Recent trends for the employment of jumbo squid (*Dosidicus gigas*) by-products as a source of bioactive compounds with nutritional, functional and preservative applications: A Review

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SUMMARY

Only 50-60% of total seafood catch is used for human consumption, seafood processing being considered as one of the main sources of by-products. Among marine species, jumbo squid (JS; *Dosidicus gigas*) represents the most important squid fishery, showing an increasing economic interest in many countries. As for any other marine species, the regular cleaning, dressing and processing produce high quantities of by-products (skin, head, fins, viscera, tentacles, unclaimed mantle etc.) that are rich in many nutrients (proteins, lipids, minerals, vitamins, enzymes, bio-polymers, etc.). This review compiles information about extraction and employment of JS by-products with the aim of enhancing their economic value and reduce environmental drawbacks. A special emphasis is given to the relevance in developing methods susceptible to transform by-products into useful and profitable products susceptible to be applied in several industries such as food, medicine, agrochemical or pharmaceutical. Future possible trends for widening this profitable use are mentioned.

Running head: Bioactive compounds from jumbo squid

Keywords: *Dosidicus gigas*; by-products; bioactive compounds; antimicrobials; antioxidants; gelatine; collagen; food preservation; human health

INTRODUCTION

Marine fish and invertebrate represent a valuable natural resource, either in the raw or in the processed form, because of their high protein content and being rich in long-chain highly unsaturated fatty acids (Simopoulos, 1997). Additionally, such species are reported to provide a profitable level of minor and trace elements such as minerals (I, F, Ca, Cu, Zn, Fe, Se, etc.) and lipid-soluble vitamins (namely, A and D) and constitute an important source of functional constituents susceptible to be incorporated in the human diet (Shahidi, 2003; Blanco *et al*., 2007).

Fishing is an ancient activity that has played an important role in the human society development. Nowadays, annual seafood captures contribute with about 100 million tons of fish and shellfish worldwide. Nonetheless, only 50 to 60% of the total catch is used for direct human consumption, seafood processing being considered as one of the main sources of by-products (Venugopal, 2009). Hence, a large and considerable volume of undesired products is obtained, constituting an important source of environmental contamination unless efforts for their recovery are attained (Arvanitoyannis & Kassaveti, 2008) and their commercial value can be enhanced via extraction of valuable constituents (Shahidi, 2007).

In general, different types and quantities of by-products are generated at different steps between capture and consumption of seafood (Rustad *et al*., 2011). Moreover, the anatomical features of species as well as both the harvesting and processing methods used determine the main type of by-products that can be obtained. The traditional marine by-products have mainly included fish meal and fish oil, these providing a convenient source of high-value proteins and lipids, respectively. Remarkably, seafood by-products are reported to also contain valuable and profitable components such as amino acids, enzymes, collagen, pigments, chitin, vitamins,

71 minerals and other bioactive compounds, which may be beneficial for the human health.
72 Furthermore, non-nutritional applications are nowadays also attracting great attention as
73 in the case of chitin and chitosan, leather, pharmaceuticals, cosmetics, fine chemicals,
74 collagen, gelatine and others (Kim & Mendis, 2006; Atef & Ojagh, 2017).

75 Among cephalopod species, giant or jumbo squid (JS; *Dosidicus gigas*)
76 represents the most important squid fishery in the world, accounting for 30% of global
77 volumes and about 4% of total squid market (FAO, 2016). Being the largest known
78 mollusc, this species, also called Humboldt squid, has captivated an increasing
79 economic interest in many countries such as Chile, Peru, China, Mexico, and Japan.
80 During its processing, high levels (up to 60% of whole weight) of by-products are
81 generated, which include heads, viscera, backbones or pens, ink, skin, fins, arms,
82 tentacles and unclaimed mantle (Figure 1). Interestingly, among the different anatomical
83 components regarded, only the beak and the pen can be considered as not edible. Thus,
84 since most of the JS body is not used, undesired products resulting from processing also
85 pose an environmental issue for this fishery, especially in areas where it is harvested the
86 most (Montaño Méndez *et al.*, 2015). Hence, great attention has been accorded in
87 converting the JS by-products into sources of bioactive compounds that could be
88 employed in human nutrition or non-nutritional applications (Kim, 2013). Information
89 regarding the most studied added-value compounds obtained from JS by-products is
90 given in Table 1.

91 This review provides information of possible uses concerning by-products
92 generated during JS processing. As for any other marine species, the regular cleaning,
93 dressing and processing produce high quantities of by-products that are rich in many
94 nutrients. The study has been focussed on the possibilities that the different anatomical
95 parts of the body have shown up to day related to the possible valorisation of discarded

biomass and led to profitable products for different industries such as food, medicine, agrochemical or pharmaceutical; thus, different valorisation strategies and goals developed for body parts different from the main one (i.e., mantle) have been compiled. Recently, all such activity has been found important to ensure the sustainability of JS resources and agree with the SFP's T75 initiative (SFP, 2017). Finally, possible improvements and future trends for this profitable use are presented and discussed.

BIOACTIVE COMPOUNDS FROM SKIN

Gelatine extraction, analysis and employment

JS skin has shown to be a good source of biopolymers as gelatine, collagen, collagen hydrolysates, as well as other compounds. Furthermore, the transformation of collagen into gelatine has shown to depend on several factors such as processing parameters, pre-treatments conditions, and preservation method applied to raw material (Johnston-Banks, 1990; Karim & Bhat, 2009).

Giménez *et al*. (2009a) analysed the physico-chemical and film-forming properties of giant squid gelatine. For it, inner and outer tunics were subjected to hydrolysis with pepsin prior to gelatine extraction (G1 gelatine) using a mild-acid procedure. Additionally, a second gelatine extraction (G2 gelatine) was performed using the collagenous residues that remained from the first extraction. As a result, G1 gelatine exhibited good gel forming ability, while G2 one showed poor viscoelastic behaviour and low gel strength. In spite of these differences, both gelatines showed good filmogenic ability and similar properties were found including the absence of colour, opacity, low-water vapour permeability and high-puncture deformation. Nevertheless, films made from G1 gelatine had a higher puncture force than films made from G2

120 gelatine as a result of a higher content of low-molecular weight components in G2
121 gelatine.

122 In a subsequent research (Giménez *et al*., 2009b), the effect of addition of
123 hydrolysates from giant squid gelatine on the antioxidant properties of gelatine films
124 prepared from giant squid skin was analysed. For it, gelatine hydrolysates were obtained
125 from gelatine by alcalase hydrolysis. Antioxidant properties of gelatine were highly
126 increased by hydrolysis, especially ABTS radical scavenging capacity, while the amino
127 acid composition closely resembled the amino acid composition of the parent gelatine.
128 Edible gelatine films with increasing percentages of gelatine hydrolysate (0-10%) gave
129 rise to increasing values in FRAP and ABTS assays, as well as changes in mechanical
130 properties (puncture force decrease and puncture deformation increase) and increase in
131 water-vapour permeability.

