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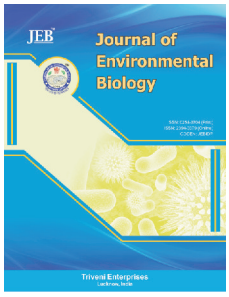

Effects of different soaking time on the extraction of gelatin from shortfin scad (*Decapterus macrosoma*) heads

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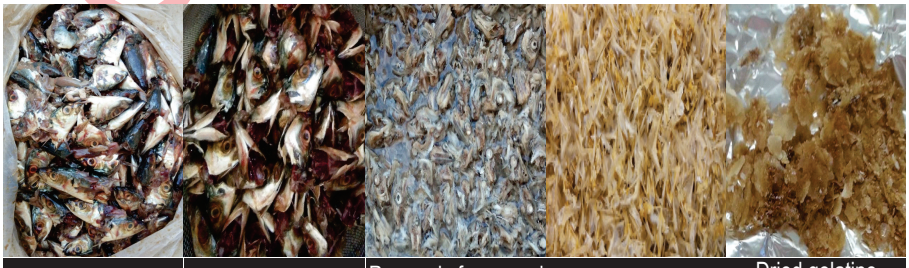
Abstract

Aim: Fish gelatin is one of the potential biopolymers that can be extracted from the connective tissues of any fish by-products such as skin, scale, bone, head, fin and frame. This study was carried out to optimise the best method of gelatin extracted from the heads of Shortfin scad (*Decapterus macrosoma*), further the effects of different soaking time on the physico-chemical properties of extracted gelatins was also determined.

Methodology: The heads of shortfin scad (*Decapterus macrosoma*) were collected from fish processing industries and were cleaned prior to extraction. The cleaned heads of *D. macrosoma* were subjected to different soaking times (1, 4, 8 or 12 hrs) in 0.1M citric acid. Prior to these soaking times, the heads were placed in boiling water to remove excess flesh and the pre-treated with 0.2M of sodium hydroxide solution for one hour, rinsed with tap water, soaked in 0.05M of sulphuric acid for one hour and were then rinsed with tap water.

Results: Results showed the optimum soaking time of *D. macrosoma* heads was obtained at 8 hr with the highest yield value of 1.70%, which was significantly higher compared to those soaked at 1, 4 or 12 hr ($p<0.05$). The moisture, crude protein, lipid and ash of the gelatins were 9.08%, 15.25%, 0.46% and 41.03%, respectively. The pH in the extracted gelatin was obtained at 3.31. Gel strength and melting point of *D. macrosoma* heads gelatin were found at 19.68 g and 16.0°C, respectively, lower than that of bovine gelatin (300.5 g; 37.0°C), respectively. The ratio of imino acids in gelatin from *D. macrosoma* heads to bovine gelatin was 46 residues to 132 residues per 1000 total amino acid residues.

Interpretation: The findings suggested that gelatin extracted from the heads of *D. macrosoma* can be potentially applied in many areas due to its comparable properties with the commercial gelatin.



Introduction

Gelatin is one of the most popular biopolymers and due to unique functional properties, it is extensively used in wide variety of applications, such as in food, pharmaceuticals, cosmetics and photographic areas (Karim and Bhat, 2009). The global demand for gelatin has been increasing over the years. Currently, the annual world output of gelatin is approximately 326,000 tons, with pig skin-derived gelatin contributing the highest (46%), followed by bovine hides (29.4%), bones (23.1%) and other sources (1.5%) (Karim and Bhat, 2009). Gelatin is water-soluble polymer that can be used as an ingredient in food industry to improve the elasticity, consistency and emulsion stability of foods (Tavakolipour, 2011). It is widely used in jelly production and as emulsifiers, micro-encapsulating agents and colloid stabilizers in food industries. In the pharmaceutical and medical fields, gelatin is widely used in the manufacture of hard and soft capsules. It is also used as a matrix for implants, plasma expanders in injectable drug delivery microspheres, and in intravenous infusions (Kim and Wijesekara, 2012). In cosmetic industry, gelatin has been used for many years as hydrolyzed animal protein in shampoos, conditioners, lipsticks and nail formulas (Kim and Wijesekara, 2012).

