Bioactive and technological functionality of a lipid extract from shrimp *(L. vanamei)* cephalothorax

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ABSTRACT

The lipid extract from shrimp cephalothorax (LES) is a potential functional food ingredient because of its astaxanthin, α-tocopherol and polyunsaturated fatty acid content. This work aims at evaluating its bioactive (anti-inflammatory and antioxidant) and technological (potential for aqueous food applications) functionalities. The LES was encapsulated by spray-drying, obtaining a powder that was readily soluble in water, the resulting solution presenting an attractive reddish color, contrary to non-encapsulated LES. Encapsulated LES exerted anti-inflammatory activity at concentrations ≥100 μg/mL, as well as lipophilic and hydrophilic antioxidant activity. On the contrary, non-encapsulated LES did not show any of these bioactivities when dissolved in water. Irrespective of processing temperature (20 °C/100 °C) or refrigerated storage time (up to 6 days at 5 ºC ± 1 ºC), the color, astaxanthin content,

and fatty acid composition of encapsulated LES were relatively stable. Gelatin gels and films containing the emulsified or encapsulated LES had a homogeneous reddish color, as well as minor physicochemical modifications (viscoelastic properties, gel strength, film water solubility, and water vapor permeability). Furthermore, encapsulation process increased the bioaccesibility of astaxanthin up to 100%, in comparison with non-emulsified LES-added samples. In conclusion, spray-dried encapsulated LES is a promising functional food ingredient, especially for aqueous applications.

Keywords

Astaxanthin; food coloring; anti-inflammatory; *L. vanamei* cephalotorax; polyunsaturated fatty acids

INTRODUCTION

Currently, consumers demand food products with a low amount of additives. This is especially true in the case of synthetic additives, so that research on new alternatives from natural origin is of great interest. A lipid extract from shrimp cephalothorax waste (LES), which contains polyunsaturated fatty acids (including DHA and EPA), α-tocopherol and astaxanthin, has been recently proposed as a natural food ingredient with potential applications as food coloring and functional ingredient (Gómez-Estaca, Calvo, Álvarez-Acero, Montero, & Gómez-Guillén, 2017). Based on their composition, these kinds of extracts may possess antioxidant and anti-inflammatory properties (Calder, 2012; Raiola, Tenore, Barone, Frusciante, & Rigano, 2015; Zhang, Sun, Sun, Chen, & Chen, 2014). Nevertheless, the food applications of the LES may be restrained because of its low solubility in water and its instability to thermal treatment or storage. Yang et al. (2015) studied the effect of thermal processing on astaxanthin in Pacific white shrimp (*Litopenaeus vannamei*) and found a total astaxanthin loos that ranged from 8 to

52% in the first 3 min, depending on the thermal processing applied (microwaving, boiling and frying). Poly-unsaturated fatty acids are also known to be sensitive to thermal treatments; Yang et al. (2017) reported that lipid oxidation of fish oil was directly related to processing temperature (25, 40 or 60 ºC), and the lipids extracted from shrimp cephalothorax have been also found to be sensitive to lipid oxidation during storage at 30 ºC (Takeungwongtrakul, Benjakul, Santoso, Trilaksani, & Nurilmala, 2015).

Spray drying is a continuous, simple and fast process in which a liquid is transformed in a powdered dry product. In the process, the liquid is atomized in a compartment and put through a flow of hot air, the quick evaporation of water keeping the temperature of the particles low. Therefore, this technique enables drying of heat-sensitive products without affecting their quality too much (Ré, 1998). This was partially true for the LES, as it was successfully encapsulated by spray-drying without astaxanthin loos, however, somewhat lipid oxidation was observed (Montero, Calvo, Gómez-Guillén, & Gómez-Estaca, 2016). Still, studies on practical food applications of lipid extracts from crustaceans, encapsulated or not, are scarce. Taksima, Limpawattana, and Klaypradit (2015) encapsulated an astaxanthin-containing lipid extract from shrimp by ultrasonic atomization, using an alginate/chitosan matrix; the lipid extract was effectively encapsulated, and, after addition of the capsules to yogurt, positive acceptance and purchase intent were found in sensory tests. In a more recent work, Abdelmalek et al. (2016) developed marinated chicken steaks containing astaxanthin extracted from shrimp by-products and found minimal lipid oxidation during storage, while microbiological quality was preserved.

Edible films and coatings have gained interest in last decades, as they are excellent carriers of food ingredients such as antioxidant, antimicrobials, colorings or flavors,

thus improving food quality and safety (Salgado, Ortiz, Musso, Di Giorgio, & Mauri, 2015). In the present work, the LES has been incorporated in gelatin gels and films, as examples of application in aqueous food matrices. Three ways of incorporation have been studied (agitation, emulsification, and encapsulation). As part of the potential of the LES for food applications, its stability to thermal treatment and refrigerated storage has been evaluated, as these processes are commonly applied during production or distribution of food products. So, the objective of this work was to evaluate the bioactive (anti-inflammatory and antioxidant) and technological (potential for aqueous food applications) functionalities of a lipid extract from shrimp waste, with special emphasis on spray-drying encapsulation as a way of improving them.

