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Effect of different extraction process on skin gelatin from Talang queenfish (*Scomberoides commersonnianus*)

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SUMMARY :

In the fish processing industry generated waste during filleting of Talang queenfish (*Scomberoides commersonnianus*) was utilized for gelatin extraction in the present investigation. These waste materials being rich in collagen are valuable raw materials for gelatin extraction. The fish skin was used for gelatin extraction by ten different methods as described by different authors namely, Grossman and Bergman (1992), Gudmundsson and Hafsteinsson (1997), Gomez-Guillen and Montero (2001), Gimenez *et al.* (2005), Zhou and Regenstein (2005), Kolodziejska *et al.* (2008), Liu *et al.* (2008), Rahman *et al.* (2008), Benjakul *et al.* (2009) and Barve (2012). The results of yield were varied between 4.12 to 12.83% and gel strength was varied in between 162.51 to 226.11 g. Based on the yield and gel strength of extracted gelatins, the method of Barve (2012) was found the best method. This method revealed that yield and gel strength were 12.83% and 226.11 g, respectively and found significantly different comparatively other methods.

KEY WORDS : Talang queenfish, Fish skin, Gelatin, Extraction process, Yield, Gel strength

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Gelatin is a biopolymer obtained from partial denaturation of collagen. It has a wide range of applications in the food and non-food (photographic, cosmetic, and pharmaceutical) industries (Regenstein and Zhou, 2007). As the global demand for gelatin has shown an increasing trend in recent years. The reports indicate that the annual world production of gelatin is nearly 326,000 tonnes, with pigskin obtained gelatin accounting for the highest output (44%), followed by bovine hides (28%), bones (27%), and one per cent from other sources (Shyni *et al.*, 2014). However, although fish gelatin has been given increasing attention

as the alternative of animal gelatin due to religious constraint. Both Judaism and Islam restricted the consumption of any pork-related products, while Hindus do not eat any cow-related products (Asher, 1999). Besides, there is an increasing concern among researchers about whether animal tissue-derived collagen and gelatin are competent of carrying pathogenic vectors such as prions (Wilesmith *et al.*, 1991). Therefore, fish gelatin is a better alternative to mammalian gelatin, especially in physical properties such as a lower melting point which results in faster dissolution in the mouth with no residual 'chewy' mouthfeel (Karim and Bhat, 2009).

Gelatin is a water-soluble protein, obtained from either bovine, pork, fish that contains collagen. Collagens get converted to gelatin by the hydrolysis process. Because of its unique properties of forming gel at room temperature, gelatin is widely used in the food, pharmaceutical, and photographic industries. Several researchers have reported that fish skins and bones could be utilized to produce a considerable amount of gelatin (Jamilah and Harvinder, 2002; Batista *et al.*, 2004). A various literature on extraction methods have been previously reported for use with fish skins (Grossman and Bergman, 1992; Gudmundsson and Hafsteinsson, 1997; Gomez-Guillen and Montero, 2001; Gimenez *et al.*, 2005; Zhou and Regenstein, 2005; Kolodziejska *et al.*, 2008; Liu *et al.*, 2008; Rahman *et al.*, 2008; Benjakul *et al.*, 2009 and Barve, 2012). A number of these methods have shown significant potential for the production of high-quality gelatin of different melting and gelling temperatures. Fish gelatin is known to vary extensively depending on the size and type of fish species, sex, habitats, and climatic conditions. Therefore, it is important to compare different extraction methods for use with fish skin to optimize the recovery of good yield and gel strength.

This study aimed to determine the best method for extraction of gelatin from fish skins resulting from the fish filleting industry using Talang queenfish. In a preliminary study, Compared among ten different extraction methods were to determine the best method based on yield and gel strength.

EXPERIMENTAL METHODS

Raw materials:

Fresh Talang queenfish (*Scomberoides commersonnianus*) locally known as "Dagol" or "Phalay" was used for the present study. It was procured from Ratnagiri fish landing center, Maharashtra, India and brought to the laboratory in ice conditions. The fishes used in the study had total length ranging from 25 to 43 cm with a weight of 2.28 to 3.92 kg. The fish skin was removed manually, cleaned, washed for removing adherent residues and stored at -18°C until needed for further experiment. Sulfuric acid, Sodium hydroxide were purchased from MERCK Chemical Company Ltd., Mumbai, India. All chemicals used were analytical grade.

