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Study on The Functional Properties Of Gelatin Extracted From The Skin Of The Fish Pacu (Piaractus Brachypomus)

Rushikulya Sahoo[1], *K. Dhanapal[1], G.V.S.Reddy[2] , A. Balasubramanian[3] and K.Sravani[1]

1: College of Fishery Science, S.V. Veterinary University, Muthukur 524 344, Nellore District, Andhra Pradesh
2: Fisheries Research Station, Palair Khammam District, A.P.
3: Fisheries Research Station, West Godavari District, A.P
*: Contact author: kdhanpal@yahoo.com

Abstract

The functional properties of the gelatin extracted from the skin of fish pacu (*Piaractus brachypomus*) were studied and compared with commercial gelatin. Higher protein content of pacu skin resulted in higher protein content in extracted gelatin, with low fat and ash content. It could be ascertained from the present study that pacu skin was a prospective source to produce gelatin in good yield with desirable functional properties comparable to commercial gelatins.

Key words: Gelatin, Pacu, Protein, Skin, functional property

Introduction

The global demand for gelatin has been increasing over years. It is reported that the annual world out-put of gelatin is nearly 3,26,000 tons which is derived from various sources like pig skin accounting for the highest (46%), followed by bovine hides (29.4%), bovine bones (23.1%) and other sources (1.5%) (Karim and Bhatt, 2009). Fish gelatin, a partially hydrolysed form of collagen, can be obtained from the skin and bones of fish. It has been given increasing attention nowadays as an alternative to land animal gelatin produced from bovine and porcine due to religious constraints. On consequences, gelatin from aquatic animals, especially from fish skins, could be a substitute for mammalian gelatin (Pranoto *et al*., 2007). The fish processing waste mainly comprising of skin and bones is one of a potential sources for gelatin production. Bones and skin together contribute 30% of the whole fish (Gomez-Guillen *et al*., 2002). Current gelatin production from fish skins and bones is in the range of 1000–1500 tons, which is less than 1% of the total gelatin production (Karim and Bhatt, 2009). The functional properties of

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gelatin depend on several factors including the method of preparation and the intrinsic characteristics of collagen (Badii and Howell, 2006).

Fish gelatin has been highlighted as a better alternative despite possessing poor qualities like faster dissolution in the mouth due to its lower melting point, absence of residual 'chewy' mouth feel, dark colour skin, and unpleasant odour of the skin. These poor qualities restricts its commercial applications to certain extend. Thus, the production of fish gelatin is still in its infancy, contributing only about 1% of the annual world gelatin production (Arnesen and Gildberg, 2006). However, the quality of gelatin from fish skins and bones is dependent on the species and the habitat (Kharyeki *et al*., 2011). As the good quality properties such as ability to form thermo reversible gels, its texture, thickening and water binding capacity of gelatin make so useful in various industries like food and pharmaceuticals, etc. (Haug and Draget, 2009). Besides, the utilization of by-catch and discards obtained from fishing and the wastes from fish processing industries for the production of gelatin fulfils the sustainable management policy of responsible fisheries.

Considering all these, the fish Pacu (*Piaractus brachypomus*), a native of South America recently introduced as an alternative species in Indian fresh water fish culture which has been cultured in larger level has been chosen, as it contains a lot of meat with less spines. Further, as there has been no attempt made to extract gelatin from the skin of this species, this study was designed to extract the gelatin from its skin, determine its functional properties and compare the gelatin with commercial one.

MATERIALS & METHODS

The fish Pacu (*Piaractus brachypomus*) purchased from the nearby fish market in fresh condition were brought to the Fish Processing Laboratory of the Institute. After washing the fishes in clean water and their length and weight were measured to nearest centimeter and gram. Later the fishes were beheaded, gutted, de-scaled and filleted manually. The skins of the fillets were separated by passing them through the de-boner. The collected skins were cleaned by scraping manually with knife to remove the adhering flesh. The cleaned skins were washed and cut into pieces in a size of 5-6 cm. The pieces were packed in polythene pack and stored at -20 °C for extraction of the gelatin.

