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Effect of phosphoric acid concentration on physicochemical properties of *Abalistes stellaris* skin gelatin

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Abstract. Due to halal and health issues, the fish by-product (waste) is one of the best sources to substitute land animal gelatin. *Abalistes stellaris* has a hard texture in skins and known as leather jacket fish species. Moreover, its skins obtained as wastes in smoked fish processing and have not been utilized. Therefore, this study aimed to analyze the physicochemical properties of gelatin extracted from *A. stellaris* skins at a different phosphoric acid concentration (0.2, 0.4, and 0.6 M). This study conducted by a completely randomized design (CRD) with three replications, followed by Duncan's post-hoc test. The different pre-treatment of phosphoric acid concentrations showed significant differences ($P<0.05$) on yield, pH, chemical composition (moisture, protein, fat, and ash), gel strength, gelling, and melting points of *A. stellaris* skins gelatin. Only the viscosity parameter and an ash content of gelatin did not show a significant effect ($P>0.05$). The yield of *A. stellaris* skins gelatin ranged from 9.48% to 10.87%. The protein, moisture, fat, and ash content of skins gelatin ranged 74.55-81.41, 8.62-12.40, 0.43-0.73, and 1,48-1,79 (dry weight basis), respectively. Also, the gel strength and viscosity of skins gelatin were in the range of 1.28 N to 2.45 N and 3.83 cP to 4.21 cP. The amino acid composition of *A. stellaris* skins gelatin was rich in glycine, proline, alanine, and glutamic acid.

1. Introduction

Abalistes stellaris is one of the economically important fish species in Indonesia. The skin of *A. stellaris* has a hard texture as a leather jacket. The weight of its skins is about 15-20% of the total weight of fish and has not been utilized. Consequently, fish skins become waste and cause environmental pollution. One of the best approaches to utilize fish skins is gelatin production [1]. Gelatin is a fibrous protein that can be produced from partial denaturation of collagen with high temperatures.

Gelatin has been widely used in food, cosmetic, pharmaceutical, and photography industries [2,3]. Gelatin has been used for emulsifying, colloidal stabilizers, foaming, gelling agents, decomposers, and microencapsulated agents [4]. Several essential characteristics of gelatin possessed by gelatin are gel strength, viscosity, gelling, and melting point [5].

Commonly, gelatin is extracted from the skin and bones of animals, such as pigs and cows. Halal and kosher issues increase the interest in replacing the gelatin source [6]. Therefore, an alternative or substitute to mammalian gelatin is fish processing wastes, especially from fish skin. Previous research studies have explored gelatin from fish processing wastes such as croaker, red snapper, grouper, tuna, and squid [2,6,7,8].

Fish processing waste is quite large, accounting for 30% of the total weight of fish consisting of skin, bones, scales, fins, and heads [1]. Total fish production in Indonesia reached 23.26 million tons in 2017. About 30% of the total weight is wastes (by-products), which represents 6.97 million tons in the form of wastes, and high recovery into fish gelatin.

A. stellaris processing waste has a potential source of halal gelatin. Scarce report on the application of *A. stellaris* waste for gelatin [9]. The extraction of *A. stellaris* skin using citric acid produced a lower quality of physicochemical characteristics. The purpose of this study was to analyze the physicochemical properties of *A. stellaris* skin gelatin using different phosphoric acid concentrations.

2. Material and methods

2.1. Material

The *A. stellaris* skins were provided from the home industry of smoked fish located in Tuban, East Java, Indonesia. The by-products were collected and put into polyethylene bags and stored into cold box (4^{o}C) during transportation. After arrived at the laboratory, the skins were washed with running water and cut around 1×1 cm^2 using a scissor. The minced samples were then put into polyethylene plastic and stored in the freezer at a temperature f -20 ^{o}C until used. All chemicals and reagents used were of the analytical grade.