Gelatin is derived from fibrous protein collagen by partial hydrolysis. Collagen can be found in skin, bones, tendons and cartilage, which consists of a triple-helix structure composed of three α-chains intertwined and bonded by inter-chained hydrogen bonds. Collagen is the major structural component of connective tissue proteins with up to 30% of the total protein in the body tissue of vertebrates and invertebrates, consisting the following amino acids such as glutamine, hydroxyproline and proline (Darmanto *et al.,* 2014). Bovine and porcine collagen are the main industrial sources, but fish collagen can be a useful alternative due to their excellent characteristics (Liu *et al.,* 2007).

Alternative sources of gelatin are gaining prominence, especially gelatin extracted from the by-products (skins, bones, heads, scales, and frames) of marine fish species (Gómez-Guillén *et al.,* 2009). This has encouraged researchers to explore this resource. Moreover, fish gelatin shows analogous characteristics to porcine gelatin, and thus can be considered as a substitute to mammalian gelatin in the food industry (Mohtar *et al.,* 2010; Kim and Venkatesan, 2014).

Fish cracker (*Keropok lekor*) is a traditional Malaysian fish product and it is a popular snack food that is widely consumed by all communities in Malaysia. It is famous, especially in the East coast of Peninsular Malaysia such as Terengganu, Kelantan and Pahang. Generally, fishes used in keropok lekor production are mainly low value pelagic fish, such as *Chirocentrus dorab*, *Decapterus macrosoma* and *Sardinella fimbriata*. According to a local keropok lekor in Terengganu, ikan *D. macrosoma* is the best fish to be used for making keropok lekor due to its sweet taste and lower amount of bones (Omar *et al.,* 2008).

High amount of waste are produced from the expanding fish processing industries. Malaysians are among the world's top fish consumers, eating at least 56.5 kg of fish per person each year (The Star Online, 2014). Utilization of fish waste to produce gelatin will increase their commercial value and help reduce the adverse environmental problems. The waste produced from fish processing industry can occupy as much as 75% of the total fish catch weight (López-Caballero *et al.,* 2013). This huge amount of fish waste disposal may cause health and environmental problems. Historically, fish by-products are simply disposed because it is recognized as being low value (Kim and Venkatesan, 2014). However, fish waste can be a source of potential ingredients application to the food and others industry. The physical and chemical properties of gelatin depends on the raw materials of extracted species and on the processing condition of gelatin manufacturing (Gómez-Guillén *et al.,* 2009). Nevertheless, the yield of gelatin may be affected by the pre-treatment and extraction process, which depends on the pH, temperature and soaking time. The important functional properties of gelatin consist of gel strength, melting temperature and viscosity, which is generally related to their gelling characteristics. From previous studies conducted on the soaking time, researchers demonstrated the importance of pre-treatment time and several condition factors on the yield and quality of gelatin as shown in grass carp (Kasankala *et al.,* 2007), shark (Cho *et al.,* 2004), cuttlefish (Ninan *et al.,* 2015), rainbow trout (Tabarestani *et al.,* 2010), as well as chicken feet (Liu *et al.,* 2011) and chicken shank bones (Puspitasari *et al.,* 2013). Therefore, it is necessary to determine the optimum pretreatment conditions, such as soaking time in citric acid solution, to extract gelatin in fish by-products. In view of the above, the present study was carried out investigate the effects of different soaking time on gelatin yield from Shortfin Scad (*Decapterus macrosoma*) heads and to determine some of the physico-chemical properties of extracted gelatin with optimum soaking time during the extraction procedure.

Materials and Methods

Raw material: A total of 50 kg of *Decapterus macrosoma* heads were purchased from a fish processing plant, Maperow Sdn. Bhd. in Terengganu, Malaysia. The heads were washed with tap water and then the attached flesh on the fish heads were removed in boiling water and then any excess remaining flesh was scraped off with a knife. The remaining samples were kept at -20ºC until further use.

Gelatin extraction: Gelatin was extracted from the cleaned samples following the method of Gudmundsson and Hafsteinsson (1997) with some modifications. Samples of cleaned fish heads were pre-treated by soaking in a 250 ml beaker containing 0.2 M of sodium hydroxide solution for one

hour with subsequent rinsing with tap water to pH 7. The treatment was repeated twice. The fish heads were then soaked with 0.05 M of sulphuric acid for one hour with subsequent rinsing with tap water. The fish heads were then soaked with 0.1 M citric acid for one hour with subsequent rinsing with tap water. The acidic solutions were drained off and the samples were washed with running water until a pH of 7 was maintained. The experiments were repeated by replacing one hour of soaking time in citric acid with 4, 8 and 12 hrs. The ratio of fish heads to washing liquid was 1kg fish heads (dry weight) to 7 l of acid or alkali solution for each treatment.

