



## Compositional properties and bioactive potential of waste material from shrimp cooking juice



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### ABSTRACT

A functional concentrate rich in proteins and lipids was obtained from an industrial shrimp cooking juice by using a centrifugal separator, and it was characterized in terms of chemical composition parameters and functional or biological activity (antioxidant and ACE-inhibitory capacities). The concentrate presented 116 g/kg protein, 135 g/kg crude fat and ash content less than 10 g/kg, with predominance of Na ions, followed by K, Ca, Mg, Cu, Fe, Zn and Mn. A relative abundance of glucose, glycerol, polyalcohols, acetate and phosphate was found. The antioxidant (as determined by ABTS, FRAP, chelating and photoluminescence assays) and ACE-inhibitory capacities of the material recovered from the shrimp cooking juice were strongly related to the presence of small peptides (1355–502 Da), with a clear predominance of Gly, Pro, Glu, Asp and Arg. Other antioxidants, such as free astaxanthin (*cis* and *trans* isomers) and derived esters, were also detected.

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### 1. Introduction

The process of cooking shellfish generates a large amount of effluent with a high organic load and generally also high salt contents, which represents an environmental hazard and is subject to wastewater treatment regulations (Cros, Lignot, Jaouen, & Bourseau, 2006). Thus, the recovery of the residual organic material present in crustacean cooking water could reduce the cost of depolluting treatments, and it would also involve a valorization of waste and a search for potentially bioactive molecules. Crustacean species such as shrimp, crab, lobster, etc. are rich sources of amino acids, peptides, protein and other useful biochemicals, such as sugars, organic acids, carotenoids, etc., which may be recovered for utilization as ingredients in various food applications (Simpson, Nayeri, Yaylayan, & Ashie, 1998). The resulting protein powders can also be used in feed formulations for farm animals and also for fabricated seafoods such as shrimp analogue or shrimp crackers in order to provide essential amino acids as well as carotenoids. Astaxanthin is the major naturally occurring carotenoid pigment in marine crustaceans and is the main compound responsible for their

typical orange-pink colouration. Carotenoids are normally found forming strong associations with proteins and fatty acids, therefore cooking treatments and enzymatic hydrolysis have been shown to be successful pre-treatments to recover free carotenoids from crustaceans (Mezzomo, Maestri, Dos Santos, Maraschin, & Ferreira, 2011; Simpson, Nayeri, Yaylayan & Ashie, 1998; Sowmya, Rathinaraj, & Sachindra, 2011).

The process of cooking shrimp and oyster has been shown to generate a large amount of effluent containing appreciable amounts of soluble components, such as peptides, amino acids, glycogen, and other organic compounds (Cambero et al., 1998; Kim et al., 2000). These effluents are also a source of seafood flavouring compounds (Cambero et al., 1998), owing to the presence of certain amino acids, nucleotide-related compounds, organic acids and Maillard reactions between free amino acids and sugars present in the cooking juice as a result of thermal hydrolysis (Mandeville, Yaylayan, & Simpson, 1992), or else favoured by enzymatic hydrolysis (Kim et al., 2000). Cooking effluents, however, may also contain high amounts of salts, and therefore a two-step membrane process, combining desalination by electrodialysis and concentration by reverse osmosis, has been reported to be technically feasible to produce aroma concentrates from shrimp cooking juices (Cros, Lignot, Jaouen & Bourseau, 2006).

Shrimp wastes subjected to enzymatic hydrolysis have been shown to be an important source of bioactive peptides with antioxidant (Guerard, Sumaya-Martínez, Laroque, Chabeaud, & Dufossé, 2007) and antihypertensive activity (inhibiting angiotensin I-converting enzyme, ACE) (Cheung, & Li-Chan, 2010). Antioxidant peptide

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sequences obtained from *Penaeus japonicus* (Suetsuna, Ukeda & Ochi, 2000) and ACE-inhibitory peptides obtained from *Acetes chinensis* (He, Chen, Sun, Zhang, & Zhou, 2006) have been identified. However, to our knowledge, bioactive peptides have not been yet reported from shrimp cooking effluents.

The aim of the present work is to obtain a functional concentrate from industrial shrimp (*Penaeus* spp.) cooking juice and to explore its bioactive potential by determining different antioxidative mechanisms and antihypertensive (ACE-inhibitory) capacity.

## 2. Materials and methods

### 2.1. Materials

Shrimp (*Penaeus* spp.) cooking juice (CJ) (90 L) was obtained from a local crustacean processing factory (Gambastar, Burgos, Spain) operating under industrial conditions, immediately before being sent to the water treatment plant. The recovered cooking juice was tempered and frozen at  $-20^{\circ}\text{C}$  until use. Nitric acid and hydrogen peroxide were purchased from Panreac (Moncada i Reixac, Barcelona, Spain). HPLC grade acetonitrile, methanol and formic acid were from VWR international Inc. (Barcelona, Spain). HPLC grade water was prepared from distilled water using a Milli-Q system (Millipore Laboratory, Bedford, MA). Methyl *t*-butyl ether (MTBE) was obtained from Labscan Ltd. (Dublin, Ireland). Vitamin C, all-trans astaxanthin, ABTS radical [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonate)], potassium persulphate,  $\text{FeCl}_3$ ,  $\text{FeSO}_4$ , ascorbic acid, Trolox and 2,4,6-tripyridyl-*s*-triazine were from Sigma–Aldrich (St. Louis, MO, USA). ACW and ACL kits, reagent 2 and reagent 3 for the photochemiluminescence assay (Photochem<sup>®</sup>) were purchased from Analytik Jena AG (Jena, Germany).

