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Effect of Transglutaminase on the Mechanical and Barrier Properties of Whey Protein/Pectin Films Prepared at Complexation pH

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ABSTRACT: The behavior of pectin and thermally denatured whey proteins at both different protein/polysaccharide ratios and different pH values was investigated. Our findings suggest the formation at pH 5.1 (complexation pH) of transglutaminase-catalyzed cross-links among soluble ionic whey protein/pectin complexes, which could be responsible for the observed increase of both tensile strength (2-fold) and elongation to break (10-fold) of films obtained in the presence of enzyme. Conversely, a significant reduction of elasticity, probably due to the formation of covalent bonds among single whey protein molecules, was observed when the films were prepared in the presence of the enzyme at pH 6.0. In addition, the presence of the enzyme at complexation pH significantly reduced film permeability. Atomic force and scanning electron microscopy revealed significant changes in the microstructure of the films prepared in the presence of TGase as well as in the morphology of their surface.

KEYWORDS: *transglutaminase, edible films, bioplastics, pHc, pectin, whey proteins*

INTRODUCTION

Protein/polysaccharide (P/P) complexes widely occur in nature as well as in a large variety of industrial products.[1] The study of P/P interactions has, hence, relevance not only for issues concerning biological systems such as living cell organization, but also to improve the drug vehicle or food processing by edible films or coatings.[2,3]

Attractive interactions between positively charged proteins and anionic polysaccharides can lead to gelation,[4] coacervation,[5] or multilayer formation,[6] and, as a consequence, the overall stability and texture of colloidal systems depend not only on the functional properties of the individual ingredients but also on the nature and strength of P/P interactions. In fact, highly structured P/P complexes may display better functionality—as hydration, interfacial, and adsorption properties—than proteins and polysaccharides alone.[1] Therefore, the manipulation of P/P interactions[7] can represent an important tool to modify the microstructure and the shelf life of the composite systems in the edible films, since the formation of a continuous network strictly depends on the biopolymer characteristics in the film-forming solution. For these reasons the understanding of stability and phase behavior of the latter solution is crucial for optimizing film performance.

During the titration of a polyanion/protein mixture from high pH, as the charge on the protein is reduced, there is a transition, experimentally probed by different techniques,[8−10] at a specific pH value called complexation pH (pHc), where a soluble complex is formed.[11,12] Then, the complex may be further stabilized through other intermolecular forces such as hydrophobic ones[13] and/or hydrogen bonds.[14]

As part of our current research to improve the features of P/P composite edible films,[15] we examined the interactions between pectin (Pec), an anionic polysaccharide, and the globular proteins contained in whey protein (WP) isolate. Pec is a polyelectrolyte generally associated with the cell wall and the intercellular regions of plants and fruits and is widely used as a gelling and stabilizing agent in foods.[16] Recently, WP-based edible films have been extensively investigated for food packaging and coatings.[17] WP isolate contains about 65% β-lactoglobulin (β-Lg), 25% α-lactalbumin (α-La), 8% bovine serum albumin, and 2% different minor proteins. Although β-Lg and α-La have quite different pI values (5.2 and 4.1, respectively), the electrostatic interactions between WP and Pec (pK_a, 4.6) are dominated by β-Lg properties since β-Lg is present in a much larger quantity. As a consequence, P/P complexation must take place at pH values between 4.6 and 5.2, at a value where Pec and β-Lg carry the majority of their opposite charges. As far as the hydrogen bonds are concerned, it has been reported that they play a significant role, especially in highly methylated Pec-containing complexes, only after the resulting electrostatic interactions.[14]

Numerous studies have previously characterized different complexes formed by Pec and β-Lg, demonstrating that it is possible to produce biopolymer systems with different properties by controlling the pH of the mixture solution.[14,18−20] When Pec and β-Lg are mixed at neutral pH and the pH is reduced, the formation of a soluble complex is first observed, then a coacervate is produced, and, finally, a precipitate is formed as the strength and number of electrostatic bonds are increased.[20] Diminishing of the net opposite charges on the macromolecular reactants reduces both the hydrophilicity and, as a consequence, the solubility of the resultant complex. Therefore, the P/P ratio in the mixture strongly affects both the

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charge balance of the complex and the behavior of the latter. Maximum complexation is obtained with a specific P/P ratio at a given condition of pH and ionic strength. Complex formation, thus, is guided not only by the characteristics of the polymers (i.e., chemical nature, charge density, molar mass, concentration, and ratio) but also by environmental conditions.[1,18] In addition, P/P complexes may be influenced by some intrinsic factors such as protein aggregation and cross-linking.[18]

In the present study we prepared WP/Pec films at pHc, cross-linked or not by a microbial transglutaminase (TGase),[21,22] to obtain possible hydrocolloid food coatings with appropriate features.

