Physicochemical and functional properties of gelatin from tuna (*Thunnus thynnus*) head bones

ANISSA HADDAR – ALI BOUGATEF – RAFIK BALTI – NABIL SOUISSI – WAEL KOCHED – MONCEF NASRI

Summary

Extraction, characteristics and some functional properties of gelatin from tuna (*Thunnus thynnus*) head bones, obtained by hydrolysing tuna heads with an alkaline protease, were investigated. The bones were desalted using 0.4 mol·l^{-1} HCl and soaked for 30 min in 9 g·l^{-1} $Ca(OH)_2$. The extraction conditions for the gelatin were 75 °C, pH 4.0 for 4 h. Yield calculated based on dry weight of tuna head bones was 18.1% ± 0.8%. The gelatin from tuna head bones (THBG) had 88.3% ± 1.2% protein and 1.1% ± 0.9% lipid. The gel strength of the gelatin gel from tuna head bones was 109 g ± 1.8 g. THBG had a lower emulsifying activity index and stability than that of a commercial halal bovine gelatin (HBG) ($p < 0.05$). THBG showed poorer foaming properties than HBG. Fat-binding capacity of THBG was slightly lower than that of HBG, but water-holding capacity was higher with THBG.

Keywords

gelatin; tuna head bones; halal bovine gelatin; gel strength; emulsification; foaming; fat-binding; water holding capacity

Gelatin is a term generally used for all the extracted collagen fractions that exceed an arbitrary minimum molecular weight of 30 kDa [1]. The collagen fractions with a lower molecular weight are not considered gelatin as they are not able to form a gel. Gelatin is obtained by partial hydrolysis of collagen through destruction of cross-linkages between polypeptide chains of collagen along with some level of breakage of polypeptide bonds [2]. It is the only hydrocolloid widely used in the food, drug and cosmetic industries that is not a saccharide [3]. The chemical composition of gelatin is similar to that of the parent molecule, collagen, and is mainly multiple repetitions of a Gly-X-Y sequence, where X is often proline (Pro) and Y is often hydroxyproline (Hyp) [4; 5]. One of the most important characteristics of gelatin is its low melting point, that is, below human body temperature, which makes it very favourable for use in the food industry [6]. Another important characteristic of gelatin is that its gel strength is usually higher than most of the common gelling agents [7]. Gelatin can be obtained from the skins, bones and scales of various animals, but most importantly from pig skin. Recent studies have shown that fish skin might be an alternative raw material for gelatin production as it provides relatively good quality gelatin and also eliminates the religious concerns of the Muslim and Jewish communities. In addition, using fish processing industry by-products for value-added products may help to overcome some disposal and environmental problems and can provide extra profit via more complete utilization of fish.

A number of studies have been devoted to the processing and functional properties of fish gelatin. The gelatins were prepared either from skin, scales, bone or cartilage. Examples of species of fish that were used for gelatin production are: channel catfish [8], lumpfish [9], tilapia [10, 11], shark [12], cod [13], nile perch [14], pollock [15], yellow fin tuna [16], skate [17], catfish [18], sin croaker and shortfin scad [19], cattlefish [20], grey triggerfish [21], grass carp fish [22] and rainbow trout [23].

The objective of this study was to extract gelatin from the head bones of tuna and to determine its physicochemical and functional characteristics.

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MATERIALS AND METHODS

Chemicals

Sodium dodecyl sulphate (SDS), acrylamide, ammonium persulphate, *N,N,N',N'*-tetramethyl ethylene diamine (TEMED), Coomassie Brilliant Blue R-250 were from Bio-Rad Laboratories (Hercules, California, USA). Food grade halal bovine gelatin (HBG), obtained with gel strength about 259 g, was obtained from Groupe EURALIS (Brive la Gaillarde, Corrèze, France). Other chemicals and reagents used were of analytical grade.

