JOURNAL OF Applied Polymer SCIENCE

Special Issue: Bio-based Packaging

Guest Editors: José M. Lagarón, Amparo López-Rubio, and María José Fabra
Institute of Agrochemistry and Food Technology of the Spanish Council for Scientific Research

EDITORIAL

REVIEWS

RESEARCH ARTICLES

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Impact of fermentation residues on the thermal, structural, and rheological properties of polyhydroxy(butyrate-*co*-valerate) produced from cheese whey and olive oil mill wastewater
L. Hilliou, D. Machado, C. S. S. Oliveira, A. R. Gouveia, M. A. M. Reis, S. Campanari, M. Villano and M. Majone, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42818

Synergistic effect of lactic acid oligomers and laminar graphene sheets on the barrier properties of polylactide nanocomposites obtained by the in situ polymerization pre-incorporation method
J. Ambrosio-Martín, A. López-Rubio, M. J. Fabra, M. A. López-Manchado, A. Sorrentino, G. Gorrasi and J. M. Lagarón, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42661

Antibacterial poly(lactic acid) (PLA) films grafted with electrospun PLA/allyl isothiocyanate fibers for food packaging
H. H. Kara, F. Xiao, M. Sarker, T. Z. Jin, A. M. M. Sousa, C.-K. Liu, P. M. Tomasula and L. Liu, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42475

Poly(L-lactide)/ZnO nanocomposites as efficient UV-shielding coatings for packaging applications
E. Lizundia, L. Ruiz-Rubio, J. L. Vilas and L. M. León, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42426

Effect of electron beam irradiation on the properties of polylactic acid/montmorillonite nanocomposites for food packaging applications
M. Salvatore, A. Marra, D. Duraccio, S. Shayanfar, S. D. Pillai, S. Cimmino and C. Silvestre, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42219

Preparation and characterization of linear and star-shaped poly L-lactide blends
M. B. Khajeheian and A. Rosling, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42231

Mechanical properties of biodegradable polylactide/poly(ether-block-amide)/thermoplastic starch blends: Effect of the crosslinking of starch
L. Zhou, G. Zhao and W. Jiang, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42297

Interaction and quantification of thymol in active PLA-based materials containing natural fibers
I. S. M. A. Tawakkal, M. J. Cran and S. W. Bigger, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42160

Graphene-modified poly(lactic acid) for packaging: Material formulation, processing, and performance
M. Barletta, M. Puopolo, V. Tagliaferri and S. Vesco, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42252

Edible films based on chia flour: Development and characterization
M. Dick, C. H. Pagno, T. M. H. Costa, A. Gomaa, M. Subirade, A. De O. Rios and S. H. Flóres, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42455

Influence of citric acid on the properties and stability of starch-polycaprolactone based films
R. Ortega-Toro, S. Collazo-Bigliardi, P. Talens and A. Chiralt, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42220

Bionanocomposites based on polysaccharides and fibrous clays for packaging applications
A. C. S. Alcântara, M. Darder, P. Aranda, A. Ayral and E. Ruiz-Hitzky, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42362

Hybrid carrageenan-based formulations for edible film preparation: Benchmarking with kappa carrageenan
F. D. S. Larotonda, M. D. Torres, M. P. Gonçalves, A. M. Sereno and L. Hilliou, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42263

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Structural and mechanical properties of clay nanocomposite foams based on cellulose for the food packaging industry
S. Ahmadzadeh, J. Keramat, A. Nasirpour, N. Hamdami, T. Behzad, L. Aranda, M. Vilasi and S. Desobry, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42079

Mechanically strong nanocomposite films based on highly filled carboxymethyl cellulose with graphene oxide
M. El Achaby, N. El Miri, A. Snik, M. Zahouily, K. Abdelouahdi, A. Fihri, A. Barakat and A. Solhy, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42356

Production and characterization of microfibrillated cellulose-reinforced thermoplastic starch composites
L. Lendvai, J. Karger-Kocsis, Á. Kmetty and S. X. Drakopoulos, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42397

Development of bioplastics based on agricultural side-stream products: Film extrusion of *Crambe abyssinica*/wheat gluten blends for packaging purposes
H. Rasel, T. Johansson, M. Gällstedt, W. Newson, E. Johansson and M. Hedenqvist, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42442

Influence of plasticizers on the mechanical and barrier properties of cast biopolymer films
V. Jost and C. Stramm, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42513

The effect of oxidized ferulic acid on physicochemical properties of bitter vetch (*Vicia ervilia*) protein-based films
A. Arabestani, M. Kadivar, M. Shahedi, S. A. H. Goli and R. Porta, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42894

Effect of hydrochloric acid on the properties of biodegradable packaging materials of carboxymethylcellulose/poly(vinyl alcohol) blends
M. D. H. Rashid, M. D. S. Rahaman, S. E. Kabir and M. A. Khan, *J. Appl. Polym. Sci.* 2015, DOI: 10.1002/app.42870

Journal of Applied Polymer Science

The effect of oxidized ferulic acid on physicochemical properties of bitter vetch (*Vicia ervilia*) protein-based films

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ABSTRACT: The aim of the present study was to investigate the effect of oxidized ferulic acid on some physicochemical features of bitter vetch protein concentrate-based films. Our results indicate that moisture content, total soluble matter, water vapor permeability, and mechanical resistance values of the films prepared in the presence of oxidized ferulic acid were significantly modified in comparison with the ones detected with films obtained in its absence. In fact, film moisture content, total soluble matter, and water vapor permeability significantly decreased when oxidized ferulic acid was added to the film forming solutions, whereas both film tensile strength and elongation at break resulted markedly higher. Moreover, atomic force and scanning electron microscopy analyses showed that film morphology was also markedly affected by the presence of oxidized ferulic acid, film surface roughness, and compactness resulting higher than the ones observed in control samples. Therefore, these findings show a marked improvement in obtaining edible films derived from cheap starting biopolymers, such as bitter vetch proteins, potentially useful for different food and pharmaceutical applications. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42894.

