Chemical characterization of wash water biomass from shrimp surimi processing and its application to develop functional edible films

Joaquín Gómez-Estaca¹ · Pilar Montero¹ · M. Carmen Gómez-Guillén¹

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Abstract The aim of this work was to recover and study the composition of the biomass obtained from the surimi processing industry, and to explore the feasibility of its valorization by developing functional edible films. A concentrate from wash water of minced shrimp muscle of low commercial value [shrimp concentrate (SC)] was obtained by alkaline solubilization and isoelectric precipitation, which had protein content of 61.8% and fat 23.6%. The protein fraction of low molecular weight (≤ 37 kDa), with maximum solubility at basic pH 9–11, and was rich in essential amino acids (296 essential amino acids/1000 residues), whereas the fat fraction showed a high degree of unsaturation (26.8% MUFA, 28.3% PUFA). SC, showed antioxidant activity measured by FRAP and ABTS assays and, was successfully incorporated into an agar film matrix (agar-to-protein ratio of 3:1). The films were translucent and brownish and maintained integrity upon immersion in water, but swelled considerably. Films were able to release protein and exert antioxidant activity in water and 95% ethanol (aqueous and fatty food simulants, respectively), the release being faster and greater in the former. In conclusion, the biomass obtained from shrimp mince/surimi processing was for developing edible films.

Keywords Edible film · Antioxidant · Shrimp · Soluble protein · Valorization

Introduction

Shrimps are much-appreciated, high-value fishery products worldwide, and particularly in Spain. In the food industry it is not uncommon to throw away shrimp shipments because of extensive melanosis or long periods of frozen storage, among other causes. The recovery of the muscle protein of these shipments by washing to produce wash mince and/or surimi may be a way to give value to this waste for the production of restructured seafood products. Furthermore, cephalothorax also contains a number of compounds with interest from a technological or nutritional point of view, such as carotenoids and polyunsaturated fatty acids that can be valorized (Gómez-Estaca et al. 2017). To produce washed muscle mince or surimi, large amount of water is used to remove water-soluble substances such as sarcoplasmic proteins, pigments, amines, vitamins, and enzymes in order to concentrate myofibrillar proteins (Montero and Gómez-Guillén 1998). As a result of these washing processes, considerable amounts of organic matter are discarded into waste streams, constituting a huge contaminating load. For both ecological and economic reasons it is very important that this biomass be recovered and valorized. In this context, not only underutilized crustaceans would be valorized, but also the waste water biomass produced during washing. Earlier were carried out worked on the recovery and/or characterization of wash water protein from different fish and crustacean species (Montero and Gómez-Guillén 1998; Stine et al. 2012), but applications of the biomass obtained have been done to a limited extent.

A possible way of giving value to water-soluble fish muscle proteins is the development of edible films (Iwata et al. 2000). The ability to provide a barrier to moisture, oxygen, carbon dioxide, oil, and/or flavor/aroma

✉ Joaquín Gómez-Estaca
joaquin.gomez@csic.es

¹ Institute of Food Science, Technology and Nutrition (CSIC),
José Antonio Novais 10, 28040 Madrid, Spain

migration between adjacent food components, and/or between the food and the environment, is the reason for the great interest in edible films in recent years. An additional benefit is the improvement of functionality by incorporating food ingredients such as antioxidants, antimicrobials, or flavors (Gomez-Estaca et al. 2009). Among all the existing biopolymers that can be employed to develop edible films, proteins offer the best prospects. Obviously, physicochemical properties of proteins affect film-forming ability and film properties. Edible films may be formulated by combining different polymers by taking both advantage of their properties. An example of combining different components to improve the properties of the resulting material is the inclusion of lipids in the formulation of edible films made from proteins or polysaccharides, thus improving the low water barrier properties of these matrices. Gómez-Estaca et al. (2015) succeeded in improving water vapor permeability and antioxidant activity of shrimp muscle protein films through incorporation of lipids extract from shrimp cephalothorax waste. Arancibia et al. (2015) developed active films of chitosan isolated by mild extraction with added protein concentrate from shrimp waste, improving mechanical and antimicrobial properties.

The objective of this work was to recover and characterize organic biomass from shrimp processing wash water, and to develop functional edible films from the same.