Materials and pretreatment

Bluefin tuna (*Thunnus thynnus*) was provided by the Institut Nationale des Sciences et Technologie de la Mer, Centre de Mahdia, Mahdia, Tunisia. It is neither a warm-blooded nor a cold-blooded fish that can live for up to 40 years and grow to over 4 m in length and 600 kg in weight. Bluefin tuna in this work were around 2.5 m long and weighed around 350 kg. Immediately after catching, the heads were removed using an electrical saw. On the same day (approximately 2 h after catching), heads were packed in polyethylene bags, placed in ice with a sample/ice ratio of approximately 1:3 (w/w) and, after landing, transported to the research laboratory within 30 min. Once received in the laboratory, fish heads were immediately ground twice using an industrial grinder FATOSA P 130, Hachoir E130 (Technocarne, La Wantzenau, France) at medium speed for 30 min. The mixed heads were frozen again at –20 °C for 24 h. Before use, heads were allowed to thaw at room temperature. Bones were obtained by hydrolysing tuna head (100 g) with an alkaline protease from *Bacillus mojavensis* A21 prepared in our laboratory according to Haddar et al. [24] at pH 10.0 at a ratio of 1000 U·g^{-1} of tuna head at 50 °C for 140 min and agitated with a mixer (Ultra-Turrax T25; IKA Werke, Staufen, Germany) at 2.5 Hz. During the reaction, pH was maintained by multiple addition of 1 mol·l^{-1} NaOH solution (approximately 6 times). Alkaline protease activity was measured by the method of Kembhavi et al. [25] using casein as a substrate. A 0.5 ml aliquot of protease, suitably diluted, was mixed with 0.5 ml 100 mmol·l^{-1} glycine–NaOH (pH 10.0) containing 1% casein, and incubated for 15 min at 60 °C. The reaction was stopped by the addition of 0.5 ml trichloroacetic acid (20%; w/v). The mixture was allowed to stand at room temperature for 15 min and then centrifuged at 10 000 × *g* using a microcentrifuge (MIKRO 20; Hettich, Tuttlingen, Germany) for 15 min to remove the precipitate. The acid-soluble material was estimated spectrophotometrically at 280 nm (T70 UV/VIS spectrometer; PG Instruments, Beijing, China). A standard curve was generated using solutions of 0–50 mg·l^{-1} tyrosine. One unit of protease activity was defined as the amount of enzyme required to liberate 1 μg of tyrosine per min under the experimental conditions used.

The mixture obtained was composed of bones and a liquid fraction of protein hydrolysates. After filtering using Whatman No. 4 filter paper (Whatman, Maidstone, United Kingdom), the solid fraction that constituted head bones, was dried by hot air at 60 °C and crushed in a mortar and pestle into pieces that were generally less than 0.5 mm in diameter.

Extraction of gelatin from tuna head bones

Gelatin from tuna head bones was extracted according to the method described by Liu et al. [8]. The dry bone powder was treated with 0.4 mol·l^{-1} HCl (1:5 w/v bone:acid) for 7.5 h at 20 °C, agitated at 2.5 Hz and the acid solution changed at 1.5 h intervals. After decantation of the supernatant, bone powder was washed with 0.1 mol·l^{-1} NaOH until the pH reached 10–11 as determining using a pH meter (Metrohm, Villebon, France), subsequently treated with 9 g·l^{-1} of $Ca(OH)_2$ (1:2 w/v bone:$CaOH_2$) for 144 h agitated at 2.5 Hz, and finally washed twice with water. Bone gelatin was extracted at 75 °C for 4 h (1:4 w/v bone:water) with continuous stirring with a mixer (Ultra-Turrax T25) at 2.5 Hz. Finally, the gelatin extracted was freeze-dried using a freeze-dryer Christ ALPHA 1-2 (Bioblock Scientific, Illkirch, France).

ANALYSIS OF GELATIN

Approximate analysis

The moisture, ash and fat contents of the freeze-dried gelatin powder were determined according to the AOAC methods number 927.05, 942.05 and 920.39 B, respectively [26]. The protein content was determined by estimating its total nitrogen content by Kjeldahl method according to the AOAC method number 984.13 [26]. A factor of 5.4 was used to convert the nitrogen value to protein. All measurements were performed in triplicate.

The yield of gelatin was calculated based on the weight of gelatin obtained per dry weight of head bones.

$$Yield\,[\%] = \frac{FDG}{HHB} \times 100 \quad (1)$$

where *FDG* is weight of freeze dried gelatin and *HHB* is the weight of dried head bones.