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The gelatin from the skins of pacu was extracted following different pretreatment methods (Table 1.) described by Gudmundsson and Hafsteinsson (1997). Of which, the following process method was adopted. Thawed pacu fish skin was thoroughly cleaned and rinsed with excess water to remove superfluous material. The cleaned materials were then sequentially soaked with 0.15 % (w/v) sodium hydroxide, 0.15% (w/v) sulphuric acid and 0.75% (w/v) citric acid for 35 minutes. After soaking treatments, the skins were washed under running tap water until they reached a pH of 7.0. The pH was measured by using a pH meter (M/S, Oakton Eutech instrument, Malaysia). Each soaking and washing treatment was repeated three times and the entire treatments were completed in a time of 2 h. The skins were further washed for 35 minutes using washing liquid in a ratio of 1:7. The skins were then subjected to a final wash with distilled water to remove any residual matter. The gelatin extraction from the cleaned skins was carried out in distilled water with a ratio of 1:3 (w/v) under controlled temperature of 50 °C for 16 hr. The clear extract obtained was filtered in a Buchner funnel using a Whatman filter paper No. 4. The filtered solution was later dried for period of 20 – 24 h in a hot air oven at 60 °C till obtaining a dry sheet of gelatin. The yield of gelatin was calculated using the following formula: Yield of gelatin (%) = (Weight of dry gelatin / Weight of fresh fish skin) × 100.

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Table 1. Different pretreatment steps and temperatures used to extract gelatin from pacu skin.

Process Variable /Range	1	2	3	4	5	G1 (40°)	G2 (50°C)	G3 (60 °C)
NaOH Conc. (Mol/L)	0.1%	0.15%	0.2%	0.25%	0.3%	0.15%	0.15%	0.15%
H_2SO_4 Conc. (Mol/L)	0.1%	0.15%	0.2%	0.25%	0.3%	0.15%	0.15%	0.15%
Citric acid Conc. (Mol/L)	0.7%	0.75%	0.8%	0.85%	0.9%	0.75%	0.75%	0.75%
Soaking time (min.)	30	35	40	45	50	35	35	35
Extraction temperature (50 °C) & Time (hr.)	14	16	18	20	22	40	50	60
Extraction time (hr)						16	16	16

Proximate and Functional properties analyses

Crude protein (TN x 6.25) and fat contents were determined by the micro-kjeldahl and soxhlet method respectively. Crude ash was determined by heating an incinerated sample in a muffle furnace (5500C for 16 hr) and moisture was determined using the method of AOAC (2000). The pH was determined by blending 10 grams of tilapia sandwich paste and fish muscle with 90 ml distilled water in a homogenizer (Kinematica AG, Polytron System PT 2100, Lucerne, Switzerland) each for 30 seconds using a digital pH meter (Oakton, Eutech Instruments, Singapore) standardized at pH 4 and 7 (APHA 1998).

Water holding capacity (WHC) of fish muscle was measured by modified centrifugation method described by Diniz and Martin (1997) and expressed in percentage (%).Hydroxyproline content of gelatin was determined according to the method of Bergman and Loxley (1963) . Bloom value was determined using the method described by Gelatin manufactures of Europe (2000). Viscosity of gelatin sample was determined according to the method of Cho *et al*. (2005) using a Brookfield digital viscometer (Model LV-DV-II, Brookfield Engineering; MA, USA)

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equipped with C-18 spindle (Model LV) at 60 rpm at 40 ± 1 °C. The melting point measured by the method described by Wainewright(1977). The method of Yasumatsu *et al.,* (1972) was used to determine emulsifying capacity and stability. The method of Miller and Goninger (1976) was used to determine foaming properties.

