

Gelatine Extraction from Skipjack Tuna (*Katsuwonus pelamis*) Head Bones by Acid-Hydrolysis Method and Its Physicochemical and Functional Characterizations

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Abstract

The purpose of this study was to extract gelatine from skipjack tuna (*Katsuwonus pelamis*) head bones, which is an abundant by-product of tuna canning factories. Acid-hydrolysis method was used. Used extraction condition was 70 °C, 90 min and 0.4% HCL. The gelatine sheets yield of raw material and gel strength were respectively 6.50% and 37.35 g. The results of chemical properties of the skipjack tuna (*K. pelamis*) head gelatine were 83.70% protein, 6.58% lipid, 6.09% moisture and 1.13% ash contents, and the pH, viscosity at 60 °C, gelling point, and melting point values were 4.7, 6.0 cP, 4-10 °C, and 25-27 °C, respectively. According to the results, skipjack tuna (*K. pelamis*) head gelatine complies with the physicochemical and functional requirements of Iranian National Standardization Organization for an edible gelatine. Therefore, skipjack tuna (*K. pelamis*) heads of tuna canning factories are a potential source for producing good quality gelatine that could be used in food and introduced for pharmaceutical applications.

Introduction

Skipjack tuna (*K. pelamis*), is a pelagic tropical fish species that is found in the tropical waters of India and the western Pacific. It is also the highest tuna catch in southern Iran. More than half of the country's tuna catches are in international waters. Iran is ranked 12th in the world in commercial tuna fishing. The top 10 countries for tuna catching are Indonesia, Japan, New Guinea, Taiwan, Spain, Ecuador, Korea, United States, Kiribati and Philippines, respectively. The commercial value of Indian Ocean tuna is the highest after the Pacific, estimated at \$ 6.8 billion (IFO, 2021). The processing of this species generates a large amount of solid by-products including heads, skins and viscera (up

to 40%) (ASRI, 2018), in consequence causes ecological problems and environmental pollution without suitable management. Shyni et al. (2014) recommend that conversion of these residues into value-added products can be beneficial for fish industry. Several studies reported the extraction and characterization of fish head bones gelatines from various commercial species, including Nile perch (*Lates niloticus*) (Muyonga et al., 2004), channel catfish (*Ictalurus punctatus*) (Liu et al., 2009), tuna (*Thunnus thynnus*) (Haddar et al., 2011), Kalamtra Sturgeon (hybrid species of *Huso dauricus* × *Acipenser scherenkii* × *Acipenser transmontanus*) (Islam et al., 2020), and *Pangasius sutchi* (Atma and Taufik, 2021). However, skipjack tuna (*K. pelamis*) head has not been studied in Iran as a source for fish gelatine

production. Muyonga et al. (2004), Cho et al. (2005) and Karim and Bhat (2009) indicated that gelatines extracted from warm-water fish had better properties (gel strength, viscosity, melting and gelling points) than cold-water fish gelatines, and they were nearly similar to those of mammalian gelatines. Gel strength and color are the most important physical properties for industry because they determine the gelatine's commercial value, whereas protein yield is related to profitability (GMIA, 2019). On the other hand, gel strength, color and protein yield depend on the extraction conditions and drying methods. Therefore, the purpose of this study was to extract high-quality gelatine from skipjack tuna (*K. pelamis*) head bones and to obtain the physicochemical and functional characteristics in order to evaluate its potential application in food and nutraceutical industries.

Materials and Methods

Materials

Skipjack tuna (*K. pelamis*) heads were prepared from Pars Kadous canning factory in Bandar Anzali. The heads were transported to the laboratory under ice. The lengths of the skipjack tuna (*K. pelamis*) heads were about 15–18 cm. The skins and residual meats were removed from head bones manually by a sharp scalper. After washing the head bones with cold tap water, they were packed in packages of 100 g and stored at freezer (-18 °C) storage until use. Chemical materials with Merck grade were purchased.