Preparation of raw materials:

The frozen skins were thawed at room temperature for 24 h before removing any remaining flesh and scales. Skins were cut into 2.5 x 2.5 cm pieces and then washed with tap water until free from the flesh. These raw materials were used for the extraction of gelatin.

Selection of gelatin extraction methods:

Ten different methods of fish gelatin extraction from published literature were considered. Among these methods, the most appropriate method of extraction was identified based on the yield and gel strength of the gelatin obtained. The following methods were used for the extraction of Talang queenfish skin gelatin.

Method of Grossman and Bergman (1992):

The procedure described by Method Grossman and Bergman (1992), originally used for extracting gelatin from Tilapia skins (*Oreochromis niloticus*). A certain modification was carried out to extract the Talang queenfish skin gelatin. The fish thawed prior and remove the skin were to the experiments. The accurately weighed 100 g of each of the fish skins were cleaned and washed with tap water to remove superfluous materials. The fish skins were soaked in a sodium hydroxide (NaOH 0.25%, w/v) for 40 minutes. After washing out sodium hydroxide, two successive acid incubations were performed, each for 40 min, first in sulfuric acid (H_2SO_4 0.25%, v/v) and then in a citric acid solution (1.1%, w/v). The acid solutions were drained and then samples were washed with cold water till neutral pH. The final extraction of gelatin was performed in distilled water at 45°C for 18 h. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1), followed by vacuum oven drying and made powder by pestle and mortar and packed in an airtight container.

Method of Gudmundsson and Hafsteinsson (1997):

The procedure described by Gudmundsson and Hafsteinsson (1997) originally used for extracting gelatin from the Cod skins (*Gadus morhua*). Following this method, the skins of Talang queenfish were used for the extraction of gelatin. Thawed skins were thoroughly cleaned and rinsed with excess water to remove superfluous material and treated by soaking with 0.2% (w/v) sodium hydroxide solution for 40 min. Then they were soaked with 0.2% (w/v) sulphuric acid for 40 min. This was followed by soaking with 1.0% (w/v) citric acid. After each soaking treatment, the skins were washed under running tap water until they had a pH of about 7. Each soaking and washing treatment was repeated three times with a total time of 2 h for each treatment. The ratio of skin to washing liquid used was 1 kg skin (wet weight) to 7 L of acid or alkali solution for each treatment. The skins were then subjected to a final wash with distilled water to remove any residual matter. The final extraction was carried out in distilled water at a controlled temperature within the range of 45°C for 12 h. The ratio used was 1 kg (weight of wet skin) to 3 L of distilled water. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1), followed by vacuum oven drying and made powder by pestle and mortar and packed in an airtight container.

Method of Gomez-Guillen and Montero (2001):

The procedure described by Gomez-Guillen and Montero (2001) originally used for extracting gelatin from the skin of Megrim (*Lepidorhombusboscii*). Following this method was used to extract the gelatin from the skin of Talang queenfish. The skins were washed with tap water (1:6 w/v) in homogenizer at 5^0C for 10 min and were rinsed with abundant running tap water. Then the skin was further cleaned with 0.8 M NaCl with ratio 1:6 (w/v), again homogenizer for 10 min at 5^0C and were rinsed with abundant running tap water. This step was repeated three times. Pretreatment of skin with 0.2 N NaOH (1:6 w/v) at 5^0C for 30 min with constant stirring and rinsing with abundant running tap water. This washing cycle was repeated three times. Then cleaned skins were constantly and slowly stirred for 16 to 18 h at 20^0C, with 0.05 M propionic acid with ratio 1:20 (w/v). The mixture with the remains of skins was then filtered in a Buchner funnel with Whatman no. 4 filter paper, and the clear filtrate was then air-dried in the convection oven at 40^0C, in the form of very thin layers, until moisture was less than 15%. The dried collagens, extracted with propionic acid were dissolved in distilled water at 45^0 C for 30 min, at a concentration of 6.67% (w/v).