Gelatin colour and gel clarity analyses

Colour measurement was made by using a Lab Scan XE spectrophotometer (Hunter Lab scan XE, USA) and it was calibrated to white and black standard sites of sample. The tristimulus L*a*b* measurement mode was used as it relates to the human eye response to colour. The L* variable represents lightness (L*=0 for black, L*=100 for white), the a* scale represents the red/green. (+a* intensity in red and –a* intensity in green) and the b* scale represents the yellow/blue (+b* intensity in yellow and –b* intensity in blue). hue angle (arcton, b*/a*), saturation index $(a^{*2}+b^{*2})^{0.5}$. Clarity was determined by measuring transmittance (%T) at 620 nm in spectrophotometer (Lovibond pc spectro, Germany) through 6.67% (w/w) gelatin solution which were heated at 60 °C for 1hr (Avena-Bustillos *et al*., 2006).

The SPSS 16 (IBM, 2010) Statistical Package for Social Sciences was used for analysis of the experimental results. Sufficient numbers of samples were carried out for each analysis. The results were expressed as mean ± standard deviation (SD). The correlation coefficients between the parameters were carried out using the same software.

Results & Discussion

Proximate Composition

The determined percentage proximate composition such as, moisture, protein, fat and ash content in the pacu skin were 76.40±0.46, 18.29±0.04, 4.14±0.04 and 1.12±0.07 respectively, with a pH of 6.91±0.0. Gelatin from pacu skin had protein content of G1: 40 °C; G2: 50 °C and G3: 60 °C were 84.33±0.04%, 87.69±0.20% and 91.39±0.03% respectively. The moisture content of G1: 40 °C; G2: 50 °C and G3: 60 °C were 4.88±0.03%, 3.32±0.02% and 3.81±002% respectively. While the fat content of G1: 40 °C; G2: 50 °C and G3: 60 °C were 1.07±0.02%, 1.16±0.02% and 1.1±0.04% respectively. The ash content of G1: 40 °C; G2: 50 °C and G3: 60 °C were 0.92±0.02%, 1.07±0.02% and 1.13±0.03% respectively. The pH of pacu skin of G1: 40

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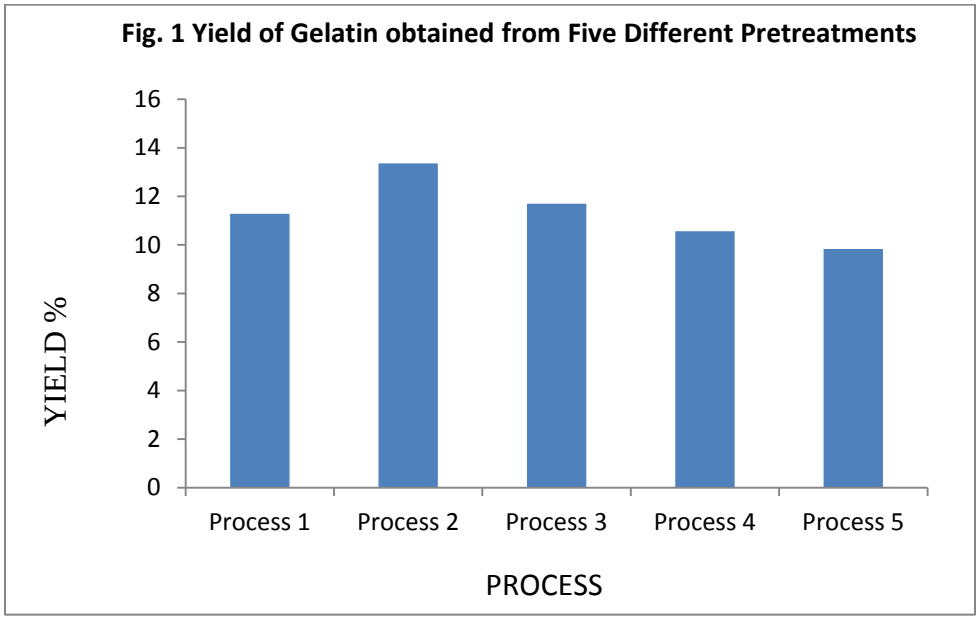
°C; G2: 50 °C and G3: 60 °C were 4.68±0.04, 4.43±0.06 and 4.34±0.03 respectively. On the other hand, the contents of the moisture, protein, fat, ash and pH of commercial gelatin (CG) were 5.26±0.03%, 92.6±0.10%, 0.71±0.05%, 0.73±0.62% and 4.38±0.02 respectively. The protein content of pacu skin indicates the maximum yield of gelatin. Gelatin yield and properties vary due to differences in proximate composition of skin, amount of soluble components in the skin and the collagen content.