The fish heads were washed with distilled water in 1:3 to remove any residual substances and were then extracted with distilled water at 45°C for 12 hrs. The mixture was filtered and dried in an oven at 60ºC overnight. The dried gelatin was crushed into powder by using a pestle and mortar and then stored in air-tight containers. All treatments were repeated three times at each soaking time.

Proximate analysis: The proximate analysis of gelatin extracted from *Decapterus macrosoma* heads were carried out according to the procedures of AOAC (2000).

Gelatin pH : The pH of extracted gelatin was determined according to See *et al.* (2010). A total of 0.667 g of dry gelatin was dissolved in 10 ml of distilled water at 60°C for 30 min. The gelatin solution was cooled to room temperature for 30 min, and a pH meter was used to determine the pH at 25°C.

Gel strength : The gel strength of extracted gelatin was determined according to BSI (1975). A 6.67% gel was prepared in a beaker by dissolving 0.334 g of dry gelatin in 5ml of distilled water at 60°C for 30 min. The solution was cooled in room temperature for 30 min and then chilled in a refrigerator at 7°C for 18 hrs. The gel strength was measured immediately after being removed from a refrigerator using a TA.XT Texture Analyzer. The texture analyzer was equipped with a load cell of 5 kg, cross-head speed 1 mm sec^{-1} and was equipped with a flat bottomed plunger that was 0.5 inches in diameter. The beaker was placed centrally under the plunger for the penetration test. The probe proceeded to penetrate the gel at 4 mm depths and the maximum force (g) was determined.

Melting point : The melting point was conducted according to Muyonga *et al.* (2004) with some modifications. Gelatin of 6.67% was prepared in a glass vial by dissolving 0.334 g of dry gelatin in 5 ml of distilled water. This mixture was dissolved at room temperature for 30 min and then heated in a water bath at 60°C for another 30 min. The dissolved samples were cooled at room temperature for 30 min, and then chilled in a refrigerator at 7°C for 18 hrs. The samples were transferred into a water bath at 10°C and inverted. The water bath was warmed gradually by adding warm water at about 1°C per min. The temperature was recorded at which the gel melted.

Amino acid composition: The amino acid composition of *Decapterus macrosoma* head and bovine (control) gelatins were analyzed following on the method of Villas-Bôas *et al.* (2003), using gas chromatography-mass spectrometry (GC-MS). Three processes involved included hydrolysis, derivatisation, and injection of methyl chloroformate derivatised gelatins on the GC-MS. A total of 5 mg of gelatin sample was hydrolysed and incubated in 1 ml of 6N hydrochloric acid at 110°C for 12 hrs. The hydrolysed gelatin was transferred into a 1.5 ml eppendorf tube and was then dried using nitrogen gas. The hydrolysed gelatin was derivatised by using methyl chloroformate method for the analysis by GC-MS. The hydrolysed gelatin was dissolved in 10 µl with 10mM internal standard (*d*4-Alanine) and 190 µl of 1M sodium hydroxide solution. This mixture was transferred to a 5ml silanised reaction tube and 167 µl of methanol and 34 µl of pyridine were added to the suspended solution. To initiate the derivatisation process, 20 µl of methyl chloroformate was added to the solution, followed by vortexing for 30 sec. Next 20 µl of MCF was then added, followed by mixing for another 30 sec. A volume of 400 µl of chloroform was immediately added to the reaction mixture and was mixed vigorously for 10 sec. This mixture was then centrifuged at 2000 rpm for 2 min at room temperature to achieve better separation of two layers. The upper aqueous layer was discarded whereas the organic lower layer was dehydrated by adding 100 mg of anhydrous sodium sulphate. An aliquot of 1 µl of dried organic phase was injected into the GC-MS.

The data were analyzed by using analysis of variance (ANOVA). Comparison of means were performed by using SPSS version 20.

Results and Discussion

Gelatin yield obtained from different soaking time in citric acid : Pre-treatment time is an important factor that may affect the gelatin yield and associated properties. Increasing pre-treatment time (soaking time) generally increases gelatin yield (Tabarestani *et al.,* 2010) and in this study, the gelatin yield increased from 1 to 8 hrs of soaking time (Fig. 1). This may be due to organic acid solubilizing the uncrosslinked collagens and breaking some of the inter-chain cross-linkages of collagens, thus leading to further collagen solubilization during extraction (Liu *et al.,* 2015). An adequate number of cross-links must be broken to convert collagen into a suitable form for extraction (Zhou and Regenstein, 2005). Meanwhile, as for 1 and 4 hrs, the cross-linkage collagen fibers may not have been broken down completely.

