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Impact of the structure of arabinoxylan gels on their rheological and protein transport properties

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Abstract

Water-extractable arabinoxylan (WEAX) gels exhibiting different structural, rheological and protein transport properties were obtained upon laccase treatment of WEAX solutions, by modifying (i) the initial ferulic acid (FA) content of WEAX from 2.3 to 1.6 μg/mg AX or (ii) the AX concentration of the WEAX solution from 0.5 to 2.0% (w/v). WEAX gels with di and tri-ferulic acid (di-FA, tri-FA) contents varying from 6.2 to 3.2 μg/ml gel and from 0.61 to 0.27 μg/ml gel, respectively, were obtained. In parallel, increases in gel mesh sizes from 201 to 331 nm and reduction of G' of gels from 160 to 12 Pa were observed. The differences in structural and rheological characteristics of WEAX gels were reflected in their capacity to load and release proteins of M_w ranging from 43 to 669 kDa. The possibility of modulating protein release from WEAX gels makes these gels potential candidates for the controlled delivery of proteins.

Keywords: Arabinoxylan gels; Ferulic acid; Diferulic acid; Triferulic acid; Mesh size; Protein release

1. Introduction

Hydrogels are hydrophilic polymer networks that are capable of imbibing large amounts of water without dissolution (Peppas & Khare, 1993; Kopecek, 2003). The water absorption property of hydrogels gives them interesting properties in applications like enzyme immobilization, chromatography and controlled release of biomolecules (Peppas, Bures, Leobandung, & Ichikawa, 2000). Hydrogels as delivery matrices are frequently used in food (Pothakamury & Barbosa-Canovas, 1995), medicine (Peppas, 1997), agronomy (Bajpai & Giri, 2003) and cosmetic industries (Valenta & Schultz, 2004). Although most studies concern hydrogels made from synthetic polymers, gellable native or tailored polysaccharides which are generally non-toxic and highly biocompatible, are receiving increasing attention (Rodriguez, Alvarez-Lorenzo, & Concheiro, 2003).

Research on the controlled release of proteins is expanding as they are becoming an increasingly important class of therapeutic agents. Nevertheless, oral delivery of proteins is complicated because of protein inactivation by digestive enzymes (Peppas et al., 2000). The colonic region, due to its lower proteolytic activity in comparison to that in the small intestine, has been considered as a possible absorption site for orally administrated peptides and proteins (Brondsted, Hovgaard, & Simonsen, 1996). Polysaccharides like dextran, guar gum and pectin forming hydrogels resistant to enzymic digestion but degraded in the presence of colonic microflora, are potential devices for colon-specific drug delivery (Peppas et al., 2000).

Arabinoxylans (AX) are non-starch polysaccharides from the cell walls of cereal endosperm (Fincher & Stone, 1986) constituted by a linear backbone of β-(1→4)-linked xylose units containing α-L-arabinofuranosyl substituents attached trough O-2 and/or O-3 (Izydorczyk & Biliaderis, 1995). Arabinoxylans have been classified as water extractable (WEAX) or water-unextractable (WUAX). AX 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). Feruloylated WEAX can gel by covalent cross-linking involving FA oxidation by some chemical or enzymatic (laccase and peroxidase/H_2O_2 system) free radicals-generating agents (Geissman & Neukom, 1973; Hoseney & Faubion, 1981; Izydorczyk,

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Biliaderis, & Bushuk, 1990; Figueroa-Espinoza & Rouau, 1998). Diferulic acids (di-FA) (Figueroa-Espinoza, Morel, & Rouau, 1998; Schooneveld-Bergmans, Dignum, Grabber, Beldman, & Voragen, 1999; Vansteenkiste, Babot, Rouau, & Micard, 2004) and tri-ferulic acid (tri-FA) (Carvajal-Millan, Guigliarelli, Belle, Rouau, & Micard, 2005a) have been identified as covalent cross-link structures in laccase gelled WEAX. Both, covalent bridges (diFA, tri-FA) and physical interactions between AX chains have been reported to be involved in the WEAX gelation process and the final gel properties (Vansteenkiste et al., 2004; Carvajal-Millan et al., 2005a; Carvajal-Millan, Landillon, Morel, Rouau, Doublier, & Micard, 2005b). WEAX gels have interesting properties like neutral taste and odour, high water absorption capacity (up to 100 g of water per gram of dry polymer) and absence of pH or electrolyte susceptibility (Izydorczyk & Biliaderis, 1995). The macroporous structure of WEAX gels with mesh sizes varying from 200 to 400 nm (Carvajal-Millan, Landillon, Morel, Rouau, Doublier, & Micard, 2005b), and the dietary fiber nature of arabinoxylans (Van Laere, Hartemink, Bosveld, Schols, & Voragen, 2000) give them potential applications for colon-specific protein delivery. In this study, the effect of feruloylation degree of WEAX and the concentration of WEAX before gelation on the structural and rheological properties of gels obtained by enzymatic way was investigated. WEAX gel structure was characterized in terms of molecular weight between cross-links, cross-linking density and mesh size determined from swelling measurements. Protein loading and release capacity of WEAX gels was then investigated and related to the gel structure.

