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Characteristics and functional properties of gelatin extracted from squid (*Loligo vulgaris*) skin

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ABSTRACT

The characteristics and functional properties of gelatin extracted from squid skin (*Loligo vulgaris*), using commercial pepsin at a level of 15 Units/g skin, were investigated. The gelatin extraction yield was 6.82 g/100 g wet basis, showing high protein content and low fat and ash contents. SDS-PAGE of squid skin gelatin showed high band intensity for the major protein components, especially, α- and β-components. The amino acid profile of squid skin gelatin showed a high percentage of imino acids, essential amino acids and hydrophobic amino acids. Fourier transformed infrared spectroscopy (FT-IR) spectra showed helical structure. Squid skin gelatin was able to form a completely thermo-reversible gel. Gelatin possessed interesting functional properties, which were governed by gelatin concentration.

1. Introduction

Gelatin is a protein compound derived from denatured collagen (Bailey & Light, 1989). In order to obtain gelatin, a pre-treatment process is required to convert the tissue collagen into a suitable form for extraction. This means the loss of the ordered structure of the native insoluble collagen, which results in a swollen, but still insoluble collagen (Latinovic, Hough, & Ou-Yang, 2010). Subsequent heating cleaves hydrogen and covalent bonds, leading to the conversion into gelatin by a helix-to-coil transition (Makareeva & Leikin, 2014). Gelatin is an important functional biopolymer that has a very broad application in many industrial fields (Karim & Bhat, 2009). Besides their basic hydration properties, such as swelling and solubility, the most important properties of gelatin can be divided into two groups: properties associated with their gelling behavior, i.e. gel formation, texturizing and water binding capacity, and properties related to their surface behavior, which include emulsion and foam formation and stabilization, adhesion and cohesion, protective colloid function, and film-forming capacity (Schrieber & Gareis, 2007). The functional properties of the gelatin are greatly influenced by the amino acid composition, the molecular weight distribution (Kolodziejska, Skierka, Sadowska, Kolodziejski, & Niecikowska, 2008; Muyonga, Cole, & Duodu, 2004a,b) and also the ratio of α/β chains contained in the gelatin (Karim & Bhat, 2009).

There is a considerable interest in the use of fish skins for gelatin production since it supposes the exploitation of by-products from fish processing industry and, from a socio-cultural standpoint, presents an alternative to mammalian gelatin whose consumption is rejected in some cultures. Numerous studies have emerged investigating the use of undervalued seafood sources and processing by-products of no or low market value to produce products for use in functional foods and nutraceuticals (Udenigwe & Aluko, 2011). Recently, skin gelatins from several marine species such as giant squid (Giménez, Gómez-Estaca, Alemán, Gómez-Guillén, & Montero, 2009), squid skin-derived (Gòmez-Guillèn et al., 2002), barbel (Sila et al., 2015), smooth hound (Bougatef et al., 2012), tuna (Haddar et al., 2012), big eye snapper (Nalinanon, Benjakul, Visesssanguan, & Kishimura, 2008) and Atlantic salmon (Arnesen & Gildberg, 2007) have been extracted and characterized.

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Different methods to extract gelatin have been described depending on the raw material used. Depending on the degree of collagen aggregation, the extraction process could also include the use of proteases (Bougatef et al., 2012; Sila et al., 2015) that together with a swelling step might lead to increases in the yield.

Squid gelatin, together with other marine gelatins, is offered as an alternative to mammalian gelatin source due to the sociocultural and safety considerations associated with the latter. Compared to fish species, a special feature of squid collagen lies in its high degree of cross-linking, due to high amount of Hydroxylysine, together with high content of Hydroxyproline (Gomez-Guillen et al., 2002). Squid has been used for industry transformation in Tunisia and the skin generated, as by-products with a low market value could be a source of many value-added products, especially gelatin. So far no gelatin has been extracted from the skin of *Loligo vulgaris* and no information on its properties is available.

Therefore, the objective of this study was to extract and characterize the gelatin from squid (*L. vulgaris*) skin using an acid protease aided process and to study its physicochemical characteristics as well as its visco-elastic and functional properties.

2. Materials and methods

2.1. Raw material

The squid *L. vulgaris* is a large squid belonging to the family Loliginidae. It is found throughout the Mediterranean and in the eastern Atlantic Ocean from the North Sea to the Gulf of Guinea. The species is extensively exploited by commercial fisheries (FAO, 2012).

2.2. Preparation of squid skin

Squid by-products were obtained from a local marine processing industry (CALEMBO) in Sfax, Tunisia. The samples were packed in polyethylene bags, placed in ice with a sample/ice ratio of approximately 1:3 (w/w), and transported to the research laboratory within 30 min. Upon arrival, the samples were washed twice with water to eliminate dark ink, which consists of a suspension of melanin granules in a viscous colorless medium. The squid byproducts were separated, and only squid outer skin was collected and stored in sealed plastic bags at −20 °C until further use for gelatin extraction and analysis.

