Arabinoxylan Networks as Affected by Ovalbumin Content

*Elizabeth Carvajal-Millan, Anne Surget, Xavier Rouau, Stéphane Guilbert, Valérie Micard**

Department of Cereal Technology and Agropolymers, ENSAM-INRA, 2 Place P. Viala 34060, Montpellier Cedex 1, France
E-mail: micard@ensam.inra.fr

Summary: Solutions (1 % w/v) of water-extractable arabinoxylans (WEAX) from wheat were mixed with ovalbumin solutions at different concentrations in order to obtain different protein/polysaccharide ratios (0, 0.6, 2.5, 5.0, 7.5 and 10.0). These mixtures were oxidized by a laccase (*Pycnoporus cinnabarinus*). The kinetics of gelation was monitored by rheological measurements of the storage modulus (G') and loss modulus (G'') at 25°C during 2 h. In the different ovalbumin/WEAX mixtures, WEAX cross-linking occurred as shown by an increase in G'. However, final G' decreased as a function of protein concentration from 17.2 Pa (WEAX) to 7.1 Pa (ovalbumin/WEAX=10), following a sigmoidal relationship. Ovalbumin is probably entrapped in a WEAX covalent network, which explains the changes in rheological properties, but it does not participate in the network formation. Ferulic acid oxidation and diferulic acid formation was delayed at ovalbumin/WEAX=5.0 until 40 min but not at the end of gelation. In the same mixture SH groups from ovalbumin oxidized more rapidly than the protein alone. Ovalbumin via SH groups are probably responsible for the FA oxidation delay and the weaker WEAX gel formed by blocking the enzymatically formed free radicals.

Keywords: arabinoxylans; laccase; networks; ovalbumin; rheology

Introduction

Arabinoxylans are non-starch polysaccharides from the cell walls of cereal grains. These polysaccharides have been classified as water extractable (WEAX) or water-unextractable (WUAX). Molecular weight varies from 10-10,000 kDa in WEAX and exceeds 10,000 kDa for WUAX.[1] Arabinoxylans consist of a linear backbone of β-(1→4)-linked D-xylopyranosyl units to which α-L-arabinofuranosyl substituents are attached through O-2 and/or O-3.[2] A particular structural feature of arabinoxylans is the ferulic acid covalently linked via an ester linkage to O-5 of the arabinose. Feruloylated WEAX can form three-dimensional covalent networks by oxidative coupling of the phenoxy radicals resulting from the oxidation of ferulic

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acid.[3] WEAX have high water absorption capacity, which increases after gelation, holding up to 100 g of water per gram of gel. As an interesting property, this hydrogel is not sensitive to electrolytes, contrary to other polysaccharide hydrogels like sugar beet pectins.[4] Ovalbumin is the most abundant protein in egg whites. This is a monomeric phosphoglycoprotein with a molecular weight of 45 kDa. Ovalbumin is rich in sulphur-containing amino acids; it contains 15 methionine residues per molecule with one disulphide bond and four sulphydryl groups (SH).[5] Native ovalbumin has the lowest index of the surface aliphatic and aromatic hydrophobicity while denatured ovalbumin has the highest index of the total surface hydrophobicity.[6] Ovalbumin is widely used in the food industry because of its ability to foam, to bind other molecules, to emulsify and to form gels. From a nutritional and functional point of view, ovalbumin belongs to the highest quality food proteins. Most neutral and ionic ligands (such as aliphatic, alcohols and vanillin) bind preferentially to the denatured form of ovalbumin.[7] Proteins and polysaccharides are key components in the structuration and stabilization of food technology. These features can explain the great scientific and industrial interest as engineered food materials.[6, 8] WEAX and proteins form no covalent linkages but two distinct networks in the presence of phenoloxidases or peroxidases.[9] The aim of this work was to investigate the rheological and structural behaviour of feruloylated arabinoxylan/ovalbumin mixtures when oxidized by the phenoloxidase laccase.

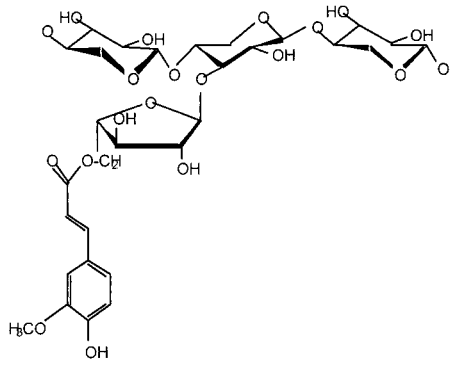

Fig. 1. Wheat feruloylated arabinoxylans.