2. Experimental

2.1. Materials

Water extractable arabinoxylans (WEAX) were obtained from endosperm of wheat kernel as previously described by Carvajal-Millan et al. (2005b). These native WEAX were partially deferuloylated by a chemical way without affecting their other structural properties (arabinose to xylose (A/X) ratio, intrinsic viscosity [η] and molecular weight (M_w)), by using the method reported by Carvajal-Millan et al. (2005b). Native and partially feruloylated WEAX (PF-WEAX) presented an A/X ratio of 0.6, a M_w of 438 kDa, and a [η] of 5.68 dL/g. The FA content of native WEAX was 2.3 μg/mg AX while in PF-WEAX it was modified to 1.8 and 1.6 μg/mg AX. Laccase (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) from *Pycnoporus cinnabarinus* was supplied by the Unité de Biotechnologie des Champignons Filamenteux-(UMR 1163 INRA-ESIL, Luminy, France). Laccase activity was measured as reported elsewhere (Carvajal-Millan et al., 2005a). Citric acid, sodium phosphate dibasic, ovalbumin (43 kDa), bovine serum albumin (67 kDa) and catalase (232 kDa) were obtained from Sigma Chemical Co. (St Louis, MO, USA). Aldolase (158 kDa), ferritine (440 kDa) and thyroglobuline (669 kDa) were from Pharmacia Biotech (Uppsala, Sweden). Amyloglucosidase (97 kDa) and sodium azide were from Fluka (Steinheim, Switzerland). Xylanase (15 nkat/μl) was from Megazyme (Wicklow, Ireland).

2.2. Methods

2.2.1. Preparation of WEAX gels

WEAX solutions at the same concentration (1.0% w/v in AX) but made from WEAX with different initial FA content (2.3, 1.8 and 1.6 μg/mg of AX, i.e. 1.0%, $1.0_{80\%}$, $1.0_{70\%}$) or with the same initial FA content (2.3 μg/mg AX) but at different concentrations (0.5, 1.0, 1.5 and 2.0% w/v) in AX were prepared in 0.05 M citrate phosphate buffer pH 5.5. 2 ml of each WEAX solution were mixed with 50 μl of laccase (1.675 nkat per mg AX). Gels were allowed to form for 2 h at 25 °C.

2.2.2. Phenolic acids content

Ferulic acid (FA), dimers of ferulic acid (di-FA) and trimer of ferulic acid (tri-FA) contents were determined in native WEAX and PF-WEAX solutions and gels by RP-HPLC after deesterification step. FA and di-FA were quantified as described by Vansteenksite et al. (2004). Response factors of di-FA determined by Saulnier et al. (1999) were used. Tri-FA (tri-FA 4-O-8′, 5′-5″dehydrotriferulic acid) was quantified as described by Rouau et al. (2003). An Alltima C18 column (250× 4.6 mm) (Alltech associates, Inc. Deerfield, IL) and a photodiode array detector Waters 996 (Millipore Co., Milford, MA) were used. Detection was followed by UV absorbance at 320 nm.

2.2.3. Rheological tests

Small amplitude oscillatory shear was used to follow the gelation process of native WEAX and PF-WEAX solutions. Cold (4 °C) WEAX solutions (1% w/v in AX) in 0.05 M citrate phosphate buffer pH 5.5 were mixed with laccase and immediately poured into the cone–plate geometry (5.0 cm in diameter and 0.04 rad in cone angle) of a strain controlled rheometer (ARES 2000, Rheometric Expansion System, Rheometric Scientific, Champ sur Marne, France) maintained at 4 °C. Exposed edges were recovered with silicone to prevent evaporation. WEAX gelation was started by a sudden increase of temperature from 4 to 25 °C and monitored at 25 °C for 2 h by recording the storage (G') and loss (G'') moduli. Measurements were carried out at 1 Hz. From strain sweep tests, WEAX gels showed a linear behaviour from 0.02 to 100% strain. 10% strain was used in all the rheological measurements. The mechanical spectra of gels were obtained by frequency sweep from 0.016 to 16 Hz at 25 °C.