2.3. Extraction of squid skin gelatin (SSG)

Gelatin was extracted according to the method of Nalinanon et al. (2008) with a slight modification. Squid skins were previously cut into small pieces and then soaked in 0.05 mol/L NaOH (10 g/100 mL) in order to remove non-collagenous proteins. The mixture was stirred for 2 h at room temperature, and the alkaline solution was changed every 30 min. The alkaline-treated skins were then washed with distilled water until neutral pH was obtained. The alkaline-treated skins were soaked in 0.2 mol/L acetic acid with a ratio of 1:10 and subjected to limited hydrolysis with commercial pepsin (Sigma Chemicals Co. (St. Louis, MO, USA)) at ph 2.0 and at a level of 15 units/g of alkaline-treated skin (Balti et al., 2011). The mixtures were stirred for 48 h. The pH of the mixtures was then raised to 7.5 using 10 mol/L NaOH. The mixtures were stirred for 1 h at 4 °C to terminate protease activity, and then incubated at 50 °C for 18 h with continuous stirring to extract the gelatin from the skin. The mixtures were centrifuged for 30 min at 10,000× *g* using a refrigerated centrifuge to remove insoluble material. The supernatant was collected and lyophilized. The powder obtained was referred as squid skin gelatin (SSG).

2.4. Characterization of squid skin gelatin

2.4.1. Chemical composition

Moisture and ash contents were determined according to the AOAC standard methods 930.15 and 942.05, respectively (AOAC, 2000). Total nitrogen content was determined using the Kjeldahl method. A factor of 6.25 and 5.55 was used to convert the nitrogen value to protein in the case of skin and gelatin respectively.

2.4.2. Determination of color

The color of the obtained gelatin powder, as well as of the gelatin gel (6.67 g/100 mL) was measured in quintuplicate using a Konica Minolta CM-3500d spectrophotometer (Konica Minolta Sensing, Inc., Osaka, Japan) set to D65 illuminant/10° observer. 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°, where L* is the lightness, 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:

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

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

2.4.3. Amino acid analysis

Squid skin gelatin was dissolved (1 mg/mL) in ultrapure water and further hydrolyzed in vacuum-sealed glass at 110 °C for 24 h in presence of continuously boiling 6 N HCl containing 0.1% phenol and norleucine as internal standard. After hydrolysis, the sample was again vacuum-dried, dissolved in application buffer, and injected onto a Biochrom 20 amino acid analyser (Pharmacia, Barcelona, Spain). Results were expressed as number of residues per 1000 residues.

2.4.4. Molecular weight profile

The molecular weight distribution of squid skin gelatin was determined by SDS-polyacrylamide gel electrophoresis. Gelatin solution was mixed with loading buffer to get a final protein concentration of 2 mg/mL. Samples were heat-denatured at 95 °C for 10 min, centrifuged and then analyzed according to Laemmli (1970) in a Mini Protean II unit (Bio-Rad Laboratories, Hercules, CA, USA) using 7.5% precast gels at 25 mA/gel. Loading volume was 15 μL in all lanes. Protein bands were stained with Coomassie brilliant blue R-250. A molecular weight protein standard also acquired from Bio-Rad Laboratories.

2.4.5. FTIR spectroscopic analysis

The absorption spectra of the samples were obtained using FTIR spectroscopy (Analect Instruments fx-6160). The FTIR spectra of the prepared materials were recorded between 400 and 4000 cm^{-1} in a NICOET spectrometer. The transmission spectra of the samples were recorded using the KBr pallet containing 0.1 g/100 g of sample.

2.4.6. Dynamic oscillatory studies

Gelatin was dissolved in distilled water (6.67 g/100 mL) and subjected to dynamic viscoelastic studies on a Bohlin CVO-100 rheometer (Bohlin Instruments Ltd., Gloucestershire, UK) using a cone-plate geometry (cone angle 4°, gap = 0.15 mm). A dynamic frequency sweep was done at 5° (after 15 min gel of cold

maturation) by applying oscillation amplitude within the linear region ($\gamma = 0.005$) over the frequency range 0.1 10 Hz. The elastic modulus (G′; Pa) and the viscous modulus (G″; Pa) were plotted as a function of frequency. To characterize the frequency dependence of G′ over the limited frequency range, the following power law was used:

$$G' = G'_0 \omega^n$$

Where G_0' is the energy stored and recovered per cycle of sinusoidal shear deformation at an angular frequency of 1 Hz, ω is the angular frequency and n is the power law exponent.