132 Gelatine obtained from giant squid inner and outer tunics was hydrolysed by
133 seven commercial proteases (Protamex, Trypsin, Neutrase, Savinase, NS37005,
134 Esperase and Alcalase) to produce bioactive hydrolysates (Alemán *et al*., 2011). As a
135 result, the Alcalase hydrolysate was found the most potent angiotensin-converting
136 enzyme inhibitor, while the Esperase hydrolysate showed the highest cytotoxic effect on
137 cancer cells. The radical scavenging capacity of gelatine increased approximately 3-fold
138 for Protamex, Neutrase and NS37005 hydrolysates and between 7 and 10-fold for
139 Trypsin, Savinase, Esperase and Alcalase hydrolysates. Furthermore, Trypsin, Savinase,
140 Esperase and Alcalase hydrolysates had a metal chelating capacity above 80%, whereas
141 Protamex, Neutrase and NS37005 hydrolysates registered a lower capacity than 25%.
142 Interestingly, the most active hydrolysates (from Alcalase and Esperase proteases) were
143 comprised mostly of peptides with molecular weights ranging from 500 to 1400 Da.

Uriarte-Montoya *et al*. (2011) extracted gelatine from giant squid skin with a yield of 7.5% (wet basis). Further analyses showed that skin gelatine had a high protein content (89%) with an amino acid profile similar to that of interstitial collagen. Infrared spectroscopy and circular dichroism confirmed the existence of specific bands of collagen and gelatine, which were modified during their thermal transition. Analysis of fluorescence spectroscopy revealed emission due to pyridinoline cross-links, while differential scanning calorimetry confirmed that skin gelatine was a weak thermo-reversible gel. Finally, scanning electron microscopy showed porous components within the skin gelatine structure, which agrees with the viscosity and water-holding capacity values obtained.

Research on preparation and molecular-weight distribution of gelatine hydrolysate from *D. gigas* was carried out by Mao *et al*. (2014). For it, four different kinds of protease were used to hydrolyse gelatine, and the antioxidant activity of gelatine peptide was measured. The optimal enzyme was alkaline protease, and the optimal hydrolysis conditions were as follows: 55 °C (temperature), 7000 U g^{-1} (enzyme concentration, enzyme/substrate), 109 min (time), 6% (substrate concentration) and 6.66 (pH). Under these conditions, the scavenging ability on the DPPH radical assay of gelatine peptide was up to 93.18%.

Nanoliposomes including hydrolysates prepared from collagen of JS were tested for their activity as angiotensin I-converting enzyme (ACE) inhibitors (Mosquera *et al*., 2016). For it, a fraction of peptides with molecular weights below 1 kDa, with reasonably high ACE-inhibitory activity (half-maximal inhibitory concentration IC_{50} = 0.096 g L^{-1}) was encapsulated in phosphatidylcholine nanoliposomes. As a result, liposomes containing ACE-inhibitory peptides were incorporated in fish gelatine without detriment to the rheological properties and thermal stability of the resulting

169 cold-induced gel. Additionally, the ACE-inhibitory activity of the peptide fraction,
170 which was not affected by the encapsulation process, conferred a marked bioactive
171 potential to the nanoliposome-containing gelatine gel.

172 Chemical and biochemical properties of gelatine from different by-products
173 (fins, arms, and skin) of JS were comparatively studied by Chan-Higuera *et al*. (2016).
174 Gelatine from skin showed the highest polar and imino amino acid contents and a
175 higher proline hydroxylation degree. These differences may explain the higher *in vitro*
176 digestion and higher antioxidant capacity (before and after digestibility) of the skin
177 gelatine. Fin gelatine decreased TAC-ORAC assay values, while all gelatines tested
178 decreased the malondialdehyde levels (antioxidant behaviour). It was concluded that JS
179 gelatine, administered during feeding, may have an inhibitory effect on the breakdown
180 of primary lipid oxidation compounds in serum.

181

182 Collagen extraction, analysis and employment

183 A wide range of studies have demonstrated that JS skin could be a profitable source of
184 collagen. Fu *et al*. (2013) isolated pepsin-soluble collagen from giant squid skin, the
185 physicochemical properties being subsequently determined. As a result, a maximum
186 absorbance at 220 nm was detected, while SDS-PAGE analysis suggested the collagen
187 containing alpha-1 and alpha-2 chains to be classified as type-I collagen. Amino acid
188 composition indicated a lower amino acid content than that of mammalian collagen.
189 Denaturation temperature of the pepsin-soluble collagen was 26.8 °C, while a relatively
190 high solubility in alkaline condition or NaCl concentrations below 2% was observed.
191 Furthermore, FTIR spectroscopy investigation showed the existence of helical
192 arrangements of collagen and a uniform and regular network structure. Results indicated
193 that giant squid skin would provide an interesting source of collagen.

Basic characterisation of acid-soluble collagen from giant squid skin was carried out by Deng *et al*. (2015). Results showed that the hydroxyproline content of this collagen was 8.03%. Its analysis by SDS-PAGE revealed that it consisted of two kinds of alpha-chains (alpha-1 and alpha-2) at least, and was characterised as type-I collagen. Furthermore, acid-soluble collagen showed a maximum absorption of UV at 228 nm and the FT-IR spectra indicated the existence of a triple-helical structure. The thermal denaturation temperature was 32.0 °C, which was a little higher than for acid-soluble collagen from common fish skin. The maximum relative solubility was at pH 2.0, while the minimum was at pH 6.0, which showed 100% and 8.57% values, respectively. Giant squid was found as a useful raw material for extracting acid-soluble collagen.

A hydroxylysyl-pyridinoline study of collagen from skin and mantle JS was carried out by Ramírez-Guerra *et al*. (2015). As a result, muscle collagen showed a higher content in glutamic acid, arginine and glycine, but lower in hydrophobic amino acids when compared with skin collagen. Lysine hydroxylation (%) was higher in muscle collagen (46.9±4.0) than in skin collagen (23.4±1.7). Carbohydrate (i.e., arabinose, glucose and xylose) content was similar for both collagens; interestingly, mannose and galactose were found only in muscle collagen. FT-IR analysis suggested major supra-organisational rearrangement in muscle collagen than in skin collagen, through presence of more stable triple-helix structures associated to higher contents on glycine, hydroxylysine, polar amino acids and carbohydrate.

Due to the intrinsic biological characteristics of collagen, JS skin collagen was investigated in a variety of medical applications. Cai *et al*. (2015a) explored its effect on enhancing the function of anti-damage in osteoblast cells (MC3T3-E1). For it, MC3T3-E1 cells were randomly divided into three groups, i.e., two of them treated with H_2O_2 and collagen, respectively, and a third one was the control. Compared with the H_2O_2

219 group, superoxide dismutase activity improved and malondialdehyde content decreased
220 in collagen group; at the same time, the JS collagen treatment also showed the ability to
221 decline the rate of osteoblast apoptosis and percentage of cells during the G0-G1 period
222 and to increase the percentage of cells during the S period. The expression of Bax was
223 weakened while the human stress protein was improved. It was concluded that H_2O_2
224 treatment would cause oxidation injury and apoptosis in MC3T3-E1 cells, but collagen
225 treatment proved the ability to repair the damage.

