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Properties and antioxidant activity of soy protein concentrate films incorporated with red grape extract processed by casting and compression molding

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ABSTRACT

Self-standing transparent soy protein concentrate (SPC) films plasticized by glycerol (30% w/w SPC dry basis) and supplemented with red grape extract (RGE) as natural antioxidant (0–10% w/w SPC dry basis) were prepared by two methods: casting and intensive mixing followed by compression molding. The influence of RGE on key film properties was analyzed at the light of the specific stabilizing interactions of SPC films. The addition of RGE had a favorable effect on moisture content, total soluble matter, water vapor permeability and percentage of elongation of casted films compare with compression molded counterparts. RGE induced a redistribution of hydrogen interactions of casted films replacing protein-protein hydrogen interactions by protein-polyphenol ones with no variations in disulfide bridges, while these last interactions were significantly reduced in compression molded films in favor of hydrophobic and hydrogen interactions, as disclosed by differential solubility assays and infrared spectroscopy. The antioxidant activity of the SPC films in terms of scavenging activity of the stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power, increased significantly irrespective of the manufacturing method, being the release of antioxidants from casted films lower than that from compression molded films in accordance with the strong interactions between SPC matrix and polyphenols.

1. Introduction

The main function of food packaging is to protect foodstuff by delaying or restraining the adverse effect of the environment on food, in order to extend its shelf-life (Marcos et al., 2014; Rooney, 2001; Woranuch, Yoksan, & Akashi, 2015). In this sense, active packaging is a concept that arose in response to consumer demands and market trends and is gaining increasing attention from researchers and food technology industry (Arrieta et al., 2014; Martucci, Gende, Neira, & Ruseckaite, 2015). These systems are based on the deliberate interaction of the packaging with the packed food or the environment and can provide certain functions lacking in conventional packaging systems, affording quality and hygiene benefits and shelf-life extension (Dainelli, Gontard, Spyropoulos, Zondervan-van den Beuken, & Tobback, 2008; Rooney, 2001). Active functions may include the removal of oxygen, moisture, antimicrobial activity, antioxidant activity, UV protection, etc. (Giménez, López de Lacey, Pérez-Santín, López-Caballero, & Montero, 2013; Robertson, 2009; Rooney, 2001).

The incorporation of antioxidants in the packaging material, that migrates from the packaging to the food, provides advantages compared to direct addition to food, since it allows one to extend the shelf-life of the product, while reducing the amount of chemical additives in direct contact with food (Li, Miao, Wu, Chen, & Zhang, 2014; Liang & Ludescher, 2011; Marcos et al., 2014). Currently, due to safety concerns associated with the use of synthetic active antioxidants in food packaging, many research works have focused on natural active substances (Giménez et al., 2013; Li et al., 2014; Marcos et al., 2014; Moreno, Atarés, & Chiralt, 2015), such as organic acids, sorbic (Ozdemir & Floros, 2001), ascorbic (Gemili, Yemenicioğlu, & Altınkaya, 2010; Le Tien, Vachon, Mateescu, & Lacroix, 2001), citric (Le Tien et al., 2001), tannic (Pyla, Kim, Silva, & Jung, 2010); α-tocopherol (Han & Krochta, 2007) and vegetal extracts and essential oils (Arancibia, López-Caballero, Gómez-

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Guillén, & Montero, 2014; Atarés, Bonilla, & Chiralt, 2010; Giménez et al., 2013; Gómez-Estaca, Bravo, Gómez-Guillén, Alemán, & Montero, 2009; Martucci et al., 2015; Moradi et al., 2012; Nie, Gong, Wang, & Meng, 2015; Pruneda et al., 2008; Tongnuanchan, Benjakul, & Prodpran, 2012). Among vegetal extracts, grape seed extracts are excellent alternatives to synthetic antioxidants, since they are recognized as GRAS (Generally Recognized as Safe) by the Food and Drug Administration US (FDA, US Food and Drug Administration) and are natural and renewable (Perumalla & Hettiarachchy, 2011). Grape extracts contains several polyphenolic compounds with antioxidant activity, including monomeric flavon-3-ols such as catechin, epicatechin and procyanidin dimmers and trimers (Chedea, Braicu, & Socaciu, 2010; Perumalla & Hettiarachchy, 2011).

One of the main challenges in the field of active packaging is the incorporation of natural active components into biogenic matrices to obtain completely bio-based active films. As a part of our continuous work on the development and characterization of soybean protein concentrate (SPC) films intended for food packaging (Ciannamea, Stefani, & Ruseckaite, 2014, 2015), our ongoing work was to evaluate the effect of the incorporation of red grape extract (RGE) as natural active agent. Besides the study on the potential activity induced by RGE, the work was focused on the possible alteration of the properties of SPC films due to the well reported interactions between proteins and polyphenols, which are the main components of RGE (Giménez et al., 2013; Sivarooban, Hettiarachchy, & Johnson, 2008; Tongnuanchan et al., 2012). Reportedly, the incorporation of phenolic compounds can alter protein film's properties, such increase the flexibility and lower water vapor permeability (Nie et al., 2015). Some phenolic compounds can react and establish covalent bonds with proteins (Hager, Vallons, & Arendt, 2012; Kroll, Rawel, & Rohn, 2003; Salgado, López-Caballero, Gómez-Guillén, Mauri, & Montero, 2012; Sivarooban et al., 2008; Tongnuanchan et al., 2012; Zhang et al., 2010). Furthermore, protein-polyphenol complexes may occur through various non-covalent interactions, such as hydrophobic interactions or hydrogen bonds (Salgado et al., 2012; Tongnuanchan et al., 2012; Zhang et al., 2010).