Both heating and cooling dynamic temperature sweeps were also performed from 2 to 25 °C and back to 2 °C at a scan rate of 1 °C/min, frequency of 0.5 Hz, and target strain $\gamma = 0.005$. G′ (Pa), G″ (Pa) and phase angle (δ; °) were obtained, as a function of temperature. Results were the average of at least two determinations.

2.5. Determination of interfacial properties

2.5.1. Foaming properties

The foam expansion (FE) and foam stability (FS) of squid skin gelatin solutions (different concentrations from 1 to 4 g/100 mL) were tested using the method described by Shahidi, Han, and Synowiecki (1995). Foam capacity was expressed as foam expansion at 0 min, which was calculated according to the following equation:

$$\text{FE}\ (\%) = \frac{V_T - V_0}{V_0} \times 100$$

Foam stability was calculated as the volume of foam remaining after 30 and 60 min using the following equation:

$$\text{FS}\ (\%) = \frac{V_t - V_0}{V_0} \times 100$$

Where V_T refers to the total volume after whipping (ml), V_0 to the volume before whipping, V_t to the total volume after leaving at room temperature for different times (5, 30 and 60 min).

2.5.2. Emulsifying properties

The emulsion activity index (EAI) and the emulsion stability index (ESI) of SSG were determined according to the method of Pearce and Kinsella (1978). The absorbance measured immediately (A_0) and 10 min (A_{10}) after emulsion formations were used to calculate the emulsifying activity index (EAI) and the emulsion stability index (ESI) as follows:

$$\text{EAI}\left(\text{m}^2\text{g}^{-1}\right) = \frac{2 \times 2.303 \times A_0}{0.25 \times \textit{proein weight}\ (\text{g})}$$

$$\text{ESI}\ (\text{min}) = \frac{\Delta A}{A_0} \times t$$

Where ΔA is ($A_0 - A_{10}$) and t = 10 min. All determinations are means of at least three measurements.

2.5.3. Microscopic observation of emulsion droplet

Emulsions were prepared by homogenizing 20 mL/100 mL sunflower oil with 80 mL/100 mL aqueous phase (containing 1 g SSG/100 mL) for 1 min at room temperature (22 ± 1 °C) using ULTRA-TURRAX T25 basic (IKA-WERKE, Germany). A positive control was prepared using SDS as emulsifier (1 g/100 mL aqueous phase), as well as a negative control without emulsifier. The mixtures were adjusted to pH 7.0 using NaOH (0.15 mol/L) with continuous stirring for 30 min to promote dispersion and adsorption of surfactant molecules on to the SSG-coated lipid droplet surfaces. A drop of emulsions were placed on a microscope slide and covered with a cover slip. The emulsions structures were then observed using an optical microscope linked to a CCD video camera controlled by an image processor. Pictures were taken at 40× magnification. These observations allowed the complementary checking of emulsion droplet formation.

2.5.4. Fat-binding and water-holding capacities

Fat-binding capacity and water-holding capacity were measured according to Lin, Humbert, and Sosulki (1974).

2.6. Statistical analysis

All experiments were carried out in triplicate, and average values with standard deviation errors are reported. Mean separation and significance were analyzed using the SPSS software package (SPSS, Chicago, IL).

3. Results and discussion

3.1. Squid skin gelatin extraction

The gelatin extraction yield was 6.82 g/100 g on the basis of wet weight. This yield was similar to that obtained for shortfin scad (*Decapterus macrosoma*) by Cheow, Norizah, Kyaw, and Howell (2007) and higher than those described by Gòmez-Guillèn et al. (2002) for squid skin-derived gelatin (2.6 g/100 g). The gelatin yield obtained for barbel was 8.36 g/100 g (Sila et al., 2015).

3.2. Proximate composition and color of SSG

As shown in Table 1, squid skin contained moisture as the major component (86.2 ± 2.51 g/100 g) and the fat content of squid skin was less than 1 g/100 g. The squid skin gelatin contained protein as the major component (89.76 ± 0.59 g/100 g). Sila et al. (2015) reported that protein content of gelatin derived from Tunisian barbel was 92.1 g/100 g. Gelatins from skins of bigeye snapper, cuttlefish, smooth hound and tuna had protein contents of 92.6, 91.35 and 97.3 g/100 g, respectively (Balti et al., 2011; Bougatef et al., 2012; Haddar et al., 2012). Skin gelatin of squid had low moisture (8.73 ± 0.15 g/100 g), fat (0.38 ± 0.03 g/100 g) and ash (0.35 ± 0.01 g/100 g) contents (Table 1), suggesting the efficient removal of fat and minerals from the skin material. Gelatin consists of mainly protein and water. So, the presence of ash, lipid and other impurity at very low contents are important for the quality of gelatins (Jones, 1977). The relatively low ash content suggests that the extracted gelatin is of good quality. In general, recommended ash content for a high quality gelatin should be less than 0.5 g/100 g (Benjakul, Oungbho, Visessanguan, Thiansilakul, & Roytrakul, 2009).