226 In a subsequent study by the same authors (Cai *et al*., 2015b), the effect of *D.*
227 *gigas* collagen peptide on enhancing the function of anti-osteoporosis in MC3T3-E1
228 cells was investigated. For it, MC3T3-E1 cells were randomly divided into three groups,
229 i.e., two of them treated with a cadmium-derivative compound and collagen,
230 respectively, and a third one was the control. It was concluded that cadmium would
231 cause injury and apoptosis in MC3T3-E1 cells, but collagen peptide had the ability to
232 enhance the anti-osteoporosis activity.

233

234 Extraction, analysis and application of lipophilic compounds

235 Pigments constitute a natural compounds group with commercial interest that can be
236 obtained from JS skin. Thus, pigment compounds obtained from JS skin with acetic
237 acid-ethanol extraction were characterised by Aubourg *et al*. (2016). As a result,
238 solubility behaviour, UV-Vis, and FT-IR spectra of the skin extract suggested that this
239 pigment might belong to the ommochrome family, thus showing a characteristic
240 xanthommatin peak (1740 cm^{-1}). Furthermore, the squid skin extract exhibited
241 scavenging activity on ABTS radical and in the ORAC assay. On the basis of a heated
242 (15, 25 and 50 ºC) cod-liver oil system (Aubourg *et al*. 2016), a marked inhibitory effect
243 on peroxide and thiobarbituric acid reactive substances formation was implied.

Additionally, an important polyene index drop could be observed in control samples corresponding to 50 ºC heating (Figure 2); interestingly, this loss was greatly inhibited in oil samples corresponding to conditions including the highest contents of skin extract tested (i.e., C-2, C-3 and C-4). As a conclusion, this extract was identified as a promising source of antioxidants to retard fish lipid oxidation.

An aqueous solution of acetic acid-ethanol extract of JS skin was tested as icing medium during the chilled storage of Atlantic mackerel (*Scomber scombrus*) (Ezquerra-Brauer *et al*., 2016). An important inhibition of trimethylamine formation was observed for the 10-13-day period in fish preserved with the icing medium with the highest content of squid skin extract tested (i.e., C-3 condition; Figure 3). In agreement with this result, a microbial activity decrease (aerobes, psychrotrophs, Enterobacteriaceae, proteolytics and lipolytics counts) was recorded in the same fish batch; furthermore, sensory analysis revealed that chilled mackerel preserved in this icing medium was still acceptable after 13 days of storage, while all other mackerel batches were found rejectable. A marked and profitable microbial activity inhibition was concluded by the presence in ice of lipophilic compounds obtained from the JS skin.

In a subsequent study, the same extract was tested as icing medium during the chilled storage of a lean fish species (European hake, *Merluccius merluccius*) (Ezquerra-Brauer *et al*., 2017). An inhibitory effect on lipid hydrolysis development (days 3 and 10; Figure 4) could be observed in fish specimens stored under the icing condition with the highest squid skin extract presence tested (i.e., C-2 batch; Figure 4), while no effect was depicted for lipid oxidation. Additionally, inhibition of microbial activity (microbial and chemical parameters) and shelf-life extension (raw and cooked descriptors) were obtained in hake preserved in C-2 batch.

The same lipophilic extract was included in the glazing system applied to frozen Atlantic chub mackerel (*Scomber colias*) (Ezquerra-Brauer *et al*., 2018). An inhibitory effect of skin extracts on lipid hydrolysis (free fatty acid formation) evolution was observed; furthermore, lower average values for lipid oxidation indices were observed in fish samples corresponding to the highest presence of the JS skin in the glazing system. Sensory quality enhancement was evident in mackerel as a result of including squid extracts in the glazing medium. The lipophilic extract showed promising antioxidant properties that could be applied to enhance the seafood quality during the commercialisation under frozen conditions.

BIOACTIVE COMPOUNDS FROM HEPATOPANCREAS

Research has been carried out on the extraction and possible employment of enzymes present in giant squid tissues. With this aim, the hepatopancreas has been the main target, being proteases reported as the main group of enzymes based on their commercial applications (Ezquerra-Brauer *et al.*, 2002). Like in other marine organisms, the activity of the enzymes (namely, trypsin, chymotrypsin, aminopeptidase, and carboxypeptidase) detected in hepatopancreas extracts from JS showed to be affected by the season of capture.

Thus, Cárdenas-López and Haard (2005) investigated the cysteine proteinase activity in hepatopancreas from JS. It could be observed that proteinase activity remained at least at 60% of the original value after 45 h at 4 ºC in the pH range of 3-8. Furthermore, activity was inhibited 70–85% when extracts were treated with cysteine proteinase specific inhibitors. The proteinases extracted from JS hepatopancreas showed to be mainly of the cysteine type and had significant activity towards a cathepsin L specific substrate.

Later on, the same authors identified a cysteine proteinase from JS hepatopancreas (Cárdenas-López & Haard, 2009). Thus, the molecular weight of the proteinase was 24 kDa in agreement with the SDS-PAGE analysis and 23.7 kDa taking into account the mass spectrometry study. The activity showed an optimum pH of 4.5 and optimum temperature of 55 °C under the assay for cathepsin L specific synthetic substrate Z-PAAFC; contrary, the cathepsin B and H specific synthetic substrates Z-AAAFC and H-AMC did not show any hydrolysis with the partially purified enzyme. Peptide mapping of trypsin digests of the 24 kDa band from SDS-PAGE showed the squid cysteine proteinase was homologous to cathepsin L from different animal sources. The activity of the partially purified fraction with the cathepsin L specific substrate Z-PAAFC was inhibited 75-89% by enzyme inhibitors specific for cysteine proteinases but was also inhibited by serine and aspartate proteinase inhibitors.

An aminopeptidase was extracted and partially purified from JS hepatopancreas by Osuna-Ruiz *et al*. (2010) with 154.24-fold and yield of 6.15%. The enzyme molecular weight was approximately 48-53 kDa as estimated by SDS-PAGE analysis. With L-leu-p-NA, it had optimum activity at pH 8.0 and 30 °C. The Km and V_{max}/K_m values of the enzymes for L-leu-p-NA were 0.326 mM and 2787 at 37 °C, respectively. The aminopeptidase showed activity against seven synthetic substrates according to the following decreasing sequence: L-proline–p-NA > L-methionine–p-NA > acid L-gamma-glutamic–p-NA > L-glycine–p-NA > L-leucine–p-NA > L-alanine–p-NA > L-lysine–p-NA. The enzyme was strongly inhibited by bestatin, partially inhibited by a metal-chelating agent and by a cysteine protease inhibitor. Zn^{++} and/or Ca^{++} seemed to be its metal cofactor(s). Interestingly, incubation of casein with the partially purified aminopeptidase resulted in a degree of hydrolysis of 6%.