Method of Gimenez et al. (2005):

Method of Gimenez *et al.* (2005) originally used for extracting gelatin from the skin of Dover sole (*Solea vulgaris*) fish. Following this method was used to extract the gelatin from the skin of Talang queenfish. The skin was washed with salt solution 0.8 M NaCl with a ratio of 1:6 (w/v) at 5^0C for 2 min. Then the excess of water was removed by draining the cleared skins and squeezing in a manual pressed. After that treated with 0.2 N with ratio 1:6 (w/v) NaOH at 5^0C for 30 min with constant stirring and rinsing with abundant running tap water. This washing cycle was repeated three times. The alkali-treated skin was treated in 0.05 M acetic acid with ratio 1:6 (w/v) at 5^0C for 30 min and constant stirring and rinsing with abundant running tap water. This washing cycle was repeated three times. The extraction was carried out in distilled water at 45^0C for overnight. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1); vacuum dried, powdered by pestle and mortar and then packed in an airtight container.

Method of Zhou and Regenstein (2005):

Method of Zhou and Regenstein (2005) was originally used for extracting gelatin from the Alaska pollock (*Theragra chalcogramma*). In this method, the skins were maintained at a temperature below 10^0C with 6 volumes (w/v) of 0.2 M $Ca(OH)_2$ for 1 h. This was followed by soaking in 6 volumes of 0.05 M acetic acid at the same temperature for 1 h. After each soaking treatment at below 10^0C, the skin waste was washed with excess cold water until a pH of about 7 was reached. The skins were subjected to final wash with distilled water to remove any residual matter. Finally, extraction was done with 3 volumes of distilled water at 50^0C for 3 h. The extract was then filtered with cheesecloth and dried in the oven at 90^0C. After drying in a trey, the sheet of gelatin formed was reconstituted in distilled water, stabilized at 90^0C for 1 h. The reconstituted gelatin was centrifuged at 3500 rpm for 15 min. filtered through Whatman (No. 1) filter paper; vacuum dried for 12 h at 70^0C, ground to powder form and packed in an airtight

container.

Method of Kolodziejska et al. (2008):

The method was originally suggested for the extraction of gelatin from Baltic cod (*Gadus morhua)*, Salmon (*Salmo solar*), and Herrings (*Clupea harengus*). Following this method, the Talang queenfish skins were stirred with 0.45 M NaCl for 3 min. at 4^0C and washed with tap water, and extraction continued in distilled water for 60 min at 45^0C. The clear extract obtained was filtered in a Buchner funnel with Whatman filter paper (No. 1), vacuum dried and made into powder by pestle and mortar and packed in an airtight container.

Liu et al. (2008):

Method of Liu *et al.* (2008) extracted gelatin from the skin of Channel catfish (*Ictalurus punctaus*). Following the same method, the skins of Talang queenfish were treated with 10 volumes (w/v) of 0.1% Ca(OH)2 solution at 15^0C for 68-76 h. The treated skin was washed with distilled water and 1 M H_2SO_4 was used to neutralize calcium hydroxide in the skin and bone. Then the skins were washed again to remove the deposition of calcium sulfate. Gelatin was subsequently extracted from skins in distilled water at temperatures from 45^0C for 6 h. The extracted solution was filtered through Whatman No. 1 filter paper, concentrated with a rotary evaporator. The remainders of concentrated solution were dried until moisture was less than 10 per cent dry gelatin was then ground and packed in an airtight container.

Method of Rahman et al. (2008):

Method of Rahman *et al.* (2008) originally used for extracting fish gelatin from the skin of Yellowfin tuna (*Thunnus albacares*). Following this method gelatin was extracted from the skins of Talang queenfish. In this method, the skins were washed in tap water and then treated with 0.5 M NaCl at 5^0C for 5 min. They were then soaked in 0.1 M NaOH for 40 min. at 20^0C, rinsed with tap water and extracted in 0.1 N acetic acid for 18 h at 50^0C. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1), vacuum dried, powdered and packed in an airtight container.