Materials and methods

Materials

The raw material used in the present work was whole shrimps (*Litopenaeus vannamei*) that had been frozen stored for 3 years, being considered a low-commercial-value batch because of spoilage. The whole shrimps were used in order to give value to lipophilic body components present in the cephalothorax too; furthermore peeling operation would not be viable from an economical point of view having in mind the low value of the raw material.

Shrimp processing

A typical washing process to obtain fish myofibrillar proteins was carried out. The shrimps were passed through a deboning machine (Baader 694, Lübeck, Germany), using an orifice diameter of 3 mm, to separate cuticles and muscle. The resulting mince was washed with cold 0.05 M NaCl (18 g NaCl/kg mince) in a tank with constant stirring for 10 min at 4 °C. After draining, excess water was removed using a screw press (Baader 523, Lübeck,

Germany) and collected. With this process myofibrillar proteins are concentrated and separated from sarcoplasmic proteins, lipids, etc., as well as from undesirable compounds such as metals, ammonium, or enzymes. The collected wash water was brought to pH 10 with 10 M NaOH and then left for 15 min with constant stirring at room temperature to achieve maximum protein solubilization. Afterwards, the pH was adjusted to 5, which was the pH of maximum precipitation as determined in a previous experiment (unpublished data), and the precipitate concentrated by centrifugation in a pilot plant vertical centrifuge (GEA Westfalia OKA 2-06-566, Düsseldorf, Germany). The above-mentioned undesirable compounds are mostly discarded in the supernatant. The biomass obtained was freeze-dried and stored at 3 ± 1 °C until analysis and use. The dry biomass is referred as shrimp concentrate (SC) all over the text.

Physicochemical properties

Proximate composition

Total ash and moisture of the SC were determined according to official methods (AOAC 2000). Crude protein was determined in a LECO FP-2000 nitrogen/protein analyzer (LECO Corp., St. Joseph, MI, USA) using a nitrogen-to-protein conversion factor of 6.25. Crude fat was determined following the method of Bligh and Dyer (1959).

Protein solubility

Protein solubility as a function of pH, was determined SC (1g) was dispersed in 100 mL of distilled water and pH was adjusted to 3, 5, 7, 9, or 11 with 5 M HCl or 5 M NaOH (Adebiyi and Aluko 2011). After 1 h of stirring at room temperature, the samples were centrifuged at $10,000 \times g$ for 15 min (Beckman Instruments Inc., Fullerton, CA, USA) and the protein in the supernatant was determined in a LECO protein analyzer. Results were reported as mean of three repetitions and are expressed as mg dissolved protein/100 mg protein.

Molecular weight profile

Molecular weight of proteins was determined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). The sample was mixed with loading buffer (2% SDS, 5% mercaptoethanol, and 0.002% bromophenol blue) to achieve a final protein concentration of 2 mg/mL. Sample was heat-denatured at 95 °C for 5 min and then centrifuged at $13,000 \times g$ for 10 min and analyzed in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA, USA) using

10% resolving gels (Bio-Rad Laboratories) at 25 mA/gel. Protein bands were stained with Coomassie brilliant blue R-250. A molecular weight protein standard acquired from Bio-Rad Laboratories (Precision Plus Protein™ Dual Xtra Standards, ref. 161-0377-MSDS) with molecular weights of 250, 150, 100, 75, 50, 37, 25, 20, 15, 10, 5, and 2 kDa was employed.

Amino acid profile

Amino acids were determined using Biochrom 20 amino acid analyzer (Pharmacia, Barcelona, Spain) after hydrolysis the samples at 108 °C for 18 h with 5.7 N HCl. Phenol and norleucine were used as internal standards. Results are expressed as number of residues per 1000 residues.

Fatty acid profile

The fatty acid profile was determined in duplicate using Agilent 7820A gas chromatograph fitted with FID detector and HP-88 (ref. 112-8867) column, after preparing fatty acid methyl esters as per Gómez-Estaca et al. (2017). Identification was accomplished by comparing retention times with standards and results were expressed as relative percentage of each fatty acid.