MATERIALS AND METHODS

Obtaining of lipid extract from shrimp waste (LES). It was obtained as previously described (Gómez-Estaca et al., 2017), using frozen shrimps (*L. vannamei*) kindly supplied by Angulas Aguinaga Burgos (Burgos, Spain). Briefly, 10 g of chopped cephalothorax were mixed with 50 mL ethyl acetate, stirred for 30 min, filtered and then evaporated under vacuum to dryness.

Encapsulation process. Maltodextrin (Manuel Riesgo S. A., Madrid, Spain) was dissolved in distilled water at 20 g/100 mL. Partially purified phosphatidylcholine from soya lecithin, obtained as per Mosquera et al. (2014), was used as emulsifier (2 g/100 g LES). Phosphatidylcholine was mixed with LES and sonicated at 50 Hz amplitude in 5 consecutive cycles of 30 secs each at 1 min intervals, immersed in an ice bath to avoid overheating. Then the LES was added to the polymer in solution (10 g/100 g polymer), and an oil-in-water emulsion was prepared by homogenizing in a T-25 Ultra-Turrax blender (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 14,000 rpm for 1 min

followed by sonication in a Qsonica sonicator, at 100 Hz amplitude, in 10 consecutive cycles of 1 min each at 1 min intervals. The sample was immersed in an ice bath during the ultrasound treatment in order to avoid overheating. The emulsion was dried in a B-290 Büchi spray-dryer (Switzerland). Processing conditions were: inlet temperature 150 °C, aspiration rate 100%, flow rate 6 mL/min, resulting in an outlet temperature of 95 ± 2 °C.

Anti-inflammatory activity. The anti-inflammatory activity was esteemed from the determination of the production of nitric oxide by the RAW 264.7 cell line in the presence of lipopolysaccharide (LPS, Sigma)(Leikert et al., 2002). Cells (2.1 x 10^3 cells/mL) were seeded in 96-well plates and cultured in DMEM (Sigma) supplemented with 10% (v/v) of foetal bovine serum, penicillin (100 units/mL) and gentamicin (50 µg/mL) at 37 ºC in an atmosphere with 95% relative humidity and a CO2 flow of 5%. Equivalent amounts of non-encapsulated and encapsulated LES were dispersed/dissolved in PBS, then added to final concentrations of 1 mg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL or 0.1 µg/mL. LPS was added at 1 µg/mL and cells incubated for 24 h prior to determination of NO_2. For this, culture medium (50 µL) was incubated with the Griess reagent (1% sulfanilamide and 0.1% N-[1-naphthy]-ethylenediamine dihydrochloridre in 5% phosphoric acid, Sigma) in the darkness at room temperature for 10 min, and the absorbance read at 540 nm. NO_2 concentration was calculated from a standard curve of sodium nitrite (20nM – 0,2µM) prepared in advance.

Antioxidant activity. A photochemiluminescence assay was conducted in order to evaluate the antioxidant activity of the LES either encapsulated or not. This assay involves the photochemical generation of superoxide (O_2 –) 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). LES and encapsulated LES were dispersed/dissolved in distilled water at the concentration of 1 mg LES/mL. 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 samples were measured in triplicate and results expressed as ng Trolox or ascorbic acid equivalents/μg lipid extract, for lipophilic and hydrophilic antioxidant activity, respectively.

Potential of encapsulated extract from shrimp for aqueous food applications. An amount of 100 mg of non-encapsulated LES (sample LE) and the equivalent amount in encapsulated form (sample EN-LE) were dispersed/dissolved in 100 mL of distilled water at 20 °C or 100 °C by magnetic stirring for 20 min. After dissolution, the sample temperature was immediately lowered to 3 ± 1 °C in an ice bath and it was placed in the refrigerator at 5 ± 2 °C. Water solubility, astaxanthin content, fatty acid profile, and color were determined both initially and after 6 days of refrigerated storage (5 ± 1 °C).

Formulation of gelatin gels. The suitability of the encapsulated LES as a food colorant and functional ingredient was evaluated in a gelatin gel, selected as a model of aqueous food. Gelatin (Kenney & Ross, Ltd., Port Saxon, Nova Scotia, Canada) was dissolved in distilled water at 40 °C for 30 min (6.67 g/100 mL) and then LES was added (2.5 g/100 g gelatin) and mechanically stirred for 5 min at room temperature (sample LE-G), or emulsified by homogenizing in a T-25 Ultra-Turrax blender (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 14,000 rpm for 2 min (sample EM-LE-G), or dissolved in encapsulated form (sample EN-LE-G). As controls, a plain gelatin gel (sample G) and a gelatin gel containing maltodextrin at the same concentration as the EN-LE-G sample (sample G-M) were prepared. The gelatin solutions were left to gel at 2 ± 1 °C overnight.

147 **Formulation of edible films.** Film-forming solutions were prepared by dissolving
148 gelatin (4 g/100 mL) and glycerol (30 g/100 g gelatin) in distilled water at 40 °C for 30
149 min. LES was added (2.5 g/100 g gelatin) and mechanically stirred for 5 min at room
150 temperature (sample LE-F), or emulsified by homogenizing in a T-25 Ultra-Turrax
151 blender (IKA-Werke GmbH & Co. KG, Staufen, Germany) at 14,000 rpm for 2 min
152 (sample EM-LE-F), or dissolved in encapsulated form (sample EN-LE-F). As controls,
153 a plain gelatin film (sample F) and a gelatin film containing maltodextrin at the same
154 concentration as the EN-LE-F sample (sample F-M) were prepared. The films were
155 obtained by casting 40 mL of film-forming solution onto 144 cm^2 Perspex plates and
156 drying at 45 °C for 15 h. Before performing the determinations, the films were
157 conditioned in desiccators over a saturated solution of NaBr at 22 ºC for 2 days.