Preparation of raw material and gelatine extraction

Skipjack tuna (*K. pelamis*) head bones were cut into small pieces (2cm) by a snipping tool. To remove minerals, the head bones were soaked in 0.1 M acetic acid solution for 4 h with constant stirring at room temperature (25±2 °C) at a ratio of 1:8 (head bone/solution, w/v). The acidic solution was drained. The head bones were rinsed with abundant tap water until the pH was neutral. Then in order to remove lipids and non-collagenous proteins, the head bones were soaked in 0.1 M NaOH solution for 4 h with constant stirring at room temperature (25±1 °C) at a ratio of 1:8 (head bone/solution, w/v). The alkaline solution was drained. The head bones were rinsed with abundant tap water until the pH was neutral. Next, gelatine was extracted from pre-treated head bones following the method of Jalili (2004) with slight modifications. Briefly, the pre-treated head bones were treated with 0.4% HCl solution for 90 min with constant stirring at 70°C in a ratio of 1:2 (head bone/solution, w/v), and then neutralized with sodium bicarbonate until the pH was near 5.5-6. After extraction, the gelatine solution was autoclaved for 1h at 120°C. The head bones residues were removed and the gelatine solution was filtered through a cotton and cleaning cloth to remove insoluble

material. The resultant filtrate was dried at 65 °C for 20-22 h in a hot-air dryer (Memmert Etuve, Lab Oven and Furnace, Germany). The dry gelatine sheets were milled to obtain a powder and stored at 4 °C until use.

Analysis of physicochemical and functional characterizations

Determining the degree of hydrolysis

The degree of enzymatic hydrolysis (DH) was measured based on the method of Hoyle and Merritt (1994). Briefly, 5 ml of the sample (6.67%) was mixed with 5 ml of 10% trichloroacetic acid (TCA). Then, the sample was vortexed for 10s and centrifuged at 6000 rpm for 15 min under room temperature. The amount of protein in the solution phase was measured by the biuret method (Gornall et al., 1949). Total protein in sample was measured by the kjeldahl method. The DH was calculated by the following equation:

$$\text{DH (\%)} = \frac{10\% \text{ TCA soluble protein in supernatant}}{\text{Total protein in sample}} \times 100$$

Proximate composition and pH

The moisture, lipid, ash and protein contents of skipjack tuna (*K. pelamis*) head bones gelatine powder were determined according to the AOAC (2000). The total protein content was calculated using a nitrogen conversion factor of 6.25. The pH of the gelatine was determined as described in the Iran Standard Organization method (INSO, 2018) by using a pH meter (WTW, pH 7110, Germany). All measurements were performed in triplicate.

Gel strength

Gel strength was determined according to Gómez-Guillén et al. (2002). Gelatine powder was dissolved in deionized water (60 °C) to obtain a final concentration of 6.67%. The solution was cooled at 4 °C for 24 h. Gel strength was determined using a Brookfield texture analyzer (CT3 Texture Analyzer, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), and the values were expressed in grams (g). The measurement was performed in triplicate.

Protein yield of gelatine sheets and gelatine solution

Protein yield of gelatine sheets was determined using the following equation:

$$\text{Protein yield of gelatine sheets (\%)} = \frac{\text{Extracted gelatine}}{\text{Total initial sample}}$$

The soluble protein concentration was determined by the Biuret method (Yang et al., 2007), using a spectrophotometer at 540 nm (UV/Vis, PerkinElmer Inc.,

Waltham, MA, USA) with bovine serum albumin (BSA, standard grade, Sigma Chemical Co., St. Louis, MO, USA) as a standard. The protein yield of gelatine solution was determined using the following equation:

$$\text{Protein yield of gelatine solution (\%)} = (P \times V \div W) \times 100$$

where P is the protein concentration (g/ml), V is the volume of the extract (ml), and W is the sample weight used for extraction (g).