Mineral content

Calcium, magnesium, sodium, potassium, zinc, iron, copper, and manganese cations were determined by atomic absorption spectroscopy (Perkin-Elmer, model 5100 PC, Norwalk, CT, USA) as described by Pérez-Santín et al. (2013). Fluoride was determined as described by Rocha et al. (2013) using a fluoride ion-selective electrode (ISE DC219-F, Mettler Toledo). Total and inorganic arsenic quantification were performed with an atomic absorption spectrometer (model 3300, Perkin-Elmer, Spain) equipped with an autosampler (AS-90, Perkin-Elmer), a flow injection-hydride generation system (FIAS-400, Perkin-Elmer), and an electrothermally heated quartz cell. Mercury quantification was performed by cold-vapor atomic fluorescence spectrometry (model PSA 10.025, Millennium Merlin, PS Analytical, UK). Procedures were the same than those described by Almela et al. (2002).

α -Tocopherol content

SC was subjected to saponification and subsequent extraction with hexane based on the method of Lanina et al. (2007). An aliquot was dried and dissolved in methanol and then injected in an Agilent 1100 high resolution liquid chromatograph equipped with a Supelcosil LC-F column with pre-column (ref. 59158) and coupled to an Agilent

G1946D simple quadrupole mass spectrometer. The mobile phases used were isocratic 1:10 water:methanol at a flow rate of 1 mL/min. Identification was accomplished by comparison with a DL- α -tocopherol standard (Supelco, ref. 4-7783) which was subjected to the same preparation steps as the samples. Results are expressed as $\mu\text{g } \alpha\text{-tocopherol/g SC}$.

Antioxidant activity

The SC was dissolved at pH 9 and then centrifuged at $10,000\times g$ for 15 min (Beckman Instruments Inc., Fullerton, CA, USA). The supernatant was employed for determination of the reducing power (FRAP method) and the radical scavenging activity (ABTS method) according to Gomez-Estaca et al. (2009). For FRAP measurement, dilutions of the extract were made up to obtain absorbance measurements falling within a readable range. An amount of 30 μL of the extract was incubated with 900 μL of FRAP reagent (containing TPTZ and FeCl_3) and 90 μL of distilled water at 37 °C. Absorbance values were read at 595 nm after 30 min of reaction. Results are expressed as $\mu\text{mol FeSO}_4\cdot 7\text{H}_2\text{O}$ equivalents/g SC. For ABTS measurement, 20 μL of diluted extract was mixed with 980 μL of ABTS reagent (containing ABTS and potassium persulfate). The mixture was then left to stand at 30 °C for 10 min, and absorbance values were read at 734 nm. Results are expressed as mg Vitamin C equivalent antioxidant capacity (VCEAC)/g SC.

Film preparation

A 2% stock solution of agar was prepared by dissolving it in boiling water and maintained at $60\text{ }^\circ\text{C} \pm 1$ in a thermostatic bath until use. A 2% stock solution of SC was prepared by dissolving it in pH 9 carbonate/bicarbonate buffer; afterwards it was centrifuged at $10,000\times g$ for 15 min (Beckman Instruments Inc., Fullerton, CA, USA) to remove insoluble protein. The supernatant, which was maintained at $60\text{ }^\circ\text{C} \pm 1$ in a thermostatic bath until use, showed no separation between water and lipid phases. For the preparation of the film-forming solution (FFS), agar stock solution, SC stock solution and warmed pH 9 carbonate/bicarbonate buffer (1.5:1:1.5 ratio) were mixed. Glycerol was added as plasticizer (50 g/100 g polymer (agar plus soluble protein) and stirred for 10 min at 60 °C. The films were obtained by casting 100 mL of the FFS onto 144-cm^2 perspex plates, which were allowed to gel, and then drying at 45 °C for 17 h. For comparative purposes a control film without SC addition was formulated; the FFS was prepared by mixing agar stock solution, pH 9 carbonate/bicarbonate buffer (1:1 ratio), and 50 g glycerol/100 g agar, and the film was obtained by casting under the

same conditions. The control batch is referred to throughout the text as AG film and the one containing SC as AG-SC film.

Characterization of the films

Thickness

Film thickness was measured using a digital micrometer (Mitutoyo, model MDC-25 M, Kanagawa, Japan) averaging ten different locations on five different films.

Composition

Total ash and moisture of the SC were determined according to official methods (AOAC 2000). Crude protein was determined in a LECO FP-2000 nitrogen/protein analyzer (LECO Corp., St. Joseph, MI, USA) using a nitrogen-to-protein conversion factor of 6.25. Crude fat was determined following the method of Bligh and Dyer (1959). Agar and glycerol contents were calculated theoretically from the formulation of the films.

