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Characterization and antioxidant activity of bitter vetch protein-based films containing pomegranate juice

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ABSTRACT

The effect of different amounts of pomegranate (*Punica granatum*, Malas Isfahan cultivar) juice, endowed with high antioxidant activity, on the bitter vetch seed protein films was investigated. We demonstrated that the films prepared in the presence of pomegranate juice exhibited significantly increased moisture content, total soluble matter, elongation at break, as well as water vapour permeability, whereas their tensile strength significantly decreased. These findings clearly suggest that pomegranate juice had a plasticizing effect on the prepared protein-based films, the antioxidant activity of which was also found significantly enhanced. Since scanning electron microscopic images indicated that film structure was undoubtedly affected, we hypothesized that interactions between phenolic compounds contained in the pomegranate juice and bitter vetch proteins are responsible for the modified properties of the obtained blended films. The potential use of pomegranate juice in improving the antioxidant activity of protein-based films is suggested.

1. Introduction

An increasing number of food packaging investigations have been recently focused to develop new biodegradable and/or edible films derived from natural polymers (Siripatrawan & Harte, 2010; Norajit, Kim, & Ryu, 2010; Di Pierro, Sorrentino, Mariniello, Giosafatto, & Porta, 2011), both to meet the needs of consumers and to counteract environmental pollution. In particular, protein-based films, prepared at low relative humidity conditions, were shown to behave as effective lipid, oxygen and aroma barriers (Bamdad, Goli, & Kadivar, 2006). Moreover, protein-based films can be used as vehicles of different additives, like antioxidants and antimicrobials, vitamins, flavours and colorants, thus acting as compound releasing packaging able to improve food quality and preservation (Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011; Salgado, Fernandez, Drago, & Mauri, 2011). Among these, active packaging endowed with antioxidant properties is receiving special attention, since food oxidation is known to represent one of the major problems affecting food quality (Lopez-de-Dicastillo, Gomez-Estaca, Catala, Gavara, & Hernandez-Munoz, 2012). But, although numerous synthetic antioxidants, like butylated-hydroxyanisole (BHA) or -hydroxytoluene (BHT), have been thus far frequently inserted into active food coatings, significant concerns related to toxicological aspects still persist (Siripatrawan & Harte, 2010). Therefore, current trend is to apply natural antioxidants in alternative to the synthetic ones (Salgado et al., 2011; Viuda-Martos et al., 2011). Moreover, several studies support the notion that the consumption of phytochemicals may provide also benefits against a number of diseases, like cancer, coronary heart disease, diabetes, as well as microbial, viral and parasitic infections (Dillard & German, 2000). Among the various natural antioxidants, special attention has been given to polyphenols (Scalbert, Johnson, & Saltmarsh. 2005; Gomez-Guillen and Montero, 2007), organic phytochemicals characterized by the presence of large multiples of phenol structural units, often derived from spices and aromatic plants. In particular, borage seeds and leaves have been employed as polyphenolic source to retard lipid oxidation in food model systems (Wettasinghe & Shahidi, 2000, 2002) as well as antioxidant additives in gelatin films (Gómez-Estaca, Giménez, Montero, Gómez-Guillén; 2009). However, the majority of the obtained crude extracts of polyphenols generally confer undesirable odours and flavours to foods.

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The occurrence of a high antioxidant activity in extracts obtained from pomegranate (*Punica granatum*, L.) peel, juice and seeds has been also reported (Singh, Murthy, & Jayaprakasha, 2002) and attributed to the high levels of phenolic compounds, such as anthocyanins, ellagic acid, punicalagin, punicalin and flavanols (Gonzalez-Molina, Moreno, & Garcia-Viguera, 2009). Pomegranate, one of the oldest edible fruits, is extensively cultivated in tropical and subtropical countries and, currently, Iran is one of the most important producers and exporters in the world (Tehranifar, Zarei, Nemati, Esfandiyari, & Vazifeshenas, 2010; Tehranifar, Selahvarzi, Kharrazi, & Jahan Bakhsh, 2011; Yasoubi, Barzegar, Sahari, & Azizi, 2007; Çama, Hısıl, & Durmaz, 2009). On the other hand, we recently demonstrated that bitter vetch (*Vicia ervilia*, BV) -an annual species of Vicia genus grown for livestock feed in non-tropical dry areas with average annual rainfall 200–350 mm (Larbi, El-Moneim, Nakkoul, Jammal, & Hassan, 2011)- is a potentially useful source for food packaging applications being an unexpensive source for protein based film production (Arabestani, Kadivar, Shahedi, Goli, & Porta, 2013; Arabestani et al., 2016; Porta et al., 2015; Sadeghi, 2011).

