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Development, properties, and stability of antioxidant shrimp muscle protein films incorporating carotenoid-containing extracts from food by-products

Joaquín Gómez-Estaca*, Marta M. Calvo, Alicia Sánchez-Faure, Pilar Montero, M. Carmen Gómez-Guillén

Instituto de Ciencia y Tecnología de Alimentos y Nutrición (ICTAN-CSIC), José Antonio Novais 10, 28040 Madrid, Spain

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ABSTRACT

In the present work various waste products from the food industry are intended to be valorized by means of the development of antioxidant edible films. Shrimp muscle proteins, which were extracted from spoiled shrimp due to long freezing storage, were used as a biopolymeric matrix in which lipid extracts obtained from shrimp cephalothorax or tomato peel waste, rich in astaxanthin and lycopene respectively, were incorporated as active ingredients. For comparative purposes a film containing commercial β-carotene was also formulated. The films' properties were determined initially and during storage. Films were transparent and easy to handle, showing high solubility in water (≈90%), and a high water vapor permeability, however, after 30 days of storage (22 °C/50% RH), they became more opaque and less water soluble (≈30%), indicating film matrix reorganizations. UV/Vis spectra, carotenoid content, and color of the films pointed to the occurrence of carotenoid degradation (32, 32, and 17% degradation after one month of storage for β-carotene, lycopene, and astaxanthin, respectively), as well as a hint of interaction of lycopene with the protein network. Antioxidant activity was very stable during storage, in spite of the carotenoid degradation, suggesting that other compounds present in the extracts contributed to the antioxidant activity.

1. Introduction

Nowadays, considerable efforts are being expended on the valorization of food industry waste or by-products, both because of environmental problems associated with their elimination and because of the high potential for the development of new products, packaging materials, and bioactive compounds (López-Caballero, Giménez, Gómez-Guillén, & Montero, 2013). The crustacean processing industry produces a high amount of waste annually, mainly cephalothorax and cuticles from peeling, muscle spoiled as a result of extensive melanosis or long frozen storage, and cooking juices (Gómez-Estaca, Montero, & Gómez-Guillén, 2014; Pérez-Santín, Calvo, López-Caballero, Montero, & Gómez-Guillén, 2013; Sanches Silva, Costa, Losada, Sendon, & Sanchez-Machado, 2010). Extraction of biopolymers to develop edible films or coatings for food packaging applications is a possible way of giving value to this waste. Among biopolymers extracted from crustacean waste, chitosan is the most important one for the production of edible films because of its high availability, stability, biocompatibility, antimicrobial properties, etc., and hence it is the one that has been most studied (Harish Prashanth & Tharanathan, 2007; Shahidi, Arachchi, & Jeon, 1999); even so, it is not the only one, as we recently indicated the possibility of developing edible films from shrimp muscle proteins (Gómez-Estaca et al., 2014). Apart from chitin/chitosan, cephalothorax and cuticles from shrimp waste contain other bioactive compounds of interest, such as astaxanthin and/or carotene-proteins (Arancibia et al., 2014; Babu, Chakrabarti, & Surya Sambasivarao, 2008). Other food industries, such as the tomato industry, also generate considerable amounts of waste rich in carotenoids. It is estimated that peel and seeds represent 20–50 g/kg of the initial weight of tomatoes (Knoblich, Anderson, & Latshaw, 2005), from which fiber and carotenoids, mainly lycopene, have been extracted (Ranveer, Patil, & Sahoo, 2013; Sarkar & Kaul, 2014).

* Corresponding author. Tel.: +34 915492300; fax: +34 915493627.
E-mail address: joaquin.gomez@csic.es (J. Gómez-Estaca).

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The consumption of carotenoids has been associated with the prevention of some diseases such as cancer, cardiovascular diseases, macular degeneration, etc., mainly ascribable to their antioxidant and anti-inflammatory properties (Britton, Liaaen-Jensen, & Pfander, 2009; Jie, Sun, Sun, Chen, & Chen, 2014; Rao & Rao, 2007). Apart from their possibilities in the design of functional foods, carotenoids or extracts containing them have interest as food additives too, thanks to their antioxidant and coloring capacities; however, dosage and manipulation are not easy. The incorporation of bioactive substances into food through the formulation of edible films containing active substances has gained much interest in the last decade (Silva-Weiss, Ihl, Sobral, Gómez-Guillén, & Bifani, 2013). Thanks to the above-mentioned biological activities of carotenoids, both astaxanthin and lycopene from shrimp and tomato waste are very good candidates to be included in the formulation of edible films. The inclusion of co-products rich in astaxanthin obtained from shrimp cephalothorax and cuticle waste in edible films made of chitosan has been shown to enhance the intrinsic antimicrobial properties of chitosan films, also conferring antioxidant properties, thus showing promising results for the development of edible coatings and films for food packaging applications (Arancibia, Alemán, López-Caballero, Gómez-Guillén, & Montero, 2015). López-Rubio and Lagaron (2011) incorporated β-carotene into edible films based on different biopolymers through stabilization with glycerol, improving the stability of the carotenoid when exposed to UV light. Other authors have incorporated carotenoids in the formulation of packaging materials from conventional polymers (polyethylene) or polylactic acid, showing good results for food packaging applications (Colín-Chávez, Soto-Valdez, & Peralta, 2014; Colín-Chávez, Vicente-Ramírez, Soto-Valdez, Peralta, & Auras, 2014; Samsudin, Soto-Valdez, & Auras, 2014).