Method of Benjakul et al. (2009):

Method of Benjakul *et al.* (2009) originally used for extracting gelatin from the skins of Bigeye snapper (*Priacanthus tayenus* and *Priacanthus macracanthus*). Following this method skins of Talang queenfish were used for extraction gelatin. To extract gelatin, initially, the skins were soaked in 0.025 M NaOH solution with the skin to solution ratio of 1:10 (w/v). Then the mixture was stirred for 2 h at room temperature 25-28°C. The alkaline solution was changed every hour to remove non-collagenous proteins and pigments. Alkaline-treated skins were then washed with tap water until neutral or faintly basic pH of wash water was obtained. The skins were then soaked in 0.2 M acetic acid with a skin solution ratio of 1:10 (w/v) for 2 h with gentle stirring. The acid solution was changed every 40 min allow to swelling of the collagenous material in the fish skin matrix. Acid-treated skins were washed thoroughly as previously described. After swelling, the swollen fish skins were soaked in 10 volumes (w/v) of distilled water (45^0C) for 12 h with an occasionally stirring. The mixture was then filtered using two layers of cheesecloth. The clear extract obtained was filtered in a Buchner funnel with a Whatman filter paper (No. 1); vacuum dried, powdered and packed in an airtight container.

Method of Barve (2012):

Method of Barve (2012) originally used for extracting gelatin from the surimi processing waste. Following this method was used to extract the gelatin from the skin of Talang queenfish. Initially, the skin was soaked in proteases enzyme 0.175% (pepsin) for 4 h at 4-15^0C. Then wash with normal water at low temperature and treated with dilute aqueous alkali 3.5% (NaOH) for 4 h at 4-15^0C. Then again washing with normal water at low temperature has about neutral pH and treated with dilute aqueous acid 3.5% (H_2SO_4) for 4 h at 4-15^0C. To acid-treated skin was washed with normal water at low temperature till the pH is almost neutral and extracted with water at about neutral pH at a temperature between (55^0C) for dissolving of hydrolyzed collagen into water. The mixture was then filtered using two layers of cheesecloth and then the obtained clear extract was purified in a Buchner funnel with a Whatman filter paper (No. 1); oven-dried at 55^0C and obtained powder of gelatin was packed in an airtight container.

Yield of extracted Gelatin:

The yield of gelatin was calculated based on wet

weight of fresh skin using the following formula:

$$\text{Yield of skin gelatin } Z \frac{\text{Weight of oven dried skin gelatin}}{\text{Wet weight of fresh skin}} \text{ x } 100$$

Determination of Gel strength:

The gelatin gel was prepared and the gel strength of gelatin gel was determined according to the method described by Wainewright (1977). The gel was prepared in bloom jar (SCHOTTGLAS. Mainz. Bloom test vessel. Product no. 2112501) by dissolving a 6.67% (w/v) dry gelatin powder in distilled water at 60^0C. Then it was cooled for 15 minutes at room temperature and kept for 18 h at 7^0C for maturation. Gel strength was determined on TexVol Texture Analyzer (Perteninstrument TVT 300XP Box 45, 26040 Viken, Sweden) according to British Standard BS 757 (BSI, 1975), with a load cell of 5 kg, cross-head speed 1 mm s^{-1} and using a 20 mm diameter, flat bottomed plunger. The standard glass bloom jar was placed centrally under the plunger and the penetration test was then performed. The maximum force (g) was determined until the probe penetrated into the gel to a depth of 4 mm. Measurements were done in triplicate using similar jars and the maximum force of penetration was recorded and used for the analysis of gel strength.

Statistical analysis:

Data regarding the different methods of gelatin extraction by identification of yield and gel strength were analyzed by one way analysis of variance (ANOVA) and Duncan's multiple-test was used for Post - hoc test to determine the significance of the difference between the means. The statistical package used in the study was SPSS version 16.0. The difference of means between pairs was resolved by means of confidence intervals using a Post Hoc test at the level of significance of $p < 0.05$. All data represented are the means of triplicates.

