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Characterization and storage stability of astaxanthin esters, fatty acid profile and α-tocopherol of lipid extract from shrimp (*L. vannamei*) waste with potential applications as food ingredient

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ABSTRACT

In this work a lipid extract from shrimp waste was obtained and characterized. The most abundant fatty acids found were C16:0, C18:2n6c, C18:1n9c, C22:6n3, and C20:5n3. The extract contained all-*trans*-astaxanthin, two *cis*-astaxanthin isomers, 5 astaxanthin monoesters, and 10 astaxanthin diesters (7 ± 1 mg astaxanthin/g). C22:6n3 and C20:5n3 were the most frequent fatty acids in the esterified forms. Appreciable amounts of α-tocopherol and cholesterol were also found (126 ± 11 mg/g and 65 ± 1 mg/g, respectively). Little lipid oxidation was observed after 120 days of storage at room temperature, revealed by a slight reduction of ω-3 fatty acids, but neither accumulation of TBARS nor formation of oxidized cholesterol forms was found. This is attributed to the antioxidant effect of astaxanthin and α-tocopherol, as their concentrations decreased as storage continued. The lipid extract obtained has interesting applications as food ingredient, owing to the coloring capacity and the presence of healthy components.

1. Introduction

Large amounts of cephalothorax and cuticle waste, which may account for up to 50–60% of animal weight, are produced annually by the crustacean processing industry (Sachindra, Bhaskar, & Mahendrakar, 2005; Treyvaud Amiguet et al., 2012). This waste has the potential to be valorized to obtain food ingredients and additives such as chitin/chitosan, proteins, lipids, or carotenoids (Arancibia et al., 2014; Takeungwongtrakul, Benjakul, & H-Kittikun, 2012; Takeungwongtrakul, Benjakul, Santoso, Trilaksani, & Nurilmala, 2015; Treyvaud Amiguet et al., 2012). Recent works of our research group have shown that the lipid extract obtained from shrimp cephalothoraxes and cuticles waste is a promising food ingredient with multiple technological applications (Gomez-Estaca, Comunian, Montero, Ferro-Furtado, & Favaro-Trindade, 2016; Gómez-Estaca, Calvo, Sánchez-Faure, Montero, & Gómez-Guillén, 2015; Montero, Calvo, Gómez-Guillén, & Gómez-Estaca, 2016). This is mainly because of its coloring capacity, provided by astaxanthin, which is improved when encapsulated by spray-drying or complex coacervation or incorporated into edible films. Moreover, astaxanthin is not the main component of the extract, as it is reported to range between 2 and 5 mg/g lipid extract (Gómez-Estaca, Montero, Fernández-Martín, Calvo, & Gómez-Guillén, 2016; Takeungwongtrakul et al., 2015). Lipid extracts of similar nature have been reported to be rich in polyunsaturated fatty acids (Chen, Zhang, & Shrestha, 2007; Takeungwongtrakul, Benjakul, & H-Kittikun, 2012), and also to contain tocopherols (Özogul, Özogul, & Kuley, 2011) and cholesterol (Hernández-Becerra, Ochoa-Flores, Soto-Rodriguez, Rodriguez-Estrada, & García, 2014). Astaxanthin, polyunsaturated fatty acids (specially eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)) and tocopherol may have health-promoting effects when consumed, mainly related to their antioxidant and/or anti-inflammatory activities. Inflammatory and oxidative mediated disorders including cancer, allergy, diabetes, neurodegenerative diseases and coronary heart diseases are the main disorders that are reduced thanks to consumption of these molecules (Calder, 2012; Zhang, Sun, Sun, Chen, & Chen, 2014; Özogul et al., 2011). For this reason, this ingredient can be considered not only a food colorant but also a functional compound for healthy food design. Multiple applications on the use of ω-3 fatty acids-rich oils or astaxanthin for the development of functional foods can be found in the literature (Lopez-Huertas, 2010; Taksima, Limpawattana, & Klaypradit, 2015). Other compounds, however, such as the

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oxidized forms of cholesterol, are not desirable (Hernández Becerra et al., 2014).

Microalgae, seaweeds, and crustaceans are the most important sources of astaxanthin (Sowmya & Sachindra, 2011). However, astaxanthin intake is low and almost limited to fish species (crustaceans and salmonids). Astaxanthin possesses strong antioxidant activity, which is higher than that of other carotenoids or α-tocopherol (Bauerfeind, Hintze, Kschonsek, Killenberg, & Böhm, 2014), emphasizing the interest on valorizing shrimp waste and developing new food ingredients from it. Indeed, the epidermis and the hepatopancreas have been found to be major sources of carotenoids in crustaceans, appearing colored or with massive, highly colored fat globules (Nègre-Sadargues, Castillo, & Segonzac, 2000). Astaxanthin is commonly found in nature either conjugated with proteins or esterified with one or two fatty acids (monoester and diester forms) (Breithaupt, 2004; Yang et al., 2015). Work on the characterization of astaxanthin molecular species can be found for the alga *H. pluvialis* (Miao, Geng, Lu, Zuo, & Li, 2013) and for some crustacean species, such as *L. vannamei*, *E. superba*, and *P. borealis* (Breithaupt, 2004; Grynbaum et al., 2005; Yang et al., 2015).