The yield and bloom value of gelatin obtained through acid /alkali process are given in Fig 1. & 2. These properties vary with the species, age of the fish, and the extraction techniques used (Songchotikunpan *et al*., 2008). The moisture content of all gelatin samples was less than 6% which is within the limit prescribed for edible gelatin (Gelatin Manufacturers of Europe (GME), (2008). The ash content in all gelatin samples was, in the range of 0.92±0.02 - 1.13±0.03% which is much less than the recommended maximum limit of 2.6% (Jones, 1977) and the limit set for edible gelatin is (2%) (GME, 2008). Chandra and Shamasundar (2014) extracted gelatin from skin of fresh water carps also reported that the protein content of the dried gelatin from skins of catla, mrigal and rohu was in the range of 92–94%. The moisture content of



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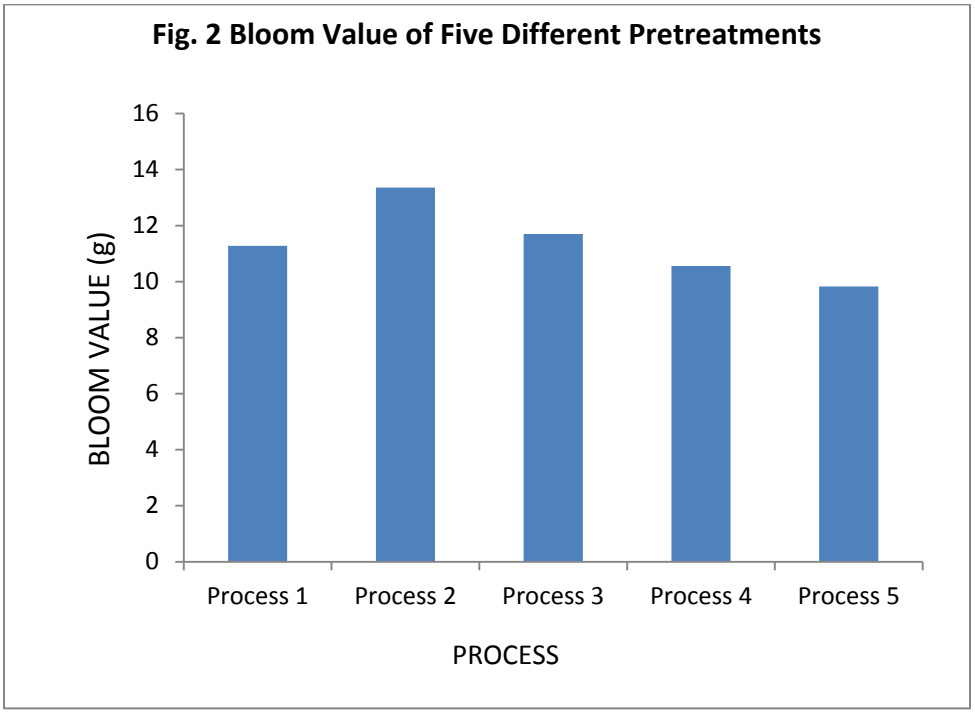