2.2.4. Swelling

After laccase addition, WEAX solutions were quickly transferred to a 5 ml tip-cut-off syringe (diameter 1.5 cm) and allowed to gel for 2 h at 25 °C. After gelation, the gels were removed from the syringes, placed in glass vials and weighted. The gels were allowed to swell in 20 ml of 0.02% (w/v) sodium azide solution to prevent microbial contamination. During 36 h 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:

$$q = (W_s - W_{AX})/W_{AX} \quad (1)$$

where W_s is the weight of swollen gels and W_{AX} is the weight of AX in the gel (Carvajal-Millan et al., 2005b).

2.2.5. Structure

From swelling measurements, the molecular weight between two cross-links (M_c), the cross-linking density (ρ_c) and the mesh size (ξ) values of the different WEAX gels were obtained as reported by Carvajal-Millan et al. (2005b). M_c was calculated using the model of Flory and Rehner (1943) modified by Peppas and Merrill (1976) for gels where the cross-links are introduced in solution (Eq. (2)).

$$\frac{1}{\mathrm{Mc}} = \frac{2}{Mn} - \frac{(\upsilon/V_1)\left(\ln(1-\upsilon_{2,s}) + \upsilon_{2,s} + \chi_1 \upsilon_{2,s}^2\right)}{\upsilon_{2,r}\left((\upsilon_{2,s}/\upsilon_{2,r})^{1/3} - \frac{1}{2}(\upsilon_{2,s}/\upsilon_{2,r})\right)} \quad (2)$$

where M_n is the number average molecular weight of WEAX (considering only the xylose backbone). The M_n value was calculated from the WEAX M_w value knowing the A/X ratio. In Eq. (2), V_1 is the molar volume of water (18 cm^3/g), $\upsilon_{2,r}$ and $\upsilon_{2,s}$ are the polymer volume fraction of the gel in a relaxed state (directly after gel formation) and at equilibrium swelling, respectively. $\upsilon_{2,r}$ and $\upsilon_{2,s}$ were calculated from the weight of the gels before exposure to the sodium azide solution and at equilibrium swelling, respectively, assuming volume additivity of water and WEAX. χ_1 is the Flory polymer–solvent interaction parameter. The arabinoxylans–water system was calculated to correspond to theta conditions (Qian, Wang, Han, & Cheng, 2001). χ_1 was therefore taken as equal to 0.5 for the present system. The partial specific volume (υ) value of AX reported by Carvajal-Millan et al. (2005b) was used (0.596 cm^3/g).

After Mc calculation by Eq. (2), the average mesh size (ξ) of the WEAX gels was obtained as reported by Peppas et al. (2000) (Eq. (3))

$$\xi = \upsilon_{2,s}^{-1/3}\left(\frac{2C_n M_c}{M_r}\right)^{1/2} l \quad (3)$$

with M_r representing the molecular weight of the repeating unit (xylose, 132 g/mol), C_n the characteristic ratio for arabinoxylans (11.5) (Picout & Ross-Murphy, 2002) and l the bond length between two xyloses (0.286 nm).

The cross-linking density in WEAX gels (ρ_c) has been calculated from M_c as previously reported by Peppas, Moynihan, and Lucht (1985) (Eq. (4)).

$$\rho_c = \frac{1}{\upsilon M_c} \quad (4)$$

2.2.6. Preparation of WEAX gels for the controlled release of proteins

As soon as laccase was added in the WEAX solutions, they were poured into a 30 ml cylindrical plastic cell (diameter 30 mm). Gels were allowed to form for 2 h at 25 °C. Gels at 0.5% (w/v) in AX were not used as they did not withstand the controlled release experiment. WEAX gel dimensions were 3 cm diameter and 0.3 cm thickness.

2.2.7. Protein loading on WEAX gels

Proteins of different M_w (ovalbumin (43 kDa), bovine serum albumin (67 kDa), amyloglucosidase (97 kDa), aldolase (158 kDa), catalase (232 kDa), ferritine (440 kDa) and thyroglobuline (669 kDa)) were loaded in WEAX gels. The protein solutions (500 μl, 10 mg/ml) in 0.05 M citrate phosphate buffer pH 5.5 were placed on the surface of the WEAX gels. Protein was allowed to diffuse into the gel during 12 h at 25 °C and 90 rpm tangential rotation. After 12 h incubation the whole protein solution appeared to be absorbed by the gel. The un-loaded protein still on the gel surface was recovered by rapidly rinsing twice with 6 ml of sodium azide solution 0.02% (w/v) for further quantification. The protein loading volume was chosen in order to restrict the WEAX gels volume change to 25% (Ritger & Peppas, 1987). The percentage of protein loaded in WEAX gels was calculated (weight of protein loaded/weight of protein placed on the gel surface).