Mechanical properties

A TA.XT2i texture analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5-kg static load cell was used to perform the tensile test. At least 10 strips 2 cm wide and 10 cm long were conditioned at 58% relative humidity (in desiccators containing a NaBr saturated solution, at 25 °C) for 1 week prior to testing. The grip separation was set at 5.8 cm and the cross-head speed at 100 mm/min. The maximum tensile strength (σ_m , MPa) and the percentage of elongation at break (ε_b , %) of the films were calculated from the plot of stress (tensile force/initial cross-section area) versus strain (elongation as a fraction of the original length).

Optical properties

For opacity determination, rectangular pieces measuring 30 mm × 9 mm were cut from the films and placed directly in a quartz spectrophotometer (UV-1601 spectrophotometer, Shimadzu, Kyoto, Japan) test cell, using an empty test cell as the reference. The opacity index of the films was calculated by the following equation:

$$O = \frac{Abs_{600}}{x} \quad (1)$$

where Abs_{600} is the value of absorbance at 600 nm and x is the film thickness (mm). Measurements were taken in triplicate.

Film color was measured using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc.,

Osaka, Japan) set to D65 illuminant/10 observer. Film specimens were measured against the surface of a standard white plate and the CIELAB color space was used to obtain the color coordinates L^* [black (0) to white (100)], a^* [green (−) to red (+)], and b^* [blue (−) to yellow (+)]. The color was expressed using the polar coordinates $L^*C^*h^\circ$, where L^* (lightness) is the same as above, C^* is the chroma, and h° is the hue angle. Simple transformations were used to convert a^* and b^* coordinates to C^* and h° chromatic parameters:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$h^\circ = \arctg\left(\frac{b^*}{a^*}\right) \quad (3)$$

Ten measurements were taken of each sample and three samples of each film were measured.

Weight loss, swelling, and dimensional stability after immersion in water

Film specimens 20 mm in diameter (initial diameter, $\emptyset^i$) and of known thickness (initial thickness, l^i) were dried in a desiccator over silica gel for 10 days, at which point moisture content close to zero was assumed. The dry films were weighed accurately (initial dry weight, W_d^i) and immersed in test tubes containing 10 mL of distilled water. The tubes were gently shaken (150 rpm) at 25 °C for 24 h. The films were then removed from the test tubes and the remaining water was eliminated from the surface with absorbent paper. Then the wet films were measured for their thickness (final thickness, $\emptyset^f$), weight (final wet weight, W_w^f), and diameter (final diameter, $\emptyset^f$). Afterwards, the films were dried at 105 °C for 24 h and the final dry weight (W_d^f) was recorded. From these data the following calculations were made: percentage weight loss (WL) or film solubility, percentage water uptake (ΔW) or swelling, diameter increase ($\Delta \emptyset$), and thickness increase (Δl), according to Eqs. 4, 5, 6, and 7, respectively.

$$WL(\%) = \frac{W_i - W_f}{W_i} \cdot 100 \quad (4)$$

$$\Delta W(\%) = \frac{W_w^f - W_d^f}{W_d^f} \cdot 100 \quad (5)$$

$$\Delta \emptyset(\%) = \frac{\emptyset^f - \emptyset^i}{\emptyset^i} \cdot 100 \quad (6)$$

$$\Delta l(\%) = \frac{l^f - l^i}{l^i} \cdot 100 \quad (7)$$

Water vapor permeability (WVP)

WVP was determined following the method described by Gómez-Estaca et al. (2015). Films were attached over the

openings of cells (permeation area = 1.54 cm²) containing silica gel, and the cells were placed in desiccators with distilled water at 22 °C. The cells were weighed daily for 7 days. Water vapor permeability was calculated using the following equation:

$$WVP = \frac{w * x}{t * A * \Delta P} \quad (8)$$

where w was weight gain (kg), x film thickness (m), t elapsed time for the weight gain (s), A permeation area (m²), and ΔP the partial vapor pressure difference between the dry atmosphere and pure water (2642 Pa at 22 °C). Determinations were performed in triplicate.