158 **Astaxanthin content.** For extraction of astaxanthin, the gelatin gels were previously
159 melted at 45 °C and the gelatin films were dissolved in water at 45 °C. Aliquots of
160 dissolved/dispersed astaxanthin in water, melted gelatin gels, or dissolved gelatin films
161 were extracted by adding hexane in a 1:1 proportion and vigorously shaking in a vortex
162 (3 consecutive cycles of 30 seconds each), followed by centrifugation at 5,000 × g for
163 15 min at room temperature. The process was repeated until the lower fraction was
164 colorless, and the hexane phases were mixed. Astaxanthin content was determined
165 according to the following equation (Britton, 1995):

$$\frac{A \cdot V \cdot P}{\varepsilon}$$

166 where A is absorbance, V is the dilution volume (mL), and P and ε the molecular weight
167 and the molar absorption coefficient of astaxanthin (597 and 125100, respectively).

Powder water solubility. The water solubility was determined by a gravimetric method. Two hundred milligrams of powder was accurately weighed and dissolved in 10 mL of distilled water by agitation in a vortex. The mixture was then centrifuged (760 × g/10 min/20 °C) and a 9 mL aliquot was dried at 105 °C for 24 h. The water solubility was calculated according to the following equation:

Color. Sample color was measured as previously described (Gómez-Estaca, Calvo, Sánchez-Faure, Montero, & Gómez-Guillén, 2015) using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan). For liquid samples, a glass sample container was filled with 10 mL.

Bioaccessibility. Samples of both gels (G, G-M, LE-G, EM-LE-G, and EN-LE-G) and films (F, F-M, LE-F, EM-LE-F, and EN-LE-F) were subjected to *in vitro* simulated gastrointestinal digestion following the static model described by Gómez-Estaca, Gavara, and Hernández-Muñoz (2015), with minor modifications. The astaxanthin present in the bioaccessible fraction was measured as described above after liquid-liquid extraction of an aliquot in hexane. The bioaccessibility results are expressed in g bioaccessible astaxanthin/100g astaxanthin present before SGID.

Dynamic viscoelastic properties. A dynamic oscillatory study of the different gelatin solutions at 6.67 g/100 mL concentration was carried out on a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle 4°, gap 0.15 mm). Cooling from 25 to 2 °C and heating back to 25 °C, after 5 min of cold maturation at 2 °C, took place at a scan rate of 1 °C/min, a frequency of 0.5 Hz, and a target stress of 3 Pa. The elastic modulus (G′; Pa), viscous modulus (G″;

190 Pa), and phase angle (°) were plotted as functions of temperature. At least two
191 determinations were performed for each sample, with an experimental error of less than
192 6% in all cases.

193 **Gel strength.** The gelatin solutions were poured into flasks 3.7 cm in diameter and 6.2
194 cm in height and left to mature in a refrigerator at 2 °C for 16–18 h. Gel strength at 2–5
195 °C was determined on a TA.XT2i universal machine (Stable Micro Systems, Surrey,
196 UK) equipped with a 5 kg load cell and a flat-faced cylindrical plunger 1.27 cm in
197 diameter, at a cross-head speed of 1 mm/s. The maximum force (g) was determined
198 when the plunger had penetrated 4 mm into the gelatin gels. Analyses were performed
199 in triplicate.

200 **Film water solubility and water vapor permeability.** They were determined as
201 described by Gomez-Estaca, Montero, Fernandez-Martin, Aleman, and Gomez-Guillen
202 (2009a). Water solubility was expressed in % and water vapor permeability in kg
203 $m/m^2/s$ Pa. Both determinations were performed in triplicate.

204 **Fatty acid profile.** It was determined in triplicate in an Agilent 7820A gas
205 chromatograph with FID detector equipped with an Agilent HP-88 (ref. 112-8867)
206 column, after preparing fatty acid methyl esters as previously described (Gómez-Estaca
207 et al., 2017). Identification was accomplished by comparison of the retention times with
208 standards and results were expressed as relative percentage of total fatty acids. The
209 polyene index was calculated using the next equation:

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210 **Statistical analysis.** Statistical tests were performed using the SPSS® computer
211 program (SPSS Statistical Software, Inc., Chicago, IL, USA). Differences between pairs

of means were assessed on the basis of confidence intervals using the Tukey-b test. The level of significance was $p \leq 0.05$. Results have been expressed as means ± standard deviation.

RESULTS AND DISCUSSION

Bioactive functionality of the LES

The potential of the LES as a functional food ingredient was evaluated through the determination of its anti-inflammatory and antioxidant activities, either in the free or encapsulated form. The anti-inflammatory activity was evaluated using the RAW 264.7 cell line, which produces NO as a response to an induced stress. So, the lower the NO production (determined as NO_2), the higher the anti-inflammatory effect of the added compound. On the one hand, the non-encapsulated sample did not exert any anti-inflammatory effect (Table 1), this fact being attributable to inefficient exposition of the cells to the LES, owing to its low water solubility. To confirm this point, the experiment was repeated dissolving the LES in DMSO, showing in that case anti-inflammatory activity (data not shown). On the other one, when the LES was encapsulated, concentration ≥100 μg/mL in water significantly ($p \leq 0.05$) reduced the production of NO_2, as compared to a control sample without addition. The anti-inflammatory activity observed is largely attributed to efficient solubilization of the LES thanks to encapsulation (97% ± 3% solubility), in contrast to the non-encapsulated LES, which was not soluble. Figure 1 clearly depicts this effect. The main active components of the LES are poly-unsaturated fatty acids, α-tocopherol and astaxanthin (Gómez-Estaca et al., 2017), all of them have been related to the prevention of inflammation-related diseases (Calder, 2012; Raiola et al., 2015; Zhang et al., 2014).