317 Márquez-Ríos *et al*. (2016) purified chymotrypsin from JS hepatopancreas with
318 2.4-fold and yield 1.9%, and characterised its molecular weight with a 31 kDa value,
319 according to the SDS-PAGE analysis. Furthermore, chymotrypsin effect over collagen
320 extracted from the mantle, fins and arms of JS was evaluated. The enzyme exhibited the
321 maximum activity at pH 7 and 65 °C using Suc-Ala-Ala-Pro-Phe-p-nitroanilide as a
322 substrate and it was identified using the specific inhibitors N-tosyl-L-
323 phenylalaninechloromethyl ketone and phenyl methyl sulfonyl fluoride, showing
324 residual activities of 6% and 0%, respectively. Furthermore, a high activity was
325 observed in the pH range of 4.0 to 8.0. The purified enzyme showed a moderate *in vitro*
326 activity using muscle collagen as a substrate. Results suggested that the enzyme had a
327 potential application where acidic or slightly alkaline conditions are needed.
328
329 **BIOACTIVE COMPOUNDS FROM FINS, TENTACLES, ARMS, AND HEADS**
330 Torres-Arreola *et al*. (2008) investigated the content and physical and chemical
331 properties of pepsin-soluble and insoluble collagen from two different by-products (i.e.,
332 fins and tentacles) of giant squid and compared it to the mantle tissue values. It was
333 observed that tentacles had the highest concentration of insoluble collagen.
334 Furthermore, analysis by scanning electron microscopy analysis showed the same
335 structural properties in soluble collagens from the three anatomical parts studied, but
336 different structural properties for the insoluble collagens. Differential scanning
337 calorimetry analysis showed that the soluble collagen had a very high transition
338 temperature (115-120 °C), while the highest T_{max} and ΔH values were measured in
339 tentacle collagen. A profitable employment of both by-products in commercial
340 processing was concluded.

Rocha-Estrada *et al*. (2010) investigated the properties of mantle and fin tissues proteins of JS by microscopic analysis of muscle fibre and SDS-PAGE of protein profiles. Thus, fins showed a higher content of connective tissue and a complex fibre arrangement, while their gels were harder than those corresponding to mantle. Myosin-heavy chains were reported to be found in sarcoplasmic, myofibril and soluble-in-alkali fractions of mantle; contrary, fin tissue only included such chains in sarcoplasmic and soluble-in-alkali fractions. Fin and mantle proteins yielded similar results in solubility tests, but differences occurred at specific pH and concentrations of salt. It was concluded that high-strength gels were formed both from squid mantle or fin muscles, although fin displayed similar or better properties than mantle in tests corresponding to functional properties.

The potential application of acid-soluble collagen (ASC) in the preparation of biofilms in composites with commercial chitosan was investigated by Arias-Moscoso *et al*. (2011). For it, JS by-products (heads, tentacles and skin) were checked as collagen source. As a result, ASC led to a structure with a less compact morphology than chitosan films and to an increase in the percentage of elongation at break and a decrease of the elastic modulus of films. Contrary, the addition of ASC to chitosan films had a negative effect on the water-barrier properties.

The comparative effect of 25 and 50 g kg^{-1} of lyophilised JS fin and mantle muscles on dough properties and baking performance of wheat flour was studied by Ramírez-Suárez *et al*. (2012). Fin muscle (25 g kg^{-1}) almost triplicated dough maximum resistance compared to control dough, while fin or mantle muscle (50 g kg^{-1}) doubled it. As animal protein increased on blend, extensibility decreased. Both fin or mantle muscle (25 g kg^{-1}) increased 2.4 and 1.8 times the control area, respectively. Addition of 50 g kg^{-1} of fin or mantle muscle affected specific loaf volume, so that a decrease was

366 produced. Sensory results showed that a low level powder addition (25 g kg^{-1}) could be
367 used for bread production.

368 The use of JS by-product (i.e., head, tentacles, and skin) hydrolysates obtained
369 by acid-enzymatic hydrolysis and by autohydrolysis as ingredients in practical diets for
370 shrimp was evaluated (González-Félix *et al*., 2014); for it, the hydrolysates were
371 included at levels of 2.5 and 5.0% (diet dry wt.). As a result, sensory analysis of cooked
372 shrimp muscle showed profitable differences for all variables evaluated (colour, odour,
373 flavour and firmness), so that a profitable effect of inclusion of current hydrolysates
374 from JS by-products into shrimp diets was obtained without affecting growth or
375 survival.

376 Arias-Moscoso *et al*. (2015a) studied the physicochemical characteristics of
377 protein hydrolysates of JS by-products (skin, head, and fins) produced by endogenous
378 proteases at two different pH values (5.0 and 7.0). As a result, the level of hydrolysis
379 increased from 3.5 to 11.2 % at pH 5.0 and from 4.8 to 17.5 % at pH 7.0. Both pH
380 treatments exhibited similar degradation patterns with progressive proteolysis and, after
381 120 min of hydrolysis, yielded hydrolysates that contained molecular masses below 45
382 kDa. A lower hydrophobic amino acid exposure for the protein hydrolysates prepared at
383 pH 5.0 was detected when compared with the hydrolysates corresponding to pH 7.0.

384 JS hydrolysates obtained by autolysis without addition of lactic acid at two
385 different pH (5 and 7) and included at 25 and 50 g kg^{-1} concentrations in a commercial
386 (indoor and outdoor conditions) shrimp (*Litopenaeus vannamei*) feed were evaluated by
387 Arias-Moscoso *et al*. (2015b). Diets containing hydrolysates from squid by-products
388 (i.e., skin, head, and fins) at both concentration levels caused a higher feed consumption
389 by shrimp. In general, shrimp fed on both kinds of hydrolysates, but particularly on that
390 prepared at pH 7, exhibited similar or better production responses (survival, biomass,

391 feed conversion ratio, and specific growth rate) compared to those fed on diets without
392 the inclusion of hydrolysates. Shrimp cultured outdoor showed a better growth
393 performance compared to those cultured indoor. Results suggested that the free amino
394 acids provided by squid hydrolysates contributed to improve the feed consumption and
395 growth performance of shrimp cultured under both indoor and outdoor conditions.

396 Márquez-Álvarez *et al*. (2015) compared and evaluated the functional properties
397 of the protein concentrates obtained from squid fins *via* alkaline dissolution and
398 subsequent isoelectric precipitation and the conventional methodology (water washing
399 muscle). The electrophoretic profile of the alkaline concentrate showed that the myosin-
400 heavy-chain band disappeared, mainly due to the denaturation induced by alkaline
401 solubilisation or activation of alkaline protease. Concerning the quality of the gels, the
402 folding test showed that gels obtained using the alkaline concentrate were better than
403 those obtained using the conventional methodology, but a texture profile analysis
404 detected fracture in the alkaline-concentrate gels. Regarding the interfacial properties,
405 low emulsifying capacity was observed for both protein concentrates, the alkaline one
406 being also better. Additionally, the foaming properties were found satisfactory for both
407 concentrates, being better for the alkaline one.

408 Hydrolysates from two different JS by-products (namely, fins and arms)
409 produced by trypsin and protease were compared on the basis of their antioxidant,
410 antimutagenic and antiproliferative activities (Suárez-Jiménez *et al*., 2015). JS arms
411 showed higher content of collagen than fins, and their hydrolysates provided the highest
412 antioxidant activity. Additionally, arm-derived collagen hydrolysed with protease
413 showed the highest antimutagenic activity.