2.2.8. Protein release from WEAX gels

The protein was released in 6 ml of distilled water placed on the surface of the protein loaded WEAX gels. The liquid medium containing sodium azide (0.02% w/v) as an antimicrobial agent, was renewed every hour from 1 to 6 h and at 9 and 24 h incubation; 1 ml was taken for protein quantification. Cells were incubated at 25 °C and 90 rpm tangential rotation during 24 h. At the end of the test, the WEAX gels were hydrolyzed by a xylanase (100 μl; 15 nkat/μl) in order to quantify un-released protein. Xylanase was added to the gel surface and incubated for 2 h at 25 °C and 90 rpm tangential rotation. Protein recovery (un-loaded protein + released protein + un-released protein) was near to 100%. The protein was quantified by using the Bradford assay (Bradford, 1976). Protein release from WEAX gels was characterized by calculating an apparent diffusion coefficient (D_m) (Eq. (5)). This D_m was estimated from the release kinetic curve, fitted by using an analytical solution of the second Fick's law, which gives the solute

concentration variation as a function of time and distance (Crank, 1975).

$$\frac{M_t}{M_o} = \frac{4}{L}\left(\frac{D_m\, t}{\pi}\right)^{0.5} \quad (5)$$

Where M_t is the accumulated mass of protein released at time t, M_o is the mass of protein in the gel at time zero, L is the sample thickness (0.3 cm) and D_m is the apparent diffusion coefficient. By plotting the relative solute mass released (M_t/M_o) at time t, versus the square root of time, a simplified determination of D_m can be made assuming that the coefficient is constant and that the sample is a plate with a thickness (L). In this study the apparent D_m was calculated from the linear part of the M_t/M_o (t) curves ($M_t/M_o < 0.6$). The percentage of protein released at the end of the test (24 h) was calculated (weight of protein released/weight of protein loaded).

2.2.9. Statistical analysis

Chemical determinations were made in duplicates and the coefficients of variation were lower than 5%. Small deformation measurements were made in triplicates and the coefficients of variation were lower than 8%. Swelling and controlled release tests were made in duplicates, coefficients of variation were lower than 10%. All results are expressed as mean values.

3. Results and discussion

3.1. WEAX gel characterization

3.1.1. Rheology and covalent cross-links

The mechanical spectra of gels made with native WEAX or PF-WEAX are showed in Fig. 1. Whatever the feruloylation degree or concentration of WEAX used, a typical gel behaviour was found with the storage modulus (G') independent of frequency and higher than the loss modulus (G'') (Doublier & Cuvelier, 1996). For gels at the same concentration in WEAX (1% w/v in AX), G' values increased from 13 to 44 Pa when the initial FA content of WEAX was from 1.6 to 2.3 μg/mg AX (Fig. 1a). Nevertheless, G'' values were almost superposed indicating that the viscous contribution to the gel structure was similar. As showed in Fig. 1b, G' and G'' modulus increased with the WEAX concentration in the gel. G' values augmented from 12 to 160 Pa when the WEAX concentration in the gel was from 0.5 to 2.0% (w/v) in AX. The increase in G'' as a function of WEAX concentration suggest a higher viscous contribution to the gel structure. From G' and G'' values in the different WEAX gels at 1 Hz (frequency used during WEAX gelation), tan δ (G''/G') values were calculated. It was found that tan δ values increased either when the WEAX initial FA content was reduced or when the WEAX concentration in the gel was increased. This augmentation in tan δ value indicates an increase in the polymer chain flexibility in the gel (Doublier & Cuvelier, 1996).