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INTRODUCTION

In recent years a huge range of oil-based polymers has been used for different packaging applications. It is well known, however, that such packaging materials represent a significant part of municipal solid wastes causing dramatic environmental concerns because they are nonbiodegradable and determine mixed levels of contamination due to complex composites difficult to recycle or reuse.[1,2] Therefore, there is an increasing interest in developing environment-friendly and biodegradable materials from renewable natural resources, such as crops, to substitute oil-derived polymers.[3] As a consequence, biopolymers including proteins and polysaccharides, or their combinations, are increasingly under investigation to prepare new biodegradable and/or edible films for packaging or coatings uses.[4,5] In particular, protein films are effective lipid, oxygen and aroma barriers at low relative humidity environmental conditions and, thus, they have been extensively investigated as possible edible packaging components.[6]

Some species of leguminous family are cheap protein sources generally used for animal feeding.[7] The *Vicia* genus is a legume with about 160 annual and perennial species distributed throughout temperate regions of Europe, western and central Asia, north Africa, and Americas.[8–11] *Vicia* seed protein content ranges from 20 to 32% and, in particular, *Vicia ervilia* (bitter vetch) is an important annual *Vicia* genus widely cultivated for the utilization of its seeds and hay.[9,11] Bitter vetch seeds, containing up to 25% of protein, are a good and inexpensive source of both protein and energy[12] and, hence, they may represent an affordable protein source to produce biodegradable films especially for food applications.[13]

Most of the protein-based films exhibit poor barrier properties against water vapour, due to their inherent hydrophilic properties[14] and weaker mechanical characteristics with respect to the ones showed by the oil-derived polymer films.[3] Conversely, polypeptide chains possess reactive side groups which can be modified via physical, chemical, or enzymatic treatments to improve both water vapour permeability (WVP) and mechanical resistance of protein-based films.[3,14,15] Polyphenols are known to react with protein side chain amino groups under oxidizing conditions, leading to the formation of covalent crosslinks.[15] In particular, ferulic acid, a phenolic acid derivative quite ubiquitously distributed in the plant kingdom as cell wall crosslinking agent, is known to be able to bind proteins

producing resonance stabilized free radical intermediates. Moreover, oxidized ferulic acid (OFA) can react with endoprotein both amino and thiol groups and, thus, it could be used as an effective crosslinking agent in the attempt to improve protein-based film features.[16,17]

Because our previous researches on bitter vetch seed protein-based films showed a relatively poor water vapour barrier capacity and a not satisfying mechanical resistance,[13] we decided to investigate the effect of OFA addition on bitter vetch seed protein film forming solution to try to obtain edible films with decreased WVP and improved mechanical properties.

EXPERIMENTAL

Materials

Bitter vetch seeds were obtained from a local market in Isfahan, Iran. All chemicals and solvents used in this study were analytical grade commercial products. Sodium hydroxide, hydrochloric acid (37%), glycerol (about 87%), and ferulic acid were purchased from the Merck Chemical Company (Darmstadt, Germany).

Preparation of Bitter Vetch Seed Proteins

Proteins were extracted from the bitter vetch seeds according to Monsoor and Yusuf[18] by milling the seeds to a fine powder (40 mesh) and soaking the latter in distilled water (1 : 10, w/v) brought at pH 11 by 0.1 N NaOH. After stirring (IKA® RH basic 2, Germany) at medium speed for 1 h at 25°C, the mixture was centrifuged at 3200 × *g* for 10 min (Sigma 2-16, Germany) and the precipitate discarded. The pH of the supernatant was then adjusted to pH 5.4 by 0.1*N* HCl addition and the resulting precipitate, collected after centrifugation at 3200 × *g* for 10 min, was finally dissolved at pH 7.0 and the solution dried in a vacuum oven at 40°C. The obtained dry concentrate of bitter vetch proteins was finally minced in a coffee grinder (Kenwood, CG 100, China). Protein content of both bitter vetch seeds and bitter vetch protein concentrate (BVPC) was determined by the Kjeldahl's method.[19]

Film Preparation

Because bitter vetch protein-based film was produced for the first time, the preparation conditions were investigated. These conditions included protein concentration (3, 5, 7, and 10 g/100 g distilled water) concentration of glycerol as plasticizer (0, 20, 30, and 50% w/w BVPC), type of heating the film forming solution (direct such as hot plate or indirect such as water bath), heating time, and temperature of the film forming solution on hot plate (60–80°C for 15–30 min) and in a water bath (70–90°C for 15–30 min) with shaking or without shaking, heating time and temperature of drying the film forming solution (for example at room temperature for 24 h or in oven with different temperatures from 30–50°C for 12–24 h). Although, we tested different conditions for preparing the BVPC films in most cases, without any additional tests, we realized that the produced film did not have the appropriate properties to allow it be handled easily. For example, when we used a protein concentration higher than 5 g/100 g distilled water or plasticizers (glycerol) at concentration lower than 50% w/w BVPC, the films were very thick and brittle, respectively. Moreover, direct heating of the film forming solutions with and without shaking and indirect heating of the film forming solutions without shaking, at temperature lower than 80°C for 30 min resulted in films that were dark in appearance (especially with direct heating of the film forming solutions using a hot plate) or insoluble particles in its structure. Therefore, based on film appearance (thickness and colour) and its mechanical properties, the optimal conditions as described in the present work were selected.

Five grams of BVPC powder was dispersed under constant stirring in ~50 mL distilled water and then glycerol (50%, w/w BVPC) was added. The total weight of solution was led to ~90 g with distilled water, the pH value was adjusted to 11 and, then, distilled water was added to reach a final weight of 100 g. The film forming solution was heated in a water bath, for 30 min at 80°C under constant stirring, then cooled at room temperature, degassed, and finally cast on a Teflon-coated glass plate (30 × 30 cm^2) at 30°C for 24 h.