MATERIALS AND METHODS

Materials. Commercial WP isolate was obtained from BioLine (New Zealand). Pec (low methoxyl) from *Citrus* fruits, sorbitol, and all other reagents were purchased from Sigma (Steinheim, Germany). Microbial TGase (Activa WM; product no. AJ301402, lot no. 00.02.03), derived from the culture of *Streptoverticillium* sp., was supplied by Ajinomoto Co. (Japan).

Methods. *Film-Forming Solutions.* WP and Pec solutions were prepared as follows: WP isolate (1.2 g) and sorbitol (0.6 g) were dissolved in 25 mL of distilled water. Separately Pec (1.2 g) was dissolved in 25 mL of distilled water as well. Both solutions were stirred for 2 h at room temperature for a complete hydration of the macromolecules. Then, the WP solution was heated, under continuous stirring, in a water bath at 80 °C for 25 min to denature the proteins. Different aliquots of Pec solution were brought to 25 mL with distilled water to obtain variously diluted polysaccharide solutions, which, after heating at 80 °C for 3 min, were then added slowly and under stirring to various WP solutions. The differently diluted Pec solutions were added to WP solution to obtain WP/Pec ratios of 2:1, 4:1, 6:1, and 8:1 (w/w), respectively, with a constant WP concentration of 20 mg/mL. Each obtained film-forming solution was heated at 80 °C for a further 2 min and finally cooled under stirring at room temperature. The pH of the film-forming solutions prepared with a WP/Pec ratio of 4:1 was adjusted to the desired value, and then TGase (8 U/g of WP isolate) was added by stirring overnight at room temperature. Finally, the film-forming solutions were cast by pipetting 30 mL of each solution into Petri dishes (150 mm × 15 mm), and the films obtained by drying at 45 °C and 30% RH.

Potentiometric Titrations and Turbidity Measurements. P/P complexation was followed by potentiometric titration of acidic and basic protein groups.[9,23,24] The potentiometric titrations were performed at 22 °C on all the film-forming solutions, obtained as described above, and on a solution containing only WP isolate at a concentration of 20 mg/mL. The initial pH was adjusted to 8.0 ± 0.05 with 0.01 N NaOH, and the solutions were titrated with 0.01 N HCl to reach pH 3.5, corresponding to a pH value lower than Pec pK_a (4.6). The pH was noted when the value was stable for at least 1 min. The reproducibility of two repeated titrations was ±0.05 pH units. The turbidity of the solutions was determined using a UV/visible spectrophotometer at 600 nm with distilled water as blank reference. The pHc was recorded as the pH value corresponding to a change in slope of both curves obtained by potentiometric and turbidometric experiments. To evaluate the influence of electrostatic interactions on the pHc, we titrated further film-forming solutions after addition of 110 mM sodium chloride.

Film Characterization. *Thickness.* Film thickness was measured using an electronic digital micrometer with a sensitivity of 2 μm (Metrocontrol, Srl, model HO62). Film strips were placed between the jaws of the micrometer, and the gap was reduced until the minimum friction was measured. Mean thickness (mm) was determined from the average of measurements at five locations.

Scanning Electron and Atomic Force Microscopy. Microstructural features of WP/Pec films were analyzed by field emission scanning electron microscopy (FE-SEM) (JEOL JSM-6335F, JEOL Ltd.). Film samples were dried in a desiccator in the presence of $CaCl_2$ (a_w = 0.113 ± 0.003) and then fragmented. Dried film strip fragments were finally mounted on specimen stubs with the cross-section oriented up and coated with a thin layer of gold by a DC sputter coater (AGAR 87340, Agar Scientific Ltd. Stansted, England). Digital images of film cross-sections were collected at a tilt angle of 0° to the electron beam using an acceleration voltage of 20 kV.

TGase-catalyzed formation of WP/Pec supramolecular complexes in the film-forming solutions and the film surface morphology were investigated by atomic force microscopy (SPM Nanoscope IIIa, Veeco Instruments Inc., USA) in tapping mode. The film-forming solution was diluted 1:50, 10 μL was stratified on the mica sheets and dried in a spin-coater at 800 rpm, and 10 μm × 10 μm images under ambient conditions were obtained. Three images were captured per formulation.