Determination of colour

The colour of the freeze dried gelatins samples was determined using a ColorFlex spectrocolorimeter (Hunter Associates Laboratory, Reston, Virginia, USA) based on three colour co-ordinates, namely L^* (lightness), a^* (redness/greenness) and b^* (yellowness/blueness) [11]. The gelatin was filled in a 64 mm glass dish and readings taken through the glass with three readings for separate samples. The white tile and black glass (Hunter Associates Laboratory) were used to standardize the equipment.

Determination of gel strength

Gel strength of gelatin was determined according to the method of Gómez-Guillén et al. [13] using 6.67% gels (w/v) prepared by mixing the freeze-dried gelatin in distilled water at 60 °C for 30 min and cooling down the solution in a refrigerator to 7 °C (maturation temperature) for 16–18 h. The gel strength of gelatin gels was determined at 7 °C using a Model TA-TX2 texture analyzer (Texture Technologies, Scarsdale, New York, USA) with a 5 kN load cell equipped with a 1.27 cm diameter flat-faced cylindrical Teflon plunger. The dimensions of the free-standing sample were 3.8 cm in diameter and 2.7 cm in height. Gel strength was expressed as the maximum force required for the plunger to press the gel in 4 mm at a rate of 0.5 mm·s^{-1} [27]. The measurement was performed in triplicate for separate samples.

Electrophoretic analysis

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was done using the method of Laemmli [28]. Each gelatin sample was dissolved in 10 mmol·l^{-1} sodium phosphate containing 1% SDS (pH 7.0). The mixture was heated at 90 °C for 5 min in a water bath to dissolve the proteins. The mixture was centrifuged at 8500 ×g for 5 min at room temperature using a microcentrifuge (MIKRO 20) to remove undissolved debris. Solubilized samples were mixed with the loading buffer (2% SDS, 5% mercaptoethanol, 0.002% bromophenol blue, at a 1:4 (v/v) sample:buffer). Samples (each containing 20 μg protein before centrifugation) were loaded onto each channel in the polyacrylamide gels comprising a 4% stacking and a 7% resolving gels and subjected to electrophoresis using a Mini Protean unit (Bio-Rad Laboratories). After electrophoresis, the gel was stained with 0.1% (w/v) Coomassie Brilliant Blue R-250 dissolved in water, methanol and trichloroacetic acid (5:4:1 v/v/v) and destained using a solution containing methanol, distilled water and acetic acid (5:4:1 v/v/v). Type I collagen from calf skin (Sigma, St. Louis, Missouri, USA) was used as a marker for α-chain and β-component mobilities.

DETERMINATION OF FUNCTIONAL PROPERTIES

Soluble protein index

The gelatin samples were separately dissolved in distilled water (1:100 gelatin:distilled water). The pH of the gelatin solution was adjusted ranging from 2.0 to 10.0 with either 1.0 mol·l^{-1} HCl or 1.0 mol·l^{-1} NaOH. The samples were centrifuged at 9000 ×g (MPW-350R/RS centrifuge; MPW, Warsaw, Poland) for 15 min at 4 °C. The final pH of the supernatant was measured. Protein concentration in the clear supernatant was determined by the buiret method [29] and was expressed as a percentage of total protein in the gelatin sample (determined by the Kjeldhal method) taking into account the volume of acid/alkali added at each pH level. Protein concentration in the supernatant was estimated using a standard curve plotted from known concentrations and absorbance of a standard protein bovine serum albumin (BSA, lyophilized powder, purity ≥ 98%; Sigma-Aldrich, St. Louis, Missouri, USA). The absorbance readings were all at 540 nm (T70 UV/VIS spectrometer).

Solubility of proteins as a function of NaCl concentration

Gelatin samples were separately dissolved in 50 mmol·l^{-1} potassium phosphate buffer pH 7.5, containing different concentration of NaCl (0–2.5 mol·l^{-1}, 1:100 gelatin:buffer). The gelatin solution was centrifuged at 9000 ×g for 15 min at 4 °C (MPW-350R/RS centrifuge). The protein concentration in the clear supernatant was determined by the buiret method [26]. A plot of protein solubility as a percentage of total protein in the gelatin sample *vs* molar concentration of NaCl was prepared.

