RESEARCH ARTICLE

Production of gelatin from Bigeye tuna (*Thunnus obesus)* skin and characterisation of gelatin properties: An alternative to minimise fish waste in tuna fish processing industries in Sri Lanka

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ABSTRACT

Fish skin that can be used to produce fish gelatin is a major by-product of fishery and aquaculture industries. Gelatin is most commonly used ingredient in food processing industry especially in dairy products. Objective of this study was adding value to the fish skin by developing a suitable processing method for extracting gelatin. Bigeye tuna (Thunnus obesus) was selected for production of gelatin due to its high commercial value and availability in the local market. Six treatments of Bigeye tuna skin samples were subjected to concentration series of alkaline pretreatments ((0.1% for 24 h (T1), 0.2% for 24 h (T2), 0.3% for 24 h (T3), 0.1% for 36 h (T4), 0.2% for 36 h (T5), 0.3% for 36 h (T6) followed by different acid extractions (0.1% for 24 h (T1), 0.2% for 24 h (T2), 0.3% for 24 h (T3), 0.1% for 36 h (T4), 0.2% for 36 h (T5), 0.3% for 36 h (T6)) under various soaking periods. Final products were analysed for different physico-chemical parameters such as yield, gel strength, melting point, color, odour, pH and proximate composition to assess the best sample. The highest gelatin extraction efficiency was recorded in the T1 sample (19.67 ±0.42) while the lowest gel yield was recorded in T6 sample (16.03±0.33). Gel strength of T1 fish gelatin sample (260 Bloom) was within the standard range; while protein (82.1%) and lipid (0.97%) contents of T1 gelatin product were within satisfactory nutritional level. The lowest melting points were recorded for all the treated samples compared to the commercial Bovine gelatin product. Lower melting point of the products signifies faster dissolution, which is a highly beneficial character in food processing industry. Bigeye tuna fish skin treated with 0.1% NaOH and H_2SO_4 and a soaking time of 48 h at 60 °C hot water extraction for 5 h was selected as the most suitable method for gelatin production, based on the physico-chemical properties of the final products. Bigeye tuna fish skin marks a novel approach in value addition sector of by-products in Sri Lanka.

Keywords*: Collagen, Alkaline pretreatment, Acidic extraction, Physico-chemical parameters, Value addition, Aquatic by-products*

INTRODUCTION

Tuna fishery and waste generation in Sri Lanka

Tuna is a significant sea food fish with a global annual production of nearly 6,000,000 mt (FAO, 2012). There are approximately 40 species existing in the Atlantic, Indian, and Pacific Oceans and in the Mediterranean Sea. Sri Lanka is one of the major tuna producing and processing countries in the Indian Ocean

and mainly Yellowfin tuna (*Thunnus albacares*), Bigeye tuna (*Thunnus obsesus*), Skipjack tuna (*Katsuwonus pelamis*), Kawakawa (*Enthynnus affinis*), Frigate tuna (*Auxis thazard*) and Bullet tuna (*Auxis rochei*) of tuna family are recorded in Sri Lanka (Joseph *et al*., 1985; Dissanayake, 2005). Total estimated catch of Tuna within Exclusive Economic Zone (EEZ) and high seas was 89,603 mt, including 2711 mt of Bigeye tuna during 2014 (Hewapathirana *et al*., 2015). Tuna is generally processed and exported as frozen, loins and steaks form with sashimi quality. However, 24% of total production is disposed as waste (Fisheries Year Book, 2003 – 2005).

During fish processing operations, waste is produced in both solid and liquid form. Rest of the raw materials after fish processing, are usually considered as residuals left after filleting (Mackie, 1974; Slizyte *et al.,* 2005; Falch *et al*., 2006a; Falch *et al*., 2006b). Fish processing leads to the generation of a large biomass of fish waste, which is generally discarded (~7.3 million mt yr^{-1}) (Kelleher, 2005). In Sri Lanka, Tuna processing industry discards nearly 40 – 50% of total tuna harvest receiving to the factory as waste or offal products (Madage, 2011). Fish skin is such a solid waste discarded by fish processing industries, without any utilization or value addition. Consequently, this discarded waste contaminates and pollute the environment. Therefore, it is vitally important to produce novel products from these discards by adding value for further utilization, to overcome waste accumulation.