gelatin was less than 6% and ash content of gelatin samples was <2%. Similarly Ninan *et al.* (2013) reported for the rohu and yellowfin tuna. Killekar *et al*. (2012) reported higher content of protein i.e. 88.72% in black kingfish skin gelatin. Koli *et al.* (2012) reported that the protein content present in the gelatin extracted under 45 °C from the skin of tiger toothed croaker was 86.45%. See *et al*. (2010) reported that the lipid content of raw skin of fresh water catfish Pangasius (10.65%) was higher than that of marine cat fish (7.29%), snakehead (4.21%) and red tilapia (2.35%) which resulted in increase of lipid content in the gelatin extracted from pangasius catfish was much higher (2.63%) than others (0.47- 0.74%). Moisture and ash content of pacu skin gelatin was much less then gelatin extracted (6.04%) from black kingfish at 45 °C and ash content (2.24%) of gelatin extracted from skin of black kingfish at temperature 45 °C. pH of pacu skin gelatin varied from 4.34±0.03 to 4.68±0.04. The pH was below the range prescribed for Type A (pH 6.0 – 9.5) and Type B gelatins (pH 4.7- 5.6). The gelatin extracted from pacu fish skin could clarified as Type B gelatin due to its lower pH. As gelatin extracted from pacu skin was rich in protein with low content of ash and fat, the process employed for extraction of gelatin was said to be more efficient. Functional and colour properties of gelatin obtained in different temperature and commercial gelatin are given in Table 2.

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Table 2: Functional and Colour properties of gelatins.

Properties/Parameters	G1 (40 °C)	G2 (50 °C)	G3 (60 °C)	Commercial gelatin (CG
Bloom value (g)	291.33±1.24^{b}	282.25±2.07^{c}	272.24±0.92	294.5±1.63^{a}
Hydroxyproline,(mg/g)	8.49±0.05^{a}	7.8±0.21^{b}	5.18±0.06^{c}	8.56±0.06^{a}
Viscosity (cP)	6.23±0.03^{b}	5.73±0.14^{c}	5.34±0.03^{d}	8.16±0.02^{a}
Meltingpoint (°C)	24.83±0.05^{b}	24.46±0.10^{c}	23.72±0.03^{d}	25.15±0.04^{a}
Water holding capacity	5.26±0.04^{b}	5.12±0.09^{c}	4.91±0.04^{d}	7.49±0.02^{a}
Emulsifyingcapacity (%)	54.65±0.10^{b}	50.80±0.04^{c}	48.2±0.08^{d}	54.9±0.04^{a}
Emulsifyingstability (%)	34.5±0.08^{b}	31.5±0.04^{c}	28.2±0.08^{d}	34.85±0.04^{a}
Foaming capacity (%)	16.70±0.06^{b}	14.00±0.08^{c}	11.50±0.06^{d}	17.06±0.06^{a}
Foaming stability (%)	13.8±0.08^{b}	11.00±0.07^{c}	8.2±0.04^{d}	13.93±0.02^{a}
L*	73.62±0.03^{b}	72.58±0.20^{c}	69.27±0.02^{d}	86.69±0.17^{a}
a*	3.62±0.05^{a}	2.45±0.11^{b}	4.14±0.05^{a}	1.61±0.02^{c}
b*	14.53±0.11^{c}	16.75±0.14^{b}	18.41±0.05^{a}	10.18±0.04^{d}
Transmittance(%)	55.62±0.07^{b}	52.97±0.16c	49.40±0.06^{d}	61.38±0.02^{a}

* Each value is represented by the mean ± SD of n=3.
^{abcd} Indicate significant difference among treatments (P <0.05).

Hydroxyproline

The hydroxyproline content of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 8.49±0.05 mg/g , 7.8±0.21 mg/g and 5.18±0.06 respectively. Hydroxyproline content of commercial gelatin was 8.56±0.06 mg/g. Proline and hydroxyproline are thought to be responsible for the stability of the triple-helix of collagen structure through hydrogen bonding between free water molecules and the hydroxyl group of the hydroxyproline in gelatin (Fernandez-Diaz *et al*., 2001). Increasing extraction temperature resulted in decreasing hydroxyproline content (Nagarajan *et al*., 2012). The hydroxyproline content of the gelatin from tigertooth croaker and pink perch were in the range of 7.41–7.77 mg/g (Koli *et al.*, 2012) which were lesser than the gelatin extracted from cod skin, 8.30 mg/gm (Gomez-Guillen *et al*., 2002). The hydroxyproline content of black kingfish skin gelatin extracted at 45 °C was found to be 8.34 mg/g (Killekar *et al.*, 2012).