Viscosity

Dynamic viscosity was determined according to Niu et al. (2013). A 6.67% gelatine solution was obtained by dissolving dried gelatine in deionized water at 60 °C, until complete solubilization. Viscosity of the solution was determined with a Brookfield DV2T viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA) equipped with an SC4-18 spindle, at 60±1 °C and 70 rpm, and the values were measured in centipoises (cP). The measurement was performed in triplicate.

Melting and gelling points

Determination of melting and gelling points was based on the method of Muyonga et al. (2004) and INSO (2018) with slight modifications. Briefly, gelatine solution (6.67%) was prepared in thin wall (12 mm × 75 mm) screw cap test tubes. For melting point determination, the dissolved gelatine powder was kept at 4 °C for 24h. Then they were transferred to an incubator (25 °C) which was warmed gradually. The first melting point of the gel was recorded. For gelling point determination, dissolved gelatine powder was cooled slowly at 4 °C and was controlled at intervals of 5min. The first gelling point was recorded. The measurement was performed in triplicate.

Water holding capacity

Water holding capacity (WHC) of gelatine powder was determined by Wassawa et al. (2007) method. Briefly, 0.5 g of the sample was added to 20 ml of distilled water in a centrifuge tube, shaken for 30s with a tube shaker and then kept at laboratory temperature for 6 h. After that was centrifuged at 6000 rpm for 30 min at room temperature. The floating phase in the tube was filtered through filter paper No. 41. The difference between the volume of initial distilled water added to the sample and the amount of water filtered from the filter paper was noted. The result was reported as the amount of water absorbed in grams.

Color

The gel color parameters such as L* (lightness), a* (greenness to redness) and b* (blueness to yellowness),

C* (Chroma), and h (the hue angle) was measured by a colorimeter (NR60CP Precision Colorimeter, 3nh, China).

Amino acid compositions

Amino acid compositions were analyzed as described by Heinrichson and Meredith (1984), using HPLC (model Chromaster, Hitachi, Ltd., Chiyoda-ku, TYO, Japan). The results were expressed as g amino acid per 100 g protein.

Protein solubility

The protein solubility of gelatine powder was determined with slight modifications similar to the method reported by Chi et al. (2014). Briefly, 200 mg of gelatine powder was dissolved in 20 ml of deionized water and the pH was adjusted to 4, 5, 6, 7, 8 and 9 by HCl (1M) or NaOH (1M). The mixture was then stirred at ambient temperature for 30 minutes. Then it was centrifuged at 6000 rpm for 30 min under room temperature. The amount of soluble protein in supernatant was measured by the biuret method. Total protein in sample was measured by the Kjeldahl method. The protein solubility was calculated by the following equation:

$$\text{Protein solubility (\%)} = (\text{Soluble protein in supernatant} \div \text{Total protein in sample}) \times 100$$

Foaming capacity and foam stability

The foaming capacity (FC) and foam stability (FS) of gelatine powder were determined by Chi et al (2014) method. Gelatine powder (0.5%) completely was dissolved at 60 °C. Then 20 ml of it was transferred to a 100 ml glass. It was homogenized at room temperature for 10 min using an ultra-thurrax (IKA T10 basic ULTRA-TURRAX). Total volume was measured at 0, 3, 10 and 30 min after homogenization. The value of FC is the same as the increase in sample volume at time zero. And FS is a steady increase in volume after 3, 10 and 30 min. The amount of volume increase due to foam formation was calculated based on the following equation:

$$\text{Foam expansion (\%)} = [(A - B) \div B] \times 100$$

where A is the volume after stirring and aerating at different times (ml) and B is the volume before stirring and aerating (ml).

Statistical analysis

The statistical analysis (One-Way ANOVA) was carried out using SAS software (Version 16.0, SAS Institute Inc., Cary, NC, USA). Duncan test ($P < 0.05$) was used to show a significance difference between the specific means. Data were reported as mean ± standard

deviation (SD). All examinations were carried out in triplicate.