Mechanical Properties. Film tensile strength, elongation to break, and Young's modulus were measured by using an Instron universal testing instrument model no. 5543A (Instron Engineering Corp., Norwood, MA, USA). Film samples were cut, using a sharp razor blade, into 10−11 mm wide and 50 mm long strips equilibrated overnight at 50 ± 5% RH and 23 ± 2 °C in an environmental chamber. Ten samples of each film type were then tested. Tensile properties were measured according to the ASTM D882[25] using Test Method A, the static weighing, constant rate-of-grip separation test. The initial grip separation was 40 mm, and the crosshead speed was 10 mm/min in tension mode.

Water Vapor Permeability. Water vapor permeability (WVP) was evaluated by a gravimetric test according to ASTM E96[26] by means of a Fisher/Payne permeability cup (Carlo Erba, Italy). Three grams of silica gel was introduced in each cup. The film samples, having a diameter of about 6 cm, were put on top of the cup and sealed by means of a top ring kept in place by three tight clamps. The film area exposed to vapor transmission was 10 cm^2. The cups containing silica gel were weighed and then placed in a desiccator containing a saturated KCl solution, which provided a constant water activity at 25 °C equal to 0.8434. The desiccator was stored in a Heareus thermostated incubator at 25.0 ± 0.1 °C. Cups were weighed at scheduled times, and the amount of water vapor transmission rate through the film was estimated by the linear portion of the diagram obtained by plotting the weight increment of the cup as a function of time. It was assumed that the steady state was reached once the regression analysis made by using the last four data points resulted in $r^2 \geq 0.998$. The WVP was calculated from the equation

$$\mathrm{WVP} = X/(A\Delta p)\ \mathrm{d}m/\mathrm{d}t$$

where $\mathrm{d}m/\mathrm{d}t$ is the slope of the cup weight versus time curve once steady state was reached, X is the film thickness, A is the film exposed area, and Δp is the water vapor pressure across the film. By assuming that the vapor pressure inside the cup, due to the presence of silica gel, can be taken equal to zero, Δp becomes equal to the vapor pressure inside the desiccator and was calculated by multiplying water activity and the water tension (P_0) at 25 °C (P_0 3167 kPa).

Oxygen Permeability. Film permeability to oxygen was determined using a modified ASTM D 3985[27] with a MultiPerm apparatus (ExtraSolution s.r.l., Pisa, Italy). Duplicate samples of each film were conditioned for 2 days at 50% RH before measurement. Aluminum masks were used to reduce the film test area to 5 cm^2, whereas the testing was performed at 25 °C under 50% RH.

Statistical Analysis. JMP software 5.0 (SAS Institute, Cary, NC, USA) was used for all statistical analyses. The data were subjected to analysis of variance, and the means were compared using the Tukey−Kramer HSD test. Differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

To produce edible WP/Pec films with acceptable mechanical and barrier properties, we preliminarily investigated the behavior of Pec in the presence of thermally denatured WP at both different P/P ratios and different pH values to determine the best experimental conditions for obtaining

soluble P/P complexes. In fact, it is well known that, according to the model proposed by Weinbreck et al.,[12] proteins and polysaccharides occur in free molecular forms when they are dissolved in water at pH values higher than their pHc, whereas at the pHc they interact by forming soluble complexes that generally aggregate and precipitate with further pH decreases. Therefore, panel A of Figure 1 shows the titration curves of

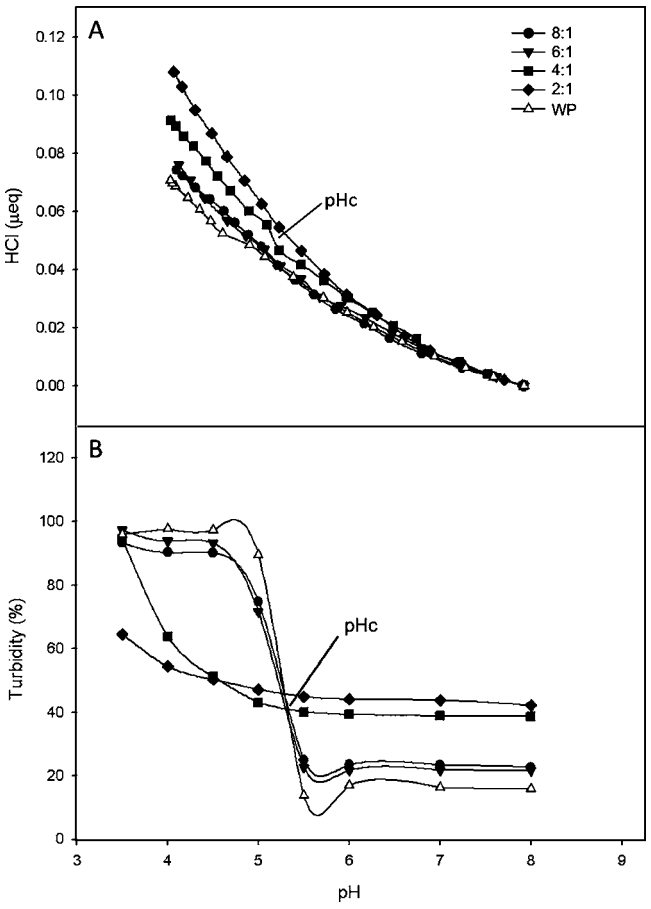

Figure 1. Titration (panel A) and turbidity (panel B) curves of WP/Pec film-forming solutions with different P/P ratios (w/w). Experimental details are given in the text.