Determination of emulsifying properties

The emulsion activity index (*EAI*) and the emulsion stability index (*ESI*) of gelatin were determined according to the method of Pearce and Kinsella [30] with a slight modification. The gelatin solutions were prepared by dissolving freeze-dried gelatin in distilled water at 60 °C for 30 min. Thirty ml of gelatin solutions at different concentrations (0.5, 1, 2 and 3%) were homogenized with 10 ml of soybean oil for 1 min at room temperature (22 °C ± 1 °C) using a Moulinex R62 homogenizer (Organotechnie, Courneuve, France). Aliquots of the emulsion (50 μl) were taken from the bottom of the container using a micropipette at 0 min and 10 min after homogenization, and diluted 100-fold

with 0.1% SDS solution. The mixtures were mixed thoroughly for 10 s using a Vortex mixer (Fisher Scientific, Strasbourg, France). The absorbance of the diluted solutions was measured at 500 nm using a spectrophotometer (T70 UV/VIS spectrometer). The absorbances measured immediately (A_0) and 10 min (A_{10}) after emulsion formation were used to calculate the *EAI* and *ESI* as follows [30]. All determinations are means of at least three measurements.

$$EAI\ [m^2 \cdot g^{-1}] = \frac{2 \times 2.303 \times A_0 \times N}{c \times \varphi \times 10000} \quad (2)$$

where N represents a dilution factor; c is the weight of protein per unit volume ($g \cdot ml^{-1}$); and φ is the oil volumetric fraction (0.25).

$$ESI\ [min] = \left(\frac{A_0}{A_0 - A_{10}} \right) \times 10 \quad (3)$$

where *ESI* stands for emulsifying stability index at 10 min, A_0 is the absorbance of emulsion determined immediately after it was prepared and ($A_0 - A_{10}$) is the value change of turbidity in 10 min.

Determination of foaming properties

Foam expansion (*FE*) and foam stability (*FS*) of gelatin solutions were obtained according to the method of Shahidi et al. [31] with a slight modification. Twenty ml of gelatin solution at different concentrations (0.5, 1, 2, and 3%) were homogenized using a Moulinex R62 homogenizer to incorporate air for 1 min at room temperature (22 °C ± 1 °C). The whipped sample was then immediately transferred into a 50 ml graduated cylinder, and the total volume was measured at 0, 30 and 60 min after whipping. Foam capacity was expressed as foam expansion at 0 min, which was calculated according to the following equation:

$$FE\ [\%] = \frac{V_T - V_0}{V_0} \times 100 \quad (4)$$

where V_T is the total volume after whipping (ml); V_0 is the volume before whipping (ml).

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

$$FS\ [\%] = \frac{V_t - V_0}{V_0} \times 100 \quad (5)$$

where V_t is the total volume (ml) after leaving at room temperature for the different times (30 min and 60 min). All determinations are means of at least two measurements.

Determination of water-holding capacity

Water-holding capacity was measured by a partially modified method of Lin et al. [32]. Gelatin (0.5 g) was placed in a centrifuge tube and weighed (tube with gelatin). Distilled water (50 ml) was added, and held at room temperature for 1 h. The gelatin solutions were mixed for 5 s every 15 min using a Moulinex R62 homogenizer. The gelatin solutions were then centrifuged at 450 ×*g* using a MPW-350R centrifuge for 20 min at 22 °C ± 1 °C. The upper phase was removed and the centrifuge tube was drained for 30 min on a sheet of filter paper after tilting at a 45° angle. Water-holding capacity was calculated as the weight of the insoluble fraction of gelatin in water after draining divided by the weight of the dried gelatin, and expressed as the weight% of the original dried gelatin.

Determination of fat-binding capacity

Fat-binding capacity was measured by a partially modified method of Lin et al. [32]. Gelatin (0.5 g) was placed in a centrifuge tube and weighed (tube with gelatin). Soybean oil (10 ml) was added, and held at room temperature for 1 h. The gelatin solutions were mixed with a Vortex mixer for 5 s every 15 min. The gelatin solutions were then centrifuged at 450 ×*g* for 20 min at 4 °C. The upper phase was removed and the centrifuge tube was drained for 30 min on a filter paper after tilting to a 45° angle. Fat-binding capacities were calculated as the weight of the insoluble fraction of gelatin in oil after draining divided by the weight of the dried gelatin, and expressed as the weight% of the original dried gelatin.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test. The SPSS statistic program version 10.0 (SPSS, Chicago, Illinois, USA) was used for data analysis.