Results

Characterization of produced skipjack tuna (*K. pelamis*) head bones gelatine including DH, yield, proximate composition, pH, and physicochemical properties are shown in Table 1. The DH is obtained from the partial hydrolysis of collagen and its conversion to gelatine. For this product, after 90 minutes of hydrolysis with acid, the percent of DH was 25.81%. Gelatine sheets yield of head bones was about 6.50%. The obtained gelatine had a high protein content of 83.70%, with low moisture, lipid, and ash values. The pH of gelatine was 4.7. This value is within the pH range of 3.8–5.5 or 3.8–7.6 for edible gelatine (GMIA, 2019; INSO, 2018). The gel strength is important as a qualitative factor in the production of gelatine. It was about 37.35

g (Table 1). INSO (2018) reported that the range of gel strength for bovine gelatine is between 80 to 120% of the amount claimed. But for fish gelatine it has not been reported the range. The protein yield of gelatine solution (6.67%) was about 72.0%. The viscosity of the extracted gelatine in this project was 6.0 cP. This value is in the range of 2.0 to 7.0 cP (Boran et al., 2010) which was reported for commercial bovine and porcine gelatines. The gelling and melting points of skipjack tuna (*K. pelamis*) head bones gelatine were 7°C and 25.6°C, respectively. Water holding capacity is another parameter that can important role in use of this product in food industry. WHC of the produced gelatine was 3.0 g. Color properties usually influence the overall acceptability of food products. The produced gel of acid-hydrolysis of skipjack tuna (*K. pelamis*) head bones (Figure 1) observed as yellowish-orange in color (Table 1). Also, C* and h were 8.40 and 36.77, respectively. Amino acid profile of skipjack tuna (*K. pelamis*) head

Table 1. Characterization of produced skipjack tuna (*K. pelamis*) head bones gelatine: DH, yield, proximate composition, pH, and physicochemical properties

	Skipjack tuna (<i>K. pelamis</i>) head bones gelatine
DH (%)	25.81 ± 6.37
Gelatine sheets yield of raw material (%)	6.50 ± 1.32
Protein (%)	83.70 ± 0.04
Moisture (%)	6.09 ± 0.11
Lipid (%)	6.58 ± 0.04
Ash (%)	1.13 ± 0.01
pH	4.7 ± 0.20
Gel strength (g)	37.35 ± 0.50
Protein yield of gelatine solution (%)	72.00 ± 0.08
Viscosity (cP)	6.0 ± 0.02
Gelling point (°C)	7 ± 0.10
Melting point (°C)	25.6 ± 0.12
WHC (g)	3.00 ± 0.00
L*	27.51 ± 2.80
a*	5.45 ± 0.56
b*	5.03 ± 1.71
C*	8.40 ± 1.23
h	36.77 ± 1.08

Data presented as mean ± standard deviation of triplicate determinations.



Figure 1. Prepared gel of the gelatine powder extracted from skipjack tuna (*K. pelamis*) head bones

bones is shown in Table 2. Glycine was the major component (16.12%), followed by proline and alanine (10.74% and 8.17%). Functional properties of produced skipjack tuna (*K. pelamis*) head bones gelatine including protein solubility, foaming capacity and foam stability are shown in Table 3. The trend of protein solubility of produced gelatine at different pH (4, 5, 6, 7, 8 and 9) was 65.82%, 62.63%, 68.86%, 72.74%, 66.92% and 66.79%, respectively ($p < 0.05$), which showed that the highest protein solubility was observed at pH of 7 and the lowest at pH of 5. Foaming capacity of produced gelatine was high and about 117.50%, which is directly related to protein solubility and protein yield in gelatine solution. Foam stability was also measured in three times intervals of 3, 10 and 30 minutes. With increasing time, the foam stability of gelatine solution gradually decreased and this difference was statistically significant ($p < 0.05$).