235 The antioxidant activity of the LES is also shown in Table 1. Non-encapsulated LES did
236 not show antioxidant activity, being also attributable to inefficient solubilization. On the
237 contrary, encapsulated LES did show antioxidant activity, especially in hydrophilic
238 media ($p \leq 0.05$), owing to its efficient solubilization. Thus, the LES in soluble form
239 would be more available to exert its activity in aqueous media. Pan, Zhong, and Baek
240 (2013) encapsulated curcumin in a sodium caseinate matrix by spray-drying, resulting
241 in a significant improvement of curcumin's water solubility and antioxidant activity,
242 measured by the ABTS radical scavenging assay.

243 **Potential of the LES for aqueous food applications: water solubility and stability**

244 Discussing the potential of the LES for aqueous food applications, it can be seen in
245 Figure 1 that the encapsulated LES was completely dispersed in water both at 20 °C and
246 at 100 °C, producing solutions with a homogeneous, red color. This is attributed to the
247 high solubility of the microcapsules (97 % ± 3%). It should be noted, however, that
248 homogenization of non-encapsulated LES at 100 °C resulted in a better dispersion than
249 at 20 °C. No significant ($p > 0.05$) differences in astaxanthin content as a function of
250 either encapsulation or dissolution temperature were found (Table 2), indicating the
251 high stability of the molecule to the thermal treatments applied (spray-drying or
252 dispersion at 100 °C). Furthermore, changes in astaxanthin content were also not found
253 after 6 days of refrigerated storage (Table 2). With regard to color measurements of the
254 various samples, the most noticeable differences are the higher chromaticity and the
255 different hue among LE and EN-LE samples, irrespective of the dissolution temperature
256 or storage ($p \leq 0.05$). This is derived from the effective dispersion of LES in the EN-LE
257 sample and is in agreement with the astaxanthin content, which is responsible for the
258 characteristic red color obtained. No color changes were observed as a result of thermal
259 treatment (100 ºC), however, after 6 days of refrigerated storage, both luminosity and

hue were significantly modified ($p \leq 0.05$). This was not true for the sample processed at 20 ºC, the color of which was stable to refrigerated storage. In any case, differences were imperceptible to naked eye.

The fatty acid profile analysis is shown in Figure 3, showing that the most abundant fatty acids were C18:1n9c (oleic acid), C16:0 (palmitic acid), and C18:2n6c (linoleic acid), although the content of C22:6n3 (DHA) and C20:5n3 (EPA) is also noticeable. Only scarce differences as a function of encapsulation, dissolving temperature, or storage time were found. Nevertheless, the polyene index was significantly lower ($p \leq 0.05$) after refrigerated storage of non-encapsulated sample (LE), irrespectively of processing temperature, whereas this was not true for the encapsulated one (EN-LE). This is indicative of the development of somewhat lipid oxidation during refrigerated storage, which was prevented thanks to encapsulation. Dissolution temperature also had an effect on polyene index, resulting in significant lower values ($p \leq 0.05$) when samples were processed at 100 ºC, for both encapsulated and non-encapsulated samples. In any case, inclusion in the diet of the LES obtained in the present work will contribute to reduce not only the PUFA/SFA ratio but also the -6/ -3 ratio, which is nowadays estimated to range between 15 and 16.7 (Simopoulos, 2002).

Application of the LES to gelatin gels and films

The gelatin gels and films produced are shown in Figure 2. As can be seen from the photographs, the method of incorporation of LES influenced its distribution in both matrices and hence their macroscopic appearance. Thus, when the LES was just mixed in a magnetic stirrer (LE-G samples), a non-homogeneous distribution was achieved. This deficient distribution is reflected by the high variability in hue and chromaticity values in film samples (Table 4), but this variability was less evident in the gelatin gels

284 owing to the concentration of the lipids at the top of the gel. However, when LES was
285 homogenized with the Ultra-Turrax (EM-LE-G and EM-LE-F samples), an emulsion
286 was formed and thus both gelatin gels and films showed a uniform reddish coloration,
287 similar to that provided by the encapsulated LES (EN-LE-G and EN-LE-F samples).
288 The objective measure of color revealed significant differences ($p \leq 0.05$) between the
289 emulsified and the encapsulated samples, especially in the film samples. The EN-LE-F
290 film showed higher lightness and lower chromaticity than the EM-LE-F one, and hue
291 was also significantly different. The hue angle was also significantly different for the
292 gelatin gels ($p \leq 0.05$), but in this case the chromaticity was similar ($p > 0.05$).