The color of the gelatin powder and the gelatin gel is shown in Table 2. To naked eye both the gelatin powder and the gelatin gel showed a brownish color that agreed with the obtained color

Table 1
Proximate composition of squid skin gelatin (SSG). Values represent averages ± standard deviations for triplicate experiments.

Composition	Squid skin	SSG
Moisture (g/100 g)	86.2 ± 2.51	8.73 ± 0.15
Protein (g/100 g)	22.96 ± 3.11	89.76 ± 0.59
Fat (g/100 g)	0.73 ± 0.08	0.38 ± 0.03
Ash (g/100 g)	3.74 ± 0.39	0.35 ± 0.01

Table 2
Lightness (L*), hue angle (h°) and chroma (C*) of squid skin gelatin powder and squid skin gelatin gel. Values represent averages ± standard deviations for triplicate experiments.

	L*	h°	C*
SSG powder	79.7 ± 0.5[b]	67.6 ± 0.6[a]	17.0 ± 0.4[a]
SSG gel	31.2 ± 0.1[a]	81.6 ± 3.3[b]	2.9 ± 0.2[b]

Different superscripts in the same column indicate the significant differences ($p < 0.05$).

parameters, the hue value being into the first quadrant (+a, +b) and showing a low chromaticity value. It is obvious that significant differences ($p \leq 0.05$) were found between the gelatin powder and gel, which are mainly attributed to a dilution effect.

3.3. Amino acid composition

The amino acid composition of squid skin gelatin expressed as residues per 1000 total amino acid residues is shown in Table 3. The squid gelatin exhibited the typical type I collagen. SSG resembled the composition of interstitial collagen, showing >30% glycine (321 residues per 1000 residues) and ~17% imino acids (180 residues per 1000 residues). The glycine content of the SSG was similar to that of cuttlefish (321 residues/1000 residues) but less than that of bovine gelatin (341 residues/1000 residues) (Balti et al., 2011). The proline and hydroxyproline contents of SSG were about 90 and 90 residues per 1000 residues, respectively. The amount of proline in the SSG was lower than that of bovine gelatin (123 residues/1000 residues) but higher than of that barbel skin gelatin (83 residues/1000 residues) (Sila et al., 2015). Gomez-Guillen et al. (2002) reported similar imino acids content for squid gelatin extracted from skins by heating at 80 °C without previous enzymatic treatment. The abundance of these amino acids in gelatin has been widely documented. Asghar and Henrickson (1982) reported that 50 60% of α-chains of collagen consist of tripeptides with the general formula glycine -XY, where X is generally proline and Y is mainly

Table 3
Amino acid composition of squid skin gelatin.

Amino acids	Number of residues/1000
Aspartic acid (Asp)	62
Threonine (Thr)	27
Serine (Ser)	52
Glutamic acid (Glu)	87
Glycine (Gly)	321
Alanine (Ala)	76
Cysteine (Cys)	2
Valine (Val)	23
Methionine (Met)	15
Isoleucine (Ile)	18
Leucine (Leu)	28
Tyrosine (Tyr)	6
Phenylalanine (Phe)	12
Hydroxylysine (OHLys)	18
Histidine (His)	6
Lysine (Lys)	14
Arginine (Arg)	53
Proline (Pro)	90
Hydroxyproline (Hyp)	90
TEAA	143
THAA	583
Imino acids	180

TEAA = ∑ Ile + Leu + Lys + Met + Phe + Thr + Val + His: total essential amino acids.
THAA = ∑ Pro + Ala + Val + Met + Gly + Ile + Leu + Phe: total hydrophobic amino acids.
Imino acids = Pro + Hyp.

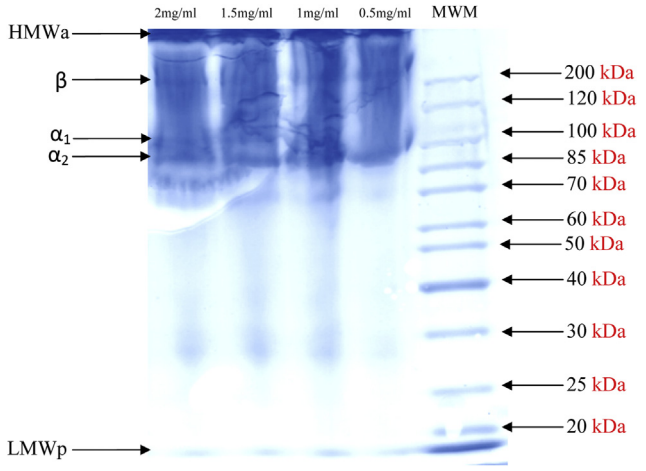

Fig. 1. Electrophoretic profile of the squid skin gelatin. MWM: molecular weight marker. HMWa: high molecular weight aggregates, LMWp: low molecular weight protein.