Release experiments

Films were cut into circles measuring 14 mm in diameter. The release experiments were carried out by placing 20 pieces of film (weighing approximately 0.4 g), which were inserted on stainless steel spikes and separated with beads in order to avoid contact between adjacent films, into sealed beakers containing 65 mL of water or 95% ethanol, which were selected as aqueous and fatty food simulants, respectively, according to UNE-EN. 1186-3 (2002). The beakers were gently shaken at 22 °C in darkness (70 rpm) using an orbital shaker; 500 µL was withdrawn from the dissolution media at various time intervals, the volume being replaced after each extraction. The release of protein to the dissolution media was determined by the BCA protein assay kit (Meridian RD, Rockford, IL, USA) and results were expressed as cumulative µg BSA released/g dry film. The antioxidant activity was determined by the ABTS method as per Gomez-Estaca et al. (2009) and results were expressed as cumulative mg VCEAC released/g dry film.

Statistical analysis

Statistical tests were performed using the SPSS® computer program (SPSS Statistical Software, Inc., Chicago, IL, USA). One-way analysis of variance (ANOVA) was carried out. 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 and discussion

Characterization of SC

The main constituents of SC were protein (61.8% ± 0.3) and fat (23.6% ± 0.8), followed by ash (6.8% ± 0.1) and moisture (2.6% ± 0.4). SDS-PAGE, showed the presence of low molecular weight proteins and peptides (<37 kDa) particularly of ≤15 kDa (Fig. 1). The protein solubility of

SC was low at lightly acidic pH (3–5) but increased significantly ($p \leq 0.05$) at extremely acid (pH 2), neutral (pH 7) and at basic pH (9–11) (Fig. 2). It is well known that protein solubility is highly dependent on pH, the lowest solubility being near the isoelectric point where proteins have a net charge close to zero, and the highest at extremely acid or basic pH. The isoelectric point found in the present work is in accordance with that of fish muscle or amaranth proteins (Nolsøe and Undeland 2009). The amino acid composition of the protein fraction is shown in Table 1. The most abundant amino acids were asparagine, glycine, and glutamine. Approximately 1/3 of the total amino acids were essential amino acids, pointing to the potential of the obtained product as a food additive to improve the nutritional quality of food. It is worth to mention the presence of taurine, which performs functions in numerous physiological processes (Hayes and Sturman 1981). The hydrophobic amino acid content was high, which was related to the emulsifying ability of a protein (Li-chan et al. 1984), and also to antioxidant power (Alemán et al. 2011).

The fatty acid composition of the SC is shown in Table 1. The high degree of unsaturation was noted, with MUFA and PUFA accounting for ≈27% and ≈28% of the total fatty acids, respectively. The most abundant fatty acids were palmitic acid (C16:0), oleic acid (C18:1n9c), docosahexaenoic acid (DHA) (C22:6n3), and eicosapentaenoic acid (EPA) (C20:5n3). Especially relevant is the high content of n-3 fatty acids, which accounted for 24.6 ± 0.1% of the total fatty acids. Furthermore, the n-6/

Fig. 1 SDS-PAGE pattern of the shrimp washing water concentrate (SC). MWM molecular weight marker

n-3 ratio was very low (0.16), so that it can be stated that SC is a very good source of n-3 fatty acids to reduce the n-6/n-3 ratio in the diet. Long-chain n-3 PUFA such as DHA and EPA provide cardioprotective benefits as they have been shown to exert potent antiarrhythmic effects, improve vascular endothelial function, and help lower blood pressure, platelet sensitivity, and serum triglyceride

levels when consumed at doses of 2–4 g/day (Wijendran and Hayes 2004).

Table 2 shows the content of some minerals present in the SC. The most abundant mineral was Na, which is attributed to the 0.05 M NaCl solution employed during washing, followed by K, Ca, and Fe. It is worth noting the presence of Hg and As; however, according to the maximum weekly intake of methylmercury (1.6 µg/kg body weight per week) and inorganic arsenic (0.3–8 µg/kg body weight per day; EFSA, European Food Safety Authority 2009, 2012) even the consumption of an amount as high as 250 g of SC per week would be safe.

The SC showed antioxidant activity, both radical scavenging activity (54.2 ± 1.1 mg VCEAC/g SC) and reducing power (79.8 ± 0.6 µmol FeSO₄·7H₂O equivalents/g SC). The antioxidant activity shown by SC should be mainly attributed to soluble proteins and peptides. The antioxidant properties of other proteins/peptides from plant or animal origin have been reported previously, including ferric reducing ability and ABTS radical scavenging activity (Gómez-Guillén et al. 2011). In this connection, the antioxidant activity of proteins has been found to increase when it is hydrolyzed; this would be consistent with the low average molecular weight of the SC protein.