293 Not only optical properties but also some other physicochemical properties may be
294 affected by the inclusion of additives in the gelatin matrices employed in the present
295 work. Among them are the rheological properties of the gels and the solubility and
296 water vapor permeability of the films. The dynamic viscoelastic properties of the gelatin
297 gels as a function of temperature are shown in Figure 3. It can be deduced from the
298 figure that all the formulations formed a gel, as G′ values increased upon cooling and
299 were considerably higher than G″ ones. The plain gelatin gel was the one that showed
300 the best viscoelastic properties, as it reached the highest G′ value at low temperature.
301 The phase angle revealed only small differences in the gelling and melting
302 temperatures; however, the plain gelatin gel gelled and melted at a slightly higher
303 temperature than the others, indicating higher thermostability. Furthermore, the results
304 obtained in the heating ramp indicate that all the gels were completely thermoreversible,
305 so irreversible bonds were presumably not formed as a consequence of incorporation of
306 the additives. The low G′ values and gelling and melting temperatures obtained are
307 characteristic of the type of gelatin employed, which was a commercial gelatin from the
308 skin of cold water marine fish (Gomez-Estaca, Montero, Fernandez-Martin, & Gomez-

309 Guillen, 2009b). The interference of the additives on the recovery of the original
310 structure of the collagen triple helix upon cooling is also not surprising, as it has
311 previously been described for gelatin gels with other additives (Gomez-Estaca, Gomez-
312 Guillen, Fernandez-Martin, & Montero, 2011; Gomez-Estaca et al., 2009a); however, it
313 is worth noting that the interference was not very marked and did not prevent gel
314 formation despite the low gelling ability of the gelatin employed. The gel strength,
315 which was measured after overnight gel maturation at 2 °C, is shown in Figure 4,
316 revealing no differences among the various gel samples analyzed ($p \leq 0.05$). Therefore,
317 the lower elastic modulus values observed in the study of viscoelastic properties for the
318 samples with additives did not lead to lower gel strength, suggesting that after cold gel
319 maturation the nucleation points initially formed in these samples had the ability to
320 grow, despite the presence of the additives.

321 With regard to the physicochemical properties of the films, with a view to their practical
322 application it is of great importance that they were completely soluble in water;
323 therefore, the release of antioxidants when applied to aqueous foods is guaranteed. The
324 reduction in the WVP of the gelatin film matrix, which was observed in the EM-LE-F
325 and EN-LE-F samples (Figure 5), is an additional beneficial property provided by the
326 inclusion of lipids in the formulation. The inclusion of edible oils in protein or
327 polysaccharide films is a possible strategy to reduce their water vapor permeability,
328 thanks to the increase in the hydrophobicity of the matrix (Pérez-Gago & Krochta,
329 2005). The WVP reduction in these samples, in comparison with the LE-F one, is
330 derived from the effective emulsion of the lipid extract in the matrices.

331 The astaxanthin bioaccessibility is shown in Table 4. Astaxanthin bioaccessibility was
332 ≈50% for both gelatin gels and films when the LES was mechanically stirred (LE-G and
333 LE-F samples). This means that only 50% of the total amount of astaxanthin added in

334 the food matrices would be available for intestinal absorption after gastrointestinal
335 digestion. When the LES was emulsified (EM-LE-G and EM-LE-F samples),
336 bioaccessibility improved significantly ($p \leq 0.05$), but the increase was even higher
337 when it was added in encapsulated form ($p \leq 0.05$). It is worth noting that astaxanthin
338 bioaccessibility was 100% when added to the gelatin gel in encapsulated form, meaning
339 that all the astaxanthin added would be available for intestinal absorption. Anarjan, Tan,
340 Nehdi, and Ling (2012) also succeeded in increasing astaxanthin solubility, antioxidant
341 activity, and bioavailability when it was nanostructured in various colloidal systems.
342 Zhang et al. (2015) encapsulated lutein by spray-drying, obtaining a powder that was
343 easily dispersed in water, also achieving an increase in lutein bioavailability when
344 evaluated *in vivo*.

345 **CONCLUSIONS**

346 The lipid extract from shrimp waste is a promising food ingredient because of its
347 composition, anti-inflammatory and antioxidant activities, coloring capacity, and
348 relative stability to thermal treatment and refrigerated storage. Furthermore, when
349 encapsulated by spray-drying, its functional properties were significantly improved;
350 being especially remarkable the water solubility, the antioxidant and anti-inflammatory
351 activities and the astaxanthin bioaccesibility, which increased up to 100%. The extract
352 may be incorporated in the formulation of a wide variety of food products including
353 soups, sauces and meat or fishery products, with bioactive and technological
354 functionalities.

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459 **Captions**

460 Figure 1. From left to right: lipid extract dispersed at 100 °C, lipid extract dispersed at
461 20 °C, encapsulated lipid extract dispersed at 100 °C, and encapsulated lipid extract
462 dispersed at 20 °C.

463 Figure 2. Gelatin gels (above) and films (below) developed. G/F: gelatin control; G-
464 M/F-M: gelatin and maltodextrin control; LE-G/LE-F: gelatin with lipid extract; EM-
465 LE-G/EM-LE-F: gelatin with emulsified lipid extract; EN-LE-G/EN-LE-F: gelatin with
466 encapsulated lipid extract.

467 Figure 3. Elastic modulus (G′, Pa), viscous modulus (G″, Pa), and phase angle (°) of
468 gelatin solutions during cooling (left) and heating (right) ramps. G, circles; G-M, dots;
469 LE-G, inverse triangles; EM-LE-G, squares; EN-LE-G, triangles.