different denatured WP/Pec film-forming solutions prepared at various P/P ratios, following the previously reported protocol used by Girard et al.[14] for β-Lg/Pec films. The detected pHc value was about 5.1. This value was quite different from the one (6.0) previously obtained with β-Lg/Pec films.[14] Moreover, Figure 1 shows that the titration curves obtained with WP/Pec ratios (w/w) of 8:1 and 6:1 were almost superimposable by decreasing the pH from 8.0 to pH 4.0, while the titration curve corresponding to a WP/Pec ratio of 4:1 changed its slope at pH 5.1, and the curve corresponding to a WP/Pec ratio of 2:1 changed its slope at pH 6.0. Thus, the valuable P/P ratio to obtain WP/Pec complexes must be 4:1.[14] This conclusion was confirmed by turbidometric experiments, even though by this method the pH value was 5.3, thus slightly different (Figure 1, panel B). To determine the nature of P/P interactions involved in the WP/Pec complexes, further titrations were carried out in 110 mM sodium chloride, as previously described for β-Lg/Pec films.[14] The results reported in Figure 2 show that the titration curve obtained with WP/Pec (4:1, w/w) film-forming solution prepared in the presence of the salt markedly shifted at pH 6.0,

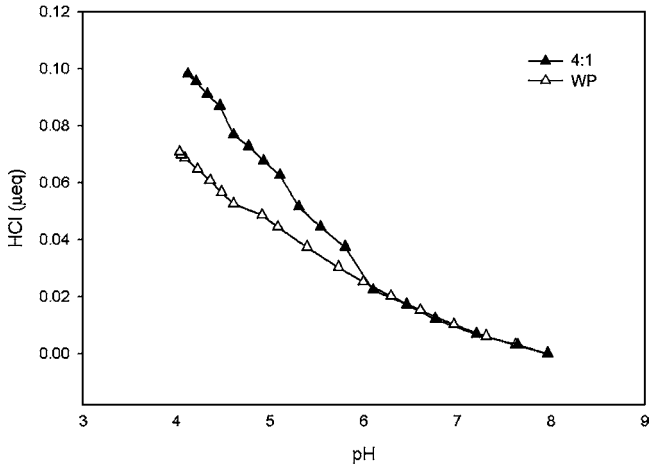

Figure 2. Titration curves of WP and WP/Pec (4:1, w/w) film-forming solutions in the presence of 110 mM NaCl. Experimental details are given in the text.

clearly indicating the occurrence of electrostatic interactions in WP/Pec complexes.[28]

To investigate the possible effects of TGase-catalyzed covalent cross-links on the mechanical and barrier properties of WP/Pec films, we prepared different film-forming solutions at pH 5.1 (pHc, when soluble P/P complexes occur) and at pH 6.0 (when the polymers are free in the solution) in both the presence and absence of the enzyme. The results reported in panel A of Figure 3 indicate that the films obtained by using WP/Pec film-forming solution prepared at pHc in the presence of TGase showed a tensile strength significantly higher than that of the corresponding films obtained with solutions made at the same pH in the absence of the enzyme, as well as at pH 6.0 in both the absence and presence of the enzyme. Moreover, it is worthy to note that a significant increase of the tensile strength was observed only at pHc, by using films made in the presence of TGase, while no significant difference was observed in the film containing the enzyme but made at pH 6.0. These findings could be explained by considering the possible formation of a supramolecular structural network during the film casting.[29] In fact, TGase-catalyzed covalent bonds among soluble complexes produced at pH 5.1 may be responsible for the improved film resistance with respect to the films prepared at pH 6.0, in which the enzyme catalyzes the formation of covalent bonds among uncomplexed WP molecules. Panels B and C of Figure 3 show, respectively, the measurements of film elongation to break, related to the capacity of the different materials to extend, and Young's modulus, which indicates the film's stiffness. From panel B of Figure 3 it is possible to conclude that, when TGase was absent in the film-forming solution, the films prepared at pH 6.0 were 2-fold more extensible than those prepared at pH 5.1. Conversely, when the films were prepared in the presence of the enzyme at pH 6.0, a significant reduction of their elasticity, due to the formation of covalent cross-links between the single soluble WP molecules, was observed. Moreover, the introduction of TGase-catalyzed covalent bonds in the WP/Pec supramolecular structural network occurring at pH 5.1 surprisingly increased the elongation to break of the obtained films about 6-fold in comparison with films obtained in the absence of the enzyme at the same pH and more than 10-fold in comparison with the ones prepared with the enzyme at pH 6.0. Finally, panel C of Figure 3 shows that the TGase-containing film prepared at pHc exhibited a reduced value of