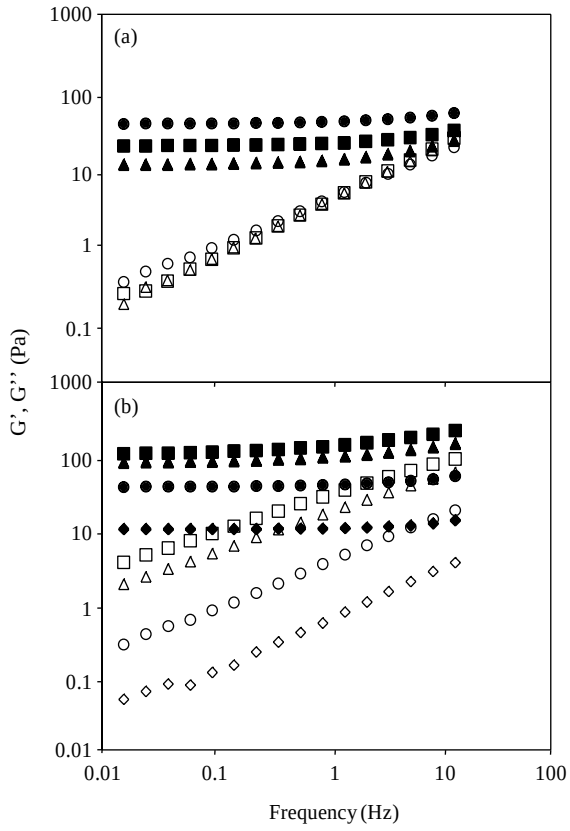

Fig. 1. Mechanical spectra of WEAX gels (a) at 1% (w/v) in AX and initial FA content of 2.3 G'(●), G''(○); 1.8 G'(■), G''(□); and 1.6 G'(▲), G''(△) μg/mg AX and (b) at variable AX concentrations (% w/v) with the same initial FA content (2.3 μg/mg AX): 0.5 G'(♦), G''(◇); 1.0 G'(●), G''(○); 1.5 G'(▲), G''(△) and 2.0 G'(■), G''(□). Data obtained at 25 °C and 10% strain.

In Table 1 are given the percentage of FA oxidized at the end of WEAX gelation (FA disappearance) and the percentage of this oxidized FA recovered as dimers and trimer of ferulic acid (di-FA + tri-FA) in the different WEAX gels. All oxidized FA was not recovered as di and tri-FA as previously observed during laccase gelation of feruloylated WEAX (Vansteenkiste et al., 2004; Carvajal-Millan et al., 2005a,b). With the exception of the gels at 1.5 and 2% (w/v) in AX, the percentages of FA oxidized ranged from 87 to 90%, while only 29–31% of the oxidized FA was recovered as di-FA and tri-FA. This result indicated that one part of the FA oxidized at the end of the WEAX gelation process was probably transformed in ferulate cross-linking structures others than di-FA and tri-FA as recently proposed (Vansteenkiste et al., 2004; Carvajal-Millan et al., 2005a,b). The amounts of di-FA and tri-FA covalent cross-links in the different WEAX gels are also presented in Table 1. For gels at the same concentration in WEAX (1.0% w/v in AX) di-FA and tri-FA content increased from 3.7 to 5.6 and from 0.38 to 0.61 μg/ml gel, respectively, when the initial FA content increased from 1.6 to 2.3 μg/mg AX. An increase in

Table 1
Characteristics of WEAX gels at different concentrations in AX or initial ferulic acid contents

(% w/v in AX)[a]	FA[b] oxidized (%)	FA[c] recovered (%)	di-FA (μg/ml gel)	tri-FA (μg/ml gel)	M_c[d] $\times 10^3$ (g/mol)	ρ_c[e] $\times 10^{-6}$ (mol/cm^3)	ξ[f] (nm)
0.5	90±1	31±1	3.2±0.2	0.00±0.00	135±0.1	12.40±0.01	319±1
1.0	90±1	30±2	5.6±0.1	0.61±0.03	132±0.1	12.71±0.01	263±1
1.0_{80}	88±2	28±2	4.1±0.1	0.48±0.01	145±0.1	11.57±0.01	305±1
1.0_{70}	87±2	29±1	3.7±0.1	0.38±0.01	149±0.1	11.25±0.01	331±1
1.5	80±1	22±2	5.7±0.9	0.34±0.02	125±0.1	13.42±0.01	222±3
2.0	67±2	21±2	6.2±0.9	0.27±0.01	119±0.1	14.05±0.01	201±1

All values are average of two repetitions.
[a] Concentration of AX in the WEAX gel.
[b] After 2 h of WEAX solution laccase exposure at 25 °C.
[c] Calculated from the percentage of oxidized FA recovered as di-FA + tri-FA in the WEAX gel.
[d] Molecular weight between two cross-links.
[e] Cross-linking density.
[f] Mesh size.