470 Figure 4. Gel strength (g) of the gelatin gels developed. G: gelatin control; G-M: gelatin
471 and maltodextrin control; LE-G: gelatin with lipid extract; EM-LE-G: gelatin with
472 emulsified lipid extract; EN-LE-G: gelatin with encapsulated lipid extract. Different
473 letters indicate significant differences.

474 Figure 5. Water vapor permeability (kg m/m^2/s Pa) of the gelatin films developed. F:
475 gelatin control; F-M: gelatin and maltodextrin control; LE-F: gelatin with lipid extract;
476 EM-LE-F: gelatin with emulsified lipid extract; EN-LE-F: gelatin with encapsulated
477 lipid extract. Different letters (a, b) indicate significant differences.

478

479

480 Table 1. Anti-inflammatory and antioxidant activities of non-encapsulated and
481 encapsulated lipid extract.

482 Table 2. Optical properties (lightness,L*; hue, h°; and chroma, C*) and astaxanthin
483 content (mg/100 mg lipid extract) of non-encapsulated (LE) and encapsulated (EN-LE)
484 lipid extract dissolved in water at 20 °C or 100 °C, at day 0 and after 6 days of
485 refrigerated storage (5 ± 1 °C).

486 Table 3. The most abundant fatty acids (% of total fatty acids) found in the non-
487 encapsulated and encapsulated lipid extract dissolved in water at 20 °C or 100 °C, at
488 day 0 and after 6 days of refrigerated storage (5 ± 1 °C).

489 Table 4. Optical properties (lightness, L*; hue, h°; and chroma, C*) and astaxanthin
490 bioaccesibility (g/100 g astaxanthin) of gelatin gel and film samples. G/F: gelatin
491 control; G-M/F-M: gelatin and maltodextrin control; LE-G/LE-F: gelatin with lipid
492 extract; EM-LE-G/EM-LE-F: gelatin with emulsified lipid extract; EN-LE-G/EN-LE-F:
493 gelatin with encapsulated lipid extract.

494

Table 1.

	Anti-inflammatory activity NO_2 (µM)			Lipophilic antioxidant activity ng trolox/µg LES	Hydrophilic antioxidant activity ng ascorbic acid/µg LES
	Control	10 µg LES/mL	100 µg LES/mL		
Non-encapsulated	42.42 ± 4.805a	45.17 ± 5.71a	42.14 ± 4.86b	ND	ND
Encapsulated	42.42 ±4.805a	38.94 ± 4.31a	27.51 ± 1.84b*	1.692 ± 0.381a	14.853 ± 0.465b

Different letters (a, b) in the same row, indicate significant differences ($p \leq 0.05$). An asterisk indicates significant differences among non-encapsulated and encapsulated samples. ND: not detected.

Table 2.

	Sample LE				Sample EN-LE			
	20 °C		100 °C		20 °C		100 °C	
	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
Astaxanthin	0.37±0.04a	0.38±0.05a	0.43±0.04a	0.41±0.05a	0.32±0.03a	0.31±0.04a	0.37±0.05a	0.35±0.04a
L*	52.5±5a	53.1±4.8a	49.1±2.7a	48.3±2.0a	32.2±0.0a	32.7±0.1a	32.0±0.0a*	34.0±0.1b
h°	30.6±6a	31.1±6.6a	33.8±2.9a	35.0±2.7a	47.7±0.2a	47.2±0.1a	47.6±0.2a*	50.3±0.2b
C*	1.8±1.5a	1.4±1.2a	2.7±1.1a	2.9±1.2a	12.0±0.0a	12.1±0.1a	11.7±0.0a*	12.6±0.0a

Different letters in the same row (a, b) indicate differences as a function of processing temperature for each sample and day of storage. An asterisk indicates significant differences as a function of storage time.

	Sample LE				Sample EN-LE			
	20 °C		100 °C		20 °C		100 °C	
Fatty acid	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6	Day 0	Day 6
C18:1n9c–oleic	19.7±0.1a*	19.2±0.1a	19.6±0.0a	19.7±0.1b	19.3±0.1a*	15.3±0.1a	19.3±0.1a	19.5±0.1b
C16:0–palmitic	16.6±0.0a*	17.3±0.1a	16.7±0.1a*	17.0±0.1a	17.0±0.1a	16.9±0.1a	17.1±0.1a*	16.8±0.0a
C18:2n6c–linoleic	14.9±0.1a*	14.4±0.1a	14.7±0.1a	14.7±0.1b	15.1±0.1a	15.3±0.1a	15.1±0.1a	15.3±0.1a
C22:6n3–docosahexaenoic	9.1±0.1a*	8.8±0.1a	9.0±0.0a	8.9±0.0a	8.9±0.1a	8.9±0.1a	8.8±0.1a	8.7±0.1a
C20:5n3–eicosapentaenoic	5.6±0.1a	5.4±0.1a	5.6±0.1a	5.5±0.1a	5.5±0.1a	5.5±0.0a	5.4±0.1a	5.4±0.1a
C18:0–stearic	4.8±0.0a*	5.1±0.1a	4.8±0.0a*	5.0±0.1a	5.0±0.0a	5.1±0.1a	5.0±0.1a	5.1±0.1a
C20:4n6–arachidonic	3.3±0.2a	3.1±0.1a	3.2±0.1a	3.2±0.0a	3.2±0.1a	3.1±0.1a	3.1±0.1a	3.1±0.0a
Polyene index[1]	0.885±0.006a*	0.820±0.005a	0.874±0.003b*	0.847±0.004b	0.847±0.005a	0.852±0.008a	0.830±0.003b	0.839±0.0.006a

Different letters in the same row (a, b) indicate differences as a function of processing temperature for each sample and day of storage. An asterisk indicates significant differences as a function of storage time.