Gelatin production using fish skin and background information

Past research findings showed that, fish skin is used on invention of new products such as fish glue by *Chanos chanos* skin *(*Archer, 2001), fish gelatin (Karim and Bhat, 2009), fish leather by Tilapia and Stingray skin (Archer, 2001) and cosmetic products. As a water soluble polypeptide (Koli *et al*. 2011), gelatin is widely used presently in food, pharmaceutical, and cosmetic applications, because of its unique functional and technological properties (Karim and Bhat, 2009). It is extensively used as an ingredient to increase the viscosity of aqueous system and form aqueous gels (Koli *et al*., 2011). In the food industry, gelatin is utilized in sweet products mainly for providing chewiness, texture, and foam stabilisation; in low-fat spreads for creaminess, fat reduction, and mouth feel; in baked products to provide gelling (Johnston-Banks, 1990; Schrieber and Gareis, 2007). Gelatin is normally recommended to enhance protein levels in foodstuffs, and especially in muscle building foods (Gans, 2007). In the pharmaceutical industry, there are reports in which live attenuated viral vaccines used for immunisation against measles, mumps, rubella, Japanese encephalitis, rabies, diphtheria, and tetanus contain gelatin as a stabilizer (Burke *et al*., 1999). Gelatin is also widely used for the manufacture of hard and soft capsules, plasma expanders, and in wound care in medical field (Koli *et al*., 2011). With these wider applications, there is a high potential to use fish gelatin as a product with a high commercial value and market demand in near future, although only small commercial

volumes are available at present (Veis 1964; Balian and Bowes 1977; Ledward 1986; Norland 1990; Schrieber and Gareis 2007).

More intensive studies have been carried out on fish gelatin production for limited number of fish species such as Cod fish (Kolodziejska *et al.*, 2008), Flounder fish (Fernandez-Diaz *et al.*, 2003), Cat fish (Yang *et al.*, 2007), Yellowfin tuna (Chiou *et al.*, 2006), Blue shark (Yoshimura *et al.*, 2000), Skate (Chao *et al.*, 2006), Atlantic Salmon (Armesen and Gildberg, 2007), Nile Perch (Muyonga *et al.*, 2004), Grass Carp (Kasankala *et al.*, 2007) and Black Tilapia (Jamilah and Harvinder, 2002) in the last decade. Major component of fish skin is collagen, which can be hydrolysed into gelatin (Karim and Bhat, 2009). However, according to past findings, fish gelatin has been extracted using a number of different methods depending on characteristics of fish skin. Gelatin production process consists with three main stages: pretreatment of the raw material, extraction of the gelatin, purification and drying (Karim and Bhat, 2009). Most appropriate method for gelatin production is assessed by modifying and changing the gelatin extraction methods, and drying stages via evaluating physico-chemical and functional properties of each product. Hence, potential of the tuna fish gelatin production of would be assessed with further improvements in this study.

Identification of research gap and aim of current study

However, there are no studies conducted on potential of producing fish gelatin using Bigeye tuna skin as a value addition method to Tuna skin in Sri Lanka. Therefore, present study aims for production of gelatin using Bigeye tuna (*Thunnus obsesus*), skin. Current study is important and widely applicable in two ways. The knowledge gained from this work would direct to promote production of the fish gelatin commercially in Sri Lanka. Hence, it allows minimising of discarding wastes through environmental friendly waste management practice. Further, large scale production of tuna fish gelatin would lead to additional economic benefit for fish processing industries, through value addition to tuna fish skin in Sri Lanka.

On the other hand, most of commercial gelatin at present is obtained from mammals, mainly bovine and porcine. Recently, foot and mouth diseases as well as Bovine Spongiform Encephalopathy (BSE) in products from mammals have strictly been concerned (Prommajak and Raviyan, 2013) and consequently, it created an effective barrier for production process of mammalian gelatin. Strong competition also exists among manufacturers for the procurement of pig skin or other mammalian sources, which have created increased demand and raised costs for gelatin production in commercial market (Karim and Bhat, 2009). Furthermore, religious concerns lead to forbid consumption of porcine or bovine products (Karim and Bhat, 2009), including mammalian gelatin. In this background, fish gelatin has been emphasised as a better substitute to mammalian gelatins, from ethical and religious point of views. Thus, the present study focuses on identifying the possibility of using tuna skin as an alternative raw material

source for producing gelatin, while overcoming serious issues related to mammalian gelatin.