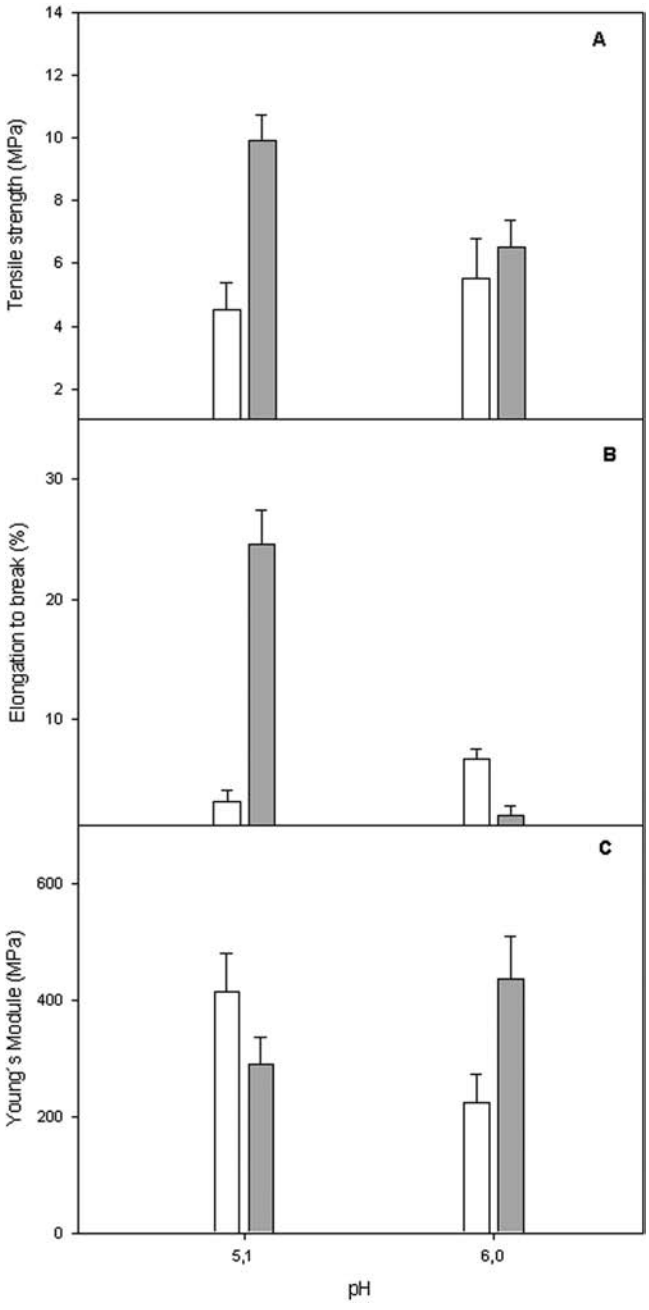

Figure 3. Tensile strength (A), elongation to break (B), and Young's modulus (C) of films obtained by casting a WP/Pec (4:1, w/w) mixture at different pH values in the presence (gray bar) or absence (open bar) of TGase (8 U/g WP isolate). Experimental details are given in the text.