hydroxyproline. The stability of the triple helical structure in renatured gelatins has been reported to be proportional to the total content of imino acids, given that proline and hydroxyproline rich regions are likely to be involved in the formation of nucleation zones (Ledward, 1986). Especially hydroxyproline is believed to play a key role in the stabilization of the triple-stranded collagen helix due to its hydrogen bonding ability through its hydroxyl group (Mizuno, Hayashi, & Bachinger, 2003).

It is worth noting the particularly high content of hydroxylysine, as compared to the works of Giménez et al. (2009) and Gomez-Estaca, Montero, Fernandez-Martin, and Gomez-Guillen (2009). As presented in Table 3, the SSG possessed a high amount of alanine (76 residues per 1000 residues). Similar results were reported by Giménez et al. (2009) in gelatin from the skin of giant squid (*Dosidicus gigas*). Together with proline and hydroxyproline, alanine is found in non-polar regions where sequences of the type glycine-proline-Y predominate, with the third position normally occupied by hydroxyproline or alanine (Ledward, 1986). Thus, in general, a gelatin preparation with high proline, hydroxyproline, and alanine content shows better viscoelastic properties than others with a lower content of these amino acids, as it has been previously reported for fish skin-derived gelatin preparations (Gomez-Guillen et al., 2002). Surprisingly, cysteine residues could be found in SSG (2 residues per 1000 residues) although cysteine does not take part in the structure of type I collagen (Morales, Moral, & Montero, 2000). The presence of cysteine in the amino acid composition could indicate that gelatin might contain a small quantity of stroma

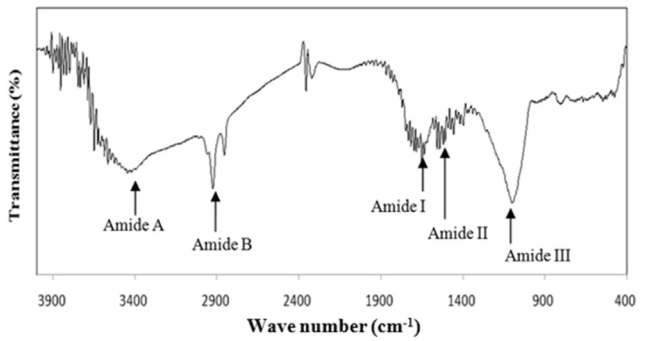

Fig. 2. Fourier transform infrared spectrum of squid skin gelatin.

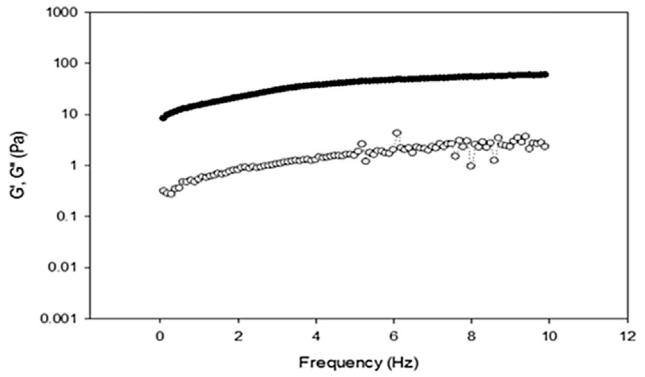

Fig. 3. Elastic modulus (G′, Pa) and viscous modulus (G′, Pa) as a function of the angular frequency of squid skin gelatin at 5 °C. $y = 18.149x^{0.5128}$ and $r^2 = 0.9627$. (G′: full circles; G″: open circles).

protein, such as elastin, which is highly insoluble and unusually stable in salt (Kim & Park, 2005). The content of hydrophobic amino acids in the squid skin gelatin was calculated, and it was found to be 58.3%. The SSG has a high percentage of essential amino acids, such as Ile, Leu Lys, Met, Phe, Thr, Val and His (143 residues per 1000 residues).

