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Development of active fish gelatin films with anthocyanins by compression molding

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ABSTRACT

The efficient use of biomass is one of the overarching objectives within sustainable development. In this context, fish gelatin and anthocyanins derived from food processing waste were used to prepare bio-based films. Anthocyanins were extracted by a simple and sustainable process and characterized before analyzing their antioxidant activity as a function of pH, in order to select the film processing conditions. In this regard, it is worth mentioning that a more sustainable process than the conventional solution casting was used in the preparation of films, since compression molding is a more rapid and less energy-consuming process. Furthermore, anthocyanins incorporated into film forming formulations maintained their antioxidant activity, as shown by the radical scavenging capacity. Additionally, the incorporation of anthocyanins decreased the water sensitivity of films, maintaining mechanical properties and, thus, highlighting the potential of these films as active packaging.

1. Introduction

Biowaste is one of the main concerns in most of the industrialized countries. In particular, food waste is not only an economic issue, but also an ethical and environmental one since it implies the waste of natural resources. Therefore, many factors must be considered through the whole food chain in order to prevent and reduce food waste, from producers to retailers and consumers (Guerrero, Arana, O'Grady, Kerry, & de la Caba, 2015; Mirabella, Castellani, & Sala, 2014). In this regard, active and intelligent packaging can play a relevant role to reduce food waste. Furthermore, considering that packaging represents a major source of solid waste, bio-based and biodegradable materials should be taken into account in order to develop ecopackaging (Cao, Liu, & Wang, 2018; Han, Yu, & Wang, 2018; Wang & Wang, 2017).

Food deterioration is an important issue that must be avoided to assure food quality and safety. Some of the most important mechanisms leading to food spoilage are the oxidation of fats and the growth of microorganisms (Muíño et al., 2017; Prasad & Kochhar, 2014). When lipid oxidation occurs, food appearance, texture, and taste change, and both food nutritional quality and shelf-life are reduced (Talón et al., 2017). In this regard, different kinds of antioxidants derived from plant extracts (Bolumar, LaPeña, Skibsted, & Orlien, 2016; Chin, Lyn, & Hanani, 2017; Wrona, Nerín, Alfonso, & Caballero, 2017) or by-products (Ferrentino, Morozova, Mosibo, Ramezani, & Scampicchio, 2018; Puga, Alves, Costa, Vinha, & Oliveira, 2017) can be used to develop active packaging in order to prevent or retard food deterioration.

Concerning the manufacturing of antioxidant packaging, in recent years, different production techniques have been employed (Tatara, 2017); however, research on industrial scale production methods, such as extrusion or compression, which are faster and more efficient than traditional casting method, is still needed. In this regard, compression molding was successfully used in this work with the aim of reducing the production times of gelatin films. Moreover, since antioxidant activity could be affected by the processing conditions used (Lin & Zhou, 2018; Wong & Siow, 2015), manufacture parameters were carefully selected.

A wide series of antioxidant compounds are used in food packaging materials (Yildirim et al., 2018). Even if most of the employed antioxidants are synthetic molecules, natural compounds can be also used, among them, anthocyanins, blue, red or purple pigments found in plants, especially in flowers, fruits and tubers (Gómez-Estaca, López-de-Dicastillo, Hernández-Muñoz, Catalá, & Gavara, 2014; Khoo, Azlan, Tang, & Lim, 2017; Stoll et al., 2017). As an example, anthocyanins contained in red cabbage, grapes, strawberries, raspberries, pomegranates, mangoes, figs and sweet potato can be mentioned (Chandrasekhar, Madhusudhan, & Raghavarao, 2012). These antioxidants are a sub-group within the flavonoids and they naturally occur as glycosides of flavylium (2-phenylbenzopyrylium) salts (Azeredo et al., 2016). Besides their antioxidant activity, phenolic antioxidants possess therapeutic benefits, such as, anticancer, anti-inflammatory and cardioprotective effects, which make them value-added bioactives (Kim, Joo, & Yoo, 2009; Yates, Ramos Gomez, Martin-Luengo, Zurdo Ibañez, & Serrano, 2017). Nevertheless, anthocyanins have high

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sensitivity to degradation reactions, which affect their stability and color (Qiu, Wang, Song, Deng, & Zhao, 2018). This color change must be considered when anthocyanins are used as pigments (Cortez, Luna-Vital, Margulis, & Mejia, 2017).