[1](C22:6n3+C20:5n3)/C16:0

Table 4.

	Gelatin gels				Gelatin films			
	L*	h°	C*	Bioaccessibility (g/100 g)	L*	h°	C*	Bioaccessibility (g/100 g)
G/F	26.8±2.0a	74.8±1.7d	1.9±0.4a	-	87.7±2.3bc	161±5c	1±0a	-
G-M/F-M	27.9±0.6a	71.8±4.8d	1.5±0.4a	-	91.8±1.1d	152±3c	2±0a	-
LE-G/LE-F	29.2±.3.0a	33.2±3.6a	2.3±0.6a	57±1a	89.0±2.1cd	72±20b	6±3a	47±5a
EM-LE-G/EM-LE-F	32.9±2.6b	41.8±1.9b	8.6±1.7b	81±1b	77.7±2.1b	56±1a	31±4b	69±4b
EN-LE-G/EN-LE-F	34.0±1.7b	45.4±1.1c	8.6±0.8b	98±2c	82.4±0.8a	59±1a	23±2c	76±7b

Different letters (a, b, c, d) in the same column indicate significant differences ($p \leq 0.05$) among samples.

Figure 1

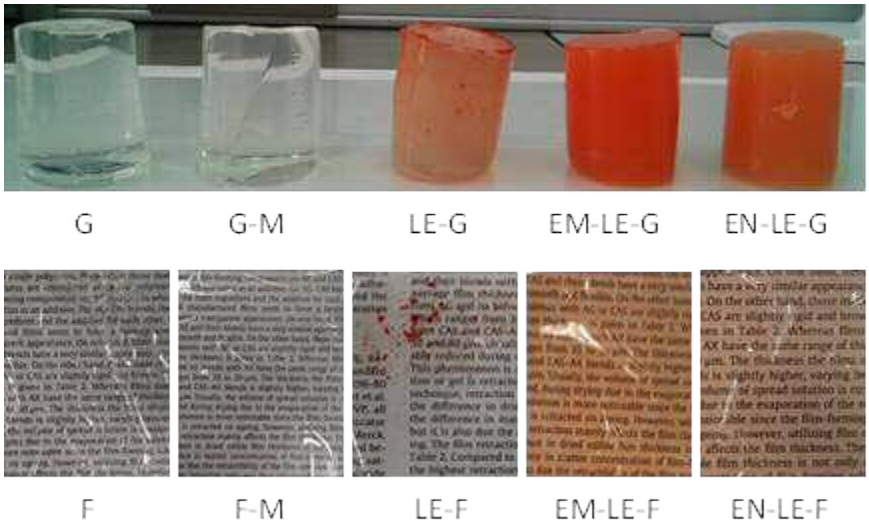

Figure 2

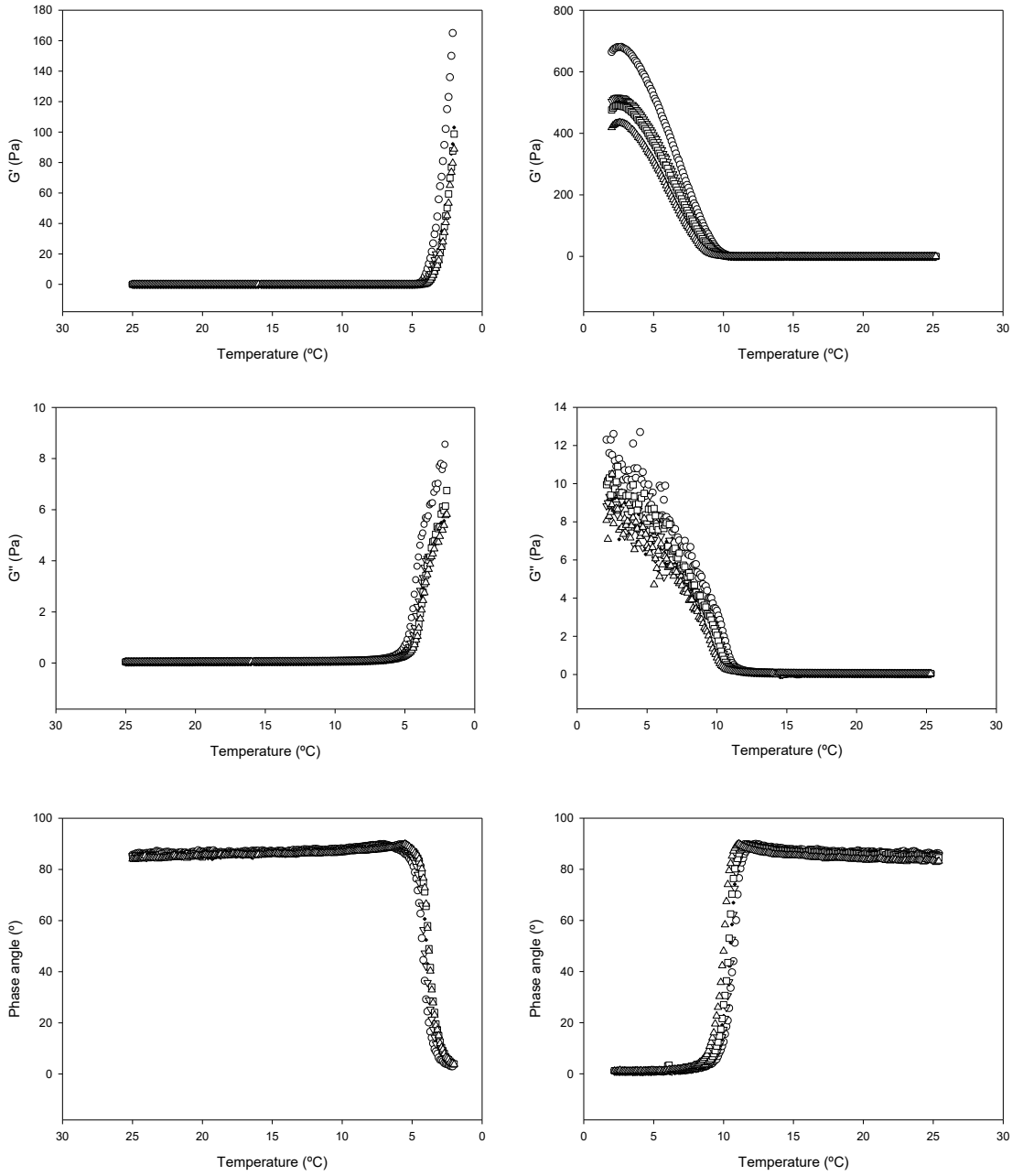
G' (Pa)
Temperature (ºC)
G'' (Pa)
Temperature (ºC)
Phase angle (º)
Temperature (ºC)