di-FA content from 3.2 to 5.6 μg/ml gel was also registered when the AX concentration in gel increased from 0.5 to 1.0% (w/v). The tri-FA structure, probably formed at low amounts, was not detected in WEAX gels at 0.5% (w/v) in AX. Further increase of WEAX solution concentration from 1 to 2% (w/v) in AX, and therefore of FA content from 23 to 46 μg/ml did not led to a significantly further production of di-FA cross-links and the tri-FA content was reduced. In gels at 1.5 and 2% in AX, lower percentages of FA oxidized (from 80 to 67%) as well as oxidized FA recovered as di-FA + tri-FA (from 22 to 21%) were found (Table 1), suggesting the possible formation in higher amounts of other ferulate cross-link structures.

3.1.2. Swelling and structure

The equilibrium swelling of WEAX gels was reached between 15 and 20 h. The swelling ratio (q, g water/g AX) in WEAX gels at 1% (w/v) in AX increased from 134 to 223 as the initial FA content in WEAX decreased from 2.3 to 1.6 μg/mg AX, while it decreased from 231 to 69 when the gel concentration ranged from 0.5 to 2.0% (w/v) in AX. The higher water uptake of gels made from PF-WEAX can be explained in terms of a decrease in covalent cross-links (di-FA, tri-FA) in the gel and therefore, the existence of longer un-cross-linked AX chains sections in the network. Uncross-linked polymer chain sections in the gel can expand easily conducting to higher amounts of water uptake. The lower q values obtained as the WEAX concentration in the gel increased can be related to the more compact network structure that limits the water absorption (Meyvis, De Smedt, Demeester, & Hennink, 2000).

The molecular weight between two cross-links (M_c), the cross-linking density (ρ_c) and the mesh size (ξ) values of the different WEAX gels are presented in Table 1. For gels made from PF-WEAX, it was found that the M_c and ξ increased as the initial FA content decreased, changing from 132 to 149×10^3 g/mol and from 263 to 331 nm, respectively when FA decreased from 2.3 to 1.6 μg/mg AX. At the same time, ρ_c decreased from 12.71 to 11.25×10^{-6} mol/cm^3. On the other hand, when the WEAX concentration increased in gel from 0.5 to 2.0% (w/v) in AX at constant FA level, M_c and ξ decreased from 135 to 119×10^3 g/mol and from 319 to 201 nm, respectively, leading to an increase in ρ_c from 12.40 to 14.05×10^{-6} mol/cm^3. As the covalent cross-links (di-FA, tri-FA) content did not increase when the concentration of AX in the gel increased from 1 to 2% (w/v), the network structure modification could be related to the formation of ferulate cross-links others than di-FA and tri-FA and/or physical interactions between AX chains, as recently reported by Vansteenkiste et al. (2004)) and Carvajal-Millan et al. (2005a,b) in laccase induced WEAX gels.

The results discussed above indicate that by tailoring the FA content of the WEAX or by modifying the concentration of polysaccharide in the network, gels with different rheological and structural properties can be obtained (Fig. 1, Table 1). These differences in the WEAX gel structure could induce changes in the functional properties of the gel. In that way, the capacity of the different WEAX gels obtained to transport proteins of different M_w was tested.

3.2. Protein transport

3.2.1. Protein loading

As showed in Table 2, the percentage of protein loading, depended on the gel characteristics (covalent cross-linking degree and AX concentration) and protein M_w, which control the transport of proteins into the gel. The percentage of protein loaded in WEAX gels ranged from 96% (ovalbumin in $1.0_{70\%}$ w/v gels) to 15% (thyroglobulin in 2% w/v gels), respectively. Gels at 1% (w/v) in AX made from PF-WEAX were able to load higher quantities of protein than those made from native WEAX. The decrease in cross-linking content in gels from PF-WEAX resulting in higher uncross-linked WEAX chain sections probably facilitated the protein movement through the gel.

Table 2
Protein loaded in WEAX gels at different concentrations in AX or initial ferulic acid contents

Protein	M_w (kDa)	Protein loaded (%)[a]				
		WEAX concentration (% w/v in AX)				
		2.0	1.5	1.0	1.0_{80}	1.0_{70}
Ovalbumin	43	78±3	80±5	83±4	93±5	96±6
BSA	67	61±2	68±2	72±1	89±3	91±5
Amyloglucosidase	97	46±1	54±1	67±2	68±2	70±3
Aldolase	158	36±3	52±3	66±1	65±4	68±4
Catalase	232	30±1	34±1	55±5	64±3	62±3
Ferritine	440	23±2	31±2	31±1	44±5	56±5
Thyroglobuline	669	15±1	30±1	28±3	34±2	34±4

All values are average from two repetitions.
[a] Weight of protein loaded/weight of total protein placed on the gel surface.