The annual world production of cabbages is approximately 68 million tones of fresh heads from 3.1 million ha, in more than 130 countries (Demirbas, 2016), and a huge amount of residues are obtained from this large-scale production. Hence, owing to its high antioxidant content and its abundance, red cabbage extract has a good potential for active packaging manufacturing (Zou et al., 2016). The aims of this work were to extract and analyze anthocyanins from red cabbage as well as to use them as antioxidant in gelatin film forming formulations. Furthermore, it is worth highlighting that films were processed by compression molding in order to scale production. Citric acid was incorporated into film forming formulations as an antibacterial agent, effect analyzed in a previous work (Uranga et al., 2018). Overall, the red cabbage extract identity and pH sensitivity were discussed, and films structural, optical, mechanical and barrier properties were studied. Furthermore, the antioxidant activity of anthocyanin powders and anthocyanin-containing films was assessed by DPPH radical scavenging, and results were compared with those obtained for α-tocopherol, a widely employed natural antioxidant.

2. Materials and methods

2.1. Materials

Fish gelatin with a 240 bloom value (Healan Ingredients, UK) was used as the main component of film forming formulations. Anhydrous citric acid (Panreac, Spain) and anthocyanins extracted from red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*) from a local market were used as active compounds. Glycerol with a purity of 99.01% (Panreac, Spain) was used as plasticizer. Water, acetonitrile and formic acid for ultrahigh performance liquid chromatography (UHPLC) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Enkephalin hydrated leucine acetate (95% purity) and 0.1 M sodium hydroxide solution were purchased from Sigma-Aldrich Chemie (Steinheim, Germany), and trifluoroacetic acid from Merck (Darmstadt, Germany). Finally, 2,2-diphenyl-1-picryl hydrazyl (DPPH) and α-tocopherol were purchased from Sigma-Aldrich (Saint-Louis, USA), ethanol from Scharlab (Barcelona, Spain), and pure methanol from VWR international (Fontenay-sous-Bois, France).

2.2. Anthocyanins extraction

Anthocyanins were extracted by a conventional solid-liquid extraction, Soxhlet extraction, employing water as solvent. Red cabbage was cut, frozen and lyophilized to obtain the powder and, thus, the filters could be filled more easily and a more efficient process could be carried out. The extraction was repeated twice and the obtained liquid, containing mostly water and anthocyanins, was frozen and then, lyophilized. The extracted anthocyanin powder was kept packaged in a desiccator protected from light.

2.3. Anthocyanins characterization

2.3.1. UHPLC-Q-TOF-MS/MS analysis

Immediately after the dilution of samples in 0.1% trifluoroacetic acid solutions in water, ultrahigh performance liquid chromatography (UHPLC) was carried out by using an ACQUITY UPLC™ system from Waters (Milford, MA, USA), equipped with a binary solvent delivery pump, an autosampler, a column compartment and a PDA detector. A reverse phase column (Acquity UPLC BEH C18 1.7 μm, 2.1 mm × 100 mm) and a precolumn (Acquity UPLC BEH C18 1.7 μm VanGuard™) from Waters (Milford, USA) were used at 40 °C for the separation of individual anthocyanins. The flow rate was 0.25 mL/min and the injection volume was 7.5 μL. Mobile phases consisted of 0.1% trifluoroacetic acid in water (A) and 0.1% trifluoroacetic acid in acetonitrile (B). Separation was carried out in 17 min under the following conditions: 0.00–3.17 min, linear gradient from 5 to 15% B; 3.17–5.43 min, 15% B; 5.43–6.00 min, linear gradient from 15 to 20% B; 6.00–12.00 min, linear gradient from 20 to 21% B; 12.00–13.00 min, linear gradient from 21 to 100% B; and finally, washing and re-equilibration of the column prior to the next injection. All samples were kept at 4 °C during the analysis. The wavelength range of the PDA detector was 210–500 nm (20 Hz, 1.2 nm resolution). Anthocyanins were monitored at 500 nm.

