

RESEARCH NOTE

## Laccase Induced Maize Bran Arabinoxylan Gels: Structural and Rheological Properties

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**Abstract** The aim of this research was to study the structural and rheological properties of gels formed by ferulated maize bran arabinoxylans (MBAX) at different concentrations. MBAX was cross-linked by a laccase leading to the formation of dimers and trimers of ferulic acid (di-FA, tri-FA) as covalent cross-link. An increase in MBAX gels elasticity (from 11 to 20 Pa) as well as lower mesh size (from 80 to 48 nm) were obtained by augmenting the MBAX concentration from 2.5 to 3.5% (w/v), respectively, but no increase in di-FA and tri-FA content was obtained (0.03 and 0.014 µg/mg MBAX, respectively).

**Keywords:** gel, arabinoxylan, maize bran, elasticity, mesh size

### Introduction

Gels are polymeric 3-dimensional networks, which swell on contact with water but do not dissolve (1). The water absorption property of gels confers those interesting properties in applications like food additives, enzyme immobilization, and controlled release devices (2-4). Although most studies concern gels made from synthetic polymers, gellable native or tailored polysaccharides, generally non-toxic and highly biocompatible, are receiving increasing attention (5). Arabinoxylans (AX) are non-starch polysaccharides from the cell walls of cereal endosperm constituted by a linear backbone of β-(1→4)-linked xylose units containing α-L-arabinofuranosyl substituents attached through O-2 and/or O-3. AX can present some of the arabinose residues ester-linked on O-5 to ferulic acid (FA) (3-methoxy, 4-hydroxy cinnamic acid) (6). Ferulated AX can gel by covalent cross-linking involving FA oxidation by some chemical or enzymatic (laccase and peroxidase/H<sub>2</sub>O<sub>2</sub> system) free radicals-generating agents (7,8). Diferulic acids (di-FA) and tri-ferulic acid (tri-FA) (9,10) have been identified as covalently cross-linked structures in laccase-gelled AX. Both, covalent bridges (diFA, tri-FA) and physical interactions between AX chains have been reported to be involved in the AX gelation process and the final gel properties (11). AX gels present interesting properties like neutral taste and odor, high water absorption capacity (up to 100 g water/g dry polymer) and absence of pH or electrolyte susceptibility (6). In this study, the effect of maize bran arabinoxylan (MBAX) concentration on the structural and rheological properties of gels obtained by enzymatic way was investigated.

### Materials and Methods

**Materials** Maize bran arabinoxylans (MBAX) were obtained and characterized as previously described (12). They contain 75% dry basis (d.b.) of pure AX. MBAX presented a ferulic acid (FA), di-FA, and tri-FA content of 0.34, 0.77, and 0.39 µg/mg of MBAX, respectively, and an A/X ratio of 0.8. The relative percentages of each di-FA structure were: 16, 21, and 63% for the 8-5', 8-O-4', and 5-5' structures, respectively. A commercial laccase (benzenediol: oxygen oxidoreductase, E.C.1.10.3.2) from *Trametes versicolor* was used as cross-linking agent obtained from Sigma-Aldrich (St. Louis, MO, USA). Laccase activity was measured as reported elsewhere (10). All chemical products were obtained from Sigma-Aldrich.

**Preparation of MBAX gels** MBAX solutions at 2.5 and 3.5% (w/v) were prepared in 0.05 M citrate phosphate buffer (pH 5.5). MBAX solutions were mixed with 50 µL of laccase (1.675 nkat/mg MBAX). Gels were allowed to form for 6 hr at 25°C.

**Phenolic acids content** FA, di-FA, and tri-FA contents were determined in MBAX solutions and gels by reverse phase-high performance liquid chromatography (RP-HPLC) after deesterification step (9,10). A Supelcosil LC-18-DB (2,504.6 mm) (Supelco, Inc., Bellefont, PA, USA) column was used. Detection was by UV absorbance at 320 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.

**Rheological tests** Rheological test were performed by small amplitude oscillatory shear by using a strain controlled rheometer (AR-1500ex; TA Instruments, New Castle, DE,

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USA) in oscillatory mode (10). Cold (4°C) solutions of 2.5 and 3.5%(w/v) MBAX were mixed with laccase (1.675 nkat/mg MBAX) and immediately placed in the cone and plate geometry (5.0 cm in diameter, 0.04 rad in cone angle) maintained at 4°C. Exposed edges of the sample were covered with mineral oil fluid to prevent evaporation during measurements. MBAX gelation kinetic was started by a sudden increase in temperature from 4 to 25°C and monitored at 25°C for 6 hr by following the storage ( $G'$ ) and loss ( $G''$ ) modulus. All measurements were carried out at 0.25 Hz and 5% strain. From strain sweep tests, MBAX gels showed a linear behaviour from 0.2 to 10% strain. The mechanical spectra of gels were obtained by frequency sweep from 0.1 to 10 Hz at 5% strain and 25°C.