Different amounts of ferulic acid were dissolved in 50 mL of 500 mg L^{-1} hydrogen peroxide solution. The mixture was kept at room temperature for 30 min to eliminate residual hydrogen peroxide, and then the pH was adjusted to 9.0. When desired, oxidized ferulic acid (OFA) solution was added to glycerol containing BVPC solution, the pH adjusted according to the method described above, and distilled water added to reach a final weight of 100 g.[16]

All the films, containing or not OFA, were manually peeled off and, prior to be tested, were conditioned at 50% RH and 25°C by placing them in a dessicator over a saturated solution of $Mg(NO_3)_2$ $6H_2O$ for 48 h. Film thickness was measured with a micrometer (Electronic digital micrometer, DC-516, sensitivity 0.001 mm) at different positions for each film specimen. At least five thickness measurements were performed with each film specimen and the mean values were considered in the different tests.

Moisture Content

Three samples (2 × 2 cm^2) were cut from each film, weighed (±0.0001 g) into aluminum dishes and dried in an air-circulating oven at 105 C for 24 h. The moisture content values were determined as the percentage of weight loss relative to original weight.

Total Soluble Matter

Film samples (2 × 2 cm^2) were weighed (±0.0001 g) and then directly immersed in beakers with 15 mL distilled water at 25°C for 24 h. The insoluble film specimens were removed from water, rinsed with distilled water, and dried in an air-circulating oven at 105°C for 24 h. The total soluble matter (TSM) percentage of the films was calculated using the following equation[20]:

$$\%\ \mathrm{TSM} = \left[\left(W_0 - W_f\right)/W_0\right] \times 100$$

where W_0 is the initial dry weight of the films and W_f is the final dry weight of the undissolved desiccated film residue. Initial dry matter values of each film were obtained from moisture content measurements for the same film.

Water Vapour Permeability

Film water vapour permeability (WVP) was evaluated by a gravimetric test according to the method used by Bamdad *et al.*[6] Protein film samples were cut into discs and then mounted on circular steel cups (5.1 cm diameter and 5.4 cm depth) containing 3 g of anhydrous calcium chloride (0% RH). The cups were weighed with their contents and placed in dessicators containing 1 L of pure water (100% RH) at 22°C. Cups were weighed every 24 h for 1 week. The water vapour transferred through the films was determined from the weight gain of the cups. Changes in the weight of the cups were recorded and plotted as a function of time. Water gain velocity (slope) was calculated by linear regression (Microsoft Office Excel 2010) and water vapour transmission rate (WVTR) was calculated from the slope (g/h) divided by the cup area (m^2). WVP was calculated as follows[21]:

$$\mathrm{WVP} = (\Delta m/\Delta t) \times X/A\Delta P$$

where $\Delta m/\Delta t$ is the weight of moisture gain per unit of time (g/h). X is the average film thickness (mm), A is the cup test mouth area (m^2), and ΔP is the water vapor pressure (kPa) difference between the two sides of the film (the vapour pressure of pure water at 22°C = 2.642 kPa).

Mechanical Properties

Film tensile strength (TS) and elongation at break (EB) were measured following the ASTM Standard Test Method D 882-91[22] using a texture analyzer (Zwick 1446-60, Germany). Each film strip (2.5 × 10 cm^2) was mounted between the grips of the texture analyzer. Initial grip separation and cross-head speed set to 5 cm and 1 mm s^{-1}, respectively.

TS value was calculated by dividing the maximum load (F_{max}) by the initial cross-sectional area (A) of the specimens. EB value was calculated as the percentage of change in the length of the specimen (ΔL) compared to the original length (L_0) between the grips.

$$\mathrm{TS} = F_{max}/A$$

$$\%\mathrm{EB} = (\Delta L/L_0)\times 100$$

Spectrophotometric Analysis

The absorption spectra of ferulic acid solution, hydrogen peroxide, ferulic acid oxidized with hydrogen peroxide, BVPC film forming solution (control), and BVPC film solution containing OFA were scanned using a UV–visible spectrophotometer (Jasco, V-530, Japan). All of the solutions adequately diluted to which their absorbance was below 1.5. Ferulic acid dissolved in ethanol, hydrogen peroxide, and film forming solutions diluted with distilled water and alkaline distilled water with pH 10, respectively.

Atomic Force and Scanning Electron Microscopies

The surface morphology of films was observed by an atomic force microscope (AFM) (Bruker, model Nanos). A sharpened Si3N4 cantilever with a spring constant of 0.2 N m^{-1} and a V-shaped tip 450-μm long was positioned over the sample and 20 × 15 μm^2 images under ambient conditions were captured. Three images were obtained per each formulation but one of them was provided in the article.

The cross section of both control and OFA containing films was examined by a Zeiss scanning electron microscope (SEM) (Germany). At first, the film samples were dried in vacuum at room temperature. Then, for cross-sectional analysis, fractured films were rested vertically on the sides of a rectangular aluminum piece and fixed on stubs using double sided adhesive tape, coated with gold and observed at a magnification of 1000×. An acceleration potential of 7 kV was used during micrograph.

Statistical Analysis

All determinations were carried out in triplicate and mean values and standard deviations were calculated. The results were analyzed using the SAS package (version 9.1). The least significant difference (LSD) test was used to describe means at the 5% significance level.

RESULTS AND DISCUSSION

Protein content values obtained in our research for flour and BVPC were 27.62% (db) and 86.45% (db), respectively. Protein content of bitter vetch seeds was similar to the ones previously reported by Haddad.[9,12,23] The method that was used for preparation of bitter vetch protein concentrate with purity of 86.45% (db) was shown to be in suitable range of pH, centrifuge, and drying conditions. The produced BVPC films resulted flexible and sufficiently strong to be handled and their thickness (0.10 ± 0.02 mm) was controlled by casting the same volume of the film forming solutions, prepared in the absence or presence of OFA, on each plate.