### 2.2. Sample preparation

The thawed cooking juice (CJ) was passed through a centrifugal separator (GEA Westfalia Separator, OKA 2-06-566, Düsseldorf, Germany) at 9560 rpm and constant flow rate (50 l/h), resulting in a liquid supernatant (CJ-L) and a pasty-looking concentrate (CJ-S). The CJ-L fraction was repeatedly extracted using a solvent mixture of ethyl acetate and water (1:1) until no colour in the organic phase was observed; the CJ-L/solvent ratio was 1:10. Extraction was performed in a separating funnel submitted to vigorous agitation for 2 min, and the aqueous (CJ-L-AP) and organic phases (CJ-L-OP) were collected. The CJ-L-AP was frozen and the CJ-L-OP was cold stored under nitrogen atmosphere and in darkness until analysis.

### 2.3. Proximate analysis and pH

Moisture and ash contents were determined in triplicate following AOAC (2000). The protein content of each sample was determined by a LECO FP-2000 nitrogen/protein analyser (Leco Corp., St. Joseph, MI, USA), using a nitrogen-to-protein conversion factor of 6.25. Fat content was evaluated (in triplicate) according to Bligh and Dyer (1959). The pH was determined using a MeterLab pHM 93 (Radiometer Analytical, Denmark) on a homogenate of 1 ml of sample in 10 ml of distilled water.

### 2.4. Total volatile basic nitrogen

The total volatile basic nitrogen (TVB-N) determination was effected according to the method of Antonacopoulos and Vyncke (1989). The results were expressed as g of TVB-N per l or kg of sample. Determinations were carried out at least in triplicate.

### 2.5. Mineral content

Samples (in triplicate) were prepared by acid digestion, in the presence of nitric acid and hydrogen peroxide (Panreac), in closed vessels, with a microwave furnace (Microwave Digestion LabStation, Milestone Inc., Shelton, USA). The minerals were quantified on an atomic absorption spectrophotometer (Perkin–Elmer, model 5100 PC, Norwalk, Connecticut, USA) with an air- and acetylene-oxidizing flame, which was used to determine calcium, magnesium, sodium, potassium, zinc, manganese, iron and copper cations.

### 2.6. Ion chromatography

For the analysis of inorganic ions (Fluoride, Chloride, Nitrate, Phosphate, Sulphate), organic acids (Citrate, Malate, Lactate, Formate, Acetate) and sugars and polyalcohols (Myo-inositol, Glycerol, Mannitol, Glucose, Sucrose), CJ and CJ-L samples were diluted in water 1:40 v/v, gently stirred for 30 min and filtered through Whatman no. 1 filter paper. The dilution of CJ-S was 1:100 w/v. The CJ-L-AP sample was diluted 7-fold in water. Diluted samples were filtered through 0.45  $\mu\text{m}$  pore size filters.

Analysis of anions and organic acids was carried out in triplicate on a Metrohm Advanced Compact Ion Chromatograph (model IC-861, Metrohm AG, Switzerland) equipped with an Advanced Conductivity Detector (IC-819, Metrohm AG). The columns employed were a Metrosep A Supp 5–250 column (250  $\times$  4 mm, 5  $\mu\text{m}$  particle size) for anion determination and Metrosep organic acids (250  $\times$  7.8 mm, 8–10  $\mu\text{m}$  particle size) for organic acids determination (Metrohm AG), using the following elution conditions: 3.2 m mol/l sodium carbonate/1 m mol/l sodium hydrogen carbonate at 0.70 ml/min as mobile phase.

Analysis of sugar and polyalcohols was carried out in triplicate on an ion chromatography 817 Bioscan (Metrohm AG, Switzerland) equipped with a pulsed amperometric detector (PAD) and a gold electrode. The column employed was Metrosep Carb (1–250/250  $\times$  4.6 mm, 5  $\mu\text{m}$  particle size). Samples were injected using an autosampler (model 838 Advanced Sample Processor, Metrohm AG) with a flow rate of 1 ml/min, using 150 m mol/l NaOH and 500 m mol/l acetate–Na in 150 m mol/l NaOH as mobile phase. Compounds were identified by comparing their retention times with those of standards (Sigma–Aldrich, St Louis, MO) and quantified by measuring the normalized peak areas.

### 2.7. Reverse phase HPLC chromatography

Carotenoid determination was performed by RP-HPLC analysis of the CJ-L-OP fraction. Analysis was performed on a Beckman System Gold binary delivery system (module 126) equipped with a UV–vis photodiode array detector (model 168, Beckman Instruments, Fullerton, CA). Analytical separations were carried out on a stainless steel (250  $\times$  4.6 mm i.d.) Develosil UG C<sub>30</sub> (5  $\mu\text{m}$  particle size) column (Nomura Chemical, Sojo, Japan) with a guard cartridge (Phenomenex, Macclesfield, U.K.) packed with ODS C<sub>18</sub>. Sample injection was performed by means of a valve (Rheodyne, Cotati, CA) with a 20  $\mu\text{l}$  peek loop. Elution was performed following a linear mobile phase gradient using methanol (4% H<sub>2</sub>O)/MTBE from 83/17 to 33/67 over 60 min at a flow rate of 1 ml/min. The column was thermostated at 22  $^{\circ}\text{C}$  on a Shimadzu CTO-10AS (Columbia, MD) column oven. The Gold Nouveau software data system was used.

Samples to be injected were dried in a rotary evaporator and dissolved in MTBE, 50  $\mu\text{l}$  was filtered through a 0.45  $\mu\text{m}$  filter and a volume of 20  $\mu\text{l}$  was injected. Commercial astaxanthin and spectral data were used to identify carotenoid peaks.

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