EXPERIMENTAL FINDINGS AND ANALYSIS

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Selection of appropriate gelatin extraction method:

Ten different methods were used for the extraction of gelatin from Talang queenfish skin. The results of yield varied between 4.12 to 12.83 per cent, while the gel strength varied between 162.51 to 226.11 g (Table 1 and Fig. 1 and 2). Since the methods of extraction were quite different, therefore both the yield and gel strength varied widely. The statistical analysis indicated that T_{10} was significantly different ($P < 0.05$) from other methods in both yield and gel strength. Further, Duncan test was applied, mean values with the same lowercase superscript letter are not significantly different ($P < 0.05$) (Table 1). Incidentally, this method was found to be the best with regards to the yield and gel strength of the skin gelatin.

According to Koli (2011) investigated that the gelatin extracted from the skin of Tiger toothed croaker and Pink perch by using ten different methods. The results examined that the yield and gel strength of Tiger toothed croaker skins were varied between 3.55 to 7.46 per cent and 128.67 to 162.16 g, respectively. The results of yield and gel strength of Pink perch skins were varied between 2.53 to 4.61 per cent and 102.53 to 133.96 g, respectively. Compared with the ten different methods, the

Table 1 : Extraction of skin gelatin from Talang queenfish by ten different methods

Treatments	Gelatin extraction methods	Yield (%)	Gel strength (g)
T_1	Grossman and Bergman (1992)	8.04 ± 0.38^{c}	221.33 ± 3.21^{gh}
T_2	Gudmundsson and Hafsteinsson (1997)	8.51 ± 0.12^{c}	215.67 ± 2.08^{fg}
T_3	Gomez-Guillen and Montero (2001)	5.57 ± 0.16^{b}	187.50 ± 4.77^{c}
T_4	Gimenez *et al.* (2005)	6.00 ± 0.29^{b}	203.68 ± 5.10^{e}
T_5	Zhou and Regenstein (2005)	9.62 ± 0.68^{d}	196.07 ± 2.53^{d}
T_6	Kolodziejska *et al.* (2008)	4.12 ± 0.25^{a}	162.51 ± 4.46^{a}
T_7	Liu *et al.* (2008)	4.32 ± 0.24^{a}	169.75 ± 2.13^{b}
T_8	Rahman *et al.* (2008)	11.17 ± 1.19^{e}	212.10 ± 2.59^{f}
T_9	Benjakul *et al.* (2009)	4.45 ± 0.22^{a}	165.56 ± 4.86^{ab}
T_{10}	Barve (2012)	12.83 ± 0.71^{f}	226.11 ± 3.54^{h}

Values are given as $\pm$ SD from triplicate determinations; Mean values with the same lowercase superscript letter are not significantly different ($P < 0.05$)

Gudmundsson and Hafsteinsson (1997) method were found the best method for both fishes of skins based on yield and gel strength. Similarly, Chavan (2018) reported that the gelatin extracted from the skins of *Pangasianodon hypophthalmus* and *Protonibea diacanthus* by using six different methods. The yield and gel strength of gelatin extracted from *P. hypophthalmus* skins varied between 5.25 to 16.96 per cent and 175.13 to 290.75 g, respectively. While yield and gel strength of *P. diacanthus* skins varied between 8.47 to 18.41 per cent and 180.11 to 290.11 g, respectively. Compared among six methods, the Grossman and Bergman (1992) were found to be the best method based on yield and gel strength of both fish species. Mohtar (2013) reported that the Hoki (*Macruronus novaezelandiae*) skin gelatin was extracted using four different methods. The results found that the yield and gel strength of gelatin extracted from different methods were varied between 1.4 to 21.2 per cent and 99.7 to 196.7 g, respectively. Among that the gelatin extracted using the method of Kolodziejska *et al.* (2008) was exhibited the highest yield and gel strength were 21.2 per cent and 196.7 g, respectively.