Discussion

The most important purpose of producing hydrolyzed collagen such as gelatine is to make optimal use of the protein part of food and increasing the absorption and digestion of final compounds. Hydrolysis process led to the reducing the size and increasing the nutritional value and biological properties of raw materials. In this regard, previously published studies have shown that the protein content for fish head bones gelatine is generally high, in the range of 77.90–98.20% (Kim et al., 1996; Liu et al., 2009; Haddar et al., 2011). Also, Muyonga et al. (2004) reported that the protein content in gelatine extracted of young Nile perch bones was of 83.30%. The results (83.70%) obtained in this study are consistent with their results. Moisture content (6.09%) was below the prescribed limit of 13%-15% for edible gelatine (GME, 2005; GMIA, 2019). INSO (2018)

Table 2. Amino acid compositions of produced skipjack tuna (*K. pelamis*) head bones gelatine and mammalian (porcine and bovine) gelatines (g/100 g protein)

Amino acid compositions	Content (g/100 g protein)		
	Skipjack tuna (<i>K. pelamis</i>) head bones gelatine	Bovine (Ninan et al., 2010)	Porcine (Ninan et al., 2010)
Aspartic acid	3.45 ± 0.01	2.50	3.01
Glutamic acid	7.02 ± 0.00	7.23	10.32
Serine	2.32 ± 0.01	2.95	3.01
Glycine	16.12 ± 0.01	29.20	27.69
Histidine	0.43 ± 0.00	0.08	0.03
Threonine	2.06 ± 0.03	2.11	2.06
Alanine	8.17 ± 0.01	11.40	11.20
Cysteine	0.00 ± 0.00	0.00	0.00
Arginine	5.49 ± 0.01	5.10	4.90
Proline	10.74 ± 0.01	11.89	12.44
Hydroxyproline	7.90 ± 0.11	11.02	11.26
Tyrosine	0.43 ± 0.00	0.11	0.08
Valine	1.60 ± 0.07	1.80	1.88
Methionine	1.23 ± 0.06	1.01	1.43
Isoleucine	1.10 ± 0.06	1.11	0.98
Leucine	2.00 ± 0.06	1.90	1.73
Phenylalanine	1.68 ± 0.07	1.60	1.20
Lysine	3.30 ± 0.04	4.01	3.29
Tryptophan	0.00 ± 0.00	0.00	0.00

Data presented as mean ± standard deviation of duplicate determinations.

Table 3. Functional properties of produced skipjack tuna (*K. pelamis*) head bones gelatine: protein solubility, foaming capacity and foam stability

Functional properties	pH					
	4	5	6	7	8	9
Protein solubility (%)	65.82 ^d ±0.46	62.63 ^a ±1.02	68.86 ^b ±0.37	72.74 ^a ±0.39	66.92 ^c ±0.51	66.79 ^{cd} ±0.38
	Time (min)					
	0	3	10	30		
Foaming capacity (%)	117.50±3.23					
Foam stability (%)	117.50 ^a ±1.32		93.75 ^b ±0.45	76.25 ^c ±0.33		

Data presented as mean ± standard deviation of duplicate determinations.

Different lowercase superscripts within the same row indicate significant difference ($p < 0.05$).