Fig. 2 Protein solubility in water of the SC as a function of pH

Table 1 Amino acid and fatty acid composition of the freeze-dried shrimp washing water concentrate (SC)

Amino acid composition (residues/1000 residues)		Fatty acid composition (%)	
Asp	126	C14:0	1.88 ± 0.03
Thr	37	C15:0	1.20 ± 0.01
Ser	66	C16:0	19.47 ± 0.1
Glu	115	C16:1	6.41 ± 0.04
Gly	118	C17:0	2.64 ± 0.01
Ala	96	C18:0	9.32 ± 0.1
Cys	10	C18:1n9c	13.99 ± 0.29
Val	48	C18:1n7c	4.32 ± 0.04
Met	22	C18:2n6c	1.55 ± 0.01
Ile	31	C18:3n3	0.49 ± 0.00
Leu	64	C20:0	0.53 ± 0.00
Tyr	29	C20:1	1.09 ± 0.11
Phe	50	C20:2	1.25 ± 0.11
His	25	C22:0	6.68 ± 0.10
Lys	56	C20:5n3	11.19 ± 0.03
Arg	43	C22:5n3	1.33 ± 0.00
Pro	47	C22:6n3	12.89 ± 0.02
Tau	19	Total SFA	43.5 ± 0.8
TEAA	296	Total MUFA	26.8 ± 0.4
THAA	358	Total PUFA	28.3 ± 0.5

The difference with respect to 100% corresponds to 10 other less abundant fatty acids (data not shown)

TEAA total essential amino acids ($\sum$ Ile + Leu + Lys + Met + Phe + Thr + Val + His), THAA total hydrophobic amino acids ($\sum$ Pro + Ala + Val + Met + Ile + Leu + Phe), SFA saturated fatty acids, MUFA monounsaturated fatty acids, PUFA polyunsaturated fatty acids

Table 2 Content of some minerals of the freeze-dried shrimp washing water concentrate (SC)

Mineral content (mg/g SC)	
Ca	1.88 ± 0.03
Mg	0.54 ± 0.01
Na	14.13 ± 1.97
K	2.55 ± 0.03
Zn	0.04 ± 0.001
Fe	1.25 ± 0.01
Cu	0.24 ± 0.01
Mn	<0.05
F	$9.95 \times 10^{-3} \pm 0.3 \times 10^{-3}$
Total As	$14.3 \times 10^{-3} \pm 0.57 \times 10^{-3}$
Inorganic As	$0.56 \times 10^{-3} \pm 0.01 \times 10^{-3}$
Hg	$0.43 \times 10^{-3} \pm 0.02 \times 10^{-3}$

The exact mechanism underlying the antioxidant activity of peptides is not fully understood, but various studies have shown that they are lipid peroxidation inhibitors, free radical scavengers and transition metal ion chelators (Gómez-Guillén et al. 2011). The values of antioxidant activity reported in the present work, while higher in absolute terms, were of the same order of magnitude as those reported in previous studies with gelatin hydrolysates (Giménez et al. 2009). The possible contribution of the low content of α -tocopherol present in the SC ($10.2 \pm 0.6 \mu\text{g/g}$ SC) to the antioxidant activity may also be taken into account.

Characterization of the films

AG- and AG-SC-containing FFSs yielded translucent, flexible, easily handled films that differed significantly ($p \leq 0.05$) in thickness (128 ± 1 and $166 \pm 1 \mu\text{m}$ for AG and AG-SC film, respectively). Film thickness is mainly dependent on the dry matter present in the film-forming solution, and according to the formulation the dry matter was higher for the AG-SC film than for the AG one. Furthermore, films made with some polymers such as agar may suffer some retraction during drying, thus increasing film thickness (Phan The et al. 2009a). The degree of film retraction was not determined in the present work; however, it was evident for both films, especially for the AG-SC film. The film composition is shown in Table 3. Moisture content of conditioned films was significantly different ($p \leq 0.05$), the AG-SC film showing a lower moisture equilibrium content. This result was expected owing to the different fat content of the films (negligible in AG film and $\approx 5\%$ in the AG-SC one), as the moisture sorption capacity of films has been found to be inversely related to the hydrophobic components in the matrix

Table 3 Composition of AG and AG-SC films, in %

	AG film	AG-SC film
Moisture ^a	40.0 ± 0.8	29.5 ± 0.6
Protein ^a	0.20 ± 0.02	11.6 ± 0.5
Fat ^a	–	5.1 ± 0.1
Ash ^a	0.10 ± 0.01	2.1 ± 0.05
Agar ^b	40.2	32.3
Glycerol ^b	19.8	21.6

^a Experimentally determined

^b Theoretically estimated, according to film formulation

(Vargas et al. 2009). The main biopolymer in the AG-SC film was agar, in a proportion of approximately 3:1 with respect to protein.