2.2. Preparation of A. stellaris skin gelatin

A. stellaris skin gelatin was extracted according to the method of Benjakul et al. [2], with slight modifications. The cut skins of *A. stellaris* were thawed by running water at room temperature (± 24 ^{o}C). The thawed samples were then soaked in 0.05 M NaOH with a skin/alkaline solution ratio of 1:5 (w/v) to remove non-collagenous proteins and pigment. The mixture was stirred for 2 h at room temperature, where the alkaline solution was changed every one hour. The treated samples were neutralized by washing with tap water until samples reached a pH 7. Next, the skins ware pretreated by adding phosphoric acid with different concentrations (0.2, 0.4, and 0.6 M), and the ratio of samples and acids were 1:5 (w/v). The mixture was stirred at room temperature for 4 h, which the acid solutions were changed every 2 hours. The swollen samples were washed by distilled water until reaching neutral pH around 7. Afterward, the samples were poured water in the ratio 1:3 (w/v), then extracted in the high temperature (55 ^{o}C) into a water bath for 4 h. The treated samples were filtered by Whatman paper, and the filtrate was dried by using an oven with a temperature of around 55 ^{o}C. The dried gelatins were stored in -20 ^{o}C until used.

2.3. Determination of yields

The yield of *A. stellaris* skins gelatin was calculated using the following equation:

$$\text{Yield (\%)} = \frac{\text{M}}{\text{Mo}} \times 100 \quad (1)$$

Where M is the weight of *A. stellaris* skin gelatin (g), and *Mo* is the weight of *A. stellaris* skin (g).

2.4. Determination of pH

The pH value of *A. stellaris* skins gelatin was determined using pH meter. About 0.2 g of skins gelatin was dissolved in 20 ml of distilled water at 80 ^{o}C. The dissolved samples were subjected to measured pH by using a pH meter at room temperature.

2.5. Determination of proximate composition

The proximate analysis of *A. stellaris* skins gelatin was measured according to the methods of AOAC [10], including moisture, protein, fat, and ash contents.

2.6. Determination of gel strength

Gelatin solution with 6.67% concentration was prepared with distilled water. The solution was stirred using a magnetic stirrer until homogeneous and heated at 60 °C for 15 minutes. The treated sample was poured into a 100 ml glass beaker with covered and kept for 2 minutes. Then, the sample was Incubated at 10 °C for 17 ± 2 hours. Furthermore, the incubated sample was measured using a digital force gauge.

2.7. Determination of viscosity

The viscosity of *A. stellaris* skins gelatin solution (6.67%, w/v) was determined by using a rheometer equipped with a cone and plate geometry. About 1.3 mL of skins gelatin solution was applied to the plate, and the excess sample was removed. The sample was covered with silicone oil to prevent evaporation during measurement. Samples were allowed to equilibrate for 2 min at 60 ^{o}C and were subjected to a programmed shear rate linearly increasing from 0.2 to 200 s^{-1} in 2 min. Shear viscosity evaluated at a shear rate of 100 s^{-1} was expressed at mPas.

2.8. Determination of melting and gelling point

Melting and gelling point of *A. stellaris* skins gelatin was determined based on the method of Suryaningrum and Utomo [10].

2.9. Determination of amino acid composition

The amino acid composition was analyzed at the Indo Genetech Saraswanti laboratory, Bogor, West Java. Amino acids were separated using ultra-performance liquid chromatography (UPLC) by following the company procedure.

2.10. Statistical data analysis

Statistical analysis was carried out using analysis of variance (ANOVA), followed by Duncan's posthoc test (SPSS 25.0 software). Statistical significance was considered at $P< 0.05$.

3. Results and Discussion

3.1. The yield of A. stellaris skins gelatin

The yield of gelatin extracted from *A. stellaris* skins at different phosphoric acid conditions was presented in Table 1. Difference phosphoric acid had a significant difference in the yields (P<0.05). The highest yield of *A. stellaris* skins gelatin was around 10.87% in the pre-treatment of 0.6 M phosphoric acid but no significant difference in the pre-treatment of 0.4 M.

Table 1. Yield, pH, and chemical parameters of *A. stellaris* skins gelatin.

Phosphoric Acid	Yield, pH and chemical parameters					
	Protein	Moisture	Fat	Ash	Yield	pH
0.2 M	74.55 ± 0.79^{a}	12.40 ± 0.61^{a}	0.73 ± 0.05^{a}	1.48 ± 0.32^{a}	9.48 ± 0.13^{a}	6.46 ± 0.33^{b}
0.4 M	78.12 ± 0.78^{b}	10.19 ± 0.59^{b}	0.43 ± 0.08^{b}	1.72 ± 0.31^{a}	10.06 ± 0.50^{ab}	6.56 ± 0.12^{b}
0.6 M	81.41 ± 0.87^{c}	8.62 ± 0.86^{c}	0.65 ± 0.08^{b}	1.79 ± 0.21^{a}	10.87 ± 0.79^{b}	5.96 ± 0.23^{a}