MATERIALS AND METHODS

Initial preparation of fish skin samples

Scales, fish muscles, fats and other residues of the fish skin samples were removed manually using a scalpel. Then fish skins were thoroughly rinsed using tap water. Cleaned samples were stored in the freezer at –18 °C of temperature until processing. Cleaned skin samples were chopped into small pieces of approximately (2 x 2 cm) and washed in running tap water for about 10 min. 18 fish skin samples were pre-prepared as each sample contains 30 g subject to have 6 different treatments (T1, T2, T3, T4, T5, and T6). Under each treatment 3 replicates were used.

Processing of gelatin by different treatments

Preliminary trials were conducted within the range of 0.05 – 0.50% of alkaline and acid medium within 6 – 48 h time period to detect the probable treatments for gelatin production. Six treatments with three different NaOH and H_2SO_4 concentrations at two different time combinations were selected for final experiment after conducting preliminary experimental trials (Table 1). Initially, six fish skin samples (T1 – T6) were soaked in three different concentrations of Sodium Hydroxide (w/v) (0.1 – 0.3% w/v) for two different time combinations separately (Table 1). Then, each pretreated skin samples were rinsed with running tap water and allowed to drain using muslin cloth. Each of the partially treated samples (T1 – T6) was again treated with different diluted H_2SO_4 concentrations 0.1 – 0.3% w/v) for two different time combinations separately (Table 1). Each treated skin samples were again rinsed with tap water and allowed to drain using muslin cloth separately. Treated samples with different acid, alkaline time combinations were placed in a water bath with distilled water (1:2 w/v) and kept separately for 5 h at 60 °C for extracting gelatin. Finally, extractions were filtered separately through two layers of muslin cloth to remove residual skin parts and filtered samples were oven dried at 90 °C for 6 h to obtain final products.

Analysis of physico-chemical parameters of final products

Final products were analyzed for physical, chemical and nutritional parameters such as yield, gel strength, melting point, colour, odour, pH and nutritional value. Yield was calculated as a percentage (%), using the weight of resulted gelatin granules and initial wet weight of used fish skin according the formula recommended by Jakhar *et al*. (2012).

For determining the gel strength, the plunger of the Texture Analyzer (53205 Digital fruit firmness tester, TR Turoni, Italy) with sample bottle including 7.50 g of gelatin sample and 105 mL of distilled water at the centre of the platform was set to move up to 4 mm of distance into the gel at the speed of 0.5 mm S^{-1},

so that the plunger contacts the sample toward the midpoint and the value (the gel strength) given by the Texture Analyzer was recorded (Bloom).

Table 1: Different acid and alkaline treatments used in the current study.

Treatment*	Applied NaOH and H_2SO_4 concentrations (w/v) for each treatment – %	Soaking time combination for the pre-treatments for each treatment – h
T1	0.1	24
T2	0.2	24
T3	0.3	24
T4	0.1	36
T5	0.2	36
T6	0.3	36

* - Three replicates were used for each treatment for validity of the results

To determine the melting point, the temperature of previously matured (refrigerated at 7 °C for 16 – 18 h), 6.67% (w/v) of gelatin solution in a water bath was increased, until the gelatin samples were dissolved.

Color variability of each gelatin sample was recorded by placing all the samples on white background and comparing each gelatin sample with control gelatin sample. Odour was determined by sensory evaluation. For determination of pH in the sample, 1% (w/v) solution of gelatin was prepared by dissolving 1 g of gelatin in 5 mL of distilled water at room temperature and the pH of the prepared sample was measured using pH meter (EUTECH 510, Eutech Instruments (Pvt.) Ltd., Singapore).

Proximate compositions of each product were analysed using standard AOAC method (1990). Moisture content was calculated by oven drying until the sample reaches up to constant weight at 105 °C for 18 h (AOAC, 1990). Nitrogen levels of each product were determined using Kjeldhal method (MBC-6/N, Raypa, Spain) and crude protein content was calculated as 6.25 × nitrogen content (AOAC 1990). Crude fat content was calculated using Petroleum Ether extraction in the Soxhlet apparatus (DNP 3000, Raypa, Spain) by allowing evaporating and drying at 100 °C to a constant weight.

Data analysis

Two factor factorial experiment was carried with a Complete Randomised Design (CRD) and data obtained for different parameters were subjected to analysis of variance (ANOVA) by MINITAB 16 statistical software at 0.05 significant levels to identify the most suitable product.

Final product, which was statistically confirmed as the best product, was compared with commercially available gelatin product in the market.