The AG-SC film showed a brownish color that differed significantly from that of the AG film for lightness, hue, and chroma (Table 4). Furthermore, the opacity of the film increased significantly ($p \leq 0.05$) as a consequence of the incorporation of SC; in spite of this, the film was quite transparent, thus allowing visualization of the food if used as wrapping (Fig. 3). Both the increase in opacity and the decrease in lightness may be attributed to a light-scattering effect of proteins and lipids present in the agar matrix (Perez-Mateos et al. 2009). A similar behavior has been previously reported for shrimp muscle protein films added with astaxanthin-containing lipid extract from shrimp cephalothorax waste (Gómez-Estaca et al. 2015).

The mechanical parameters of the AG and AG-SC films are shown in Table 4. The incorporation of SC in the agar matrix caused a significant ($p \leq 0.05$) reduction in σ_m , and increase in ε_b , which may be attributed to the presence of low molecular weight proteins and lipids in the agar matrix. Agar forms films with a compact and rigid structured network (Tian et al. 2011), whereas SC contains low molecular weight proteins and lipids that were unable to form a continuous film matrix, as shown in previous experiments. Consequently, SC proteins and lipids were likely to be incorporated in the agar film matrix between adjacent polymer chains, reducing the polymer cohesion forces and thus the film's resistance to breaking. The increase in ε_b of the AG-SC film as compared to the AG one is attributed to a plasticizing effect of low molecular weight proteins and peptides (Giménez et al. 2009). Similar behavior for both m and ε_b was reported for agar or cassava starch films incorporating emulsified hydrogenated vegetable oil (Phan The et al. 2009b).

Table 4 summarizes the results for the WVP of the films. The AG film showed a high water vapor permeability, which is characteristic of edible films made from polysaccharides or proteins (Pérez-Gago and Krochta 2005). The incorporation of SC caused a significant

Table 4 Some physicochemical properties of AG and AG-SC films

	AG film	AG-SC film
L^*	$87.0 \pm 1.9a$	$67.7 \pm 2.5b$
h°	$100.3 \pm 3.3a$	$72.7 \pm 3.5b$
C^*	$19.1 \pm 5.6a$	$38.8 \pm 1.2b$
O	$2.7 \pm 0.1a$	$3.5 \pm 0.1b$
σ_m (MPa)	$11.9 \pm 1.3a$	$7.1 \pm 1.2b$
ε_b (%)	$52.6 \pm 1.7a$	$61.9 \pm 5.4b$
WVP ($\text{kg m/m}^2 \text{ s Pa}$)	$4.0 \times 10^{-13} \pm 6.4 \times 10^{-14}a$	$5.1 \times 10^{-14} \pm 6.8 \times 10^{-15}b$
WL (%)	$56.1 \pm 1.4a$	$61.0 \pm 1.2b$
ΔW (%)	$729 \pm 50a$	$1395 \pm 175b$
$\Delta\emptyset$ (%)	$2.1 \pm 0.6a$	$1.6 \pm 0.3a$
Δl (%)	$302 \pm 14a$	$405 \pm 18b$

Different letters in the same row (a, b) indicate significant differences

WL weight loss, ΔW swelling, $\Delta\emptyset$ diameter increase, Δl thickness increase, σ_m tensile strength, ε_b elongation at break, WVP water vapor permeability, L^* lightness, h° hue, C^* chroma, O opacity

Fig. 3 Image of the AG and AG-SC films

($p \leq 0.05$) reduction in film WVP of one order of magnitude. This is attributed to the water vapor barrier property of lipids from the SC, which represented approximately 5% of the conditioned film in weight (Table 3). The reduction of film WVP in the AG-SC film may be a result of both a

lower moisture sorption and a slower diffusiveness through the film matrix, owing to the higher lipophilicity of the film matrix. Similar results have been found in films formulated with a wide range of biopolymers and fats (Pérez-Gago and Krochta 2005).