The influence of the addition of RGE (5 and 10% w/w SPC) on the formation of SPC based films and their physical, chemical, mechanical and barrier properties was studied. The effect of incorporating the RGE in films processed by the casting method and intensive mixing followed by compression molding was analyzed. Antioxidant capacity of the films through the ability to reduce iron (III) and radical scavenging using the stable radical DPPH was studied.

2. Materials and methods

2.1. Materials

Soy protein concentrate (SPC) Solcom S110, with 69% of protein, 7% of moisture, 1% fat, 3% fiber, 5% ash and approximately 15% of non-starch polysaccharides, was provided by Cordis S.A. (Villa Luzuriaga, Buenos Aires, Argentina). Glycerol (Gly, 99% Anedra, Argentina) was used as a plasticizer without any prior purification. Food grade red grape extract (RGE) was obtained from a local pharmacy (Mar del Plata, Argentina) and was used as - received as antioxidant film additive. Phosphate buffer solution pH 10 (Anedra, Buenos Aires, Argentina) was used in film manufacturing processes. Calcium chloride ($CaCl_2$; Aldrich, St. Louis, USA) was used as desiccant for water vapor permeability tests. TRIZMA/hydrochloric acid, glycine and Na_2EDTA (Biopack, Buenos Aires, Argentina), sodium dodecyl sulfate (SDS) and urea (Anedra, Buenos Aires, Argentina), 2-mercaptoethanol (2-ME, Aldrich, St. Louis, USA) and trichloroacetic acid (TCA, Biopack, Buenos Aires, Argentina) were used for differential solubility test. Sodium azide (Na_3N, Anedra, Buenos Aires, Argentina) solution was used to prevent microbial growth during total soluble matter assays. Folin-Ciocalteu reagent (Sigma-Aldrich) was used to determine the total phenolic content of the RGE. Potassium hexacyanoferrate (III) ($K_3Fe(CN)_6$), Iron (III) chloride ($FeCl_3$) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were used for antioxidant activity assays.

2.2. Film processing

Films were obtained by two different processing methods, solution casting and intensive mixing followed by compression molding, according to the procedures reported in a previous work, with some modifications (Ciannamea et al., 2014). For thermoplastic processing SPC was manually mixed with Gly (30 and 40% w/w on dry SPC basis) and 50% of pH 10 buffer solution (pH > isoionic point, pI ~ 4.5) for 15 min. RGE was incorporated (0–10% w/w on dry SPC basis) to the premixes in a lab-scale roller mixer at 70 °C, 50 RPM for 30 min. Afterwards RGE-added mixtures were thermo-compressed in a three – step operation (150 °C for 5 min at 10 kg/cm^2, 150 °C for 2 min at 100 kg/cm^2 and finally cooling up to 30 °C at 100 kg/cm^2 for about 30 min) by using an hydraulic hot-press (EMS, Argentina). Solution casting films were prepared by mixing SPC with Gly (30–40% w/w on dry SPC basis) and RGE (0–10% w/w SPC) for 15 min. The premixes were then dispersed in pH 10 phosphate buffer solution at 70 °C under constant magnetic stirring for 30 min (Cole-Parmer, USA) to obtain 5% film-forming solutions (5 g SPC/100 cm^3 solution). After degassing under ultrasonic bath (Testlab, 160 W, 40 KHz) for 15 min, the film-forming solutions were poured onto leveled Teflon-coated Petri dishes (15 cm^2 area), left to dry in an air-circulating oven (Memmert, Germany) until reaching constant moisture content (about 24 h) and peeled-off from the plates. To control film thickness, the solid content casted on each plate was fixed.

Film samples were cut into the desired shapes and preconditioned in laboratory environmental chamber at 25 ± 2 °C and 65 ± 2% RH for 48 h prior testing. Casting and compression molding films were labeled as CSPC-XGly-YRGE and MSPC-XGly-YRGE, respectively, were X refers to glycerol and Y to RGE percentages, respectively (% w/w SPC).

2.3. Thickness

Film thickness was measured with a manual micrometer (0–25 ± 0.01 mm, Bta. China). Measurements were done at ten random points along the films. For tensile tests, opacity and moisture absorption experiments, four measurements were done on each specimen.

2.4. Attenuated total reflectance – Fourier transformed infrared (ATR-FTIR)

ATR-FTIR spectra were recorded on a Thermo Scientific Nicolet 6700 spectrometer (Wisconsin, USA). All runs were performed between 400 and 4000 cm^{-1} using an attenuated total reflectance accessory (ATR) with a diamond ATR crystal and 32 scans with resolution of 4 cm^{-1} resolution. Each assay was carried out by triplicate in random points along the preconditioned films.

2.5. Differential solubility tests

Samples were immersed in specific buffer solutions according to the procedure described in the literature (Ciannamea, Stefani, & Ruseckaite, 2015; Hager, 1984). Each solution is known to disrupt