The protein loaded in the WEAX gels decreased as the concentration of WEAX in the gel increased. The latter can be explained by the presence of a more compact network resulting from a higher amount of WEAX chains and possible ferulate cross-link structures others than di-FA and tri-FA in the gel, which slowed down the protein transport through the network.

The low protein loading percentages obtained for the highest M_w proteins tested (440 and 669 kDa) can not be related to a pore size obstruction effect since ξ calculated in WEAX gels were from 201 to 331 nm, which are larger than the hydrodynamic diameter of these proteins (12 and 17 nm) (Häubler, 2003; Cole, Diener, Himelblau, Beech, & Fuster, 1998). Nevertheless, it must be considered that WEAX gels would present a heterogeneous structure due to the movement of AX chains in the network (as suggested by the increase in G'' as a function of frequency, Fig. 1), which could decrease the network free space for protein transport.

The quantity of protein loaded in the different WEAX gels ranged from 0.5 to 3 mg per ml gel. These amounts of protein loaded were similar to those reported in the literature either for synthetic or polysaccharide gels. In polyacrylamide gel 2.87 mg BSA per ml gel were loaded after 72 h incubation (Shalaby, Abdallah, Park, & Park, 1993). In the present study, a similar protein loading was obtained after only 12 h of gel incubation. By using 9% dextran gels, Gehrke, Uhden, & McBride (1998) loaded 8 mg α-amylase per ml gel but a higher protein solution concentration was used.

3.2.2. Protein release

The release of proteins (M_w from 43 to 669 kDa) from WEAX gels at 1% (w/v) in AX and different initial FA content or from gels at different native WEAX concentrations was studied. Fig. 2 gave a representative protein release profile obtained with BSA (67 kDa). As showed in Fig. 2(a) at the same WEAX concentration in the gel (1.0% w/v in AX), higher BSA release rates were obtained when the initial FA content in the WEAX was reduced. Concerning native WEAX, the increase in AX concentration in gel reduced the BSA release rate. These results indicated that different BSA release rates from WEAX gels can be obtained by modifying the network characteristics either by changing the WEAX initial FA content or the WEAX concentration before gelation. As showed in Fig. 2(b), linear relationships between M_t/M_o and the square root of time were found for BSA release from the different WEAX gels tested, allowing the calculation of the apparent diffusion coefficients (D_m) of BSA in the WEAX gels, according to Eq. (5). By using proteins with a M_w lower than 232 kDa (catalase) similar results were obtained. For proteins of higher M_w (440–669 kDa), the effect of WEAX gel characteristics was less clear.

The D_m values for the different proteins tested in the present study are given in Table 3. The diffusion coefficients of these proteins in water (D_o) obtained from the literature (Amsden, 1998; Cole et al., 1998; Garcia de la Torre, Huertas, & Carrasco, 2000; Häubler, 2003) are given for comparison. As expected, in a WEAX gel with the same structural characteristics, the D_m values decreased as

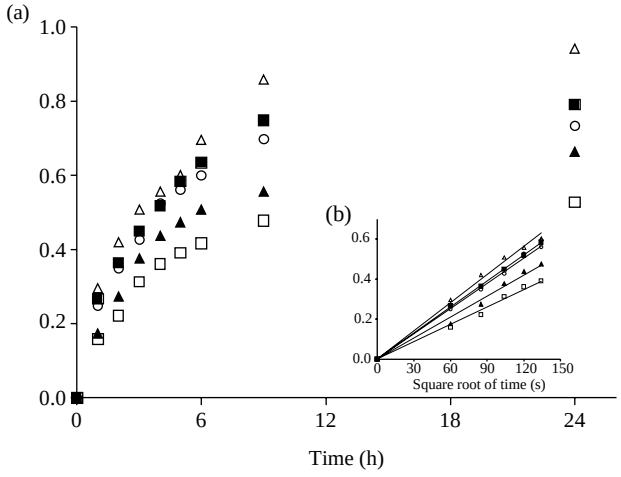

Fig. 2. Cumulative release of BSA from WEAX gels (a) as a function of time and (b) as a function of root time. WEAX gels were at 1% (w/v) in AX and initial FA content of 2.3 (○), 1.8 (■) and 1.6 (△) μg/mg AX or at 1.0 (○), 1.5 (▲) and 2.0 (□)% (w/v) in AX and same initial FA content (2.3 μg/mg AX). Protein release was followed at 25 °C and 90 rpm during 24 h.

