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Detecting astaxanthin

in shrimp by-products for active packaging

Cover Story

Regulars

Astaxanthin from Shrimp By-products for Active Packaging Systems

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The two year international project "Preparation of active packaging with antioxidant and antimicrobial activity based on astaxanthin and chitosan",[1] which started in 2009, was funded by FONCYCIT (Fund for International Cooperation of Science and Technology between the European Union and Mexico). Within this project, new methodologies for the extraction and characterization of astaxanthin and chitosan from shrimp waste were optimized. Moreover, incorporation of these compounds into different polymeric matrices was performed to obtain active packaging with antioxidant and antimicrobial properties. Finally, control-release studies were done to determine the quantity of active compounds that could migrate into foodstuffs.

In this project, the fermentation of shrimp by-products was performed and the silage was centrifuged resulting in three phases: the solid phase (from which chitin is obtained); the liquid phase with proteins, minerals and free amino acids; and the lipid phase that contains astaxanthin.

Chitosan is obtained from chitin by deacetylation and it has numerous applications, such as an adsorbent of dyes, a drug delivery system and an antimicrobial agent. To determine chitosan, this molecule was converted into the respective glucosamine units by acid hydrolysis (110 °C, 4 h with HCl 8 M). Glucosamine has no chromophore, therefore, to determine the molecule by ultra-high pressure liquid chromatography with an ultraviolet detector, a derivatization was performed with 9-fluorenylmethyl chloroformate (Fmoc-Cl).[2] Water and ethanol 95% (v/v) were the selected food simulants for the migration tests of films prepared with chitosan.[2]

Astaxanthin (Figure 1) is a carotenoid with an antioxidant activity ten and 100–500 times higher than β-carotene and vitamin E, respectively. This

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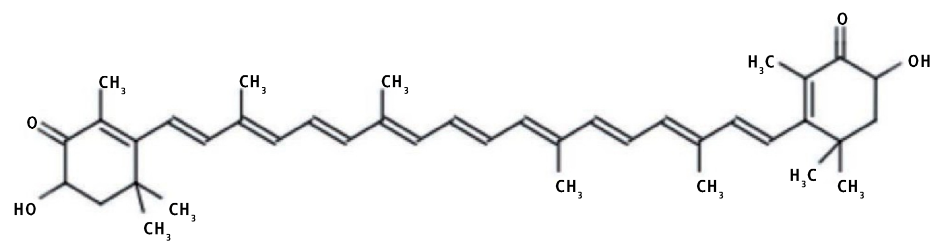
antioxidant activity allows protection against cardiovascular, inflammatory, neurodegenerative and immune diseases. Moreover, this xanthophyl is responsible for the pink-red colour of salmonids and shellfish.

An ultra-high pressure liquid chromatograph coupled with a diode array detector (DAD) was used to develop and optimize a method to determine astaxanthin in shrimp by-products. The method was also applied to determine the migration of astaxanthin from polymeric films containing different amounts of fermented shrimp waste to food simulants.

Astaxanthin was determined in the lipid fraction of the fermented shrimp by-products. Samples were prepared according to a modification of the method described by López-Cervantes et al.[3] The lipid fraction of shrimp by-products was extracted with methanol, mixed in a vortex and kept in an ultrasonic bath for 10 min to allow complete extraction. After filtration, it was injected into the chromatographic system. Excellent separation was achieved (Figure 2).

The chromatographic method uses a UPLC BEH guard column (2.1 × 5 mm, 1.7 µm particle size) and a UPLC BEH analytical column (2.1 × 50 mm, 1.7 µm particle size). A gradient mobile phase consisting of (a) acetonitrile/methanol (containing 0.05 M ammonium acetate)/dichloromethane (75:20:5, v/v/v) and (b) ultrapure water was used. The astaxanthin calibration curve was linear over the concentration range of 0.16–2.1 µg/mL. The determination coefficient (r^2) was 0.9993, indicating suitability for astaxanthin quantification. Six determinations of the same sample were performed using the same reagents and apparatus in the same day to evaluate method repeatability, on the basis of the relative

Figure 1: Astaxanthin (3,3′-dihydroxy-β-β′-carotene-4-4′-dione) chemical structure.