The objective of the present work is to develop and characterize shrimp muscle protein antioxidant edible films incorporating carotenoids from various sources, namely, a lipid extract rich in astaxanthin obtained from shrimp waste, a tomato extract rich in lycopene from tomato peel waste, and commercial β-carotene, and also to study the stability of the films during storage.

2. Materials and methods

2.1. Extraction of protein

Ten kilograms of frozen shrimp (*Litopenaeus vannamei*), kindly provided by Angulas Aguinaga Burgos (Burgos, Spain) were thawed at room temperature and peeled manually. An aliquot of 1 kg of muscle was homogenized with 5 L of cold distilled water using a mixer (Braun, Barcelona, Spain), and the pH was adjusted to 3 with 5 M HCl. The mixture was then left in an ice bath under constant stirring for 60 min to ensure complete protein solubilization. The mixture was centrifuged at 15,800 × g/4 °C/30 min (Sorvall Evolution RC Centrifuge, Thermo Fisher Scientific Inc., Landsmeer, The Netherlands), and the supernatant was collected. The shrimp protein isolate (SPI) was obtained by isoelectric precipitation after adjusting the supernatant pH to 5.0 and centrifuging at 15,800 × g/4 °C/10 min. Then the SPI was vacuum-packed and stored at −80 °C until use.

2.2. Extraction of carotenoids

Cephalothorax, cuticles, pleopods, and tails from shrimp (*L. vannamei*) were homogenized to a particle size of ≈5 mm. Aliquots of the homogenate (10 g) were mixed with ethyl acetate (50 mL) and stirred at room temperature in darkness for 30 min; after extraction the sample was filtered through Whatman No. 1 filter paper, and the filtrate was evaporated under vacuum to dryness. The lipid extract obtained was stored at −30 °C in darkness.

Tomatoes bought in a local market were dipped in boiling water for 2 min and then peeled, and the peels were freeze-dried. Afterwards, freeze-dried tomato peels were ground with a mill (0.05–0.02 mm particle size) and stored in bottles protected from light at −20 °C until extraction. One gram of dried peel was mixed with ethyl acetate (50 mL) and stirred at room temperature in darkness for 15 min. After extraction the sample was filtered through Whatman No. 1 filter paper; extraction was performed twice; the solvent was evaporated under vacuum to dryness and the lipid extract obtained was stored at −30 °C in darkness.

Commercial β-carotene was purchased from Sigma–Aldrich (Madrid, Spain).

2.3. Quantification of carotenoids

The lipid extracts from shrimp waste and tomatoes and the commercial β-carotene were diluted with hexane, petroleum ether, and ethanol, respectively, to a known volume, and the absorbance of the appropriately diluted extract was measured at 470 (astaxanthin and lycopene) and 450 (β-carotene) nm using a Beckman Coulter DU 800 spectrophotometer (Beckman Coulter, Indianapolis, USA). The carotenoid concentration was calculated using the equation described by Britton (1995),

$$Carotenoid\ (mg) = \frac{A*V*P}{\varepsilon} \quad (1)$$

where A is absorbance, V is the dilution volume (mL), P is the molecular weight of the carotenoid, and ε is the molar absorption coefficient reported by Britton (1995) for astaxanthin, lycopene, and β-carotene dissolved in the solvents indicated (125,100, 184,900, and 140,400, respectively).

2.4. Antioxidant activity of carotenoid extracts

A photochemiluminescence assay was conducted in order to evaluate the antioxidant activity of the carotenoid extracts. This assay involves the photochemical generation of superoxide ($O_2^{-\cdot}$) free radical combined with chemiluminescence detection of luminol, which acts as a photosensitizer and also as an oxygen radical detection reagent. This reaction takes place in the PHOTOCHEM® (Analytik Jena AG). The ACL and ACW kits provided by the manufacturer were used to measure lipophilic and hydrophilic antioxidant capacity, using Trolox or ascorbic acid as the calibration reagents, respectively. The results were expressed as μg Trolox or ascorbic acid equivalents/mg carotenoids in the extracts. All samples were measured in duplicate.

2.5. Film formation and experimental design

The film-forming solutions (FFSs) were prepared at a protein concentration of 2 g/100 mL. Previously, the protein content of the SPI was determined with a LECO FP-2000 nitrogen/protein analyzer (LECO Corp., St. Joseph, MI, USA). The SPI was homogenized with distilled water for 1 min using a T25 Ultra-Turrax blender (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 14,000 rpm and then the pH of the FFS was adjusted to 2. Glycerol (25 g/100 g protein) was added as a plasticizer and Tween 80 (2.3 g/100 g protein) as emulsifier. A small volume (up to 5% of the final FFS volume) of the carotenoid extracts dissolved in organic solvent was slowly added to the film-forming solutions during homogenization with a T25 Ultra-Turrax