Therefore, we decided to test pomegranate juice as a natural antioxidant source to realize an active packaging by using BV seed protein films, investigating also pomegranate juice effects on the morphological, mechanical and water vapour permeability (WVP) properties of the prepared films.

2. Materials and methods

2.1. Materials

BV was obtained from a local market in Isfahan, Iran. Pomegranate (Malas Isfahan cultivar) was supplied by Isfahan Agricultural Research Organization. All chemicals and solvents used in this study were analytical grade commercial products. Sodium hydroxide, hydrochloric acid (37%), glycerol (about 87%), Folin–Ciocalteau reagent, tannic acid, sodium carbonate and methanol were purchased from Merck Chemical Company (Darmstadt, Germany). 2,2-diphenyl-1-picrylhydrazy (DPPH) was provided by Sigma-Aldrich.

2.2. Preparation of BV seed proteins and pomegranate juice

Proteins were extracted from the BV seeds according to Monsoor and Yusuf (2002) by their solubilization (fine powder (mesh 40): alkaline water 1:10 w/v) at pH 11.0 and by stirring the solution with a magnetic stirrer (IKA® RH basic 2, Germany) at medium speed for 1 h at room temperature. After centrifugation at 3200 g for 10 min, the supernatant was collected and the pH adjusted to 5.4 by 0.1 N HCl to form a precipitate which was separated by centrifugation at 3200 g for 10 min. The pellet was finally dissolved at pH 7, the deriving solution dried at 40 °C in a vacuum oven, and the dry protein concentrate ground in a coffee grinder (Kenwood, CG 100, China). The protein content of both BV seeds and protein concentrate (BVPC) was determined by the Kjeldahl's method (AACC, 2003).

Healthy pomegranate fruits of uniform size and appearance were washed and dried. Pomegranate juice was obtained by a manual extractor and analyzed for moisture content, dry matter, pH, total phenolics and its antioxidant activity.

2.3. Determination of pomegranate juice moisture content, total soluble solids and pH

Juice samples were weighed (±0.0001 g) into aluminum dishes and dried in an oven at 105 °C to constant weight. The moisture content value was quantified as the percentage of initial juice weight lost during drying. The total soluble solids (TSS) were determined with a digital handheld refractometer (kruss, DR201-95, Germany) calibrated by distilled water. The pH measurement was performed by a digital pH meter (Jenway, 3330) at room temperature.

2.4. Determination of pomegranate juice total phenolics and antioxidant activity

Total phenolics were determined according to Singleton and Rossi (1965) by using Folin-Ciocalteau method. Briefly, 300 μL of diluted pomegranate juice (1:100 with methanol:water, 6:4) were mixed with 1.5 mL of 10-fold diluted Folin-Ciocalteau reagent and 1.2 mL of 7.5% sodium carbonate. The mixture was shaken vigorously and left to stand for 1.5 h at room temperature. Then, the absorbance was measured by a spectrophotometer (Unico, UV-2100, USA) at 760 nm. Tannic acid was used as a standard and the results were expressed as mg tannic acid/1 L of juice.

Antioxidant activity was assessed by using Brand-Williams, Cuvelier, and Berset (1995) method. 100 μL of pomegranate juice, diluted (1:100) with methanol:water (6:4), was mixed with 2 mL of 0.1 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. The mixture was vigorously shaken, then allowed to stand for 30 min at room temperature and the absorbance was finally measured at 517 nm by a spectrophotometer (Unico, UV-2100, USA). Also the absorbance value of the methanolic solution of DPPH was measured at 517 nm. The reaction mixture without DPPH was used for the background correction.

$$\text{DPPH scavenging activity } (\%) = (\text{DPPH absorbance} - \text{extract absorbance}/\text{DPPH absorbance}) \times 100.$$

2.5. BVPC film preparation

BVPC powder (5 g) was dispersed under constant stirring in approximately 50 mL of distilled water and then glycerol was added (50%, w/w). The total weight of the solution was led to approximately 90 g by distilled water addition. The pH value was adjusted to 10 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 Teflon–coated glass plate (30 × 30 cm) for 24 h at 35 °C.

For active packaging, the obtained pomegranate juice was added at different concentrations (2, 5, 10, 15 and 20%, w/w) to the film forming solutions and the pH adjusted to pH 10 by 1 N NaOH. The solutions were stirred for 5 min and degassed again before casting. At the end all the films were manually peeled off and characterized, except the ones containing the highest pomegranate juice concentrations (15 and 20%), resulting highly sticky and hard to peel off