414 Luo *et al*. (2016) investigated the optimum parameters for enzymatic hydrolysis
415 of collagen from *D. gigas* tentacle and evaluated the antioxidant activity of the

corresponding hydrolysate. As a result, the optimal hydrolysis parameters were 50 °C, 3.2% (substrate/protein content), 3.7 h, 3000 U g^{-1} (enzyme dosage), and 7.4 (initial pH); such conditions led to a degree of hydrolysis value of 37.23±0.08 % and a scavenging rate of DPPH radical of 43.61±0.09 %. The half inhibitory concentrations for ABTS radical and hydroxyl radical scavenging ability (IC_{50}) of the hydrolysate obtained were 0.37 and 0.41 mg mL^{-1}, respectively, so that a profitable antioxidant activity was implied. Moreover, the product showed a strong reducing capacity. SDS-PAGE analysis reflected that most collagen was hydrolysed into small peptides with molecular weights of 1-5 kDa as determined by gel-exclusion chromatography.

The chemical structure, thermal denaturation and nanostructure of collagen obtained from mantle, fins and tentacles of JS were comparatively studied by Sarabia-Sainz *et al*. (2017). As a result, tentacles required a greater shear force and its collagen presented a higher temperature and enthalpy of transition than the mantle and fins. The tentacle firmness could be explained by the relatively higher imino amino acid content, proline and lysine hydroxylation degrees and pyroglutamic acid content of its collagen. Moreover, among the regions studied, the collagen from the tentacles showed a more intense beta-band chain. Also, the FT-IR and Raman spectra implied that the collagen in the tentacles was more intermolecularly ordered than its counterpart from mantle and fins and sustained a higher muscle firmness.

BIOACTIVE COMPOUNDS FROM INK AND VISCERA

The fatty acid composition of several *D. gigas* by-products tissues (digestive gland, testis, arms and integument) was analysed by Saito *et al*. (2014). As a result, docosahexaenoic acid showed to be the most abundant fatty acid in all tissue triglycerides while eicosapentaenoic and docosahexaenoic acids followed by

araquidonic one were found as the most abundant in phospholipid classes. Consequently, by-products were recognised as being healthfully and susceptible to be employed as sources of polyunsaturated fatty acids (PUFA).

In the search for value-added foods, the chemical composition of giant squid meal obtained from several body parts (viscera, pens, tentacles, mantle, and mouth) was studied (Calvo *et al*., 2016). Results indicated a high content on protein (77.7%), lysine and glutamic acid (10.16 and 14.53 g amino acid/100g protein respectively), sulphur amino acids and hydrophobic amino acids. The content of fat fraction (6.3%) was low and crude fibre (2.7%), reported as chitin, reflected a low caloric score (4 kcal g^{-1}). Concerning the fatty acids profile, the ratio of saturated, monounsaturated and polyunsaturated fatty acids was 1.66:1:1.08 and the n-6/n-3 ratio was 1:1.35. Giant squid meal was considered as a profitable ingredient with potential use in different kinds of foods such as bread, crackers, seasonings, and dressings.

The effect of giant squid ink polysaccharides on intestinal microflora community in mice was explored by Lu *et al*. (2016). For it, the mouse model was chemotherapy injured by cyclophosphamide injection. The results showed that squid ink polysaccharides had a positive role on the intestinal microflora in mice.

Replacement of fishmeal using viscera meals obtained from cooked-dried giant squid in the diet for white shrimp (*Litopenaeus vannamei*) was checked (Toyes-Vargas *et al*., 2017); its effect on growth, feed utilisation and muscle PUFA and sterol composition was assessed in a 45-day feeding trial. As a result, growth and feed intake were higher in shrimp fed diets containing squid meal. Furthermore, fatty acid profile of shrimp muscle was improved for human consumption when compared with the diet containing common fish meal.

BIOACTIVE COMPOUNDS FROM PENS AND CARTILAGE

The antibacterial activity of beta-chitosan from JS pens was studied by Jung and Zhao (2013) and compared to the activity of alpha-chitosan from shrimp shells at different degrees of deacetylation (DDA) and molecular weight. As a result, both forms of chitosan showed more inhibition against *E. coli* than against *L. innocua*. No difference against *L. innocua* could be outlined between the two forms of chitosan; however, a different level of antibacterial activity against *E. coli* was implied. Thus, 75% DDA/31 kDa beta-chitosan demonstrated higher inhibition (lower minimal inhibition concentration) than that of 75% DDA/31 kDa alpha-chitosan, whereas 90% DDA/74-76 kDa alpha-chitosan had a higher inhibition ratio than that of 90% DDA/74-76 kDa of beta-chitosan. A great interest on the functionality and potential for food preparations was signalled for beta-chitosan obtained from squid pen.

In a subsequent study, the same authors analysed the changes produced in beta-chitin extracted from JS pens as a result of alkali or acid treatments (Jung & Zhao, 2014). It could be observed that beta-chitin was converted into the alpha-form after 3 h in 40% NaOH or 1-3 h in 40% HCl solution, while alpha-chitin obtained from NaOH treatment had higher moisture absorption ability than had the native alpha-chitin; contrary, induced alpha-chitin from acid treatment of beta-chitin showed few polymorphic modifications, showing no significant changes in moisture absorption ability. It could be concluded that alkali- or acid-treated beta-chitin retained good biological activity for use as a natural antioxidant and antimicrobial substance for food applications.

D. gigas cartilage was explored as a new source of chondroitin sulphate (Li *et al*., 2016). After the purification process by enzymolysis, filtration and ethanol precipitation, chondroitin sulphate provided a yield about 3.2%, a specific rotation of –

491 25.2 ºC mL g^{-1} dm^{-1}, a viscosity-average molecular of 157000, and the E-type
492 disulphated disaccharides being 17% in all chondroitin sulphate extracts. It was
493 concluded that *D. gigas* cartilage could be employed as a profitable source of
494 chondroitin sulphate.
495
496 **FINAL REMARKS AND FUTURE TRENDS**
497 A large and considerable volume of undesired products are obtained as a result of JS
498 processing, constituting an important source of environmental contamination unless
499 efforts for their recovery are carried out and their commercial value can be enhanced via
500 extraction of valuable constituents. Recent results have expanded the utilisation of JS
501 by-products, showing their potential for different kinds of industries such as food,
502 medicine, agrochemical or pharmaceutical. Furthermore, new eco-friendly technologies
503 are constantly emerging, so that alternative applications for seafood by-products may
504 potentially produce significant revenues. However, before these options become a
505 feasible reality, there are still practical and commercial issues to be studied and
506 resolved. In order to develop the practical employment of JS by-products, several
507 aspects ought to be taken into account in the next years. With this aim in mind, the
508 following recommendations could be outlined:
509
510 *Quality of raw by-products*
511 As for any marine product, JS by-products can deteriorate rapidly on the basis of
512 different damage pathways. Consequently, on-board, in-land and post-harvest handling
513 should be carried out as carefully as possible. Furthermore, time elapsing between
514 preliminary handling and cooling steps should be minimised, while the cold chain ought