However, the yield obtained for *D. macrosoma* heads did not show an increasing trend with increasing soaking time in citric acid. Gelatin yields of *D. macrosoma* heads reached a maximum gelatin yield at 8 hrs of soaking time and declined at 12 hrs. Liu *et al.* (2001) also reported that gelatin yield did not increase continuously with increasing the soaking time in acetic acid, citric acid, hydrochloric acid or lactic acid. Meanwhile, a study on

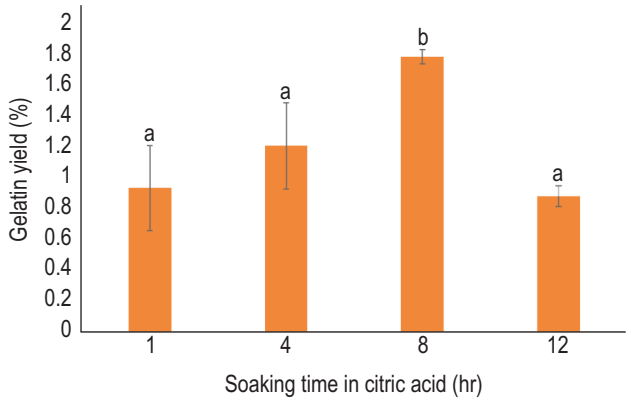


Fig. 1 : Mean gelatin yield obtained with different soaking times in citric acid. Values within a row with different letters are significantly different (p<0.05)

soaking chicken shank bones at different soaking times in hydrochloric acid solutions showed that the longest soaking time led to a lower collagen yield (Puspitasari *et al.,* 2013). Low collagen yield will simultaneously lead to a low gelatin yield. The highest gelatin yield was obtained at 8 hrs of soaking in citric acid, and the yield was significantly higher (p<0.05) compared to other soaking times.

Gelatin yield : The gelatin yield obtained at 1, 4, 8 and 12 hrs of soaking in citric acid were 0.89%, 1.15%, 1.70% and 0.84%, respectively (Table 1). Although, 10 g of fish heads were used at each soaking time, less than 0.2 g of gelatin was produced. A total of 1034.5 g cleaned fish heads were further pretreated for 8 hrs in citric acid for the characterization of gelatin and 17 g of gelatin was obtained. The yield obtained from *D. macrosoma* heads was 1.6%, indicating a low yield, but was similar to the findings of Silva *et al.* (2011) who reported 1.54% gelatin yield from the carp head bones. A higher yield of gelatin extracted from *D. macrosoma* skin was 7.3% (Cheow *et al.,* 2007). These differences in the yield could possibly be due to the differences in the amount of collagen and associated composition in the skin, bones, as well as their matrix components (Shakila *et al.,* 2012).

A low gelatin yield exhibited in this study may be due to collagen leaching during the washing treatments, loss of collagen during extraction or incomplete conversion process of collagen to gelatine strands (Jamilah and Harvinder, 2002). The species and tissue not only affects the yield and quality of the gelatin, but also by the extraction process itself (Montero and Gómez-Guillén, 2000; Karim and Bhat, 2009). A higher gelatin yield and better gel characteristics were exhibited on extracted gelatin from Alaska pollock skin using a combination of alkaline pretreatments followed by acid pretreatments (Zhou and Regenstein, 2005). The obtained yield was also influenced by concentrations of sodium hydroxide, sulphuric acid and citric acid solutions used in the preliminary treatment of raw materials (Gudmundsson and Hafsteinsson, 1997). Furthermore, the method of Gudmundsson and Hafsteinsson (1997) may not be optimal to extract gelatin from *D. macrosoma* heads and more research in this area is needed.