Film Moisture Content and Total Soluble Matter

The moisture content of BVPC films prepared in the presence of various OFA concentrations (50, 100, 150 mg of OFA/100 g of film forming solution containing 5 g of BVPC) is significantly lower (about 25%) than that observed by testing the control samples prepared in the absence of OFA (Table I). It is well known that phenols interact with proteins either reversibly by hydrogen bonds or irreversibly by covalent linkages.[24] Thus, the differences observed between the moisture content of the films obtained in the presence or absence of OFA might be dependent on the occurrence of covalent crosslinkings between OFA and reactive moieties of proteins inside the films. These linkages, by limiting protein–water interactions by hydrogen binding, may cause a remarkable decrease of the moisture content in the OFA-containing films compared to that observed in the control samples. As shown in Table I, OFA concentration of 50 mg/100 g of film forming solution is sufficient to reach the maximal effect.

Moreover, the data shown in Table I, indicate a parallel quantitative decrease in the total soluble matter (TSM) values when the films were prepared in the presence of OFA. Also in this case OFA concentration of 50 mg/100 g of film forming solution resulted sufficient to obtain the maximal effect. TSM decrease could be explained with the formation of a more stable protein network due to the OFA-mediated cross linkages.[16]

Film Water Vapor Permeability

Figure 1 reports that the WVP of films containing 50 mg of OFA is significantly reduced (about 30%) with respect to that exhibited by the control samples, no further decrease being

Table I. Moisture Content, Total Soluble Matter, and Roughness of BVPC Films Containing Different Concentrations of OFA

OFA concentration (mg/100 g)	Moisture content (%)	Total soluble matter (%)	Roughness, rms (nm)
0	27.69 ± 1.18^a	36.57 ± 2.08^a	10.63 ± 1.18^b
50	21.28 ± 0.37^b	27.74 ± 1.53^b	45.93 ± 1.22^a
100	21.22 ± 1.85^b	30.97 ± 1.68^b	46.33 ± 2.74^a
150	21.10 ± 1.06^b	30.53 ± 0.98^b	46.82 ± 3.02^a

Values with similar letter in each column are not significantly different ($P < 0.05$).

observed by adding higher OFA concentrations (100 or 150 mg/100 g). Reduced WVP values confirm the occurrence of cross-linkages in the BVPC films prepared in the presence of OFA. In fact, ferulic acid and its oxide, quinoid ferulic acid, could react with endo-protein amino and thiol groups and promote cross linkings.[25] However, WVP of the films slightly increased as the concentration of OFA increased from 50 to 100 and 150 mg/100 g. The effect of OFA on the film WVP could depend on OFA concentration inside the film. It seems that OFA is desirable for improving film properties up to a critical point. In fact, since bitter vetch protein is deficient in sulfur amino acids[9] which are able to interact with quinines, an excess of OFA might interact together resulting in increasing of inter-chain space in the protein and leading to weaker barrier properties compared to the film containing 50 mg of OFA. Ou *et al.*[16] previously reported similar results showing that, whereas ferulic acid was not able to change WVP of soy protein isolate films, OFA significantly decreased it, even though in their experiments the optimal OFA concentration was found to be higher (100 mg/100 g of film forming solution). Furthermore, Mathew and Abraham[25] showed that also WVP of OFA-containing starch-chitosan blend films resulted considerably decreased, while Fabra *et al.*[26] obtained same results by using sodium caseinate films containing ferulic acid at a concentration higher than 40 mg. Conversely, Nuthong *et al.*[15] showed that the addition of ferulic acid at levels of 1–3% (w/w of protein) significantly increased WVP of porcine plasma protein-based film.

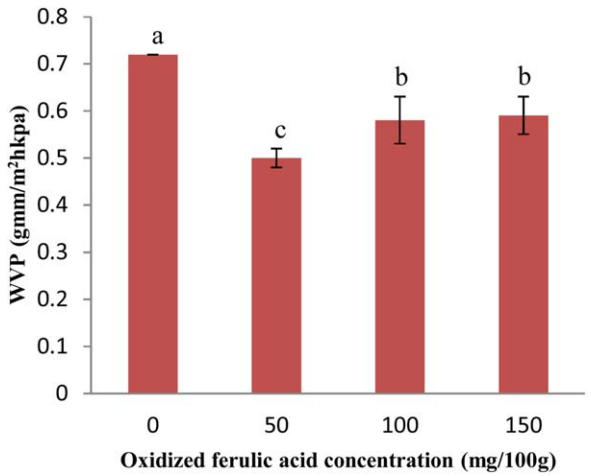

Figure 1. WVP of BVPC films prepared in the absence or presence of different concentrations of OFA. Values superscripted with dissimilar letters (a, b, c) are significantly different ($P < 0.05$) from the control values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Film Mechanical Properties

Panels a and b of Figure 2 illustrate the tensile strength (TS) and the elongation at break (EB) values of BVPC films prepared in the absence or presence of OFA. Our results indicate that OFA addition (50 mg/100 g) to the film forming solution significantly increases both TS (about 35%) and EB (about 20%) of the obtained films. No further increase was observed by adding higher OFA concentrations (100 or 150 mg/100 g). Also in these cases, covalent crosslinkings between OFA and some reactive protein side chain reactive groups could be responsible for the significant differences observed between the mechanical properties values of the control films and the ones of the films prepared in the presence of OFA.