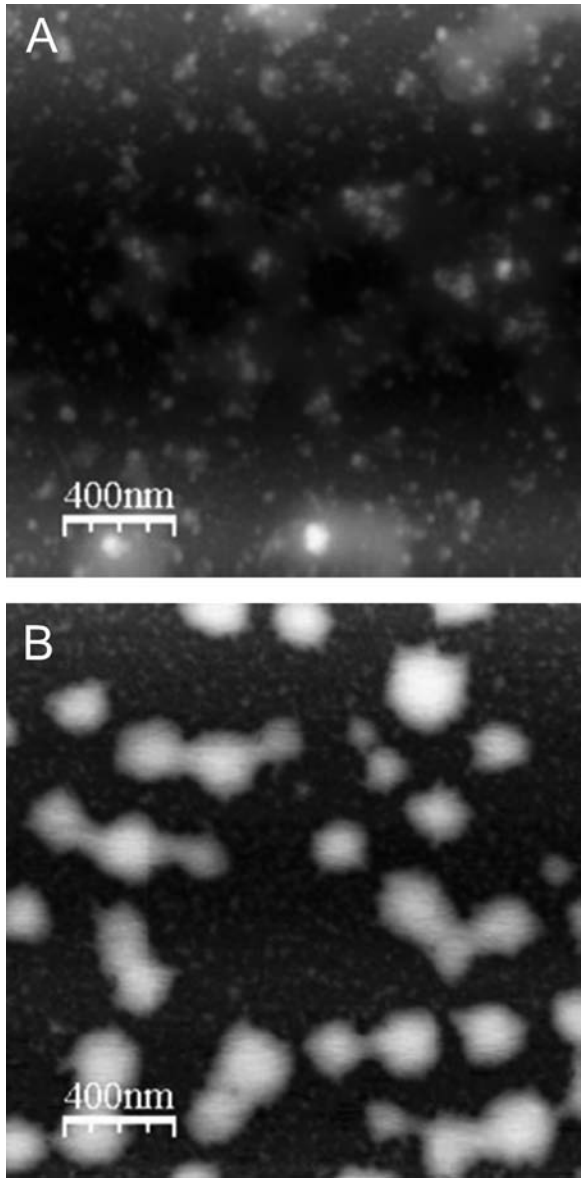

Figure 4. Atomic force microscope images of WP/Pec (4:1) film-forming solution prepared at pHc (5.1) in the absence (panel A) or presence (panel B) of TGase. Experimental details are given in the text.

Young's modulus, an index of a more flexible material, with respect to the film made in the presence of the enzyme at pH 6.0. The observed high elasticity and low Young's modulus lead to define the films derived from TGase-cross-linked WP/Pec complexes as typical elastomers.[30]

The formation of the hypothesized WP/Pec supramolecular complexes cross-linked by TGase was confirmed by AFM experiments carried out with film-forming solutions prepared at pHc in both the absence and presence of enzyme (Figure 4). Panel B of Figure 4 clearly indicates the presence of these supramolecular complexes only when TGase occurred in the film-forming solution, suggesting the production of enzymatically catalyzed cross-links among the P/P soluble electrostatic aggregates.

Finally, to evaluate the possible influence of TGase on the film barrier properties, we investigated both water vapor and oxygen permeability of the films obtained at pHc in the presence or absence of the enzyme. It is well known that several polymer properties concur to influence the barrier capability of a material to water vapor and different gases.[31] Among the various factors, the polymer chemical nature and the specific processing conditions are recognized, however, as the most relevant ones.[32] Previous studies, in fact, have shown that an increase in the crystallinity, density, orientation, molecular weight, and also cross-linking of the different materials tested is generally responsible for a decreased film permeability.[31,33] The results of our experiments, reported in Table 1, clearly show that the production of TGase-catalyzed cross-links among WP/Pec supramolecular soluble complexes obtained at pHc

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Table 1. Barrier Properties and Roughness of WP/Pec (4:1, w/w) Films Obtained at pHc in the Absence and Presence of TGase[a]

film	thickness (mm)	water vapor (cm^3 mm/m^2 day kPa)	oxygen (cm^3 mm/m^2 day kPa)	rms roughness (nm)
WP/Pec	0.152 ± 0.012	15.38 ± 0.19	0.025 ± 0.006	69.05 ± 2.60
WP/Pec + TGase	0.216 ± 0.021	9.90 ± 0.45	0.016 ± 0.002	424.90 ± 22.31

[a]Mean ± SD of 10 samples. Experimental details are given in the text.

significantly reduced film permeability to both water vapor and oxygen. The different structures of both transversal sections and surfaces of the films, observed by SEM and AFM, respectively, could explain the changes of properties of the enzyme-modified WP/Pec films. In fact, an increased homogeneity and compactness of the microstructure of samples prepared in the presence of TGase were quite evident following SEM experiments (Figure 5, lower panels). Similar SEM results