515 to be maintained till delivery of whole specimens or separated by-products into the
516 processing factory.
517
518 *Optimisation of processing conditions for each kind of by-product*
519 A different response can be expected to be produced according to the kind of JS by-
520 product available. Additionally, the kind of technological process (refrigeration,
521 heating, salting, etc.) to be applied for its further employment as well as the biological
522 aspects of specimens (capture season, maturity, sex, eating state, etc.) ought to be taken
523 into account. Processing conditions ought to be optimised in each case to enhance the
524 sensory and nutritional values, rather than extrapolating the findings made with other JS
525 by-product or any other marine by-product.
526
527 *Safety of by-products*
528 In general, public health concerns have become an issue requiring careful attention as
529 the major challenge faced by seafood trade and technologists. Before practical and
530 commercial utilisation, chemical composition of JS by-products ought to agree with
531 international regulations concerning health risks at every stage in the chain, from
532 primary handling till retail and consumer use. Similarly, microbial safety of by-products
533 should be guaranteed according to the corresponding regulations. Safety analyses of by-
534 products extracts ought to be complemented by clinical trials carried out on human
535 and/or animals.
536
537 *Detailed analysis of chemical changes*
538 In general, chemical changes occurring in processed seafood can have a decisive effect
539 on quality loss. Since most attention is normally accorded to the main product (in this

case the mantle), chemical modifications should also be considered especially important in by-products. This effect can be especially relevant when human digestibility and nutritional aspects are considered. In order to maintain the profitable activity of biomolecules included in by-products, the combination of traditional and advanced processing/storage conditions would be mandatory.

Source of feeds for aquaculture

Recent applications of JS by-products (namely, fins and arms) have focused on aquaculture feeding. Thus, protein hydrolysates obtained by endogenous enzymes have been used successfully in shrimp diets, according to the resulting improvement in organism production (survival, biomass, feed conversion ratio and specific growth). To increase the development of this issue, the possibility of employing all kinds of JS by-products ought to be checked. Furthermore, the possibility of applying a wider range of hydrolysation strategies ought to be analysed.

New and attractive products for consumer

By-products provide the possibility of offering the consumer novel and attractive ready-to-eat (RTE) products. The establishment of commercial seafood arising from JS by-products would require a full appreciation of the microbial, chemical and physical aspects and have to be considered in relation to the safety and nutritional value. Such future preparations ought to focus on the development of attractive products that fulfil the consumer's expectations for odour, colour, taste, flavour and general appearance. Interestingly, a great attention ought to be focused on possible interactions among food components when JS by-products are included as ingredient in a RTE food mixture such as bread, crackers, seasonings and dressings.

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758

FIGURE LEGENDS

Figure 1: Schematic drawing of jumbo squid anatomical regions susceptible to be employed as source of profitable and healthy compounds.

Figure 2: Polyene index assessment* in a heated (15, 25 and 50 ºC for 30 days) marine-oil system including different concentrations** of ethanolic jumbo squid extract.

* Average values of three replicates (n = 3); standard deviations are indicated by bars. Values accompanied by different letters indicate significant ($p < 0.05$) differences. Adapted from Aubourg *et al*. (2016).

** Concentrations tested of the squid extract increase in the sequence: C-1<C-2<C-3<C-4; C-0 represents the control (no squid extract present).

Figure 3: Trimethylamine-nitrogen content (mg kg^{-1} muscle)* in mackerel muscle submitted to an icing medium including different quantities of jumbo squid extract**.

* Average values of three replicates (n = 3); standard deviations are indicated by bars. Values accompanied by different letters indicate significant ($p < 0.05$) differences. Adapted from Ezquerra-Brauer *et al*. (2016).

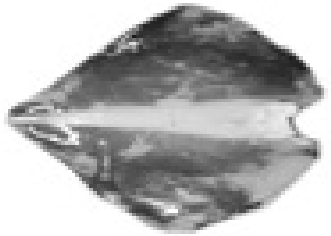
** Concentrations tested of the squid extract increase in the sequence: C-1<C-2<C-3; C-0 represents the control (no squid extract present).

Figure 4: Free fatty acids formation (mg kg^{-1} muscle)* in hake muscle submitted to an icing medium including different quantities of jumbo squid extract**.

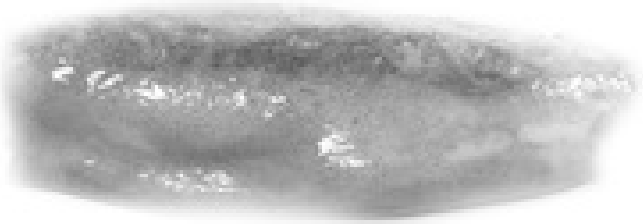
* Average values of three replicates (n = 3); standard deviations are indicated by bars. Values accompanied by different letters indicate significant ($p < 0.05$) differences. Adapted from Ezquerra-Brauer *et al*. (2017).

** Concentrations tested of the squid extract increase in the sequence: C-1<C-2; C-0 represents the control (no squid extract present).