Materials and Methods

WEAX from wheat were obtained at UTCA laboratory (ENSAM-INRA, Montpellier, France) with a content of 72.5% in arabinoxylans. Ovalbumin (albumin chicken egg, grade V, 98%) was obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO). Laccase (*Pycnoporus cinnabarinus*) (benzenediol:oxygen oxidoreductase, E.C.1.10.3.2) was supplied by Unité de Biotechnologie des Champignons Filamenteux (INRA, Marseille, France). Laccase activity (0.04 U/µl) was measured using syringaldazine as substrate.[10]

Cross-linking process. An aqueous solution of WEAX (1% w/v) and ovalbumin solutions (1.25, 5.0, 10.0, 15.0 and 20.0% w/v) in 0.1M citrate phosphate buffer pH=5.5 were prepared. One ml of WEAX solution and one ml of each ovalbumin solution were hand-mixed in order to prepare six ovalbumin/WEAX ratios: 0, 0.6, 2.5, 5.0, 7.5 and 10.0. 2U of laccase were added to the system as a cross-linking agent.

Rheology. The kinetics of network formation and the mechanical spectra of the gel were observed using small deformation oscillatory measurements. A strain-controlled rheometer (ARES 2000, Rheometric Scientific) with cone and flat plates (4.0 cm in diameter, 0.04 rad in cone angle) at 25°C was used. Time sweep measurements were conducted for 2 hours at 1.0 Hz and 10% strain. The mechanical spectrum was obtained at frequencies varying from 0.16 to 16.0 Hz and at 10% strain after 2 hours of laccase treatment. The storage modulus (G') and the loss modulus (G'') were monitored in both studies. Before the beginning of the rheological measurements, the solutions were kept at 4°C in order to delay the enzymatic reaction.

Biochemical determinations. Ferulic acid (FA) and diferulic acid (diFA) contents in WEAX and ovalbumin/WEAX=5.0 were monitored by high performance liquid chromatography (HPLC) during gelation on saponified samples at 0, 10 20, 30, 40 and 120 minutes after laccase treatment as described elsewhere.[10] An Alltima C_{18} column 250 × 4.6 mm (Alltech associates, Inc. Deerfield, IL) and a photodiode array detector Waters 996 (Millipore Co., Milford, MA) were used. Free sulphydryl groups (SH) were determined in ovalbumin (5.0 % w/v) and ovalbumin/WEAX=5.0 at 0, 10 20, 30, 40 and 120 minutes after laccase addition. SH quantification was made using Ellman's reagent 5-5'-dithiobis-2-nitrobenzoic acid (DNTB) which reacts with SH.[11] This reaction was detected at 412 nm using a spectrophotometer

(Ultraspec 2000 UV/VIS, Pharmacia Biotech).

Statistical analysis. All determinations were made in duplicate. Results are expressed as mean values.

Results and Discussion

WEAX solution treated with laccase underwent oxidative gelation as reported before.[10] Figure 2 shows the rheological response of a WEAX solution during gelation with a rapid initial rise in storage modulus (G') followed by a plateau region reaching a value of 17.2 ± 0.7, a loss modulus (G'') value prevailing over G' and a mechanical spectrum typical of a solid-like material. This behaviour reflects an initial formation of covalent linkages between ferulic acid of adjacent WEAX molecules producing a three-dimensional network. Once sufficient cross-links have formed, movement of chains is impeded by the rigidity of the gel.[12]

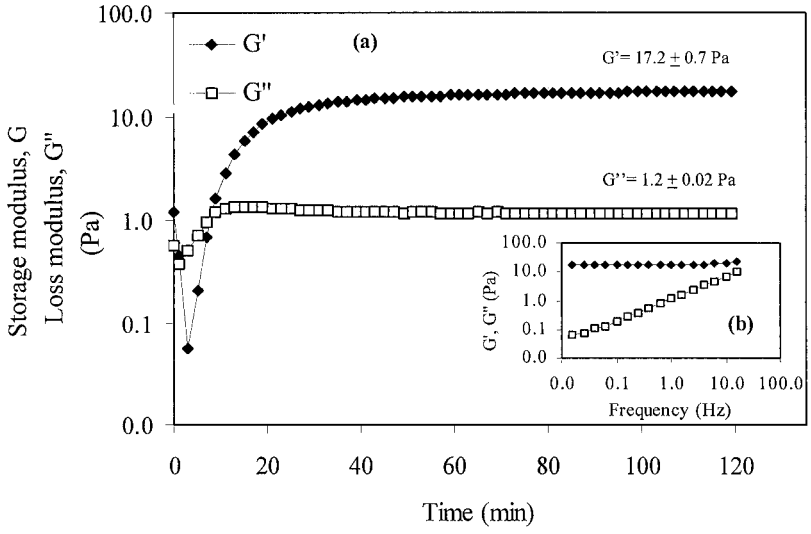

Fig. 2. Storage modulus (G') and loss modulus (G'') during gelation (a) and mechanical spectrum after gelation (b) for 1% (w/v) WEAX solution treated with 2U of laccase at 25°C. Data obtained at 1.0 Hz and 10% strain.