RESULTS AND DISCUSSION

Current study reveals the potential of using Bigeye tuna fish skin as a raw material of gelatin production. Gomez-Guillen *et al.* (2011) identified bones, cartilages, hides, tendons and skin as the common ingredient for collagen and gelatin production. During processing of fish gelatin, fibrous collagen of the tuna skin is subjected to hydrolysis, which results in cleavage of intra-molecular and inter-molecular cross linkages in collagen protein and eventually form gelatin product (Karim and Bhat, 2009).

Resulted gelatin yields of all final samples subjected to different treatments were significantly different at 0.05 level (Table 2). The highest yield (19.67%) was recorded with 0.1% w/v NaOH and H_2SO_4 subjected to soak for 24 h (T1) and the lowest yield (16.03%) was recorded with 0.3% w/v NaOH and H_2SO_4 subjected to soak for 36 h (T6) (Table 02).

Table 02: Final gelatin yield of the products as affected by different treatments.

Treatment	Gelatin yield (wet weight basis) (mL)	Gelatin extraction efficiency (%)
T1	5.90	19.67 ± 0.42^{a}
T2	5.74	19.00 ± 0.94^{ab}
T3	4.96	16.53 ± 0.40^{c}
T4	5.70	19.13 ± 0.82^{ab}
T5	5.25	17.50 ± 1.06^{bc}
T6	4.81	16.03 ± 0.32^{c}

Values are mean±SD for triplicate samples and values with different superscripts records the significant difference in yield between treatments ($P<0.05$).
SD – standard deviation

The gelatin yields and qualitative properties have been reported to vary based on the gelatin extraction process and fish species, mainly due to the differences in collagen content, the compositions of skin as well as the skin matrix (Montero & Gomez-Guillen, 2000; Foegeding, *et al.*, 1996). Further, high degree of cross linking via covalent bonds in the extraction medium can cause decrease in solubility of collagen and leads to the decrease of extractable gelatin yield (Foegeding *et al.*, 1996). Gelatin process of current study has involved use of alkaline and acid pre-treatment combinations. Alkaline pre-treatment followed by acid treatment can eliminate non-collagen protein compounds, while providing optimum pH for the gelatin extraction medium: that results in relatively a high yield of gelatin (Zhou and Regenstein, 2005). Nature and concentration of acid used in gelatin processing has an effect on the pH of the extracted solution and breakage of non-covalent bonds in the collagen cross links

of the raw material (Montero *et al.,* 1990; Norland, 1990). Therefore, suitable mild acid pre-treatment is recommended for gelatin extraction.

Treating of cod fish skin with NaOH and H_2SO_4 having the concentration (w/v) higher than 0.2% followed by citric acid can decrease the yield of gelatin and gel strength (Gudmundsson and Haffsteinsson, 1997). Result of the current study also reveals that gelatin yield has been decreased with the increasing concentrations of NAOH and H_2SO_4 at two selected time combinations (Table 2). When increasing acid and alkaline concentrations, efficiency of the breaking rate of collagen bonds in the extraction medium, becomes gradually slow (Montero *et al.,* 1990; Norland, 1990). As a result, gelatin extraction efficiency and yield decline at high concentrations of NAOH and H_2SO_4. Different yield values for the gelatins extracted from other kind of fish skins are reported by several past studies. Gelatin yield extracted by young Nile perch is recorded as 12.3% while the yield for adult Nile perch is 16.0% (Muyonga *et al*., 2004). Also, gelatin yield for Bigeye snapper and brown stripe red snapper were recorded as 6.5% and 9.4%, respectively (Jongjareonrak *et al*., 2006). Yellow fin tuna gelatin yield was reported as 18% (Rahman *et al*., 2008). The highest yield sample (T1) (19.67%) from Bigeye tuna in the present study is comparatively higher compared to that of the recorded other species. That is commercially advantageous for extracting maximum yield of gelatin.

Gel strength is the major physical property of gelatin. There was a significant difference between the gel strength and final gelatin samples at 0.05 level. The highest gel strength (260 Bloom) was recorded for T1, while the lowest gel strength (30 Bloom) was obtained for T6 (Table 3). According to the results, gel strength of final treatments decreased with increasing the concentration of acid and alkaline solutions. According to research finding of Sarabia *et al*. (2000), gel strength of the gelatin also becomes higher when hydroxyproline content of product is higher. In the present study, hydroxyproline content of samples may have declined with increasing acid and alkaline concentrations, during processing. As a result, there is a potential to decline gel strength with greater acid and alkaline concentrations of treatments. Bloomvalue of gelatin produced using yellow fin tuna skin has reported as 426 (Cho *et al.*, 2005). This value is superior compared to all final products of present study. According to Karim and Bhat (2009), a wide range of Bloomvalues has been found for the various gelatin products arises from differences in proline and hydroxyproline content in collagen composition of different species, and habitat temperature of the animals. The highest gel strengths obtained in the present study was in the similar range with the studies reported by Grossman and Bergman, (1992) for Tilapia (263 bloom) and Kasankala, *et al.* (2007) for Grass carp (267 Bloom), which are concerned as tropical fish species. Holzer (1996) has revealed that gelatins with Bloomvalues of 250 – 260 are the most desirable. Since only one final product (T1) is within the desirable range, this treatment can be considered as a suitable treatment for gelatin production. Bigeye tuna could be an appropriate resource of gelatin extraction given its desirable gel strength.