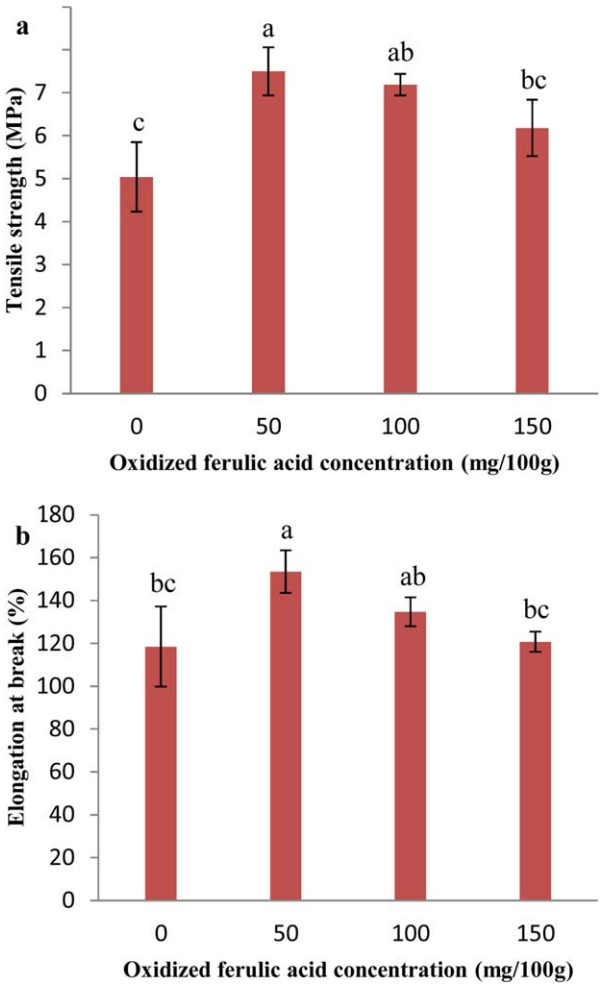

Figure 2. Tensile strength (a) and elongation at break (b) of BVPC films prepared in the absence or presence of different concentrations of OFA. Values superscripted with dissimilar letters (a, b, c) are significantly different ($P < 0.05$) from the control values. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

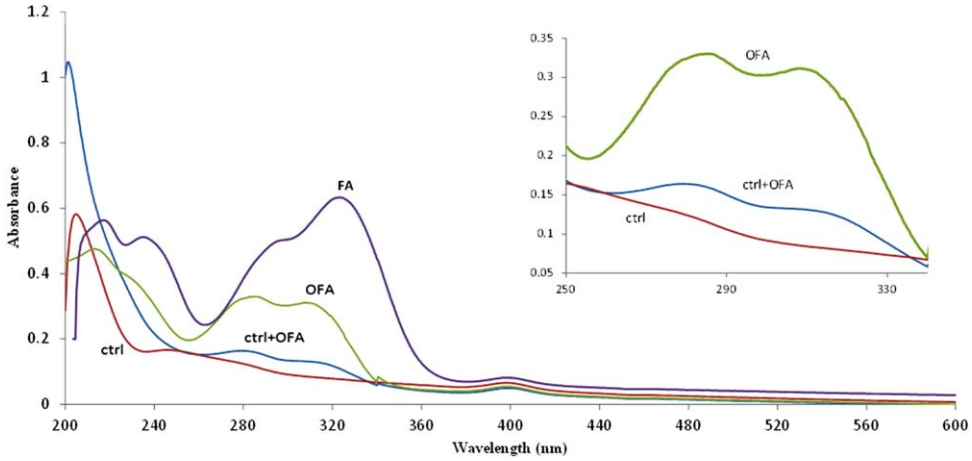

Figure 3. Absorbance spectra of BVPC solution (ctrl), BVPC solution containing oxidized ferulic acid (ctrl+ OFA), ferulic acid (FA) and oxidized ferulic aicd (OFA). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Phenolic compounds, under oxidizing conditions, could be converted to quinone, which is able to form covalent crosslinks with proteins reacting with their both amino and thiol groups.[24] The free radical deriving from ferulic acid could react also with tyrosyl protein residues and with itself to form diferulic acid by producing a molecular bridge between different protein chains.[27] Therefore, this phenomenon could explain the development of a more rigid and stable protein network inside the film[15] determining an increase of its TS and EB. Previous researches showed that also TS and EB of soy protein isolate films treated with ferulic acid (50–200 mg/100 g) significantly increased.[16] In contrast, Mathew and Abraham[25] found enhanced only TS of starch-chitosan blends prepared in the presence of OFA, whereas their EB values decreased as a consequence of the formation of a too much rigid protein network. They reported that the optimal concentration of OFA was 75 mg/100 g of blend film. Furthermore, the addition of ferulic acid to zein films was shown to lead to the production of films with a decreased TS and an increased EB.[28] The increased flexibility observed by testing zein films prepared in the presence of phenolic compounds seems to depend on phenolic compound binding onto zein surface and on the consequent increase of film matrix-free volume. The hydrophilic groups of phenolic compounds could also determine a reduced hydrophobic interaction among zein molecules and a consequent decrease of the zein film strength.

Emmambux *et al.*[29] reported an antiplasticizing effect of tannic acid (a phenolic compound) on sorghum kafiran film. They suggested that this effect could be due to the molecular properties of tannic acid, since this phenolic compound contains several hydroxyl groups able to form hydrogen bonds with carbonyl groups of proteins with the consequence to bind very tightly the polypeptide chains and to markedly reduce their mobility within the film matrix. Nuthong *et al.*[15] observed no changes in TS of porcine plasma protein based films incorporating ferulic acid at a concentration of 1–2% (w/w of protein) even though, when higher amounts of ferulic acid was added, film TS significantly increased. Conversely, addition of 1–2% ferulic acid resulted in an EB enhancement, whereas at higher ferulic acid concentrations film EB was observed to decrease again.

In conclusion, all these findings suggest that the effect of phenolic compounds on the protein-based film mechanical properties strictly depend on the type and amount of both protein and phenolic compound used. In fact, our results indicate that since bitter vetch proteins are poor in amino acids able to interact with quinones, such as tyrosine and thiol-containing amino acids,[8,11] low concentrations of OFA (50 mg/100 g) resulted sufficient to produce strong and flexible BVPC films. To the best of our knowledge, there is only a report about the improvement of BVPC film properties with calcium chloride (0.1-1% w/w BVPC).[13] Generally, BVPC films containing OFA (50 mg/100 g) showed better barrier and mechanical properties compared to BVPC films containing calcium chloride as a cross linking agent.