However, in ovalbumin/WEAX mixtures, the elasticity of the network at the end of gelation was affected by the protein concentration (Figure 3). Increasing ovalbumin/WEAX ratio slowed down the cross-linking process and decreased the final G' value of the gel in a sigmoidal relationship from 17.2 ± 0.7 to 7.1 ± 1.0 Pa. Ovalbumin/WEAX ratios from 0 to 2.5 have less detrimental an effect on the G' value at the end of gelation than ratios between 5.0 and 10.0. It is known that soluble macromolecular complexes can lead to the formation of gel structures or to phase separation because of thermodynamic incompatibility, depending on the balance of hydrophobic and electrostatic interactions between the molecules. The concentration of the polymers, the pH, ionic strength and temperature are also important factors. [8,13] Homogeneous solutions of unlike polymers can be stable in a certain concentration range over which they can separate into two phases, with each phase containing predominantly one of the components.[14]

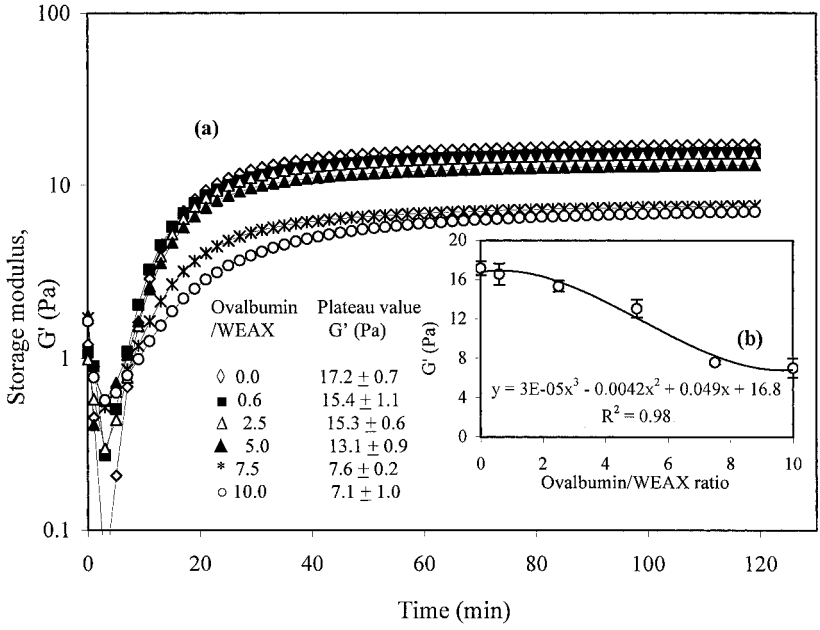

Fig. 3. Effect of ovalbumin/WEAX ratios in the development of cross-linking and in the storage modulus (G') plateau value of gels (a). Final G' values follow a sigmoidal relationship with protein/WEAX ratio (b). Data obtained at 1.0 Hz and 10% strain.

Interestingly, if gelling takes places after or during the demixing process, complex multiphase gel microstructures are formed, where, depending on the system, one phase is observed to be dispersed through the other.[15]

Interactions between charged polysaccharides and proteins have been widely studied. These interactions especially involve electrostatic interactions, which are very influenced by pH and ionic strength of the medium. Conversely, protein-neutral polysaccharide systems are less studied and exhibit interesting rheological properties that are explained as resulting from volume exclusion effects and phase separation.[16] WEAX are neutral polysaccharides and, in the medium used (pH=5.5), ovalbumin was close to the isoelectric point (Ip=4.5), so that its net electrical charge was not very high. On the other hand, as polysaccharide dispersions often show higher values of viscosity than protein dispersions, it is thus likely that the protein/polysaccharide ratio modified the viscosity of complex dispersions. In a patent application involving protein-starch complexes, a reduction of viscosity was found on increasing the protein/polysaccharide ratio from 1/5 to 2/3.[17] Similarly, a delay in the thickening of a WEAX/reduced BSA solution has been reported. This delay was proportional to the protein concentration in the mixture.[18]

FA present in WEAX molecules is considered to be responsible for oxidative gelation and several hypotheses concerning the mechanism of this reaction have been developed.[19-20] In fact, FA consumption and DiFA formation in oxidized WEAX systems can be used to follow the cross-linking process.[21] As reported by these authors, WEAX gelation was characterized by a decrease in FA content and an increase in diFA formation as a function of time (Figure 4). On the other hand, in the ovalbumin/WEAX=5.0 mixture, FA consumption and diFA formation were delayed for the first 40 min but reached similar values at the end of gelation (120 min). It is known that ovalbumin is rich in sulphur-containing amino acids.[6] As suggested earlier,[18- 20] this initial gelation inhibition was therefore attributed to the SH groups from the protein.