There was a significant difference (P<0.05) among the average melting points of final gelatin samples. The greatest melting point (24.03 °C) was recorded for T1 gelatin sample while the lowest melting point (19.00 °C) was reported for T6 (Table 3). According to previous research findings, there is a considerable variation between melting point of mammalian and fish gelatins. Fish gelatins have lower melting temperatures due to the main differences between the properties of mammalian and fish gelatins (Leuenberger, 1991), confirming current results. The greatest melting point (24.03 °C) of present study (T1) is similar to previous result (Cho *et al.*, 2005) recorded for melting point (24.30 °C) of gelatin extracted using yellow fin tuna skin.

Table 3: Gel strength and melting point of the Bigeye tuna gelatin samples.

Character	T1	T2	T3	T4	T5	T6
Gel strength (Bloom)	260.00 ±10.00	123.34 ±5.77	83.34 ±11.55	76.67 ±5.77	33.34 ±5.77	30.00 ±0.00
Melting point (°C)	24.03 ±0.21	21.67 ±0.31	21.33 ±0.31	22.00 ±0.50	19.30 ±0.30	19.00 ±0.46

Values are mean±SD for triplicate samples. (T1 – 0.1% for 24 h, T2 – 0.2% for 24 h, T3 – 0.3% for 24 h, T4 – 0.1% for 36 h, T5 – 0.2% for 36 h, T6 – 0.3% for 36 h)

Gelatin samples, which were obtained after heat treatment had different color variation from pale yellow color to amber color. However, there were no prominent color variations between different samples of gelatin solution. The amber color was observed for T1, while the pale yellow color was recorded for T2 and T6. Also, brownish red color was observed for T3. The color of the gelatin depends on the nature of raw material used for the extraction (Ockerman and Hansen, 1999). Commercial gelatin solution is not colorless, but with a color range from very pale yellow to dark amber (Siebert, 1992). The color of the gelatin is a one factor affecting on customer attraction of the product. Most of general public believes that colorless condition of gelatin is associated with purity; hence, gelatin with pale color has high demand compared to gelatin with darker color. In the present study, all final gelatin products prepared were found to have a mild but easily perceivable fishy odour. The reason for the fishy odour of the final products is due to Trimethylamine compound in fish skin. Strong fishy odour has reported for freeze-dried gelatin prepared from the skin of Black Tilapia (Jamilah and Harvinder, 2002). According to Choi and Regenstein (2000), fish gelatins have better aroma than pork gelatins on sensory evaluation.

pH values of all the resulted gelatin samples were slightly acidic and ranged between nearly 4.00 – 6.00 with significant difference at 0.05 level (Table 04). The highest pH (5.53) was obtained for the T1 and the lowest pH (4.94) was recorded for the T3. Acidic pH of gelatin is due to partial treatment using diluted H_2SO_4 (w/v) solution. Maximum moisture content (15.63%) and protein level (82.17%) were recorded for T2 and T1, respectively while the minimum moisture

level (13.6%) and protein percentage (74.32%) was found in S3. Protein level of Tilapia gelatin was reported as 89.30%, which is higher compared to the results of the current study (Jamilah and Harvinder, 2002). Haug *et al.* (2004) have reported 12.9% of moisture content for gelatin produced using cod fish skin. Moisture content of cod fish gelatin is relatively lower compared to the moisture content of Bigeye tuna gelatin in the current study (Table 4). The variations of moisture levels in different gelatin products are due to the differences in drying methods used in gelatin extraction (Jakhar *et al.*, 2012). The greatest lipid content (1.27%) was reported for T5 and the minimum (0.97%) was for T1 (Table 4). As recorded by Cheow *et al.* (2007), crude fat level of gelatin processed from sin croaker fish: *Johnius dussumieri* was 7.99%, which is superior to the lipid content of Bigeye tuna gelatin (Table 04). As Jakhar *et al*. (2012) emphasised; the lower fat level of the gelatin product signifies that the relevant gelatin extraction procedure is acceptable for production of gelatin with standard quality. Since the crude lipid level of the Bigeye tuna gelatin ranges at lower level (0.97 – 1.27%), gelatin extraction method used in this study is applicable for production of super quality gelatin.