Due to the interest and applications of the shrimp lipid extract obtained from cephalothoraxes and cuticles waste, which was previously demonstrated in our previous works, the objective of the present one has been to study in deep the composition of such extract, with special emphasis on studying the astaxanthin molecular species, and also its stability when stored at room temperature.

2. Materials and methods

2.1. Chemicals

All-*trans*-astaxanthin, cholesterol, fatty acid standards, and 1,1,3,3-tetraethoxypropane were from Sigma-Aldrich Química, S.A. (Madrid, Spain). DL-α-tocopherol was obtained from Supelco (Madrid, Spain) All HPLC-grade solvents, including methanol and methyl t-butyl ether (MTBE), were obtained from Labscan Ltd. (Dublin, Ireland). All other reagents were of analytical grade and supplied by Panreac Química S.A. (Madrid, Spain).

2.2. Obtaining the lipid extract from shrimp waste

Ten kilograms of frozen shrimp (*L. vannamei*), kindly provided by Angulas Aguinaga Burgos (Burgos, Spain), was thawed at room temperature and peeled manually. Shrimp waste (cephalothorax, cuticles, tails, and pleopods) was homogenized to a particle size of ≈5 mm. Aliquots of 10 g of the homogenate were mixed with ethyl acetate (50 mL each) and stirred for 30 min at room temperature in darkness; after extraction the samples were filtered through Whatman No. 1 filter paper and all the aliquots were mixed together.

2.3. Analysis of carotenoids

The carotenoid species contained in the lipid extract were quantified by RP-HPLC-DAD using a Develosil UG C30 column (5 μm particle size) (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, UK) packed with ODS C18. Aliquots of the lipid extract were dried and dissolved in a mixture of methanol/MTBE at 25:75 (v:v) ratio, and filtered through a 0.45 μm syringe filter, and then a 20 μL volume was injected. For elution, the linear mobile phase gradient was methanol 4% H_2O: MTBE from 83:17 to 33:67 over 60 min at a flow rate of 1 mL/min, at 22 °C. Commercial astaxanthin and spectral data were used to assign all-*trans*-astaxanthin isomers; all-*trans*-astaxanthin was quantified by means of a calibration curve following the method described by Gómez-Prieto, Caja, and Santa-María (2002). A DU-70 spectrophotometer (Beckman Instruments) was routinely used to check the concentration of the working standard solution, the concentration being calculated using the extinction coefficient 125,100 (Britton, 1995). Mono- and diesters of astaxanthin were quantified from the same calibration curve owing to the absence of patterns. Tentative identification of the mono- and diesters of astaxanthin was accomplished by RP-HPLC-MS (Agilent 6530 Accurate-Mass Q-TOF LC/MS) according to the exact masses expected for each compound, in the same conditions as described for RP-HPLC-DAD.

2.4. Fatty acid profile

Lipid extract (10 mg) was derivatized into fatty acid methyl esters (FAMEs) in triplicate using 0.5 M sodium methoxide in anhydrous methanol and acetyl chloride in anhydrous methanol. FAMEs were extracted with 4 mL hexane and used for GC analysis (1 μL). The fatty acid profile was determined in an Agilent 7820A gas chromatograph with FID detector. Separation was carried out in an Agilent HP-88 column (60 m, 0.32 mm i.d, 0.25 μm film thickness Ref. 112-8867) with split injection (40:1) and Helium at a constant flow of 1.2 mL/min. Detector temperature was set at 260 °C and injector temperature at 250 °C. The temperature profile of the oven was 125 °C for 1 min which then increased by 8 °C/min to 145 °C for 26 min, then increased by 2 °C to 220 °C for 5 min. Identification was accomplished by comparison of the retention times with standards, and results were expressed as relative percentage of the identified fatty acids. The fatty acids identified accounted for 94% of the total amount of fat in weight.

2.5. α-Tocopherol and cholesterol quantification

The lipid extract was subjected to saponification and subsequent extraction with hexane. An aliquot was dried and dissolved in methanol and then injected in an Agilent 1100 high resolution liquid chromatograph equipped with a Supelcosil LC-F column with pre-column (Ref. 59158) and coupled to an Agilent G1946D simple quadrupole mass spectrometer. The mobile phases used were isocratic 1:10 water:methanol at a flow rate of 1 mL/min. Identification and quantification were accomplished by comparison with patterns, which were subjected to the same preparation steps as the samples.

2.6. Thiobarbituric acid reactive substances

A modified version of the method described by Gudipati, Sandra, McClements, and Decker (2010) was adopted. The lipid extract was dissolved in absolute ethanol, and aliquots were mixed with a reagent solution containing 15% trichloroacetic acid and 0.375% thiobarbituric acid, and then incubated in a water bath for 15 min at 90 °C. The reaction was stopped in an ice bath and the malondialdehyde–thiobarbituric acid complex was extracted with 1-butanol by vigorous agitation in a vortex mixer. The mixture was centrifuged (5000 × g/10 min/22 °C), and the absorbance of the upper phase was read at 532 nm. TBARS concentrations were calculated from a standard curve prepared with 1,1,3,3-tetraethoxypropane.

2.7. Stability study

Aliquots of the lipid extract in ethyl acetate were transferred into 25 mL amber glass vials and dried under nitrogen flow (result-