also reported a maximum moisture content of 15% for gelatine powder or gelatine sheets. Lipid content (6.58%) was high that can be due to the use of weak acetic acid in the pre-treatment that was not likely efficient to defatted the raw material. This result was consistent with studies by Liu et al (2009) and Muyonga et al (2004), but did not match the results of Haddar et al. (2011) studies. The ash content (1.13%) was below the maximum limit (2.00%) (INSO, 2018). Generally, gelatines with ash content below 2.00% are acceptable for food applications (Kasankala et al., 2007; GMIA, 2019). Various pH values were obtained in extracted gelatines from bone as reported by Choi and Regenstein (2000) for pork bone gelatine (5.5), Silva et al. (2011) for common carp head bones gelatine (3.6-5.3) and Alfaro et al. (2009) for king weakfish bones gelatine (4.05-4.44). According to the results, the pH of skipjack tuna (*K. pelamis*) head bones gelatine complied with the requirements for an edible gelatine (Table 1). The gel strength of skipjack tuna (*K. pelamis*) head bones gelatine was greater than cold-water fish gelatines as reported by Kim et al. (1996) for cod bone (21 g), although it was lower than gel strength reported by Arnesen and Gildberg (2006) for cod head (90.90 g). The gel strength of skipjack tuna (*K. pelamis*) head bones gelatine was also lower than warm-water fish gelatines as reported by Haddar et al., (2011) for tuna (*T. thynnus*) head bones (109 g), Muyonga et al. (2004) for Nile perch head bones (179 g), Silva et al. (2011) for common carp head bones (128-131 g) and Alfaro et al. (2009) for king weakfish bones (200 g), Liu et al., (2009) for channel catfish head bones (282 g) and Atma and Taufik (2021) for *Pangasius sutchi* bones (451 g). In comparison with mammalian gelatines, the gel strength of skipjack tuna (*K. pelamis*) head bones gelatine was lower than bovine gelatines, with values of 200–221 g (Cho et al., 2005; Muyonga et al., 2004), and porcine gelatines, with values of 240–295 g (Karim and Bhat, 2009; Cho et al., 2005). Difference in gel strength depends on molecular weight range, amino acids profile, and gelatine pH (Fan et al., 2017; Karim and Bhat, 2009). Arnesen and Gildberg (2006) also reported that the extraction temperature increase can be responsible for the gel strength value decrease. The protein yield of the extracted gelatine sheets (6.50%) was higher than the yield obtained from common carp head bones (4.23-4.86%) using distilled water for extraction (Silva et al., 2007), hybrid kalamtra sturgeon head (5.01%) using HCl (Islam et al., 2020), cod head bones (5.70%) using HCl (Arnesen and Gildberg, 2006), cod head bones (5.70%) using $\text{Ca}(\text{OH})_2$ (Kim et al., 1996), greater lizardfish bones (5.08%) using warm distilled water (Taheri et al., 2009), and channel catfish head bones (3.95-8.43%) using HCl (Liu et al., 2009). But, it was lower than extracted gelatine from (*T. thynnus*) head bones (18.10%) using distilled water (Haddar et al., 2011). Variation in the protein yield value depends on age, size and structural collagen conformation on the species which the gelatine is extracted; likewise, the conditions prior to extraction