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Bloom value

The bloom value of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 291.33±1.24 gf, 282.25±2.07 gf and 272.24±0.92 gf respectively. Bloom value of commercial gelatin was 294.5±1.63 gf. The results indicated that increasing extraction temperature resulted in lower bloom value. Nagarajan *et al.* (2012) observed similar result for splendid squid skin gelatin bloom value which decreased with increasing extraction temperature. They also had lower gel strength compared to sole, megrim, cod and hake gelatins which were extracted at lower temperature of 45 °C (Gomez-Guillen *et al*., 2002). Killekar *et al*. (2012) reported that bloom values of black kingfish skin gelatin extracted at 45 °C was found to be 222 gf. The bloom value obtained in this study from pacu skin was higher to that of tilapia (Jamilah and Harvinder, 2002), sin croaker and short fin scad (Cheow *et al*., 2007) and lower than Indian Major Carps catla and mrigal except rohu (Chandra and Shamasundar 2014). Muyonga *et al*. (2004) and Cho *et al*. (2005) reported that Nile perch and yellow fin tuna skin gelatin extracted at higher temperatures exhibited a lower gel strength. Kittiphattanabawon *et al*. (2010) found that bloom strength of gelatin gels from brown banded bamboo shark and black strip shark was decreased with increase in the extraction temperature (45-75 °C) and time (6-12 h). Strength of gelatin gel is influenced by amino acids composition and molecular weight distribution of the gelatin itself. The strength of gelatin also varies with gelatin concentration, thermal history (gel maturation temperature and time).

Viscosity

The viscosity of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 6.23±0.03 cP, 5.73±0.14 cP and 5.34±0.03 cP respectively. Viscosity of commercial gelatin was 8.16±0.02 cP. The viscosity of pacu gelatin was relatively low compared to other kinds of gelatin obtained from cod (6.2 to 12.4 cP) (Gudmundsson and Hafsteinsson,1997), skate (22.5 cP) (Cho *et al*., 2005), rohu (6.06 cp) and yellowfin tuna (7.17 cp) (Ninan *et al.*, 2013). The viscosity of black kingfish skin gelatin extracted at 45 °C was found to be 13.53 cP (Killekar *et al*., 2012). Ninan *et al.* (2009) reported that the viscosity of the gelatin for the samples tested were in the range of 5.96 - 7.07 and it was significantly higher ($p<0.05$) for Grass carp followed by rohu and common carp. But higher viscosity was secured in pacu skin gelatin when compared with other

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kinds of gelatin from red tilapia (3.2 cP) (Jamilah and Harvinder, 2002). Whereas for channel catfish the optimum value predicted was 3.23 cP (Yang, *et al*., 2007).

Melting point

The melting point of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 24.83±0.05 °C, 24.46±0.10 °C and 23.72±0.03 °C respectively. Melting point of commercial gelatin was 25.15±0.04 °C .The melting points observed in the present study are higher than those reported for cold water fishes such as cod, hake (Gomez-Guillen *et al*., 2002). The melting point of gelatins extracted from tiger-toothed croaker and Pink perch skins were 20.36 °C and 19.23 °C respectively whereas the respective melting point for bones were 19.5 °C and 19.0 °C (Koli *et al*., 2012). Melting point of black kingfish skin gelatin extracted at 45 °C was found to be 22.1 °C (Killekar *et al*., 2012). Ninan *et al*. (2013) reported that in rohu skin and yellowfin tuna skin the melting point were 28.23 °C and 29.27 °C respectively

Water holding capacity

The water holding capacity of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 5.26±0.04 (ml/g) **,** 5.12±0.09 (ml/g) and 4.91±0.04 (ml/g) respectively. Water holding capacity of commercial gelatin was 7.49±0.02 (ml/g). Water holding capacity in pacu skin gelatin was found to be higher than black kingfish gelatin which was 4.43 ml/g when extracted at 45 °C (Killekar *et al*., 2012). Koli *et al*. (2012) reported that water holding capacity of tiger-toothed croaker skin (4.50 ml/g) and bone (3.00 ml/g) was higher as compared with Pink perch skin (2.36 ml/g) and bone (1.50 ml/g) gelatin.