Absorption Spectra

The UV–visible spectrum of ferulic acid revealed an absorption peak at 326 nm (Figure 3) while hydrogen peroxide showed no absorption maximum in the wavelength range 200–600 nm. Removal of the electron donating hydroxyl group after oxidation of ferulic acid with hydrogen peroxide caused a decrease in the wavelength of maximum absorbance (λ_{max} (of ferulic acid benzene ring from 218 to 212 nm, and from 238 to 228 nm due to interruption in the aromatic system (Figure 3). In addition, the λ_{max} corresponded to the carboxyl group of ferulic acid that decreased after oxidation (from 300 to 285 nm and from 326 to 313 nm). Loss of resonance in ferulic acid due to the conversion of hydroxyl to carbonyl group could be the main reason for the observed blue shift in λ_{max} of ferulic acid (ferulic acid and oxidized ferulic acid structures are reported in Figure 4). BVPC film forming solution (control) revealed an absorption peak at 205 nm while BVPC solution containing OFA had two absorption peak at 279.5 and 310 nm corresponding to the esterified ferulic acid.[25] In comparison with the OFA solution, the spectrum of BVPC solution containing OFA was slightly shifted from 285 to 279.5 nm. This shift could be due to the decrease in resonance of the carboxyl group with the double

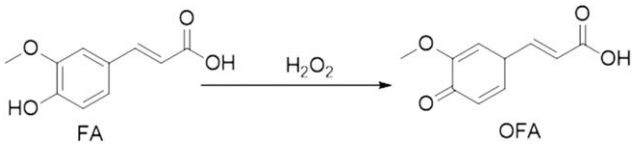

Figure 4. Ferulic acid (FA) and oxidized ferulic acid (OFA) structures.

bond in OFA caused probably by interaction of carboxyl group in OFA with amino groups (or other polar functional groups) in protein solution. These results suggested that OFA was reacted with bitter vetch proteins in the film forming solutions. Other researchers also reported interaction of ferulic acid and OFA with proteins and polysaccharides in the biodegradable film forming solutions through spectrophotometric analysis.[16,25]

Film Morphology

Figures 5 and 6 show surface topography and three-dimensional AFM images of BVPC films prepared in the absence or presence of 50 mg/100 g OFA of film forming solution. According to these images, control films present a relatively smooth and continuous matrix with a low roughness (rms value of 10.63 nm, Table I). Conversely, both surface topography and three dimensional images of films prepared in the presence of a concentration of 50 mg OFA/100 g exhibited a marked heterogeneity with a 4.5-fold higher rms value compared to control samples. The cross-linking between OFA and functional groups of the protein matrix could be responsible for the significant difference observed between the surface roughness values of the control film and the ones prepared in the presence of OFA. It is worthy to note that increases in OFA concentrations were not able to further enhance the roughness values of film surfaces (Table I). These data are in agreement with previous results obtained by other Authors. In fact, Mathew and Abraham[25] reported that OFA incorporation into starch-chitosan blend films caused an

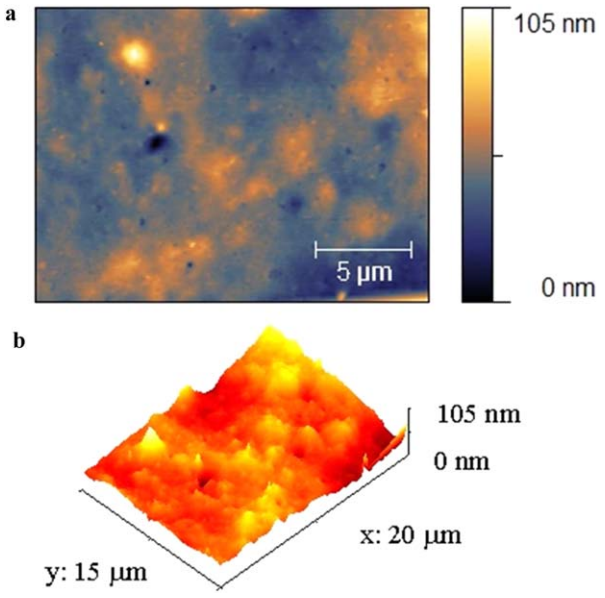

Figure 5. Surface topography (a) and three-dimensional AFM images (b) of BVPC film prepared in the absence of OFA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

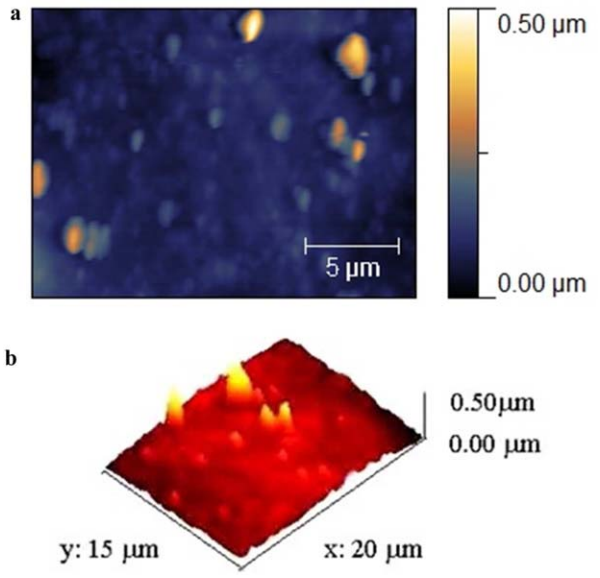

Figure 6. Surface topography (a) and three-dimensional AFM images (b) of BVPC film prepared in the presence of OFA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