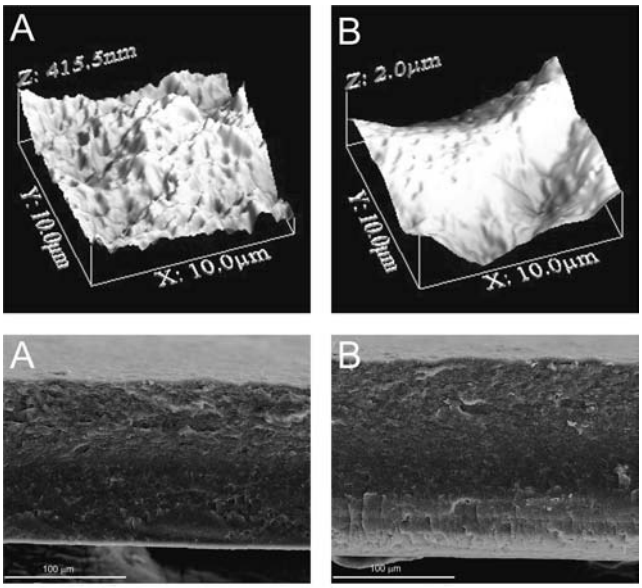

Figure 5. Atomic force (upper panels) and scanning electron (lower panels) microscope images of WP/Pec (4:1) films prepared at pHc (5.1) in the absence (A) or presence (B) of TGase. Experimental details are given in the text.

were previously obtained with phaseolin/fennel waste-based films cross-linked by TGase.[34] Moreover, also the morphology of the film surface was markedly affected, appearing in the AFM images much more rougher, by the presence of the enzyme (Figure 5, upper panels; Table1).

In conclusion our study strongly confirms that TGase is a very useful tool to produce composite bioplastics from renewable biomass sources with improved mechanical and barrier characteristics. In particular, we demonstrated the crucial importance of the pH value of the film-forming solution, which is able to dramatically influence the supramolecular structure of soluble P/P complexes able to act as enzyme substrates. Therefore, the described composite film, constituted by TGase-cross-linked WP/Pec-soluble complexes, may represent a new possible candidate as a substitute for nonedible coating materials for both food and pharmaceutical applications.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Ye, A. Complexation between milk proteins and polysaccharides via electrostatic interaction: principles and applications—a review. *Int. J. Food Sci. Technol.* **2008**, *43*, 406–415.

(2) Doublier, K. L.; Garnier, C.; Renard, D.; Sanchez, C. Protein-polysaccharide interactions. *Curr. Opin. Colloid Interface Sci.* **2000**, *5*, 202–214.

(3) De Kruif, C. G.; Tuinier, R. Polysaccharide protein interactions. *Food Hydrocolloids* **2001**, *15*, 555–563.

(4) MacDougall, A. J.; Brett, G. M.; Morris, V. J.; Rigby, N. M.; Ring, S. G. The effect of peptide behaviour–pectin interactions on the gelation of a plant cell wall pectin. *Carbohydr. Res.* **2001**, *335*, 115–126.

(5) Turgeon, S. L.; Beaulieu, M.; Schmitt, C.; Sanchez, C. Protein-polysaccharide interactions: phase ordering kinetics thermodynamic and structural aspects. *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 401–414.

(6) Decher, G. Fuzzy nanoassemblies: toward layered polymeric multicomposites. *Science* **1997**, *277*, 1232–1237.

(7) Dickinson, E. Interfacial structure and stability of food emulsions as affected by protein–polysaccharide interactions. *Soft Matter* **2008**, *4*, 932–942.

(8) Li, Y.; Mattison, K. W.; Dubin, P. L.; Havel, H. A.; Edwards, S. L. Light scattering studies of the binding of bovine serum albumin to a cationic polyelectrolyte. *Biopolymers* **1996**, *38*, 527–533.

(9) Wen, Y. P.; Dubin, P. L. Potentiometric studies of the interaction of bovine serum albumin and poly(dimethyldiallylammonium chloride). *Macromolecules* **1997**, *30*, 7856–7861.

(10) Hattori, T. H.; Kimura, K.; Seyrek, E.; Dubin, P. L. Binding of bovine serum albumin to heparin determined by turbidimetric titration and frontal analysis continuous capillary electrophoresis. *Anal. Biochem.* **2001**, *295*, 158–167.

(11) Weinbreck, F.; De Kruif, C. G. Complex coacervation of globular proteins and gum Arabic. In *Food Colloids Biopolymers and Materials*; Royal Society of Chemistry: Cambridge, UK, 2003; pp 337–344.