RESULTS AND DISCUSSION

Extraction and characterization of gelatin from tuna head bones

The approximate composition, based on fresh ground tuna head, minced twice using an industrial mixer at medium speed, gave a protein content of 7.0% ± 0.8%, moisture content of 78% ± 1.1% and lipid content of 3.2% ± 0.5% (Tab. 1).

After freeze-drying, the moisture content in the gelatin was 7.7% ± 0.1%. The Kjeldahl-based protein content in the freeze-dried gelatin was

Tab. 1. Approximate composition of tuna head bones and THBG.

Composition [%]	Tuna head bones	THBG
Moisture	78 ± 1.1	7.7 ± 0.13
Protein	7.0 ± 0.8	88.0 ± 1.2
Fat	3.2 ± 0.5	1.1 ± 0.9
Ash	5.7 ± 0.23	1.4 ± 0.3
Colour		
*L**	–	57.0 ± 1.2
*a**	–	–0.3 ± 0.05
*b**	–	8.3 ± 1.0
Solubility* [%]		94.0 ± 1.0
Gel strength [g]		109 ± 1.7

Values are given as mean ± standard deviation from triplicate determinations, expressed as % of wet weight.
* – solubility in phosphate buffer (50 mmol·l^{-1}) containing 0.5 mol·l^{-1} NaCl (pH 7.5) expressed as % of total protein.

88.3% and more than 92% of the protein was soluble in phosphate buffer (50 mol·l^{-1}) containing 0.5 mol·l^{-1} NaCl (pH 7.5) (Tab. 1). MUYONGA et al. [14] reported that the protein content in their gelatin preparations derived from skins and bones of young Nile perch were 88.8% and 83.3%, respectively, whereas adult Nile perch gelatin had protein content of 88.0% and 78.4%, respectively. Additionally, gelatin from skins of bigeye snapper and cuttlefish preparations had protein contents of 87.9% and 91.3%, respectively [20; 33].

The presence of ash, lipid and other impurity at very low concentrations are important for the quality of gelatins [34]. Usually ash contents up to 2.0% can be accepted in food applications. Gelatin from tuna head bones contained lower ash content (1.4% ± 0.3%) than the recommended maximum. Yield calculated based on dry weight of tuna head bones was 18.1% ± 0.8%. The gelatin yields obtained for channel catfish, greater lizardfish and Nile perch head bones were in the range of 3–11% [8, 14, 35].

The gelatin yields vary among fish species, mainly due to the differences in collagen content, the compositions of the skin as well as the skin matrix, and preparation methods used. Yields of gelatin obtained from fish bones have been reported for greater lizardfish (5.1%) [35] and channel catfish (3.9%) [8]. Gelatin yields were also reported for fish skins such as sin croaker (14.3%), shortfin scad (7.2%) [19], bigeye snapper (6.5%), brownstripe red snapper (9.4%) [33], cuttlefish (7.8%) [20], Atlantic salmon (15.3%) [36], grey triggerfish [21], grass carp fish [22] and rainbow trout [23]. Furthermore, a high degree of cross-linking via covalent bonds may decrease the solubility of collagen and might lead to a lower amount of extractable gelatin [37]. The extraction conditions, including solvent, temperature and extraction time, might also affect the yield of extracted gelatin.

These findings are in line with previous results reported by ARNESEN and GILDBERG [38], and LIU et al., [8] who reported a small yield of gelatin extracted from cod and channel catfish head bones, respectively.

The colour of the gelatin depends on the raw material used for the extraction and also whether it is obtained from the first stage, second stage or subsequent stages of extraction. However, colour does not influence other functional properties [39]. THBG showed significantly lower value for lightness (‘*L**) (57.3 ± 1.2) than the Halal bovine gelatin, which was 82.2 ± 1.61. The *a** value for gelatin samples showed negative value indicating a shift of colour towards green. The *b** value was positive indicating a degree of yellowness. HBG showed *b** values (19 ± 0.5) higher than those of THBG.