Table 4 shows the weight loss, swelling, diameter, and thickness of the films. Both films maintained their integrity after immersion in water but swelled considerably. Swelling was significantly higher ($p \leq 0.05$) for the AG-SC film, owing to the presence of low molecular weight proteins and lipids interrupting the compact, continuous network of the AG film, thus increasing the free volume and favoring water uptake. Similar results were found by Balaguer et al. (2014) for cross-linked gliadin films incorporated with natamycin. The increase in film water uptake was accompanied by a dimensional stability change: the greater the water uptake, the more the thickness increased; however, film diameter varied to a lower extent ($\approx 2\%$ increase), without significant differences as a function of SC incorporation. The high swelling is a very interesting property of the developed films, as they will be able to absorb exudates when disposed at the top or the bottom of the product, without losing film integrity. Both films lost more than 50% of their weight after 24 h immersion, a phenomenon that was mainly attributed to release of glycerol, which accounted for 50% (w/w) of the total polymer weight. Cuq et al. (1997) found a linear relationship between film weight loss upon immersion in water and glycerol content for fish myofibrillar protein films. There was a significant difference ($p \leq 0.05$) in weight loss between AG and AG-SC film, the latter losing more weight, which was attributed to the release of low molecular weight water-soluble proteins. SC proteins were negatively charged at the basic pH employed to dissolve them, and agar was mainly neutral or slightly anionic

Fig. 4 Cumulative release of protein (circles) and ABTS radical scavenging activity (squares) of the AG-SC film in water (filled symbols) and 95% ethanol (open symbols) as a function of time

(Stanley 2006), so little interaction via electrostatic bonds between the two molecules was expected.

Figure 4 shows the cumulative release of protein and the ABTS radical scavenging activity of the AG-SC film in distilled water (aqueous food simulant) and in 95% ethanol (fatty food simulant) as a function of time. Both the release of protein and the ABTS scavenging activity showed an “exponential growth to a maximum” type profile, the extent and kinetic of the release depending on the food simulant. Thus, protein release was faster and greater in water than in 95% ethanol, and the same was also true for the ABTS radical scavenging activity. It is now generally recognized that mechanisms of drug release from a polymer matrix can be categorized in three main processes or systems (Arifin et al. 2006): (1) diffusion from the non-degraded polymer (diffusion-controlled system); (2) enhanced drug diffusion due to polymer swelling (swelling-controlled system); (3) release by polymer degradation and erosion (erosion-controlled system). According to the characteristics of our system and bearing in mind the high swelling of the film, the most feasible mechanism of protein release in water is a swelling-mediated one. In swollen networks the drug is in an aqueous environment, so its water solubility becomes an important consideration and compounds with appreciable water solubility will rapidly leach out, independently of the matrix erosion rate (Chen et al. 2008); this is the case with the AG-SC film, owing to the high water solubility of low molecular weight proteins and peptides. As mentioned above, the release of protein in 95% ethanol was slower than in water, as shown by the less steep slope (0.096 vs. 0.456 for 95% ethanol and water,

respectively) in the earlier stage of the release process. In this case the release mechanism is more likely to be a diffusion-mediated one than a swelling-mediated one, as swelling in 95% ethanol was tenfold lower than in water (data not shown). Furthermore, the extent of protein release and the radical scavenging activity were lower in the fatty food simulant than in the aqueous one, which is also attributed to the different compatibility between the migrant and the simulant (Lopez-de-Dicastillo et al. 2012). According to these results, the antioxidant films developed would be more efficient on delaying lipid oxidation when applied to aqueous foods, such as meat or fish, than to fatty ones, such as oil, owing to the dependence on swelling to release the antioxidant compounds.

In conclusion, a concentrate rich in protein and lipids was obtained from the wash water biomass from shrimp surimi processing. It showed good nutritional quality, evidenced from amino acid and fatty acid profile, as well as antioxidant activity. Films based on agar incorporated with this biomass (agar-to-protein ratio of 3:1) showed good performance on the basis of mechanical, water vapor permeability, swelling and antioxidant properties. The nutritional quality and antioxidant activity of the wash water biomass from shrimp wash mince/surimi processing revealed its usefulness for developing functional edible films, as well as its potential for other food applications.

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