Emulsifying capacity and stability

The Emulsifying capacity of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 54.65±0.10 %, 50.80±0.04% and 48.2±0.08% respectively. Emulsifying stability of G1: 40 °C; G2: 50 °C and G3: 60 °C were 34.5±0.08%, 31.5±0.04% and 28.2±0.0% respectively. Emulsifying capacity and Emulsifying stability of commercial gelatin were 54.9±0.04% and 34.85±0.04 % respectively. The emulsifying capacity and stability of the extracted gelatin from pacu skin gelatin extracted at lower temperature (40 °C) showed higher emulsifying capacity then extracted at higher temperature (60 °C). Similar results were observed by Nagarajan *et al*. (2012) in splendid squid skin gelatin. Killekar *et al*. (2012) reported that

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emulsifying capacity of black kingfish gelatin extracted at the temperature of 45 °C was found to be 55.66% and emulsifying stability was 32.5%.

Foaming capacity and stability

The Foaming capacity of pacu fish skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 16.70±0.06%, 14.00±0.08% and 11.50±0.06 % respectively. Foaming stability of G1: 40 °C; G2: 50 °C and G3: 60 °C were 13.8±0.08%, 11.00±0.07 % and 8.2±0.04% respectively. Foaming capacity and Foaming stability of commercial gelatin were 17.06±0.06% and 13.93±0.02% respectively. The foaming capacity and stability of the extracted gelatin from pacu skin gelatin extracted at lower temperature (40 °C) showed higher foaming capacity then extracted at higher temperature (60 °C). Similar results were observed by Nagarajan *et al.*, 2012 in splendid squid skin gelatin. Ninan *et al*. (2013) reported that foam forming ability of yellow fin tuna gelatin was significantly lower than rohu gelatin despite it was reverse in foam stability. The reduced foam formation ability in the present study may be due to the aggregation of proteins which interfere with the interactions between the protein and water (Kinsella, 1977).

Gelatin colour and Gel clarity

Lightness (L*) of the pacu skin gelatin of G1: 40 °C; G2: 50 °C and G3: 60 °C were 73.62±0.03, 72.58±0.20 and 69.27±0.02 respectively while their respective Redness (a*) were 3.62±0.05, 2.45±0.11and 2.45±0.11. Respective Yellowness (b*) of the pacu skin for the G1, G2 and G3 were 14.53±0.11, 16.75±0.14 and 18.41±0.05 and the Transmittance (%) were 55.62±0.07, 52.97±0.16 and 49.40±0.06 respectively. Nevertheless the colour of commercial gelatin for Lightness (L*), Redness (a*), Yellowness (b*) and transmittance (%) were 86.69±0.17, 1.61±0.02, 10.17±0.04 and 61.38±0.02 respectively. The change in colour of gelatin increased with increasing temperatures. However, Transmittance also decreased with increasing temperature. G1 (40 °C) showed higher transmittance than G3 (60 °C). The turbidity and dark colour of gelatin is commonly caused by inorganic, protein and muco-substance contaminants introduced or not removed during its extraction (Eastoe and Leach, 1977). It indicated that lightness of gelatin decreased with increase in redness and yellowness when the extraction temperature increased. Similar results were observed for splendid squid skin gelatin by Nagarajan *et al*. (2012). Koli *et al*. (2012) reported that tiger-toothed croaker skin gelatin showed the greatest lightness value (*L**) and the gelatins from skin samples had higher values

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compared to bone gelatins. Similar results were found in the present study also for redness (*a**) and there was no significant difference with respect to yellowness (*b**). On account of these, it can be concluded that factors such as fish species and raw material, influence the colour characteristics of extracted gelatin.

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