Mackerel (*Scomberomorus commersonii*), lethrinidae fish (*Lethrinus* sp.), giant featherback (*Chitala lopis*), red tilapia (*Oreochromis nilotica*) and black tilapia (*Oreochromis mosambicus*), and blue whiting (*Micromesistius poutassou*) had lower yields than that of A. stellaris yield [12-16]. Moreover, the yield of *A. stellaris* skins gelatin was in accordance with the gelatin extracted from *Pangasius hypophthalmus*. Phosphoric acids-extracted gelatin is a lower yield than that of citric acids-extracted gelatin [9, 17,18]. The yield affected by raw materials and extract solution [19].

3.2. the pH of A. stellaris skins gelatin

The pH of *A. stellaris* skins gelatins is shown in Table 1. The concentration of phosphoric acid significantly affected pH ($P<0.05$). the pH values ranged from 5.96 to 6.56. The pH value of *A. stellaris* skins gelatin increased as the acid concentration increased. The pH values of dog shark (*Scoliodon sorrakowah*) and skipjack tuna (*Katsuwonus pelamis*) skins gelatin are around 5.14, 5.45, 5.47, 4.73, 5.6, 4.3 and 4.3, respectively [18,20]. The pH value was similar to Asian redtail catfish (5.9) and cuttlefish (6.1) [21, 22].

3.3. Proximate composition

The proximate composition of *A. stellaris* gelatin is tabulated in Table 1. Almost all parameters showed a significant difference ($P<0.05$). For protein content, the concentration of 0.6 M had the highest composition, followed by 0.4, and 0.2 M of phosphoric acids. Several research studies have been reported the protein content in catfish (*Pangasius hypophthalmus*), snapper (*Lutjanus* sp.), lethrinidae fish (*Lethrinus* sp.), dog shark (*Scoliodon sorrakowah*) and skipjack tuna (*Katsuwonus pelamis*), accounted for 91.92%, 88.88%, 85.83%, 90%, and 88%, respectively [13,17,18,20]. The protein of *A. stellaris* skins gelatin relatively low. However, *A. stellaris* skins gelatin extracted by different phosphoric acids showed higher protein content that those from croaker (*Johnius dussumieri*) (69%), shortfin scad (*Decapterus macrosoma*) (69%), and kumakuma (*Brachyplatystoma filamentosum*) (73%) skins gelatin is higher [7,23]. It could be concluded that fish species resulted in a difference in the protein [24].

3.4. Gel strength

Gel strength is the most important commercial property of gelatin [25]. The gel strength of *A. Stellaris* is tabulated in Table 2. *A. stellaris* skins gelatin had gel strength within the range of 1.28 N to 2.45 N, and their values of gel strength were significantly different ($P<0.05$) in each treatment of acid concentrations. The gel strength in this present study increased as the phosphoric acid concentrations increased. The gel strength of three spot gourami (*Trichopodus trichopterus*) skin gelatin was 1.47 N, grouper (*Ephinephelus* sp.) was 14.17- 24.73 N, and lethrinidae fish (*Lethrinus* sp.) 32.40 N was in in the range of the gel strength of *A. stellaris* gelatin [26, 27].

3.5. Viscosity

Viscosity is the second most important characteristic of gelatin [25]. The viscosity values for the *A. stellaris* skins gelatin at different phosphoric acid conditions were in the range of 3.83 cP to 4.21 cP (Table 2.). The viscosity values of black tilapia (*Oreochromis mosambicus*), catfish (*Pangasius hypophthalmus*), mackerel (*Scomberomorus commersonii*), lethrinidae fish (*Lethrinus* sp.), skins gelatin possed viscosity values were higher than A. stellaris gelatin [12,13,15,20]. Those mentioned studies had greater viscosity values compared to the viscosity from *A. stellaris* skins gelatin at different phosphoric acid conditions, on the other hand, *A. stellaris* skins gelatin had higher viscosity that those from giant featherback (*Chitala lopis*) (2.5 cP) [14] and red tilapia (*Oreochromis nilotica*) (3.2 cP) [15]. This finding was in line with the viscosity of skipjack tuna (*Katsuwonus pelamis*) skin gelatin reported by Shyni et al. [18]. The above results indicated that natural variations in the viscosity due to the difference of fish species, although the methods of skins gelatin play a role during extraction.