3.4. Molecular weight profile of SSG (SDS-PAGE)

The physical properties of gelatin depend not only on the amino acid composition, but also on the relative content of α-chains, β-components and higher molecular weight aggregates, as well as on the presence of lower molecular weight protein fragments (Johnston-Banks, 1990). The molecular weight profile of SSG was analyzed by SDS-PAGE (Fig. 1). The electrophoretic profile mainly showed smear protein bands that are products of hydrolysis of elementary chains of collagen. SSG contained α_1 and α_2-chains as the major components (≈100 kDa each one). Moreover, β-chains (≈200 kDa) were observed in SSG, as well as high molecular weight aggregates (>200 kDa) that did not enter the gel. Smudged bands with molecular weight lower than 70 kDa appeared below α-chain, which could be attributed to partially cleavage of gelatin by pepsin during swelling; however the amount of protein in these bands was very low. The obtained results are in agreement with those previously described by Sila et al. (2015). According to Gomez-Guillen et al. (2002), damage or partial loss of α_1-chains can occur during the extraction procedure.

3.5. Infra-red spectroscopic analysis

The Fourier transform infra-red (FT-IR) spectrum of squid skin gelatin is shown in Fig. 2. FT-IR spectroscopy was used to monitor the functional groups and secondary structure of gelatin (Muyonga et al., 2004a,b). SSG showed the major peaks in the amide region. The spectrum of the gelatin dispersions demonstrated the characteristic pattern reflecting the amide III band at 1114 cm^{-1}, the amide II band at 1504 cm^{-1}, and the amide I band at 1646 cm^{-1}.While the amide I band is often associated with a secondary structure of protein, the amide III band is associated with a helical structure (Surewicz & Mantsch, 1988). The amide B band was registered at 2910 cm^{-1}, where the amide B band of collagen always appeared (Kaminska & Sionkowska, 1996). The amide A band was recorded at

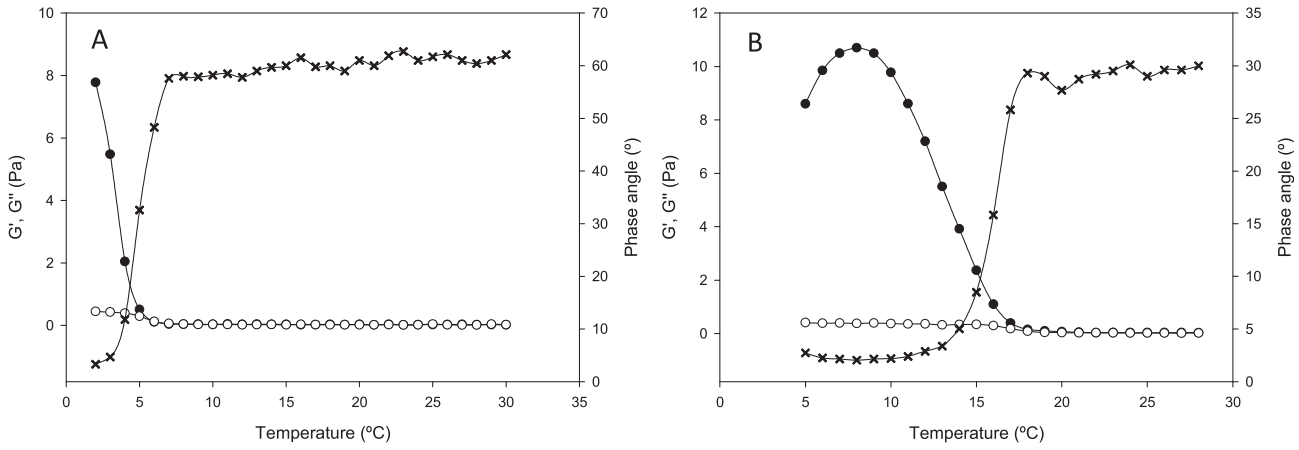

Fig. 4. Dynamic viscoelastic properties (G′, full circles; G″, open circles; phase angle, crosses) of squid skin gelatin during cooling (A) at heating (B) ramps.

Table 4
Emulsifying and foaming properties of squid skin gelatin at different concentrations. Values are given as mean ± SD from triplicate determinations. Different superscripts in the same row indicate significant differences ($p < 0.05$).

Concentration (g/100 mL)	1	2	3	4
FE (%)	114.6 ± 1.3^a	147.9 ± 1.2^b	150 ± 0.7^b	155.1 ± 1^c
FS (%)				
5 min	113.4 ± 0.3^a	147.3 ± 0.4^b	148.5 ± 0.6^b	154.9 ± 0.9^c
30 min	112.1 ± 0.1^a	146.5 ± 0.1^b	147 ± 1.1^b	151.2 ± 1^c
60 min	110 ± 0.1^a	144.1 ± 1.3^b	146.6 ± 1.8^b	150.3 ± 0.5^c
EAI (m^2/g)	11.60 ± 0.8^a	17.60 ± 0.3^b	28.33 ± 1^c	35.48 ± 0.3^d
ESI (min)	32.94 ± 0.1^d	28.33 ± 1^c	24.17 ± 0.8^c	16.33 ± 0.2^a

FE = foam expansions.
FS = foam stability.
EAI = emulsifying activity index.
ESI = emulsion stability index.

