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The effect of high-pressure treatment on functional components of shrimp (*Litopenaeus vannamei*) cephalothorax

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ABSTRACT

High-pressure (HP) technology has been applied to extend the shelf life of shrimps by inhibiting enzymes with PPO activity or microorganisms. However, there is very little information on its effect on relevant compounds from a nutritional or functional point of view, such as fatty acids, α-tocopherol, astaxanthin, and hemocyanin, which constitutes the main objective of the present work. Shrimp cephalothoraxes were HP processed at 200, 400, or 600 MPa/18 °C/15 min or three consecutive 5 min cycles. It was found that hemocyanin was partially denatured at pressures up to 400 MPa, resulting in lower PPO activity, and it was totally denatured at 600 MPa, although 20% residual PPO activity remained. Astaxanthin, α-tocopherol, and total antioxidant activity were stable whichever HP treatment was applied, whereas 600 MPa caused a slight reduction of eicosapentaenoic acid (C20:5n3, EPA) and docosahexaenoic acid (C22:6n3, DHA). Despite this reduction, the ω-6/ω-3 fatty acids ratio was very low (1).

Industrial relevance: Shrimps are high-value fishery products with a very short shelf life under refrigeration, mainly because of microbial growth and development of melanosis. Thermal treatment is effective for extending shelf life, but it affects the nutritional quality of shrimps through degradation of bioactives such as polyunsaturated fatty acids, tocopherols, or astaxanthin, which are mainly located in the cephalothorax. High pressure is a non-thermal processing technology that has been proved to extend shrimp shelf life, but very little information can be found on its effect on the above-mentioned compounds as well as on the melanosis-inducing hemocyanin. Such basic knowledge is very important for industrial application of high-pressure technology to extend the shelf life of shrimps.

1. Introduction

Shrimps are much-appreciated, high-value fishery products worldwide. In particular, Pacific white shrimp (*Litopenaeus vannamei*) is one of the most important aquaculture species, mostly imported to Europe in a frozen state from farms located in Ecuador and Thailand. Shrimps are appreciated not only from a sensory point of view but also because they are a source of high biological value proteins (high digestibility, essential amino acids), polyunsaturated fatty acids, carotenoids, and α-tocopherol (Bono et al., 2012; Dararat, Lomthaisong, & Sanoamuang, 2012; Özogul, Özogul, & Kuley, 2011). Indeed, most of the health benefits provided by frequent seafood consumption come from the adequate uptake of ω-3 (specially eicosapentaenoic (EPA) and docosahexaenoic acids (DHA)) and ω-6 polyunsaturated fatty acids and antioxidants (Barros, Poppe, & Bondan, 2014). Astaxanthin is the most abundant carotenoid found in aquatic animals such as salmon, trout, shrimp, and lobster, and its potential beneficial effects are associated with its antioxidant activity (Higuera-Ciapara, Félix-Valenzuela, & Goycoolea, 2006), which is even higher than that of α-tocopherol (Sowmya & Sachindra, 2012).

Shrimps are highly perishable fishery products, mainly as a result of microbial growth coupled with the onset of melanosis or black spot formation. Rapid melanosis development in the cephalothorax of Pacific white shrimp (*L. vannamei*) has been related to the presence of highly stable, active polyphenol oxidase (PPO) (Nirmal & Benjakul, 2012). PPO in crustaceans, normally involved in cuticle sclerotization and immune response *in vivo*, is mainly responsible for catalyzing postmortem oxidation of phenolic substrates to quinones. After non-enzymatic polymerization of quinones, an accumulation of black, high molecular weight pigments (melanins) in cephalothorax and cuticle takes place. Although these pigments are apparently harmless to consumers, they may cause an important loss of market value (Montero, Ávalos, & Pérez-Mateos, 2001b). Moreover, shrimps (*L. vannamei*) subjected to repeated freeze–thawing cycles, which often occur in retail shops, have been shown to suffer pronounced melanosis promoted by PPO activation (Manheem, Benjakul, Kijroongrojana, & Visessanguan, 2013).

Hemocyanin (Hc), which is a copper-containing respiratory protein, is the main constituent protein in crustacean hemolymph, comprising 90–95% of the total plasma proteins (Adachi, Endo, Watanabe,

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Nishioka, & Hirata, 2005). Hc has been found to acquire diphenoloxidase activity in deepwater pink shrimp (*P. longirostris*) upon postmortem protease-induced activation (Martínez-Alvarez, Gómez-Guillén, & Montero, 2008). The conversion of Hc to an active PPO form by the action of trypsin or a denaturing treatment with SDS has also been reported in whiteleg shrimp (*P. vannamei*) (García-Carreño, Cota, & Del Toro, 2008). Both PPO and Hc, which have been described as complex, high molecular weight proteins with a strong resemblance to each other, are composed of several subunits capable of aggregating into dimers, trimers, hexamers, or dodecamers, playing a crucial role in melanosis development (García-Carreño et al., 2008; Martínez-Alvarez et al., 2008). The two main strategies currently employed to reduce melanosis and/or microbial growth in order to extend the shelf life of shrimps are the addition of preservatives (antioxidants, antimicrobials, reducing agents, specific inhibitors, etc.) and the application of preservation technologies, either thermal or non-thermal (Huang et al., 2014). However, on the one hand, consumers increasingly demand the marketing of foods with lower amounts of added preservatives, and, on the other, the use of thermal treatments affects the nutritional value of shrimps as well as their sensory properties (Delfieh et al., 2013; Jantakoson, Kijroongrojana, & Benjakul, 2012). These are the reasons why the application of novel non-thermal food preservation technologies such as modified atmosphere packaging, dense high carbon dioxide processing, pulsed electric field, or high-pressure processing have been gaining interest in recent years (Huang et al., 2014).