Figure 3

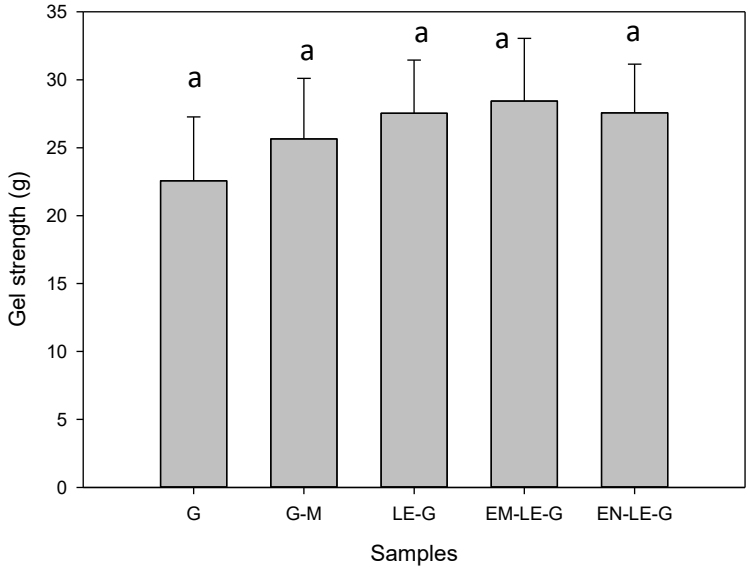
35
30
25
20
15
10
5
0
Gel strength (g)
a
a
a
a
a
G
G-M
LE-G
EM-LE-G
EN-LE-G
Samples

Figure 4

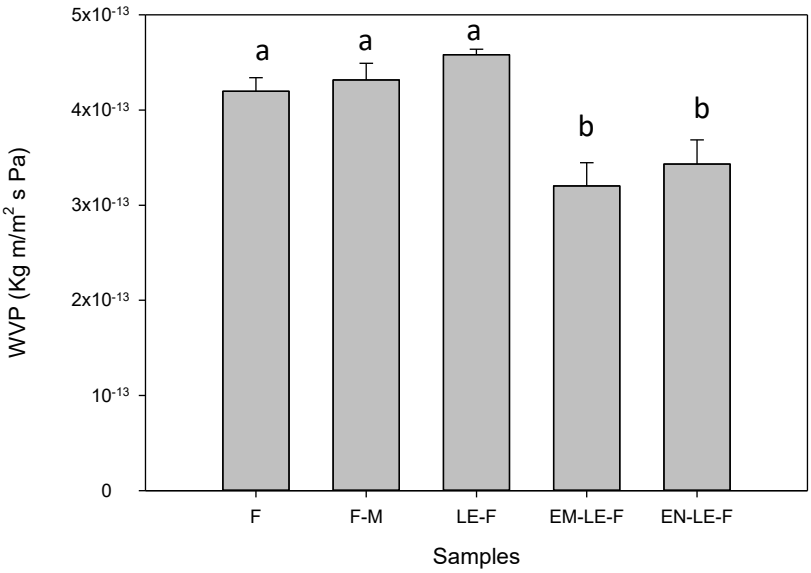
5x10-13
4x10-13
3x10-13
2x10-13
10-13
0
WVP (Kg m/m2 s Pa)
a
a
a
b
b
F
F-M
LE-F
EM-LE-F
EN-LE-F
Samples

Figure 5