**MBAX gel swelling** After laccase addition, 2 mL MBAX solutions were quickly transferred to a 5-mL tip-cut-off syringe (diameter 1.5-cm) and allowed to gel for 6 hr at 25°C. After gelation, the gels were removed from the syringes, placed in glass vials and weighed. The gels were allowed to swell in 20 mL of 0.02%(w/v) sodium azide solution to prevent microbial contamination. During 36 hr the samples were blotted and weighed. After weighing, a new aliquot of sodium azide solution was added to the gels. Gels were maintained at 25°C during the test. The equilibrium swelling was reached when the weight of the samples changed by no more than 3% (0.06 g). The swelling ratio ( $q$ ) was calculated as:

$$q = (W_s - W_d) / W_d$$

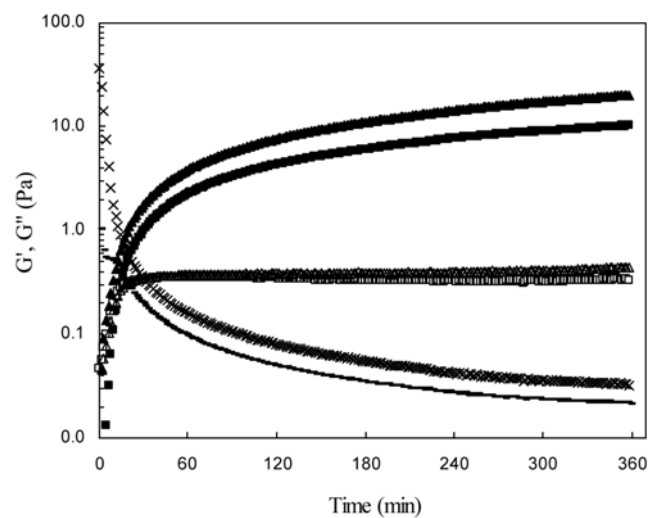
where  $W_s$  is the weight of swollen gels and  $W_d$  is the weight of MBAX in the gel.

**MBAX gel structure** From swelling measurements, the molecular weight between 2 cross-links ( $M_c$ ), the cross-linking density ( $\rho_c$ ), and the mesh size ( $\xi$ ) values of the different MBAX gels were obtained as described before (11).

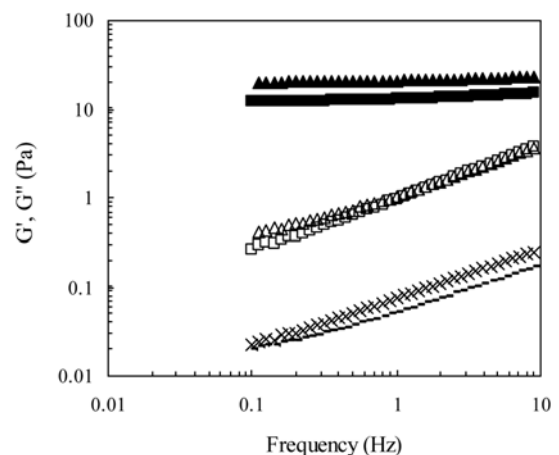
**Statistical analysis** The experimental data were analyzed with the Statistical Analysis System software (SAS Institute, Cary, NC, USA). The significance of difference was calculated using Tuckey's test ( $p \leq 0.05$ ).

## Results and Discussion

**MBAX gelation** The formation of MBAX gels over time was rheologically investigated by small amplitude oscillatory shear (Fig. 1). For all samples the storage ( $G'$ ) and loss ( $G''$ ) modulus rose to reach a plateau. The final  $G'$  values augmented from 11 to 20 Pa when the MBAX concentration in the gel was from 2.5 to 3.5%(w/v). The  $\tan \delta$  ( $G''/G'$ ) values decreased during MBAX gelation (Fig. 1) indicating the formation of a more elastic material. The mechanical spectrum of MBAX gels after 6 hr gelation was typical of solid-like materials with a linear  $G'$  independent of frequency and  $G''$  much smaller than  $G'$  and dependent on frequency (13). This behavior is similar to that previously reported for AX gels cross-linked by laccase or peroxidase/ $H_2O_2$  system (9,10). It was found that  $\tan \delta$  values in the gels decreased when the MBAX concentration augmented (Fig. 2), indicating a more elastic



**Fig. 1.** Laccase induced gelation of MBAX solutions at 2.5 [ $G'$  ▲,  $G''$  △,  $\tan \delta$  (X)] and 3.5 [ $G'$  ■,  $G''$  □,  $\tan \delta$  (-)]%(w/v). Rheological measurements made at 25°C, 0.25 Hz, and 5% strain.