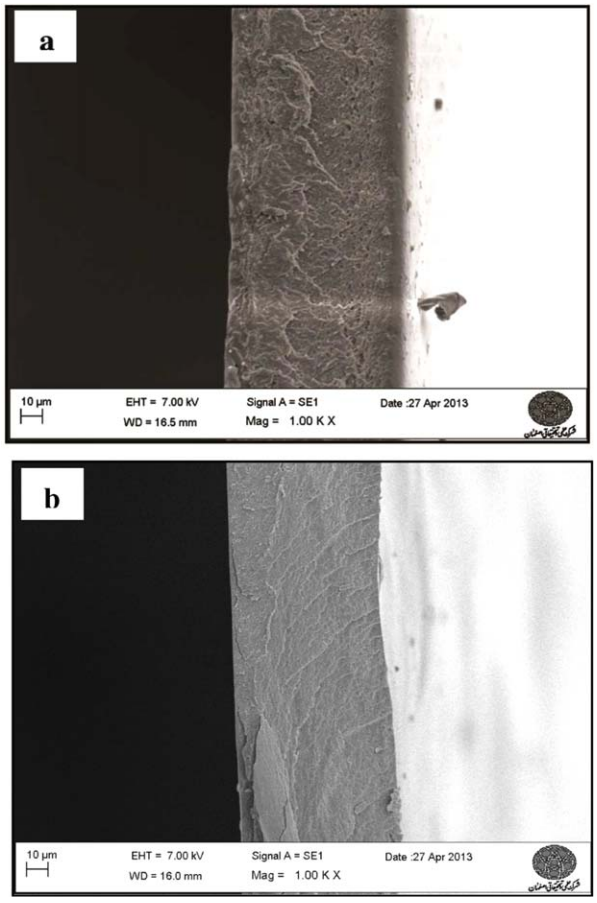

Figure 7. Cross-sectional SEM analysis (at 1000× magnification) of BVPC films prepared in the absence or presence of OFA. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table II. The Comparison of Some Properties of BVPC Film with Other Plant Biodegradable Films

Film	Thickness (mm)	TS (MPa)	EB (%)	WVP (gmm/m^{2}hkPa)	MC (%)	TSM (%)
BVPC	0.1 ± 0.02	5.04 ± 0.81	118.49 ± 18.71	0.72 ± 0.00	27.69 ± 1.18	36.57 ± 2.08
BVPC + OFA	0.1 ± 0.02	7.5 ± 0.56	153.49 ± 10.02	0.50 ± 0.02	21.28 ± 0.37	27.74 ± 1.53
NaCas	≈ 0.025	27.19 ± 1.60	3.10 ± 0.10	0.61 ± 0.01	-	-
SPI[a]	0.071 ± 0.002	1.60 ± 0.06	156.30 ± 7.10	0.096	-	-
SPI[b]	0.094 ± 0.005	2.21 ± 0.25	159.87 ± 9.20	1.24 ± 0.01	27.34 ± 2.62	40.12 ± 3.45
CZ	0.132 ± 0.002	10.19 ± 0.83	3.34 ± 0.66	-	-	-
PPP	0.068 ± 0.001	1.23 ± 0.06	67.08 ± 3.84	1.01	-	-
STCH	0.074 ± 0.004	≈ 45	29.32	0.014	-	-
PUP	-	0.86-6.56	22.2-196.61	-	10.47-20.49	22.7-89.52
SFP	0.07 ± 0.01	2.3 ± 0.4	32.0 ± 4.0	-	28.6 ± 1.5	93.2 ± 1.7
PEP	-	7.5 ± 0.5	88.0 ± 16.0		10.4 ± 0.9	96.6 ± 2.1

TS: tensile strength, EB: elongation at break, WVP: water vapour permeability, MC: moisture content, TSM: total soluble matter, BVPC: bitter vetch protein concentrate, BVPC + OFA: bitter vetch protein concentrate film containing oxidized ferulic acid (50 mg/100 g), NaCas: sodium caseinate.[26] SPI[a]: soy protein isolate.[16] SPI[b]: soy protein isolate.[20] CZ: corn zein.[28] PPP: Porcine plasma protein.[15]STCH: Starch-chitosan blend film.[25] PUP: pumpkin protein.[30] SFP: sunflower protein.[31] PEP: peanut protein.[32]

increase of film surface roughness and, more recently, Fabra *et al.*[26] showed that surface roughness of sodium caseinate films obtained in the presence of different ferulic acid concentrations was significantly higher than the one observed in the control samples.

Furthermore, we also carried out the cross-sectional SEM analysis of the BVPC films prepared in the presence of low OFA concentrations (50 mg/100 g) to observe possible morphological changes in the protein network with respect to the control films. Figure 7 shows that, whereas control sample presented discontinuous zones and some small pores (panel a), the OFA containing film was clearly more compact showing continuous zones due to the OFA-determined protein crosslinkings (panel b).

In Table II BVPC film properties were compared to previously reported biodegradable films obtained from protein or polysaccharide sources. Regarding to film thickness, starch-chitosan films show the lowest WVP and the highest TS values, whereas SPI and porcine plasma protein films exhibit the highest WVP and the lowest TS. BPVC film properties such as MC, TSM, TS and WVP are generally comparable with the ones observed with films obtained from other plant proteins,[13] although some of them, like EB, are similar to the ones of soy protein isolate film and higher than that of other biodegradable films (Table II).

The majority of protein films, due to the high proportion of hydrophilic amino acids in their structure, have low tensile strength and poor moisture barrier properties. Also, the presence of some additives such as hydrophilic plasticizers is effective on these properties.[14] It can be found that the lowest TS and the highest WVP of SPI and porcin plasma protein films might be due to the high hydroxyl groups in the films which could interact strongly with migrating water.

Other important factors affecting the protein film characteristics are pH and temperature. Alkaline conditions and heating above 60°C during film preparation can alter the three-dimensional structure through unfolding of polypeptide chains and promote protein polymerization leading to the improvement in the mechanical properties and water vapour permeability.[33] Generally, the properties of protein films depend strongly on source and amino acid profile of protein, the type and amount of plasticizers, pH and temperature in the film forming solutions.

However, improvement in TS and WVP characteristics of BVPC films determined by different treatments, such as protein cross-linking, could suggest their applications in food packaging.