Table 1 : Gelatin yield for each soaking time in citric acid

Soaking time in citric acid	Gelatin yield (%)
1 hr	0.89 ± 0.2623[a]
4 hr	1.15 ± 0.2665[a]
8 hr	1.70 ± 0.0451[b]
12 hr	0.84 ± 0.0643[a]

Values are mean of replicates ± SD; Different letters indicate significant differences (p < 0.05)

Proximate composition and pH : The proximate composition of *D. macrosoma* heads for moisture, crude protein, crude fat and ash were 9.08%, 15.25%, 0.46% and 41.03%, respectively. The moisture content obtained from *D. macrosoma* heads conducted in this study was similar to common carp head bones, as well as *D. macrosoma* skins and channel catfish head bones in previous studies (Silva *et al.,* 2011; Cheow *et al.,* 2007; Liu *et al.,* 2009). Commercial gelatins have moisture content between 8% and 13% (GMIA, 2012), as well as the moisture content of edible gelatin is between 8% and 15% (GME, 2005). The ideal humidity in gelatin is 8-12% and a moisture content above 16% is not desirable due to the risk of microbial growth (Almeida and Lannes, 2013). The moisture content of gelatin from *D. macrosoma* heads was 9.08%, which was within the range of GMIA and GME standards. Gelatin extracted from *D. macrosoma* heads exhibited relatively low protein content when compared with other studies as shown in the Table 2. The gelatin extracted from *D. macrosoma* heads was almost free of fat (<0.5%). The low lipid content in the gelatin from *D. macrosoma* heads may be related to the alkaline treatment, which might have been enough to remove lipids (Silva *et al.,* 2011). The ash content obtained from *D. macrosoma* heads gelatin was high and this result is similar to common carp head bones reported in previous study (Silva *et al.,* 2011). The high ash content in the gelatin obtained may be due to the combination of higher mineral content (Shakila *et al.,* 2012) and low concentration of the acid used (Saneai *et al.,* 2013). The bones usually contained higher ash content and lower moisture content than skins.

The pH of gelatin solution obtained was strongly acidic (3.31) and, this may be affected by the washing treatment (Jakhar *et al.,* 2012). Cheow *et al.* (2007) reported that the skin gelatin *D. macrosoma* had pH 4.87 which was acidic. The pH obtained in this study was also strongly acidic and demonstrated slight difference compared to previous studies.

Gel strength, melting point and amino acid composition : Gel strength and gel melting point are important properties of gelatin in determining the quality of the products. High gel strength values of gelatin correspond to its high economic value. The gel

Table 2 : Comparison of proximate composition of gelatin from *D. macrosoma* heads with other fish species (Common carp heads, *D. macrosoma* skin and channel catfish head bones)

Proximate composition (%)	*D. macrosoma* heads[a]	Common carp head bones[b]	*D. macrosoma* skin[c]	Channel catfish head bones[d]
Moisture	9.08 ± 0.36	15.1±0.9	11.3 ± 0.42	8.3 ± 1.9
Protein	15.25 ± 0.11	28.3±1.3	68.7 ± 0.15	77.9 ± 1.2
Lipid	0.46 ± 0.06	3.3±0.5	0.22 ± 0.02	10.3 ± 0.9
Ash	41.03 ± 0.74	51.2±1.7	1.15 ± 0.13	1.3 ± 0.5
pH	3.31 ± 0.02	5.30	4.87	-

[a]Values obtained from current study; [b]Values from Silva *et al.* (2011); [c]Values from Cheow *et al.* (2007); [d]Values from Liu *et al.* (2009)

Table 3 : Comparison of gel strength and melting point of *D. macrosoma* heads gelatine with bovine (control) and other fish species

Gelatine	Gel strength (g)	Melting point (°C)	References
D. macrosoma heads[a]	19.68	16	Current study
Bovine (control)[b]	300.5	37	Current study
D. macrosoma skins	176.92	18.5	Cheow *et al.* (2007)
Common carp heads	54.7-131.5	24.6-27.8	Silva *et al.* (2011)
Channel catfish head bones	117-282	23-27	Liu *et al.* (2009)

[a-b]Values are the mean of duplicate samples

strength obtained from *D. macrosoma* heads gelatin conducted in this study was 19.68 g (Table 3). It was relatively lower than those found in *D. macrosoma* skin (Cheow *et al.,* 2007), common carp head bones (Silva *et al.,* 2011) and channel catfish head bones (Liu *et al.,* 2009) conducted in previous studies as well as bovine gelatin, which acted as a control in this study. The low gel strength may be influenced by the pH that was 3.31 in this study because gel strength of all the gelatins decrease below pH 4 and slightly above pH 8 (Choi and Regenstein, 2000). The pH of gelatin may also be affected by the washing treatment (Jakhar *et al.,* 2012). Factors that influenced the gel strength values also include the molecular weight distribution of gelatin, size of protein chains and composition of amino acids (Sanaei *et al.,* 2013), as well as the concentration of gelatin solution, temperature, aging time and pH (Kasankala *et al.,* 2007). These factors should be further explored to improve gel strength of gelatin from *D. macrosoma* heads.