3437 cm^{-1}, which merged with the CH_2 stretch peak when carboxylic acid groups existed in stable dimeric associations (Muyonga et al., 2004a,b). Fourier transformed infrared spectroscopy spectra showed helical arrangements in the structure of squid skin gelatin and indicated that the triple helical structure was affected by pepsin treatment. Muyonga et al. (2004a,b) reported that a disorder of molecular structure due to the transformation of an a-helical to a random coil structure obtained during enzymatic hydrolysis and these changes were associated with loss of triple-helix state as a result of denaturation of collagen to gelatin.

3.6. Dynamic viscoelastic properties

The gelatin was able to form a gel at low temperature, as can be deduced from the higher value of G′ as compared to G″ (Fig. 3). The gelatin gels, which fitted well to the power law ($r^2 = 0.9627$), showed a high exponent ($n = 0.538$); for gels exhibiting an ideal elastic behavior, n value should be near-zero (Giménez, Gómez-Guillén, López-Caballero, Gómez-Estaca, & Montero, 2012). The gelling temperature was 5 °C, and the gel was completely thermo-reversible, as shown by the high phase angle value recorded in the heating ramp, the melting temperature being 16 °C (Fig. 4). In general, gelatins from cephalopods produce gels with worse thermo-stability and viscoelastic properties than those from mammalians and fish (Giménez et al., 2009).

3.7. Interfacial properties of squid skin gelatin

3.7.1. Foam capacity and foam stability

Foam expansion (FE) and foam stability (FS) at 5, 30 and 60 min after whipping were determined to evaluate the foam capacity and foam stability of SSG. FE and FS of SSG at various concentrations (1, 2, 3 and 4 g/100 mL) are depicted in Table 4. Foam expansion and foam stability values of squid skin gelatin increased with increasing gelatin concentration. This is in accordance with the works by Sila et al. (2015) and Bougatef et al. (2012) who reported an increase of FE and FS values with increasing barbel gelatin and smooth hound gelatin concentrations, respectively. For adsorption at the air—water interface, molecules should contain hydrophobic regions which become more exposed upon protein unfolding, thus facilitating foam formation and stabilization (Townsend & Nakai, 1983). FE and FS of SSG were believed to be affected by the amount of hydrophobic amino acids (583 residues/1000 residues). At all concentrations used, foaming stability decreased with time. Foam stability improved by greater protein concentration because this increases viscosity and facilitates formation of a multilayer cohesive protein film at the interface. The stability of foams depends on various parameters, such as the rate of attaining equilibrium surface tension, bulk and surface viscosities, steric stabilization, and electrical repulsion between the two sides of the foam lamella (Liu, Xu, Yuan, & Jiang, 2003).

3.7.2. Emulsifying properties

The EAI (emulsifying activity index) and ESI (emulsion stability index) of SSG at different concentrations (1–4 g/100 mL) are also shown in Table 4. The EAI of squid skin gelatin was noted to increase with the increase of gelatin concentration, as previously reported by Sila et al. (2015) and Haddar et al. (2012) for gelatins extracted from Tunisian barbel skin and Atlantic Bluefin tuna skin, respectively. Protein at high concentrations facilitated more protein adsorption at the interface (Yamauchi, Schimizu, & Kamiya, 1980). On the contrary, the emulsion stability decreased as the SSG concentration increased. At a concentration of 4 g/100 mL the emulsion stability index of squid skin gelatin was 16.33 min, which is lower than that of bovine gelatin at the same concentration (Balti et al., 2011). This may be partially attributed to gelatin molecular weight, as Surh, Decker, and McClements (2006), found that the oil-in-water emulsion prepared with high molecular weight fish gelatin (120 kDa) was more stable than that prepared with low molecular weight fish gelatin (50 kDa).