**Fig. 2.** Mechanical spectra of MBAX gels at 2.5 [ $G'$  ▲,  $G''$  △,  $\tan \delta$  (X)] and 3.5 [ $G'$  ■,  $G''$  □,  $\tan \delta$  (-)]%(w/v). Rheological measurements made at 25°C and 5% strain.

property of this material (13).

At the end of gelation, 73 and 72% of the FA initially present in MBAX was oxidized while only 17 and 18% of oxidized FA was recovered as di and tri-FA for gels at 2.5 to 3.5%(w/v), respectively (Table 1). As a fact, the di- and tri-FA content in MBAX did not increase after laccase induced gelation, they rather decreased from 0.77 to 0.03 and from 0.390 to 0.014  $\mu\text{g}/\text{mg}$  MBAX for both MBAX concentrations in the gel. Similar results were previously reported (14) with no increase in di-FA content of maize bran heteroxylans after peroxidase gelation, in spite of 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 peroxidase induced MBAX gel. This decrease in FA content without a proportional formation of di- and tri-FA structures has been also reported in laccase induced wheat and maize arabinoxylan gels (9,10). These results were

**Table 2. Structural characteristics of gels at different concentrations in MBAX<sup>1)</sup>**

| MBAX (% w/v) | Swelling ratio (q, g water/g MBAX) | Mc×10 <sup>3</sup> (g/mol) | ρ <sub>c</sub> ×10 <sup>-6</sup> (mol/cm <sup>3</sup> ) | ξ (nm) |
|--------------|------------------------------------|----------------------------|---|--------|
| 2.5          | 82 a                               | 31 a                       | 49 b  | 80 a   |
| 3.5          | 20 b                               | 20 b                       | 75 a  | 48 b   |

<sup>1)</sup>Mc, molecular weight between 2 cross-links; ρ<sub>c</sub>, cross-linking density; ξ, mesh size; All results are obtained from triplicates.

related to the formation of non reported di- and tri-FA or higher ferulate structures and/or to physical interactions between arabinoxylan chains. In the present study, the relative percentages of di-FA structures (isomeric forms of di-FA) found in MBAX gels were different to those found in the MBAX before gelation. In gelled MBAX only the 5-5' and 8-O-4' di-FA isomeric structures were detected at relative percentages of 50% while in MBAX before gelation the relative percentages of each di-FA structure were: 16, 21, and 63% for the 8-5' (mainly in the benzofuran form), 8-O-4' and 5-5' structures, respectively.

**MBAX gel structure** The equilibrium swelling of MBAX gels was reached between 15-20 hr. The swelling ratio (q, g water/g MBAX) in MBAX gels decreased from 82 to 20 when the MBAX concentration in the gel increased from 2.5 to 3.5%(w/v) (Table 1). The lower swelling ratio values obtained as the MBAX concentration in the gel increased can be related to the more compact polymeric structure that limits the water absorption (11). The molecular weight between 2 cross-links (Mc), the cross-linking density (ρ<sub>c</sub>), and the mesh size (ξ) values of the different MBAX gels are presented in Table 1. When the MBAX concentration increased in gel from 2.5 to 3.5%(w/v), Mc and ξ decreased from 31×10<sup>3</sup> to 20×10<sup>3</sup> g/mol and from 80 to 48 nm, respectively, leading to an increase in ρ<sub>c</sub> from 49×10<sup>-6</sup> to 75×10<sup>-6</sup> mol/cm<sup>3</sup>. Higher mesh sizes values (200-400 nm) have been reported in laccase induced wheat AX gels at similar AX concentrations (11). The latter could be related to a high molecular weight in AX from wheat (400-600 kDa) in comparison to AX from maize (200-300 kDa) (12). As the covalent cross-links (di-FA, tri-FA) content did not increase when the concentration of MBAX in the gel augmented, the involvement of physical interactions between AX chains and possible higher oligomers of ferulate in the final MBAX gel structure could be responsible of this evolution as reported before (11,12) in laccase induced wheat arabinoxylan gels. Different MBAX gel structural characteristics (covalent bonds and physical interactions proportions) were therefore obtained by modifying the MBAX concentration in the gel.

Increase in G' value of MBAX gels as well as lower ξ can be obtained by augmenting the MBAX concentration from 2.5 to 3.5%(w/v) but no increase in known covalent cross-linking (di-FA, tri-FA) content was obtained. These results suggest the implication of physical interactions to the MBAX gel structure or the presence of possible higher oligomers of ferulate. MBAX gels with different rheological and structural characteristics can be obtained by modifying the MBAX concentration before gelation. These differences could induce changes in the functional properties of the gel.

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