The melting point of gelatin obtained from the heads of *D. macrosoma* was 16°C (Table 3) and this value was found relatively close to the *D. macrosoma* skin (18.5°C) as reported by Cheow *et al.* (2007). However, the melting point of *D. macrosoma* head gelatin was lower compared to bovine (control) in this study, as well as common carp heads and channel catfish head bones as reported in previous studies (Liu *et al.,* 2009; Silva *et al.,* 2011). Low melting point values at low pH were reported by Choi and Regenstein (2000), as well as Pang *et al.* (2014) who showed that melting points of gelatin decreased markedly at pH values of less than 4.0. In addition, the maturing temperature and concentration of gelatin gel have potential to affect the melting point of gelatin (Mariod and Adam, 2013).

Fish gelatins are known to have lower gel strength and melting point than mammalian gelatin, and the gel strength of fish gelatin ranges from 0 to 200 g (Karim and Bhat, 2009). The gelatin melted below body temperature to ensure its melt-in-mouth property (Pang *et al.,* 2014). The lower melting point and gel strength of *D. macrosoma* heads compared to bovine (control) and other fish species may be due to lower imino acid contents. Lower imino content in gelatin leads to less proline hydroxylation (Karim and Bhat, 2009). Gelatin with low melting points can be used in microencapsulation applications, since the microencapsulation process can be carried out at lower temperatures (Karim and Bhat, 2009). Fish gelatin with low melting points can also be used in dry products such as microencapsulation of vitamins (Benjakul *et al.,* 2012). Besides, fish gelatin that cannot form gel at room temperature can be utilized in other application that do not require high gel strength, especially in short-life products such as frozen or chilled foods to prevent syneresis and changing food texture (Benjakul *et al.,* 2012).

Functional properties of gelatin are also associated with their chemical properties. The gel strength and melting point of gelatin depends on their molecular weight distribution and amino acid composition (See *et al.,* 2010). The amino acid composition of *D. macrosoma* head gelatin was expressed as residues per 1000 total amino acid residues. From Table 4, the ratio of imino acids (hydroxyproline and proline) of the *D. macrosoma* head gelatin was lower than bovine gelatin, which was about 46 residues per 1000 total amino acid residues to 132 residues per 1000 total amino acid residues. Fish gelatins have lower gelling and melting temperature compared to mammalian gelatin (Karim

Table 4 : Amino acid composition of *D. macrosoma* head and bovine gelatins (residues per 1000 total amino acid residue)

Amino acid	Source of gelatin	
	***D. macrosoma* head**	**Bovine (control)**
Glutamic acid	89	97
Phenylalanine	29	10
Tyrosine	17	11
Glycine	450	453
Proline	32	82
Alanine	67	50
Methionine	18	13
Hydroxyproline	14	50
Aspartic acid	38	20
Valine	18	35
Isoleucine	12	10
Leucine	13	15
Lysine	109	106
Serine	68	40
Histidine	34	8
Total	1000	1000

Values are means of three replicates

and Bhat, 2009). In addition, imino acids are imperative in the renaturation of gelatin subunits during gelling (Mariod and Adam, 2013). A low amount of imino acids may indicate poor gelling properties and posess a weaker gel network (Sanaei *et al.,* 2013).

The optimal soaking time of *Decapterus macrosoma* heads in 0.10 M citric acid was 8 hrs that yielded 1.7% gelatin, which was significantly different as compared to others. The moisture, crude protein, crude fat and ash were 9.08%, 15.25%, 0.46% and 41.03%, respectively, while pH was 3.31. The gel strength and melting point of *D. macrosoma* head gelatin was 19.68 g and 16.0°C, respectively, whereas values obtained from bovine gelatin were 300.5 g and 37°C, respectively. The imino acid ratio in *D. macrosoma* head gelatin was much lower (46 residues/1000 total amino acid residues) than bovine gelatin (132 residues/1000 total amino acid residues). Extracted *D. macrosoma* head gelatin is not suitable in applications that require higher gel strength and higher melting point. However, it can be used in application such as microencapsulation and frozen products. Different methods should be applied to extract gelatin from *D. macrosoma* heads to investigate the optimum extraction method that leads to the highest yield and gel strength.

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