and extraction process (Karim and Bhat, 2009; Yang et al., 2007). The viscosity of the extracted gelatine in this study (Table 1) was greater than obtained value from other species such as *Pangasius sutchi* (3.17 cP) (Atma and Taufik, 2021), cod bone (3.87 cP) (Kim et al., 1996), dog shark skin (5.60 cP), and skipjack tuna skin (4.40 cP) (Shyni et al., 2014) and lower than obtained value from cod head bones (24.00 cP) (Arnesen and Gildberg., 2006) and grass carp skin (7.10 cP) (Ninan et al., 2014). In addition, Shyni et al. (2014) found that viscosity is partially controlled by molecular weight and molecular size distribution. Furthermore, Alfaro et al. (2014) reported that the use of low extraction temperatures led to the formation of high molecular weight compounds, increasing the viscosity. Gelling point determined in this study were lower than reported gelatines from cold-water fish (11–12°C) and warm-water fish (15.5–20.5°C) (Gómez-Guillén et al., 2002). The extracted gelatine from skipjack tuna (*K. pelamis*) head bones was formed a gel at a temperature below 10°C for less than 2 hours. Melting point were comparable to those of warm-water fish gelatines (24.3–29.1°C) (Silva et al., 2011; Muyonga et al., 2004; Boran et al., 2010; Cho et al., 2005; Ninan et al., 2014). The gel obtained from gelatine powder extracted from skipjack tuna (*K. pelamis*) head bones was melted in less than 30 minutes at the room temperature. However, skipjack tuna (*K. pelamis*) head bones gelatine similar to gelatine reported of other studies had lower gelling and melting points than mammalian gelatines (23.4–31.8°C and 31.4–36.5°C, respectively) (Boran et al., 2010; Cho et al., 2005; Karim and Bhat, 2009; Ninan et al., 2014). In general, gelatines from bovine and porcine sources have higher gelling and melting points than warm-water and cold-water fish gelatines (Ninan et al., 2014; Karim and Bhat, 2009). About this, Muyonga et al. (2004) and Ninan et al. (2010) reported the content of imino acids is approximately 23.0–24.0% in mammalian gelatines, 18.0–21.0% in warm-water fish gelatines, and 16.0–17.0% in cold-water fish gelatines. It is established that the proportion of the imino acids correlate with gelling and melting points of gelatine. The differences between the gelling and melting points of skipjack tuna (*K. pelamis*) head bones gelatine and those of cold-water and warm-water fish and mammals can be explained by differences in the habitat temperature of the various species (Nikoo et al., 2014). Water-holding capacity (Table 1) is closely related to sample texture and reflect the interactions between water with other components. The amount of hydrophilic amino acids can be affected on it (Haddar et al., 2011). This test only measures this property for that component of the gelatine that was insoluble under the conditions of the measurement. Several studies have found that fish hydrolysates have good water holding capacity and can increase the yield of cook when added to meat compounds (Shahidi et al., 1995; Kristinsson and Rasco, 2000). On the other hand, the presence of polar groups such as carboxyl and amino increase during acidic hydrolysis and can have a substantial effect on the

water absorbent amount (Kristinsson and Rasco, 2000). Fish gelatine is one of the most popular natural polymers that is widely used in the food industry and can be considered as a suitable alternative to prevent food water leakage. Gelatine color is the great commercial importance as a visual feature. However, there is no universally accepted technique for evaluating it (Cole and Roberts, 1997). But the color of gelatine depends on the raw materials and the extraction method for removing inorganic, proteinaceous and mucosubstances compounds (Eastoe and Leach, 1977). Also from what stage it is obtained: the first, second or next steps of extraction. However, color does not influence other functional properties (Ockerman and Hansen, 1999). Gelatine color values vary from pale yellow to dark amber (Cole and Roberts, 1997). The color of the gel prepared from gelatine powder extracted from skipjack tuna (*K. pelamis*) head bones (Table 1) were lower than the range reported for fish and bovine gelatines (Lueyot et al., 2021). It showed significantly lower value for lightness (L^*) (27.51) than the fish and bovine gelatines, which were 94.28 and 68.80, respectively. The a^* value showed positive value indicating a shift of color towards red. The b^* value was also positive indicating a degree of yellowness. The hue (h) value was low compared to the value of fish and bovine gelatines reported by Lueyot et al., (2021). The Chroma (C^*) of the resulting sample may have been developed by the Maillard reaction, in fact a non-enzymatic browning reaction between amino acid and reducing sugar (Van Boekel, 1998). Bleaching for gel transparency can be a practical method, but it reduces the gel-like properties. For example, squid gelatine was bleached with hydrogen peroxide (2%) that caused the increase in L^* value and decrease in gel strength (Johnston-Banks, 1990). Muyonga et al. (2004) also reported that the filtration process during extraction affected the gelatine clarity. According to the result reported by (Alfaro et al., 2014), king weakfish bones gelatine was significantly lighter than this sample, tuna (*T. thynnus*) head bones gelatine reported by Haddar et al., (2011) was the greenest and it was significantly ($p < 0.05$) more yellowish when compared to gelatine extracted from this study. The extracted gelatine in this study had relatively low contents of glycine and imino acids (proline and hydroxyproline), compared with reported by Liu et al. (2009) and Muyonga et al. (2004). Cysteine and tryptophan were absent. However, both cysteine and tryptophan are not usually present in collagen and gelatine (Muyonga et al., 2004; Shyni et al., 2014). The content of imino acid was 18.64%. Similar percentages have been observed in warm or tropical species (Alfaro et al., 2009; Taheri et al., 2009), while in cold-water fish they are lower (Kim et al., 1996; Arnesen and Gildberg, 2006). Other researchers such as Ninan et al. (2010) with study on the extraction of fish skin gelatine reported that the imino acid values varied from 19.20 to 20.90%, whereas the gelatines extracted from bovine and porcine are 22.90 and 23.70%, respectively