SDS-PAGE

Functional properties of gelatin are influenced by the amino acid composition, the distribution of the molecular weights, structure and composition of its subunits.

Gelatin from tuna head bones was analysed by SDS-PAGE. Calf skin collagen type I was used as a marker for α-chains (α_1, α_2) and its polymers, mainly β-components. As shown in Fig. 1, THBG contained α_1- and α_2-chains as the major components, similar to the standard collagen used. At the

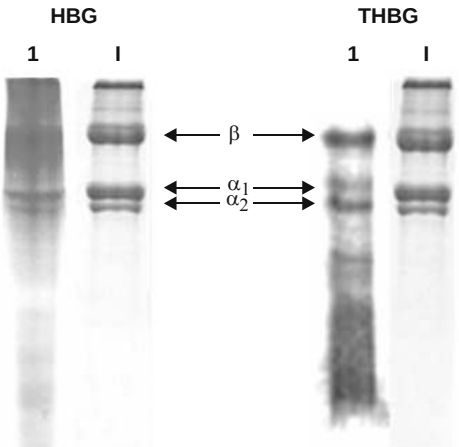

Fig. 1. SDS-PAGE patterns of HBG and THBG.

1 – 20 μg·ml^{-1} gelatin; I – calf skin collagen type I; β – β-chain; α_1 – α_1-chain; α_2 – α_2-chain.

same time, β-chains were also observed in THBG. In addition, many of the the protein bands that were generally observed in THBG were smeared over a wide molecular weight range probably due to the hydrolysis.

According to YAU et al. [40], a wide molecular weight distribution negatively affects some functional properties of macromolecules. MUYONGA et al. [14] reported that Nile perch skin gelatin was generally found to contain a higher proportion of β fractions than the Nile perch bone gelatin. They concluded that the bone gelatin had a consistently higher incidence and/or stability of cross-links than found in the skin collagen, resulting in more cleavage of peptide bonds during the manufacture of bone gelatins.

Gel strength

Gel strength is one of the most important functional properties of gelatin. Fish gelatin typically has lower gel strength than mammalian gelatin. Gel strength is a function of complex interactions determined by amino acid composition and, to some extent, by the ratio of α-chain and the amount of β-component. The gel structure of gelatin is more stable when the imino acid content is higher, and the amount of aggregates with high molecular weight is less [13]. It is well known that the hydrogen bonds between the water molecules and free hydroxyl groups of amino acids in gelatin are essential for gelatin gel strength [41].

The quality of gelatin is generally determined by the gel strength, which are characterized as low (< 150 g), medium (150–220 g) and high gel strength (220–300 g) [42]. Gelatins with different gel strengths were reported for Atlantic salmon (108 g), cod (71 g) [36], sin croaker (125 g), short-fin scad (177 g) [19], cuttlefish (181 g) [20], bigeye snapper (106 g), brownstripe red snapper (219 g) [33], and young and adult Nile perch (217 g and 240 g, respectively) [14]. The difference in gel strength among species was possibly due to the different composition, particularly in terms of amino acid composition and size of the protein chains [14], as well as the influence of the living habitat of the species [43] and the parameters of the method used for the measurement.

FUNCTIONAL PROPERTIES OF THBG

Soluble protein index and solubility as a function of NaCl concentration

The soluble protein index of gelatin showed minimum solubility of 55.3% at pH 6.0 (Fig. 2). On either side of this pH, solubility increased and a maximum solubility of 98.5% at pH 2.0 was determined. The minimum solubility at pH 6.0, which may be nearer to its isoelectric point, is due to higher electrostatic interaction because of net charges of the molecules being close to equal [44]. Gelatin is an amphoteric protein with an isoelectric point between 5 and 9 depending on raw material and method of manufacture [45, 46]. At pH values below and above the isoelectric point, proteins tend to carry more net charges, thereby enhancing hydration [47].