Because bitter vetch is able to grow in poor soils of low rainfall areas (200–350 mm annually),[8,10] its seed proteins could be economically advantageous compared to the ones extracted from other plants such as lentil, pea, bean, peanut, pumpkin and soy or sodium caseinate and corn zein. In addition, the present results indicate that OFA is able to confer to the BVPC films high resistance coupled with a high deformability.

CONCLUSION

Since we have previously shown that bitter vetch proteins have a good potential in edible film production, we investigated new ways to improve the properties of these films by testing the addition of oxidized ferulic acid to the film forming solution as protein cross-linking agent. Our findings indicate that the oxidized ferulic acid-containing films showed decreased water vapour permeability, moisture content, and total soluble matter, while exhibited an increased tensile strength and elongation at break compared to the control samples. Moreover, also the surface and the internal morphology of the oxidized ferulic acid crosslinked films resulted affected being increased their roughness and compactness.

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REFERENCES

1. Davis, G.; Song, J. H. *Ind. Crops Prod.* **2006**, *23*, 147.
2. Croisier, F.; Jerom, C. *Eur. Polym. J.* **2013**, *49*, 780.
3. Tang, C. H.; Xiao, M.-L.; Chen, Z.; Yang, X.-Q. *J. Appl. Polym. Sci.* **2011**, *122*, 789.
4. Cho, S. Y.; Park, J.-W.; Rhee, C. *Lebensm.-Wiss. U.-Technol.* **2002**, *35*, 135.
5. Porta, R.; Mariniello, L.; Di Pierro, P.; Sorrentino, A.; Giosafatto, C. V. L. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 223.
6. Bamdad, F.; Goli, A. H.; Kadivar, M. *Food Res. Int.* **2006**, *39*, 106.
7. Saki, A. A.; Pourhesabi, G.; Yaghobfar, A.; Mosavi, M. A.; Tabatabai, M. M.; Abbasinezhad, M. *J. Biol. Sci.* **2008**, *8*, 663.
8. Larbi, A.; El-Moneim, A. M. A.; Nakkoul, H.; Jammal, B.; Hassan, S. *Anim. Feed Sci. Technol.* **2011**, *165*, 278.
9. Pastor-Cavada, E.; Juan, R.; Pastor, J. E.; Alaiz, M.; Vioque, J. *J. Food Sci.* **2011**, *76*, 1118.
10. Sadeghi, G. H. *Trop. Anim. Health Prod.* **2011**, *43*, 259.
11. Reisi, K.; Zamani, F.; Vatankhah, M.; Rahimiyan, Y. *Global Vet.* **2011**, *7*, 405.
12. Sadeghi, G. H.; Pourreza, J.; Samei, A.; Rahmani, H. *Trop. Anim. Health Prod.* **2009**, *41*, 85.
13. Arabestani, A.; Kadivar, M.; Shahedi, M.; Goli, S. A. H.; Porta, R. *Int. J. Biol. Macromol.* **2013**, *57*, 118.
14. Tang, C. H.; Jiang, Y. *Food Res. Int.* **2007**, *40*, 504.
15. Nuthong, P.; Benjakul, S.; Prodpran, T. *Food Hydrocolloid.* **2009**, *23*, 736.
16. Ou, S.; Wang, Y.; Tang, S.; Huang, C.; Jackson, M. G. *J. Food Eng.* **2005**, *70*, 205.
17. Ou, S.; Kowk, K.-C. *J. Sci. Food Agric.* **2004**, *84*, 1261.
18. Monsoor, M. A.; Yusuf, H. K. M. *Int. J. Food Sci. Technol.* **2002**, *37*, 97.
19. American Association of Cereal Chemists. Approved Methods of AACC; The Association: St. Paul, MN, **2003**.
20. Tang, C. H.; Jiang, Y.; Wen, Q.-B.; Yang, X.-Q. *J. Biotechnol.* **2005**, *120*, 296.
21. Kaya, S.; Kaya, A. *J. Food Eng.* **2000**, *43*, 91.
22. ASTM. Annual Book of ASTM Standards; American Society for Testing and Materials: Pennsylvania, **2003**.
23. Haddad, S. G. *Livest. Sci.* **2006**, *99*, 221.
24. Figueroa-Espinoza, M. C.; Morel, M.-H.; Surget, A.; Asther, M.; Moukha, S.; Sigoillot, J.-C.; Rouau, X. *Food Hydrocolloid.* **1999**, *13*, 65.
25. Mathew, S.; Abraham, T. E. *Food Hydrocolloid.* **2008**, *22*, 826.
26. Fabra, M. J.; Hambleton, A.; Talens, P.; Debeaufort, F.; Chiralt, A. *Food Hydrocolloid.* **2011**, *25*, 1441.
27. Oudgenoeg, G.; Hilhorst, R.; Piersma, S. R.; Boeriu, C. G.; Gruppen, H.; Hessing, M.; Voragen, A. G.; Laane, C. *J. Agric. Food Chem.* **2001**, *49*, 2503.
28. Arcan, I.; Yemenicioglu, A. *Food Res. Int.* **2011**, *44*, 550.
29. Emmambux, M. N.; Stading, M.; Talor, J. R. N. *J. Cereal Sci.* **2004**, *40*, 127.
30. Popović, S.; Periććin, D.; Vaštag, Z.; Lazić, V.; Popović, L. *J. Food Eng.* **2012**, *110*, 374.
31. Salgado, P. R.; López-Caballero, M. E.; Gómez-Guillén, M. C.; Mauri, A. N.; Montero, M. P. *Food Hydrocolloid.* **2013**, *33*, 74.
32. Lin, W.; Liu, H.; Shi, A.; Liu, L.; Adhikari, B.; Wang, Q. *Int. J. Food Sci. Technol.* **2015**, *50*, 1538.
33. Popović, S.; Periććin, D.; Vaštag, Z.; Popović, L.; Lazić, V. *Food Hydrocolloid.* **2011**, *25*, 476.