Figure 5 compares changes in the SH groups induced by laccase in the 5.0 % (w/v) ovalbumin solution and the ovalbumin/WEAX=5.0 mixture during gelation. The ovalbumin/WEAX mixture oxidized more rapidly than the protein alone. However, as FA consumption from WEAX was delayed in presence of ovalbumin, a direct chemical reaction between FA and the SH groups in the protein was not likely. Laccase catalyzes the hydroxylation of monophenols to

p-diphenols in the presence of oxygen and their further oxidation to *p*-benzoquinones.[22] This enzyme leads to phenoxy radicals as the primary oxidation products, forming three intermediate radicals.[23] Since ovalbumin contains four SH groups from cysteine, antioxidant properties of ovalbumin via SH groups are probably responsible for the FA oxidation delay. This functional group may be able to furnish a proton, blocking the enzymatically formed free radicals. This reaction generally results in the formation of disulphides while phenoxy radicals are regenerated in the original FA.

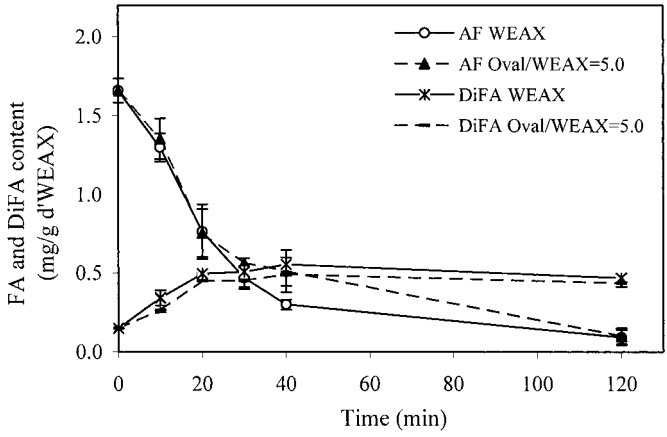

Fig. 4. Changes in FA and diFA content in 1% (w/v) WEAX solution and ovalbumin/WEAX=5.0 during gel formation at 25°C by laccase.

Conclusions

Mixing ovalbumin with WEAX results in a delay in the kinetics of gelation and a decrease in the strength of gels obtained compared to WEAX solutions alone. The sigmoidal relationship between protein concentration and G' of the mixed gel suggests initial molecular aggregation, with however phase separation at protein/polysaccharide ratios higher than 5. At this ratio, FA consumption and diFA formation from WEAX was delayed at the beginning of the cross-linking reaction. The faster oxidation of SH groups from ovalbumin in presence of WEAX indicates that this functional group may be capable of furnishing a proton, blocking the enzymatically formed free radicals.

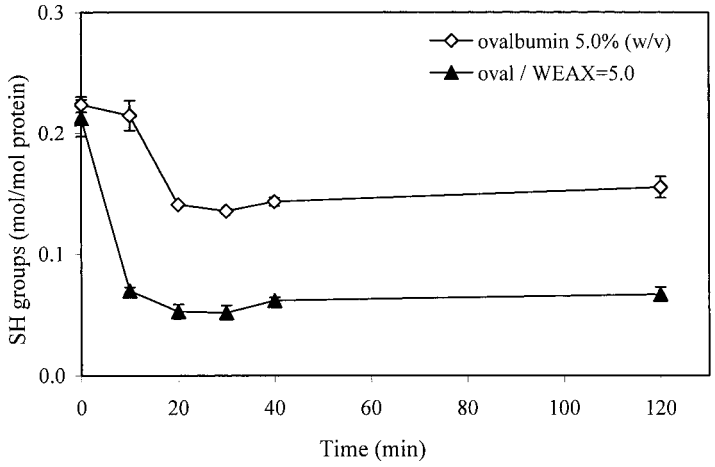

Fig. 5. Effect of 1% (w/v) WEAX solution on oxidation of sulphydryl groups from ovalbumin solution at 5% (w/v) and pH=5.5 through the cross-linking process at 25°C by laccase.

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