All mass spectrometry (MS) data acquisitions were performed on a SYNAPT™ G2 HDMS with a quadrupole time of flight (Q-TOF) configuration (Waters, Milford, MA, USA), equipped with an electro-spray ionization (ESI) source operating in positive mode. The capillary voltage was set to 1.0 kV. Nitrogen was used as the desolvation and cone gas at flow rates of 1000 L/h and 10 L/h, respectively. The source temperature was 120 °C, and the desolvation temperature was 400 °C. A leucine-enkephalin solution (2 ng/μL) in acetonitrile:water (50:50 (v/v) + 0.1% formic acid) was utilized for the lock mass correction and the ions at mass-to-charge ratio (m/z) 556.2771 and 278.1141 in the positive ionization mode from this solution were monitorized (0.3 s scan time, 10 s interval, 3 average scans, ± 0.5 Da mass window, 30 V cone voltage, 10 μL/min flow rate). Data acquisition took place over the 50–2000 u mass range in resolution mode (FWHM ≈ 20,000) with a scan time of 0.1 s and an interscan delay of 0.024 s. All the acquired spectra were automatically corrected during acquisition based on the lock mass. Before analysis, the mass spectrometer was calibrated with a sodium iodide solution.

To perform MS^E mode analysis, the cone voltage was set to 20 V (ESI +) and the first quadrupole (Q1) operated in a wide band RF mode only. Two discrete and independent interleaved acquisition functions were automatically created. The first function, typically set at 6 eV in trap cell of the T-Wave, collected low energy or unfragmented data, while the second function collected high energy or fragmented data, using 6 eV in trap cell and a collision ramp of 10–40 eV in transfer cell. In both cases, argon gas was used for collision-induced dissociation (CID) and data were recorded in centroid mode.

MS^2 product ion spectra was performed using the protonated molecule $[M]^+$ as precursor ion at a cone voltage of 20 V. A collision energy ramp from 10 to 40 eV in trap cell and of 6 eV in transfer cell was used with the aim of acquiring spectra with different fragmentation degrees from the precursor ion and, thus, obtaining as much structural information as possible. MS/MS data were collected at a range of 50–2000 m/z in centroid mode in the same conditions as described above.

The identification of the anthocyanin compounds was carried out using the UV–Vis spectrum to assign the phenolic class (Abad-García, Berrueta, Garmón-Lobato, Gallo, & Vicente, 2009), the low collision energy MS^E spectrum in positive mode to determine the molecular weight, the high collision energy MS^E and MS^2 product ion spectra to assign the protonated aglycone $[Y_0]^+$ and observed fragmentations in order to elucidate other structural details. The nomenclature proposed by Domon and Costello (1988) for glycoconjugates was adopted to denote the fragment ions.

2.3.2. DPPH radical scavenging activity

Anthocyanin aqueous solutions (2 mM) were prepared at three different pHs: acid (2.5), basic (10.0) and non-modified (6.0) pHs to analyze the effect of pH on the anthocyanin activity. DPPH radical scavenging activity was measured for each solution according to the method of Etxabide, Coma, Guerrero, Gardrat, and de la Caba (2017). Briefly, 2 mL of anthocyanin solution were mixed with 2 mL of DPPH solution (75 μM). The mixture was vigorously shaken and then, allowed to stand at room temperature in the dark for 30 min. The inhibition values (I) were determined by the absorbance decrease at 517 nm as