The solubility profile of gelatin as a function of NaCl concentration indicated a maximum solubil-

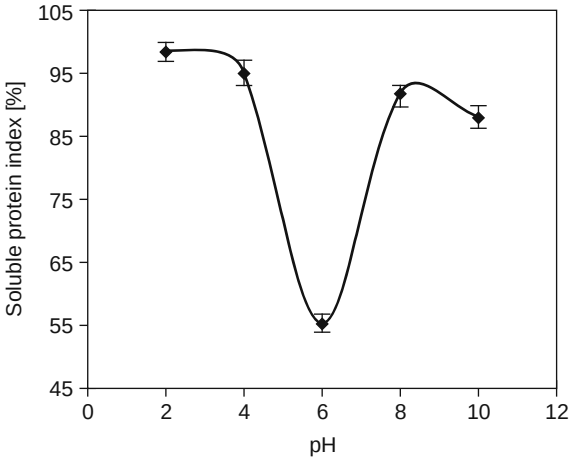

Fig. 2. Soluble protein index of gelatin from tuna head bones with distilled water as solvent in the pH range of 2–10.

Soluble protein index is expressed as percentage of total proteins.

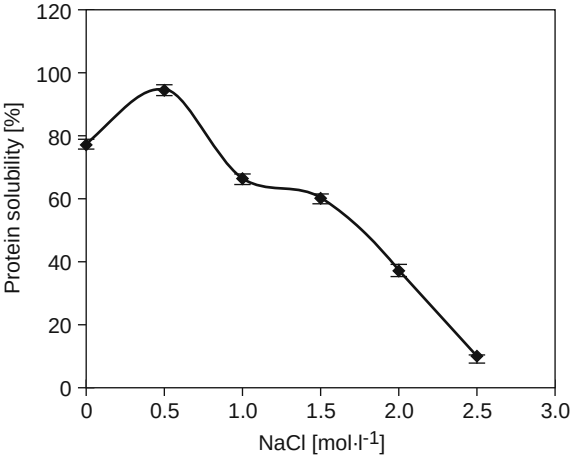

Fig. 3. Protein solubility of gelatin as a function of molar concentration of NaCl in phosphate buffer (50 mmol·l^{-1}, pH 7.5).

Protein solubility is expressed as percentage of total protein. Values are given as mean ± standard deviation from triplicate determinations.

ity at 0.5 mol·l^{-1} NaCl (Fig. 3). Nearly 77% of the total protein was found to be soluble at 0 mol·l^{-1} concentration. With an increase in NaCl concentration, the solubility of gelatin increased up to 0.5 mol·l^{-1}, beyond which solubility decreased. Generally, solubility increases with increase in salt concentration up to a certain level (salting 'in') and with a further increase in salt concentration, the solubility decreases (salting 'out') [48]. At 0.5 mol·l^{-1} concentration, nearly 94% of the gelatin was found to be soluble.

Emulsifying properties of gelatin

Gelatin is used as a foaming, emulsifying and wetting agent in food, pharmaceutical, medical and technical applications due to its surface-active properties.

Emulsion activity index (*EAI*) and emulsion stability index (*ESI*) of THBG and HBG at different concentrations are shown in Tab. 2. *EAI* of both gelatins increased with the increase of gelatin concentration. Protein at high concentrations facilitated more protein adsorption at interfaces [49]. At the same concentrations used, *EAI* of HBG was higher than that of THBG ($p < 0.05$). This possibly resulted from the difference in the intrinsic properties, amino acid composition and conformation of the gelatin proteins from the two sources [50]. High solubility of the protein in the dispersing phase increases the emulsifying efficiency, because the protein molecules should be able to migrate to the surface of the lipid droplets rapidly [51]. Kim et al. [52] reported that emulsifying properties of gelatin from cod bone were similar to those of commercial emulsifiers, such as Tween 80.

Emulsions containing THBG were more stable than those of HBG ($p < 0.05$) (Tab. 2). Surh et al. [53] found that the oil-in-water emulsion prepared with high average molecular weight fish gelatin (~120 kDa) was more stable than that prepared with low average molecular weight fish gelatin (~50 kDa). The thickness of an adsorbed gelatin membrane increased with increasing molecular weight. This was associated with the increased stability of emulsions against coalescence during homogenization [54].

Tab. 2. The emulsifying properties of tuna head bones gelatin (THBG) and halal bovine gelatin (HBG) at different concentrations.