(Table 2). Generally, these two amino acids thermally stabilize the collagen triple helix. Animals with low body temperatures is less necessary to proline and hydroxyproline (Khiari et al., 2015). Protein solubility (Table 3) plays an important role in functional properties of gelatine such as foaming properties because rapid migration and adsorption of the peptides at the interface are critical (Chobert et al., 1988). Gelatine is an amphoteric protein with an isoelectric point between 5 and 9 depending on raw material and preparation method (Johnston-Banks, 1990; Poppe, 1992). At pH values below and above the isoelectric point, proteins tend to carry more net charges, thereby enhancing hydration (Kinsella et al., 1984). Protein solubility of skipjack tuna (*K. pelamis*) head bones was 65.82% at pH 4, 72.74% at pH 7 and 66.79% at pH 9. In general, proteins and protein hydrolysates show the lowest solubility at their isoelectric points and the highest when maximally charged (Chobert et al., 1988; Kristinsson and Rasco, 2000). The high protein solubility has been also reported for other fish hydrolysates at the pH 7 (Gbogouri et al., 2004). The solution pH generally affects the charge on the weakly acidic and basic side-chain groups. In fact, the solubility of gelatine was due to the generation of moderate molecular weight peptides by acidic hydrolysis, which are expected to have more polar residues than the parent proteins as well as the ability to form hydrogen bonds with water and increase in solubility (Gbogouri et al., 2004). The foam capacity and foam stability of extracted gelatine in this study (Table 3) were high in zero time. But over time, the foam stability decreased and its amount was higher than other protein hydrolysates extracted from round scad (10% at time of 10 min), Spanish mackerel (54.16% and 25.04% at times of 3 and 10 min), sole (35% and 19% at times of 10 and 30 min), and squid (79% and 50% at times of 10 and 30 min) skins at the concentration of 0.5% (Chi et al., 2014). But, there was no information on the foam capacity and the stability of gelatines produced from bone or head bones. In the foam formation, protein has the ability to quickly absorb to the interface and lowering the surface tension. Therefore, one of the most important factors for foam formation is the adsorption rate and the ability to unfold and rearranging at the interface (Martin et al., 2002). Foam stability has been also related with the flexibility of protein, peptide structure, molecular size and hydrophobicity (Klompong et al., 2007; Martin et al., 2002). Foam stability mainly depends on the extent of protein-protein interactions within the matrix of the films surrounding the air bubbles (Mutilangi et al., 1996). As time goes on, this protein flexibility decreases, resulting in a decrease in foam stability.

Conclusion

Skipjack tuna (*K. pelamis*) head bones could be a suitable source for producing good quality gelatine along with functional and physicochemical properties.

So that it can be introduced as the competitive replace for the gelatine from mammals or applied as a new sample of the animal gelatine. Since, the head bone is a non-consumable initial material, after fish processing is usually discarded as industrial waste. Therefore, the gelatine production from them will provide a way to generate a more sustainable industry based on the integral utilization of tuna fish in Iran.

Ethical Statement

Not applicable

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Author Contribution

First Author: Conceptualization, Writing -review and editing

Second Author: Data Curation, Formal Analysis, Investigation, Methodology, Visualization and Writing - original draft

Third Author: Review and editing

Fourth Author: Review.

Conflict of Interest

There is no conflict of interest.

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