Table 3
Apparent diffusion coefficients of proteins of different molecular weight in WEAX gels at different concentrations in AX or initial ferulic acid contents

Protein	M_w (kDa)	D_m[a] $\times 10^{-7}$ (cm^2/s)					
		WEAX concentration (% w/v in AX)					
		D_o[b] $\times 10^{-7}$ (cm^2/s)	2.0	1.5	1.0	1.0_{80}	1.0_{70}
Ovalbumin	43	8.40	3.00±0.26	4.60±0.60	5.50±0.52	6.00±0.37	6.80±0.54
BSA	67	6.80	1.90±0.40	2.80±0.47	4.30±0.31	4.70±0.40	5.90±0.35
Amyloglucosidase	97	5.80	1.40±0.22	2.20±0.15	2.50±0.50	2.80±0.21	3.60±0.40
Aldolase	158	4.70	1.40±0.23	1.60±0.29	1.80±0.18	72.50±0.34	3.20±0.50
Catalase	232	4.10	1.20±0.20	1.20±0.20	1.30±0.51	2.20±0.31	2.70±0.62
Ferritine	440	2.50	1.20±0.28	1.15±0.30	1.50±0.44	2.00±0.30	1.90±0.74
Thyroglobuline	669	2.60	0.95±0.44	1.12±0.39	1.30±0.71	1.67±0.46	1.85±0.65

All values are average from two repetitions.
[a] Apparent diffusion coefficient of proteins in the WEAX gels.
[b] Diffusion coefficient of proteins in water from the literature (Amsden, 1998; Cole et al., 1998; Garcia de la Torre et al., 2000; Häubler, 2003).

the protein M_w increased. For each protein tested, the D_m value increased when the initial FA content in gels at 1% (w/v) in AX decreased, while lower D_m values were found as the WEAX concentration in gel increased. The D_m values were in the range of order for proteins in hydrated gels (Gehrke et al., 1998). In 9% dextran gels Gehrke et al. (1998) reported a D_m value of 1×10^{-7} cm^2/s for ovalbumin, which is lower than the ovalbumin D_m values obtained in the present study with WEAX gels (from 3 to 6.8×10^{-7} cm^2/s). The latter could be attributed to the differences in the structural and mechanical properties between dextran and WEAX gels. In dextran gels, elasticity and mesh size values from 12 to 28 kPa and from 18 to 15 nm have been reported (de Jong, van Eerdenbrugh, van Nostrum, Kettenes-van den Bosch, & Hennink, 2001), which indicated a more compact network structure in comparison to the WEAX gels used in the present study (G' and mesh size from 12 to 160 Pa and from 331 to 201 nm, respectively).

These differences in D_m values for the different WEAX gels were reflected in the percentage of protein released at the end of gels incubation (24 h at 25 °C). Higher protein release percentages were obtained for WEAX gels at 1% (w/v) in AX with lower initial FA contents. The percentages of protein released were from 97% (ovalbumin in $1.0_{70\%}$ w/v gels) to 31% (thyroglobuline, 2% w/v gels).

4. Conclusions

WEAX gels with different rheological and structural characteristics can be obtained by modifying either the initial FA content of WEAX or the WEAX concentration before gelation. The partial deferuloylation of WEAX reduced the WEAX gel covalent cross-link content (di-FA, tri-FA and probably other ferulate cross-link forms) being reflected by a decrease in elasticity (G') and an increase in mesh size (ξ). An increase in di-FA and tri-FA content and G' value of WEAX gels as well as lower ξ can be obtained by increasing the WEAX concentration up to 1% (w/v) in AX. At higher AX concentrations, lower amounts of FA were oxidized at the end of WEAX gelation and no increase in known covalent cross-linking (di-FA, tri-FA) content was obtained. However, G' of WEAX gels continued to increase and ξ to decrease, suggesting the implication of ferulate covalent cross-links structures others than di-FA and tri-FA and/or physical interactions to the WEAX gel structure. The differences in structural and rheological characteristics of WEAX gels were reflected in their capacity to load and release proteins of molecular weight (M_w) ranging from 43 to 669 kDa. The apparent diffusion coefficient (D_m) of proteins from WEAX gels decreased as the cross-linking degree and the AX concentration in the gels increased. The possibility to modulate protein release from WEAX gels makes these hydrogels potential candidates for the controlled delivery of proteins.

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