Pa) of NAX gels was lower than those reported for laccase-induced wheat arabinoxylan gels (20–40 Pa) at lower polysaccharide concentrations (1–2% w/v) (Carvajal-Millán et al., 2005a). Such behaviour might have its origin in the structural and/or conformational characteristics of these macromolecules. Clearly, further studies on the distribution of arabinose and feruloyl groups along the polymer chain backbone are needed to establish relationships between the molecular structure and gelling ability of NAX. The di and tri-FA cross-linking contents of NAX gels at 4% (w/v) were 0.02 and 0.01 $\mu\text{g}/\text{mg}$ NAX, respectively. At the end of gelation, 74% of the FA initially present in 4% (w/v) NAX solutions was oxidised, while only 18% was recovered as di and tri-FA. Interestingly, the di and tri-FA contents in NAX did not increase after laccase-induced gelation; in fact, they decreased from 0.58 to 0.02 and from 0.30 to 0.01 $\mu\text{g}/\text{mg}$ NAX, respectively. In similar studies, Lapiere et al. (2001) and Carvajal-Millán et al. (2007) reported that, in maize bran arabinoxylans, di-FA and tri-FA contents did not increase after peroxidase or laccase gelation, despite a decrease in FA content. These authors attributed this result to the formation of ferulate cross-linking structures which can not be released by mild alkaline hydrolysis and/or to the participation of lignin residues in the formation of the arabinoxylan gel. In accordance, Grabber, Ralph, and Hatfield (1988) have already reported the propensity of ferulate esters to copolymerise with lignin units. The relative percentages of di-FA structures found in NAX gels were different from those found in the NAX gum, as only the 5-5' and 8-0-4' di-FA structures were detected and at relative percentages of 50%. Similar results were found in maize bran arabinoxylan gels induced by laccase (Carvajal-Millán et al., 2007) while Lapiere et al. (2001), reported that the di-FA profile of maize bran arabinoxylans was not altered by peroxidase gelation, the 8-8' structure being predominant.

4. Conclusions

Arabinoxylans recovered from nejayote (NAX) presented a ferulic acid content high enough to allow the formation of laccase-in-

duced covalent gels. The covalent cross-linking structures (di and tri-FA) quantified in NAX gels only represent 18% of the FA initially present in 4% (w/v) NAX solutions. This result suggests that covalent linkages, through di and tri-FA quantified structures, are only partially involved in the NAX gel formation. Other covalent cross-links might result from the strong oxidising treatment and/or physical interactions which participate in the NAX gel structure. The NAX presented in this study could be a potential texturing agent for the food industry. Besides, recuperation of this NAX from nejayote, generated from tortilla-making industries, could represent a commercial advantage over other gums commonly used in the food industry.

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