Bloom value or gel strength of extracted gelatin was mainly depending ontemperature of extraction, concentrations of acid and alkali and the molecular weight (Ockerman and Hansen, 1988). Gel strength is one of the most important functional properties of gelatin and fish gelatin typically has lower gel strength than mammalian gelatin (Gilsenan and Ross Murphy, 2000). According to Holzer (1996), the gel strength of commercial gelatin expressed as bloom value, ranges from 100 to 300 g but gelatin with bloom values in the range of 250–260 g are most desired. Results from the present study show that skin of Talang queenfish yielded gelatins with different bloom value (Table 1). The strongest with a bloom strength value of in the T_{10} (226.11 g) and weak bloom value of in the T_6 (162.51 g) was obtained from the Talang queenfish skin.

Several researchers have reported indicate typical bloom values of 100 to 200 g for gelatin obtained from skin of hake, cod and megrim (Gudmundsson and Hafsteinsson, 1997; Fernandez- Diaz *et al.*, 2001). The bloom values of different fish species obtained in this study were comparable to the values of fish gelatin previously reported. Jamilah and Harvinder (2002) reported that gelatins from red tilapia and black tilapia skin possessed the bloom strengths of 128.10 g and 180.8 g, respectively. The variation in the reported gel strength or bloom strength could be explained by differences in the molecular weight distribution rather than in amino acid composition without disregarding the existence of additional factors that may influence these parameters. Though these parameters have not been evaluated in this study, it is well established that fish gelatin has a lower melting point than mammalian gelatins (Norland, 1990) and melting point of gelatins increase with increasing in molecular weight (Ward and Courts, 1977). It is also well established that hydrogen bonds between water molecules and free hydroxyl groups of amino acid in gelatin are essential for the gelatin's bloom value (Arnesen and Gildberg, 2002).

Conclusion:

Based on the present study, it was concluded that skin waste of Talang queenfish could be utilized for the extraction of gelatin. Among these ten methods, the most

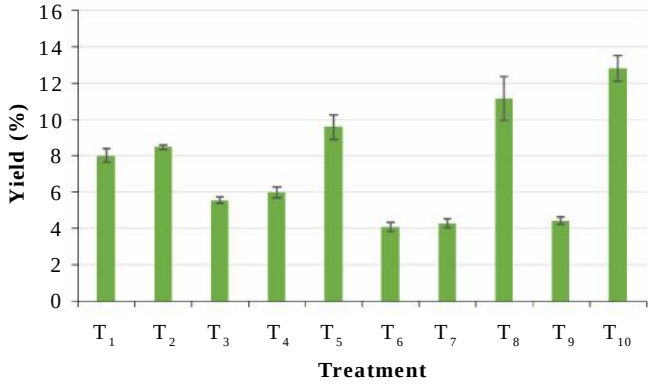

Fig. 1 : Yield of skin gelatin extracted by ten different methods

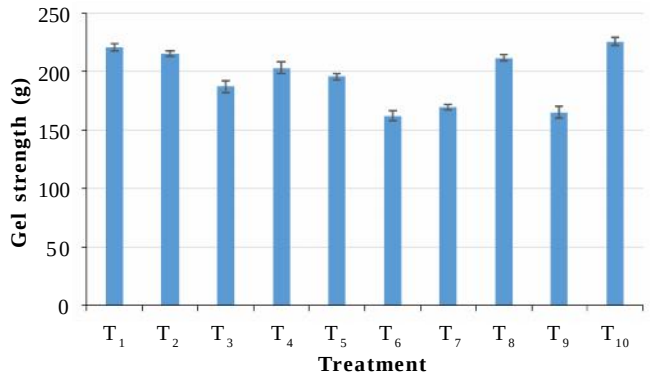

Fig. 2 : Gel strength of skin gelatin extracted by ten different methods

appropriate method of extraction was identified based on the yield and gel strength of the gelatin. By employing optimum parameters for extraction of good quality gelatin could be extracted successfully from Talang queenfish skin waste. By changing the extraction conditions, the bloom values of the gelatin could be controlled to yield the desired properties of fish gelatin made suitable for various applications.

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