FINS

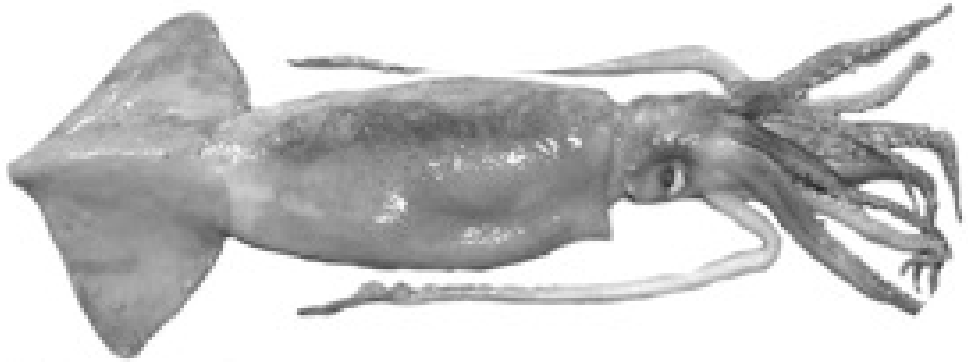


SKIN

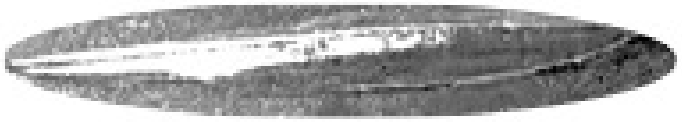


HEAD, TENTACLES AND ARMS

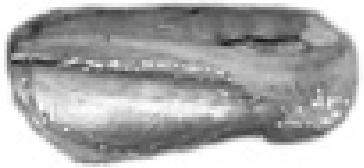
VISCERA



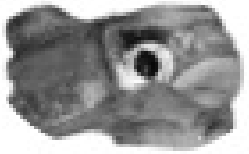
CARTILAGE/PENS



INK

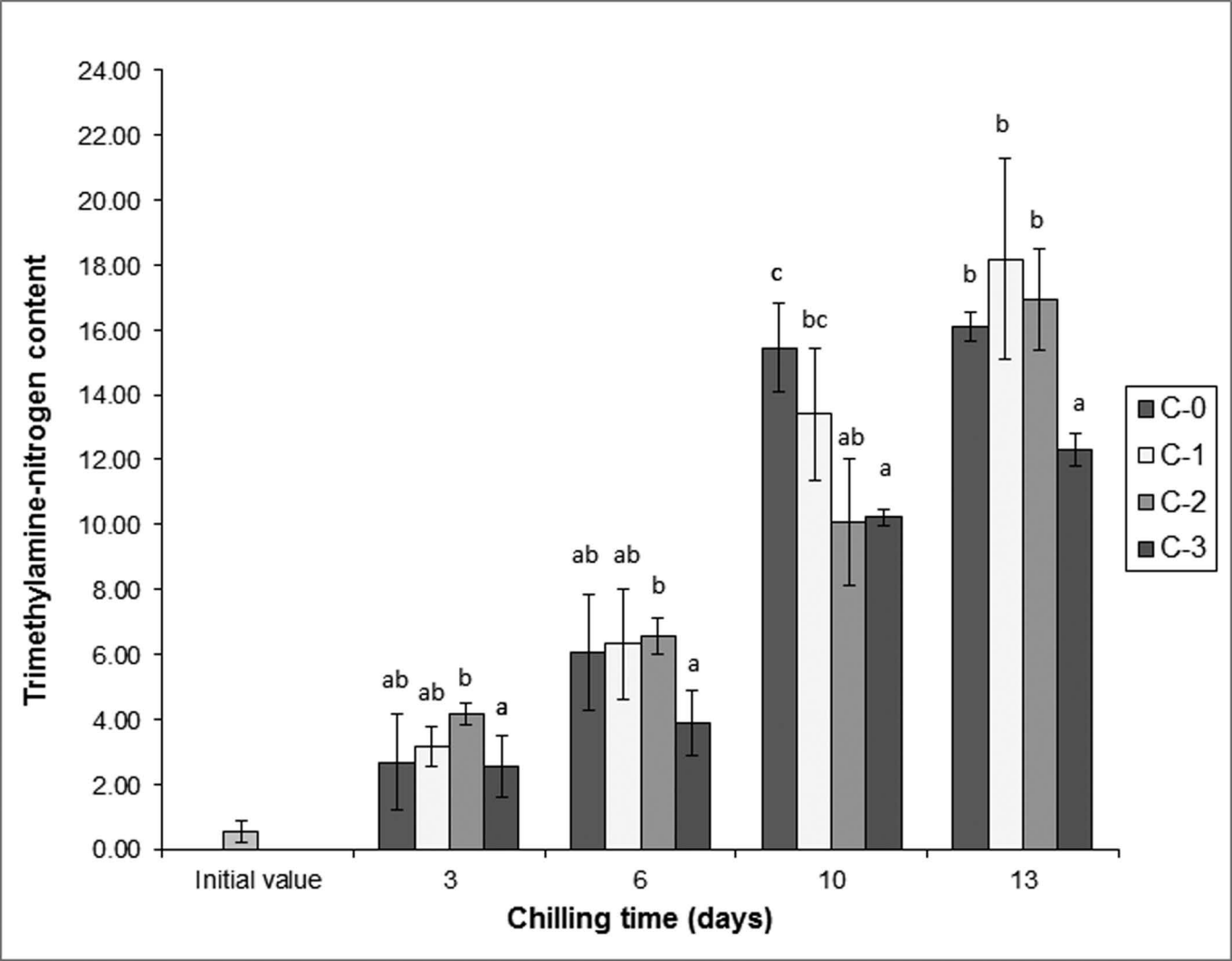


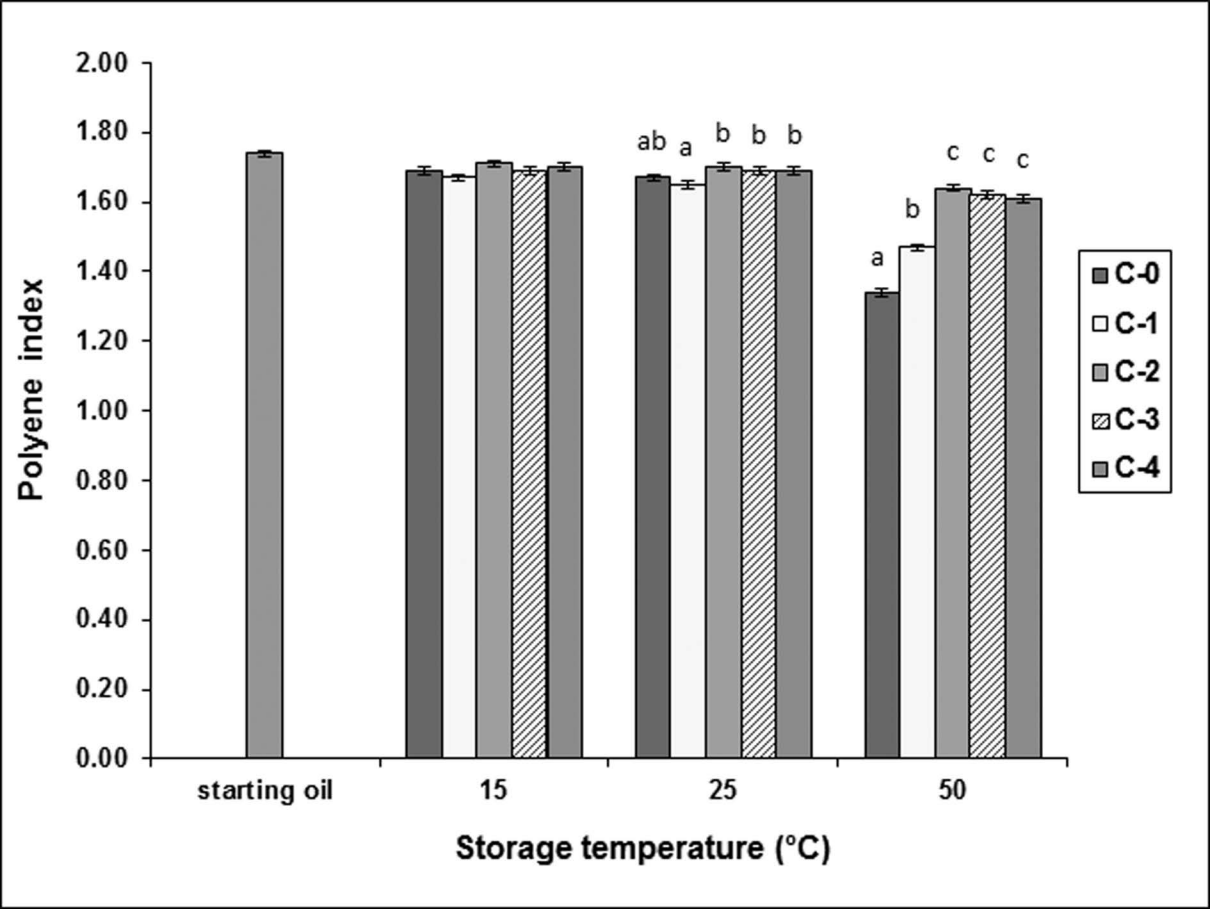
HEPATOPANCREAS



EYES

MOUTH


24.00
22.00
20.00
18.00
16.00
14.00
12.00
10.00
8.00
6.00
4.00
2.00
0.00
Trimethylamine-nitrogen content
ab
ab
b
a
ab
ab
b
a
c
bc
ab
a
b
b
b
a
Initial value
3
6
10
13
Chilling time (days)
C-0
C-1
C-2
C-3