High pressure has been applied to shrimps, although the amount of work found is much lower than for meat, vegetables, or fish species. For example, Jantakoson et al. (2012) applied pressures up to 800 MPa/20 min/28 °C on black tiger shrimp (*Penaeus monodon* Fabricius) that caused lower modifications (weight loss, texture, color) than heat treatment at 100 °C for 2 min. With regard to enzymatic activity, results from the literature reveal that pressurizing a PPO enzyme extract from *L. vannamei* up to 300 MPa/2 min/25 °C increased enzyme activity, whereas pressures of 400–600 MPa/2 min/25 °C inactivated it (Huang et al., 2014). Despite this, when pressurizing whole shrimps (400–600 MPa/2 min/25 °C or 400 MPa/10 min/7 °C), no melanosis inhibition was observed, or it was even higher than in unpressurized samples (Huang et al., 2014; Montero, Lopez-Caballero, & Perez-Mateos, 2001a). It is evident, and also previously documented, that the effect of high pressure is very dependent on the pressure/processing time/temperature trinomial as well as on the application on food extracts, whole or minimally processed foods, etc. (Van der Plancken, Grauwet, Oey, Van Loey, & Hendrickx, 2008). With regard to the antimicrobial action of high pressure, it has been reported to extend the shelf life of shrimps effectively (200 or 400 MPa/10 min/7 °C) (Lopez-Caballero, Perez-Mateos, Borderias, & Montero, 2000). High-pressure technology has also been proposed as a means for improving astaxanthin extraction from shrimp waste consisting of cephalothoraxes and cuticles (Du, He, Yu, Zhu, & Li, 2013). Although no differences were observed in comparison with a sample processed at atmospheric pressure, results suggest that astaxanthin was stable to high-pressure treatment. Therefore, the few works found in the literature on the application of high-pressure treatment on shrimps deal with the effect on muscle proteins, sensory properties, microorganisms, or PPO activity/melanosis development, but there is very little information on its effect on relevant compounds from a nutritional or functional point of view, such as fatty acids, α-tocopherols, astaxanthin, and hemocyanin, which constitutes the main objective of the present work.

2. Materials and methods

2.1. Sample preparation and high-pressure (HP) treatment

Among the different shrimp body parts, cephalothorax contains the greatest amount of carotenoids, lipids, and hemolymph proteins (Sachindra, Bhaskar, & Mahendrakar, 2005); for this reason, previously separated cephalothorax was employed for the study of the effects of HP treatment, considering also, from a methodological point of view, that it may represent an intermediate material type between unprotected extracts and fully protected whole shrimps. Ten kilograms of frozen shrimps (*L. vannamei*), kindly provided by Angulas Aguinaga Burgos (Burgos, Spain), was thawed at room temperature and peeled manually. Cephalothoraxes were homogenized to a particle size of ≈5 mm. Aliquots of the homogenate (20 g) were mixed with 10 mL of distilled water and vacuum packed in flexible bags (type BB4L, Cryovac, Barcelona, Spain). HP treatment was performed using a Stansted Fluid Power Iso-lab 900 High Pressure Food Processor (Model: FPG7100:9/2C, Stansted Fluid Power Ltd., Harlow, Essex, UK) at 200, 400, or 600 MPa for 15 min in one cycle (samples named as 200, 400, or 600, respectively) or three consecutive cycles of 5 min each without waiting time between cycles (samples named as 200C, 400C, or 600C, respectively) at 18 °C. A control sample was held at atmospheric pressure for comparative purposes (sample AP). The samples were freeze-dried in a BenchTop 6 KB lyophilizer (VirTis Co., Gardiner, NY, USA) and subsequently pulverized in a grinder (Moulinex, Madrid, Spain).

2.2. Molecular weight profile

The molecular weight profile of the cephalothorax protein material was determined by SDS–polyacrylamide gel electrophoresis (SDS–PAGE). The freeze-dried sample powders were mixed with loading buffer (2% SDS, 5% mercaptoethanol, and 0.002% bromophenol blue) to achieve a final protein concentration of ≈2 mg/mL. Sample was heat-denatured at 95 °C for 5 min and then centrifuged at 13,000×*g* for 10 min and analyzed according to Laemmli (1970) in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA, USA) using 7.5% resolving gels (Bio-Rad Laboratories) at 25 mA/gel. Protein bands were stained with Coomassie brilliant blue R-250. A molecular weight protein standard acquired from Bio-Rad Laboratories (Precision Plus Protein™ Dual Xtra Standards, ref. 161–0377-MSDS) with molecular weights of 250, 150, 100, 75, 50, 37, 25, 20, 15, 10, 5, and 2 kDa was employed.

2.3. Thermal behavior

It was determined with a model TA-Q1000 Differential Scanning Calorimeter (DSC) (TA Instruments, New Castle, DE, USA). The freeze-dried powders were reduced to ~1 mm particle size in a mortar for proper sampling. Samples of approximately 5–8 mg (± 0.002 mg) were weighed out using a model ME235S electronic balance (Sartorius, Goettingen, Germany), tightly encapsulated in hermetic aluminum pans and scanned under dry nitrogen (50 mL/min) purge. An empty capsule was used as reference. Samples were heated from 5 °C to 100 °C at 10 °C/min, and a second scan was occasionally run to check whether protein denaturation was already complete. Peak temperatures (T_{peak}, °C) and transition enthalpies (ΔH, kJ/g_{dm}) by linear baseline integration were calculated after normalization to dry matter content by encapsulated-sample desiccation. Samples were analyzed in triplicate.

2.4. Lipid extraction

Aliquots of 2 g of the freeze-dried powders 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; afterward it was stored at −30 °C in darkness. The extraction yield was calculated and expressed as g/100 g dry sample.