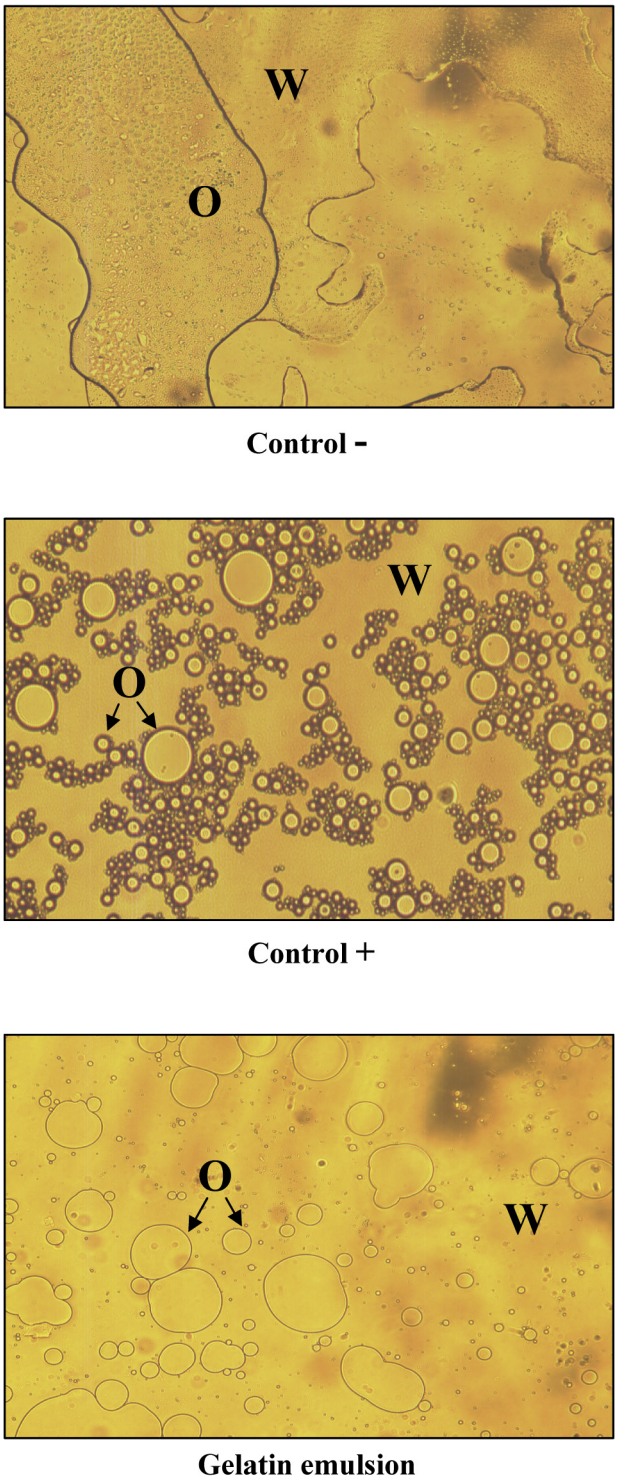

Fig. 5. Microscopic images (40×) of emulsion droplet formation (W: water and O: sunflower oil). Control-: Water + sunflower oil; Control+: SDS solution (1%) + sunflower oil; Gelatin emulsion: Squid skin gelatin solution (1%) + sunflower oil.

3.7.3. Influence of squid gelatin on emulsion formation

The influence of squid gelatin on the formation of sunflower oil-in-water emulsion was examined (Fig. 5). Optical microscopy indicated that there were some large droplets in squid gelatin formed emulsion. Sodium dodecyl sulfate (SDS) was used as an anionic surfactant. SDS rapidly adsorbs to the surfaces of lipid droplets formed during homogenization and produce primary emulsion containing small anionic droplets. The ability of proteins to form and stabilize emulsions is dependent on their ability to adsorb to interfaces and on the amount of protein required to saturate the interface (McClements, 2005). This study shows the ability to generate emulsion containing squid gelatin as emulsifier used in the development of food products with improved stability of emulsion systems.

3.7.4. Fat-binding capacities and water-holding capacities

Fat-binding and water-holding capacities are interfacial properties that are closely related to texture by the interaction between components such as water, oil and other components. Fat-binding capacity (FBC) and water-holding capacity (WHC) of the SSG gelatin were 9.2 g oil/g protein and 6.8 g water/g protein, respectively. Water-holding capacity is believed to be affected by the amount of hydrophilic amino acids and by the existence of a great number of pores and voids within the gelatin structure (Kaewruanga, Benjakul, & Prodpran, 2014). Fat-binding capacity depends to the presence of non-covalent bonds, such as hydrophobic, electrostatic and hydrogen bonding forces that are involved in lipideprotein interactions (Lawal, 2004).

4. Conclusion

An enzyme-aided process was successfully applied to obtain gelatin from squid (*L. vulgaris*). The gelatin extraction yield was 6.82 g/100 g. The gelatin, which showed a high average molecular weight and relatively high imino acids (180 residues per 1000 residues) and total hydrophobic amino acids contents, was able to form a completely thermo-reversible gel. The extracted gelatin also exhibited other desirable interfacial properties such as foaming, emulsifying, fat-binding and water-holding capacities. Overall, the findings indicate that the squid skin gelatin obtained in the present study has a number of attractive properties that point to its potential for future application as an additive in the food industry.

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