2.00
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
Polyene index
ab a b b b
a b c c c
C-0
C-1
C-2
C-3
C-4
starting oil
15
25
50
Storage temperature (°C)

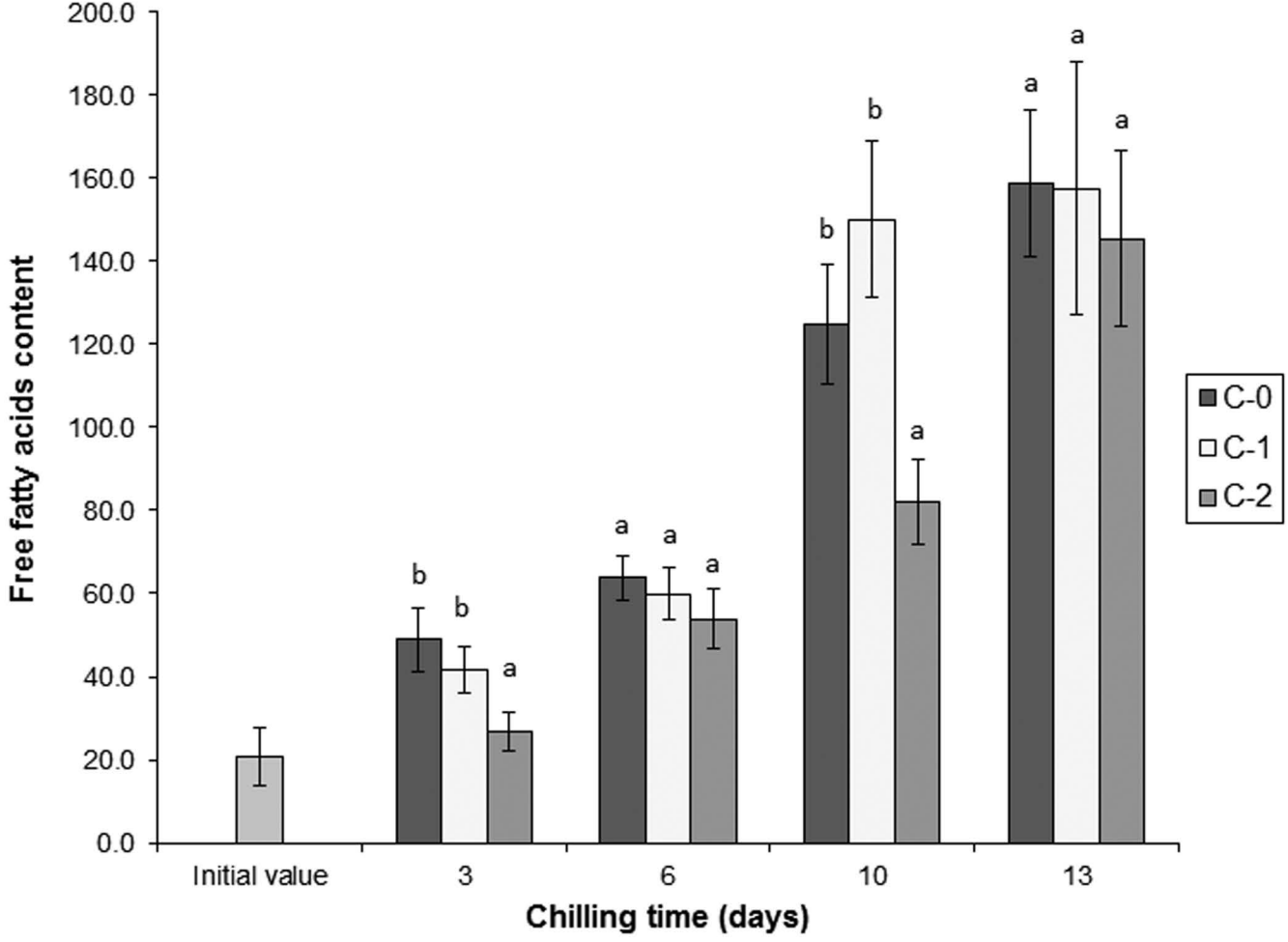

200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
Free fatty acids content
b
b
a
a
a
a
b
b
a
a
a
a
C-0
C-1
C-2
Initial value
3
6
10
13
Chilling time (days)

TABLE 1

Potential added-value compounds from jumbo squid by-products

Added-value compounds	By-product	Reference
Collagen and gelatine	Arms, fins, head, tentacles, skin	Chan-Higuera *et al.* (2016) Deng *et al.* (2015) Fu *et al.* (2013) Giménez *et al.* (2009a) Giménez *et al.* (2009b) Suárez-Jiménez *et al.* (2015) Uriarte-Montoya *et al.* (2011)
Bioactive compounds	Arms, fins, head, ink, skin, tentacles	Alemán *et al.* (2011) Cai *et al.* (2015a) Chan-Higuera *et al.* (2016) Lu *et al.* (2016) Mosquera *et al.* (2016)
Bio-plasticizer	Head, fins, tentacles, skin	Arias-Moscoso *et al.* (2011) Giménez *et al.* (2009a) Giménez *et al*. (2009b)
Chitin	Cartilage, pens	Jung & Zhao (2013) Jung & Zhao (2014) Li *et al*. (2016)
Eicosapentaenoic and docosahexaenoic acids	Digestive gland, testis, arms, integument	Saito *et al.* (2014)
Food additives	Arms, head, mouth, pens, viscera, pens, tentacles	Calvo *et al*. (2016) Rocha-Estrada *et al.* (2010) Ramírez-Suárez *et al.* (2012)
Pigments as preservative agents	Skin	Aubourg *et al.* (2016) Ezquerra-Brauer *et al*. (2016) Ezquerra-Brauer *et al.* (2017) Ezquerra-Brauer *et al.* (2018)
Proteases	Hepatopancreas	Cárdenas-López & Haard (2005) Ezquerra-Brauer *et al.* (2002) Márquez-Rios *et al.* (2016) Osuna-Ruíz *et al.* (2010)
Protein concentrates	Fins	Márquez-Álvarez *et al*. (2015)
Replacement fish meal	Arms, head, tentacles, viscera	Arias-Moscoso *et al.* (2015b) González-Félix *et al*. (2014) Toyes-Vargas *et al.* (2017)