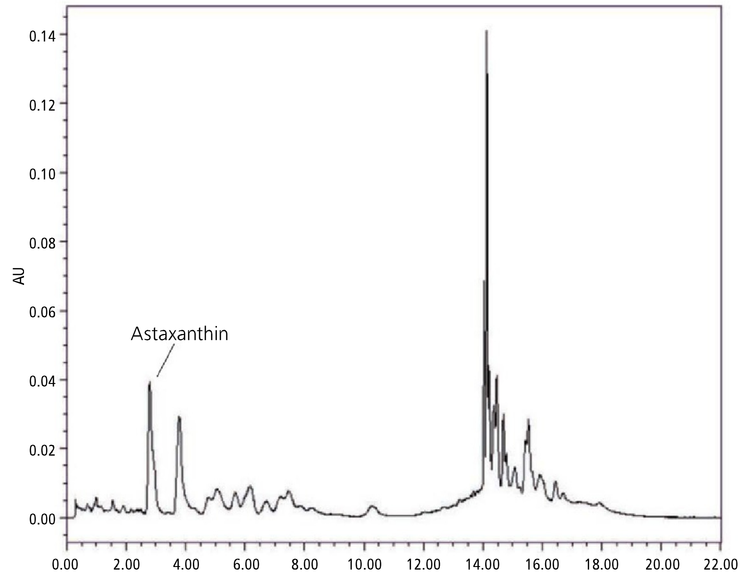


standard deviation (RSD). The method presented good intra-assay precision (RSD = 3.6%) and also good inter-assay precision (RSD = 4.7%), evaluated by analysis of three independent samples in three consecutive days.

The limit of detection (signal three times the height of the noise level) was 0.054 μg/mL and the limit of quantification (signal ten times the height of the noise level) was 0.16 μg/mL. Astaxanthin was found in the lipid fraction of fermented shrimp by-products in a concentration of 453.8 ± 16.3 μg/g. However, noteworthy differences between samples from different batches were found because of the different availability of the pigment in crustaceans' diets.

Low density polyethylene films were prepared with different amounts of the lipid fraction of fermented shrimp waste by extrusion and migration was evaluated into food simulants (isooctane and ethanol 95%, v/v). Migration tests were performed at 40 °C for 10 days and at 70 °C for two days with isooctane and ethanol

Figure 2: Ultra-high pressure liquid chromatogram of the lipid fraction of the fermented shrimp by-products.



95% (v/v). Strips of films were fully immersed in the simulant. Isooctane extracts were evaporated to dryness in a rotary evaporator and then redissolved in methanol, while ethanol 95% (v/v) extracts were directly injected into the chromatograph. For each film, three independent migration tests were performed.

Shrimp by-products are a good source of astaxanthin and the new ultra-high pressure liquid chromatographic method allows detection of astaxanthin, which is a potent antioxidant, in shrimp by-products at low detection levels. The active low density polyethylene films produced by extrusion did not cause astaxanthin migration into the tested fatty food simulants and conditions.

References

1. A. Sanches-Silva, H.S. Costa, P.P. Losada, R. Sendón, D.I. Sánchez-Machado, H.S. Valdez, I.A. Varona & J. López-Cervantes, *Preparation of active packaging with antioxidant and antimicrobial activity based on astaxanthin and chitosan*, British Nutrition Foundation, *Nutrition Bulletin*, **35**, 268–271 (2010).
2. A. Sanches-Silva, T. Ribeiro, T.G. Albuquerque, P. Paseiro, R. Sendón, A. Bernaldo de Quirós, J. López-Cervantes, D.I. Sánchez-Machado, H. Soto Valdez, I. Angulo, G.P. Aurrekoetxea & H.S. Costa, Ultra-high pressure LC determination of glucosamine in shrimp by-products and migration tests of chitosan films, *Journal of Separation Science*, (2011) in press.
3. J. López-Cervantes, D.I. Sánchez-Machado, M.A. Guitiérrez-Coronado & N.J. Rios-Vázquez, Quantification of astaxanthin in shrimp waste hydrolysate by HPLC, *Biomedical Chromatography*, **20** 981–984 (2006).

Ana Sanches-Silva graduated in Pharmacy from the University of Coimbra, Portugal, in 2001 and in January 2005 received her PhD in Pharmacy from the University of Santiago de Compostela, Spain. Before joining the National Health Institute Dr. Ricardo Jorge in July 2007 as a postdoctorate, she was a postdoctorate at the Analytical Chemistry, Nutrition and Food Science Department of the University of Santiago de Compostela. Since May 2008 she has been a researcher at the Food and Nutrition Department of the National Health Institute Dr. Ricardo Jorge.

Ana Sanches-Silva's research focuses on the development of chromatographic methods for the analysis of food components and contaminants. Moreover, her special interest is the study of food-packaging interactions, especially the migration of food additives from packaging to food.

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