Table 4: pH values and proximate composition of Bigeye tuna skin gelatin samples.

Concentration (%) and time (h)	pH	Moisture content (%)	Crude protein (%)	Crude lipid (%)
T1	5.53±0.04	15.20±0.20	82.10±0.47	0.97±0.07
T2	5.13±0.06	15.63±0.35	77.10±2.07	1.15±0.06
T3	4.94±0.13	13.61±0.44	74.32±0.66	1.14±0.04
T4	5.29±0.06	14.77±0.25	80.51±2.00	1.10±0.11
T5	5.15±0.02	14.90±0.70	77.33±1.06	1.27±0.04
T6	5.00±0.10	14.10±0.36	76.49±0.89	1.23±0.08

Values are mean±SD for triplicate samples. (T1 – 0.1% for 24 h, T2 – 0.2% for 24 h, T3 – 0.3% for 24 h, T4 – 0.1% for 36 h, T5 – 0.2% for 36 h, T6 – 0.3% for 36 h)

According to results of physicochemical and functional properties of final gelatin products, mainly gel strengths, final yields and nutritional quality are not in satisfactory level for all the final products except one treatment. Thus, Bigeye tuna fish skin treated using 0.1% NaOH and H_2SO_4, concentration with a soaking time of 24 h at 60 °C hot water extraction for 05 h is recommended as the best treatment for gelatin production, due to its desirable gel strength, maximum yield, the highest melting point and satisfactory nutritional quality (also, the highest protein level and the lowest lipid content).

When compared to commercially available bovine gelatin, crude protein level was significantly (P<0.05) lower in the gelatin extracted with Bigeye tuna skin

(Table 5). This treatment also recorded comparatively higher lipid content over the market available gelatin. Average value of pH of fish gelatin of present study was slightly acidic (5.53) compared to mammalian gelatin.

Table 5: The physical, chemical and functional properties of Bigeye tuna gelatin compared to bovine gelatin.

Properties	Bigeye tuna gelatin	Bovine gelatin
Gel strength (Bloom)	260.0	200.0
Melting point (°C)	24.0	33.8
Moisture content (%)	15.2	14.0
Crude protein (%)	82.2	88.4
Crude lipid (%)	0.97	0.26
pH	5.53	6.50
Odor	Mild fishy odor	No odor
Color	Amber	Pale yellow

Although, the nutritional composition of Bigeye tuna gelatin was not at a satisfactory level, the gel strength was greater than that of the bovine gelatin. The melting point of Bigeye tuna gelatin was comparatively inferior (Table 05). Given the lower melting point of this product, it may result a faster dissolution in the mouth with no residual 'chewy' mouth feel during food processing (Karim and Bhat, 2009). It is a beneficial character in food industry.

Overall gelatin yield extraction efficiency using Bigeye tuna fish skin for all the samples (at the range of 16.03 - 19.67%) are higher compared to the gelatin products of cattle hide wastes (13.60%) (Barbooti *et al.,* 2008). Therefore, Bigeye tuna fish skin can be considered as highly profitable ingredient in gelatin processing industry. Production cost of food grade Bovine gelatin and tuna fish gelatin unit price (1 kg) is approximately Rs. 1500.00 and 900.00, respectively. Since fish skin is generally discarded as waste by processing plants, cost of raw material for Bigeye tuna gelatin is lower compared to commercial gelatin. Moreover, utilization of Bigeye tuna fish skin for gelatin industry is an environmental friendly solution to overcome the waste accumulation in terrestrial and aquatic environments. Further investigations are needed to improve nutritional quality and physical properties of Bigeye tuna gelatin.

CONCLUSIONS

According to the present findigs, Bigeye tuna fish skin treated with concentration of 0.1% NaOH and H_2SO_4, with a soaking time of 24 h at 60 °C hot water extraction for 5 h, is recommended as the most appropriate method for gelatin production. Bigeye tuna fish skin is cost-effective ingredient in gelatin processing industry as it results significantly higher gelatin yield. Therefore, Bigeye tuna fish skin can be highlighted as a new alternative to mammalian gelatin industry with further developments in future.

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