Gelatin	Concentration [%]	*EAI* [m^2.g^{-1}]	*ESI* [min]
THBG	0.5	12.0 ± 0.4	43.0 ± 2.2
	1	25.0 ± 0.1	41.0 ± 2.2
	2	31.0 ± 0.1	34.0 ± 0.2
	3	32.0 ± 0.04	31.0 ± 0.04
HBG	0.5	19.0 ± 0.5	41.0 ± 0.7
	1	27.0 ± 2.5	40.0 ± 1.4
	2	34.0 ± 1.3	34.0 ± 1.7
	3	39.0 ± 0.6	30.0 ± 2.9

Values are given as mean ± standard deviation from triplicate determinations.

Foam capacity and foam stability of gelatin

Foam formation ability is another important property of gelatin for commonly used foods such as marshmallows. Foam expansion at 0, 30 and 60 min after whipping was determined by evaluating the foam capacity and foam stability of both gelatins.

FE and *FS* of THBG and HBG at various concentrations are shown in Tab. 3. FE and FS of both

Tab. 3. The foaming properties of tuna head bone gelatin (THBG) and halal beef gelatin (HBG) at different concentrations.

Gelatin	Concentration [%]	*FE* [%]	*FS* [%]	
			30 min	60 min
THBG	0.5	64.0 ± 0.1	41.0 ± 0.3	37.0 ± 0.5
	1	68.0 ± 0.5	48.0 ± 0.5	46.0 ± 1.0
	2	73.0 ± 0.8	55.0 ± 0.7	51.0 ± 1.8
	3	80.0 ± 0.1	60.0 ± 1.0	54.0 ± 1.1
HBG	0.5	110 ± 0.7	80.0 ± 0.7	70.0 ± 1.5
	1	119 ± 1.3	101 ± 1.8	82.0 ± 0.9
	2	131 ± 1.5	106 ± 1.4	99.0 ± 0.5
	3	138 ± 1.1	114 ± 0.02	102 ± 1.7

Values are given as mean ± standard deviation from triplicate determinations.

Tab. 4. Water-holding and fat-binding capacities of tuna head bones gelatine (THBG) and halal beef gelatin (HBG).

	THBG	HBG
oil/gelatin [g·g^{-1}]	2.5 ± 0.8	2.8 ± 0.1
water/gelatin [g·g^{-1}]	3.5 ± 0.3	3.1 ± 0.5

Values are given as mean ± standard deviation from triplicate determinations.

gelatins increased with increasing gelatin concentrations from 0.5% to 3%. Foams with higher concentration of proteins were denser and more stable because of an increase in the thickness of interfacial films [55]. Foam formation is generally controlled by transportation, penetration and reorganization of protein molecules at the air-water interface. A protein must be capable of migrating rapidly to the air-water interface, and unfolding and re-arranging at the interface to show good foaming ability. A positive correlation between hydrophobicity of unfolded proteins and foaming characteristics has been reported [56].

When comparing the foaming properties between both gelatins, *FE* and *FS* of HBG were higher ($p < 0.05$) than those of THBG. Foaming properties of protein might be influenced by the source of the protein, intrinsic properties of protein, the compositions and conformations of the protein in solution and at the air/water interface [55, 57, 58].

Water-holding and fat-binding capacities of gelatin

Water-holding and fat-binding capacities are functional properties that are closely related to texture and reflect the interactions between water and oil with other components. Water-holding capacity and fat-binding capacities of both gelatins are shown in Tab. 4. THBG and HBG had similar fat-binding and water-holding capacities. Fat-binding capacity depends on the degree of exposure of the hydrophobic residues inside gelatin. Water-holding capacity is believed to be affected by the amount of hydrophilic amino acids. Both tests only measure these properties for that component of the gelatin that was insoluble under the conditions of the measurement.

CONCLUSION

THBG could be extracted after the head was pretreated with alkaline protease, HCl and $Ca(OH)_2$. The THBG had medium gel strength, which is of commercial significance, considering the potential applications in foods and edible film preparations.

The functional properties, including *ESI* and water-binding capacity of THBG, were greater than those of HBG. On the other hand, *EAI, FE* and *FS*, fat-binding capacity and gel strengh were slightly lower. THBG contained α- and β-components.

It is likely that gelatin from tuna head bones may be applicable in the food industry.

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