(12) Weinbreck, F.; de Vries, R.; Schrooyen, P.; de Kruif, C. G. Complex coacervation of whey proteins and gum Arabic. *Biomacromolecules* **2003**, *4*, 293−303.
(13) Hallberg, R. K.; Dubin, P. L. Effect of pH on the binding of beta-lactoglobulin to sodium polystyrenesulfonate. *J. Phys. Chem.* **1998**, *102*, 8629−8633.
(14) Girard, M.; Turgeon, S. L.; Gauthier, S. F. Interbiopolymer complexing between beta-lactoglobulin and low- and high-methylated pectin measured by potentiometric titration and ultrafiltration. *Food Hydrocolloids* **2002**, *16*, 585−591.
(15) Porta, R.; Mariniello, L.; Di Pierro, P.; Sorrentino, A.; Giosafatto, C. V. L. Transglutaminase-crosslinked chitosan and pectin based edible films: a review. *Crit Rev. Food Sci. Nutr.* **2011**, *51*, 222−238.
(16) Schols, H. A.; Voragen, A. G. J. The chemical structure of pectins. In *Pectins and Their Manipulation*; CRC Press: Boca Raton, FL, 2002; pp 1−29
(17) Regalado-González, C.; Pérez-Pérez, C.; Cortés-Lara, E.; García-Almendárez, B. E. Whey protein based edible food packaging films and coatings. In *Advances in Agricultural and Food Biotechnology*; E Guevára-González, R. G., Torres-Pachecho, I., Eds.; Research Signpost: India, 2006; pp 237−261.
(18) Bedie′, G. K.; Turgeon, S. L.; Makhlouf, J. Formation of native whey protein isolate−low methoxyl pectin complexes as a matrix for hydro-soluble food ingredient entrapment in acidic foods. *Food Hydrocolloids* **2008**, *22*, 836−844.
(19) Sperber, B. L. H. M.; Schols, H. A.; Cohen Stuart, M. A.; Norde, W.; Voragen, A. G. J. Influence of the overall charge and local charge density of pectin on the complex formation between pectin and *β*-lactoglobulin. *Food Hydrocolloids* **2009**, *23*, 765−772.
(20) Jones, O. G.; Decker, E. A.; McClements, D. J. Formation of biopolymer particles by thermal treatment of b-lactoglobulin−pectin complexes. *Food Hydrocolloids* **2009**, *23*, 1312−1321.
(21) Aeschlimann, D.; Paulsson, M. Transglutaminases: protein cross-linking enzymes in tissues and body fluids. *Thromb. Haemostasis* **1994**, *71*, 402−415.
(22) Mariniello, L.; Porta, R. Transglutaminases as biotechnological tools. In Transglutaminases; Mehta, K., Eckert, R., Eds.; *Prog. Exp. Tumor. Res.* **2005**, 174−191.
(23) Mattison, K. W.; Dubin, P. L.; Brittain, I. J. Complex formation between bovine serum albumin and strong polyelectrolytes. Effects of polymer charge density. *J. Phys. Chem. B* **1998**, *102*, 3830−3836.
(24) ASTM D882-97. In *Annual Book of ASTM Standards*; American Society for Testing and Materials: Philadelphia, PA, 1997; p 159.
(25) ASTM, E96-93. In *Annual Book of ASTM Standards*; American Society for Testing and Materials: Philadelphia, PA, 1993; p 701.
(26) ASTM, D3985-81. In *Annual Book of ASTM Standards*; American Society for Testing and Materials: Philadelphia, PA, 1981; p 534
(27) Tolstoguzov, V. B. Protein-polysaccharide interactions. In *Food Proteins and Their Application*; Domodaran, S., Paraf, A., Eds.; Marcel Dekker: New York, 1997; pp 71−198.
(28) Dubin, P. L.; Gao, J.; Mattison, K. W. Protein purification by selective phase separation with polyelectrolytes. *Sep. Purif. Methods* **1994**, *23*, 1−16.
(29) Ashby, M.; Jones, D. R. H. *Engineering Materials 1: An Introduction to Properties, Applications, and Design*, 3rd ed.; Michael and William Andrew Publishing, 2005; pp 29−93.
(30) Han, J. H.; Scanlon, M. G. Mass transfer of gas and solute through packaging materials. In *Food Packaging*; Han, J. H., Ed.; Elsevier Science & Technology: Oxford, 2005; pp 12−23.
(31) Jasse, B.; Seuvre, A. M.; Mathloutht, M. Permeability and Structure in Polymeric Packaging Materials. In *Food Packaging and Preservation*; Mathlouthr, M., Ed.; Blackie, 1994; pp l−22.
(32) Salame, M. Barrier Polymers. In *The Wiley Encyclopedia of Packaging Technology*; Bakker, M., Ed.; John Wiley & Sons: New York, 1986; pp 48−54.
(33) Miller, K. S.; Krochta, J. M. Oxygen and aroma barrier properties of edible films: A review. *Trends Food Sci. Technol.* **1997**, *8*, 228−237.
(34) Mariniello, L.; Giosafatto, C. V. L.; Moschetti, G.; Aponte, M.; Masi, P.; Sorrentino, A.; Porta, R. Fennel waste-based films suitable for protecting cultivations. *Biomacromolecules* **2007**, *8*, 3008−3014.