Table 2. Physical parameters of *A. stellaris* skins gelatin.

Phosphoric Acid	Physical Parameter			
	Viscosity	Gel Strength	Gelling Point	Melting Point
0.2 M	3.83 ± 0.75^{a}	1.28 ± 0.38^{a}	15.05 ± 0.33^{a}	19.46 ± 0.72^{a}
0.4 M	4.08 ± 0.66^{a}	1.93 ± 0.29^{b}	14.27 ± 0.73^{b}	20.13 ± 0.53^{ab}

0.6 M	4.21 ± 0.31^{a}	2.45 ± 0.51^{c}	11.78 ± 0.72^{c}	20.46 ± 0.77^{b}

3.6. Gelling point

The gelling points of the gelatin extracted from *A. stellaris* skins at different phosphoric acid concentrations were determined, and the results are presented in Table 2. Generally, the gelling points observed in the *A. stellaris* skins gelatin was significantly different ($P<0.05$), and their values decreased when the phosphoric acid concentrations increased. The gelling points in the present study were in the range of 11.78 ^{o}C to 15.05 ^{o}C, and when compared to other studies, including snapper (*Lutjanus* sp.) (10.15 ^{o}C) [12], bigeye snapper (*Priacanthus hamrur*) (10 ^{o}C) [28] and giant featherback (*Chitala lopis*) (5.5 ^{o}C) [13], the *A. stellaris* skins gelatin had higher gelling point. Nevertheless, the gelling points were lower in the gelatin extracted from *A. stellaris* skins than those in Lethrinidae fish skin (*Lethrinus* sp.) (15.50 ^{o}C) [13], yellowfin tuna (*Thunnus albacares*) (18.7 ^{o}C) [29] and silver carp (18.7 ^{o}C) [31]. These results might be due to the raw materials (extracted from different fish species) used for gelatin production, which may have a difference in habitats[1].

3.7. Melting point

The melting point property is an indicator of gelatin quality [31]. Mammalian gelatins have higher melting points than that of fish species gelatins. The melting points for bovine and porcine were 29.7 ^{o}C and 32.3 ^{o}C, respectively [32,33]. The melting points of gelatin extracted from *A. stellaris* skins at different phosphoric acid conditions were observed, and the data are presented in Table 2. In general, the melting points of *A. stellaris* skins gelatin showed significant difference ($P<0.05$) and ranged from 19.46 ^{o}C to 20.46 ^{o}C. The highest point was in the 0.6 M of phosphoric acid and indicated the melting point enhanced as the concentration of phosphoric acid increased. When compared to previous research studies had noted that melting points of 27.26 ^{o}C, 24.5 ^{o}C, 25.8^{o}C and 24.2^{o}C, for snapper (*Lutjanus* sp.), giant featherback (*Chitala lopis*), dog shark (*Scoliodon sorrakowah*) and skipjack tuna (*Katsuwonus pelamis*) [14,17,18], respectively, the *A. stellaris* skins gelatin had lower in melting points. However, the melting points observed in the present study are in accordance with the sole (19.4 ^{o}C) and grass carp (19.5 ^{o}C)[1]. The difference in melting point might be related to fish species and fishing season along with the effect of the preparation.

3.8. Amino acid composition

The amino acid composition of *A. stellaris* skins gelatin extracted with different phosphoric acid concentrations is tabulated in Table 3. The *A. stellaris* gelatin was rich in glycine, proline, alanine, and glutamic acid. Moreover, the gelatin had low concentrations of tyrosine, histidine, and isoleucine. Meanwhile, tryptophan and cysteine were not detected after acid hydrolysis. The glycine residue of *A. stellaris* gelatin was slightly lower compared to those observed from dog shark (*Scoliodon sorrakowah*) and skipjack tuna (*Katsuwonus pelamis*).

In contrast, the proline residue found in *A. stellaris* gelatin had higher in comparison with reported by [19]. It might be indicated that glycine is the most critical amino acid property in gelatin, which typically repeat in the form of Glycine-X-Y-Glycine-X-Y-Glycine-X-Y, and X-Y are mostly proline and hydroxyproline. However, in the present study, it was not observed.

Table 3. Amino acid composition of *A. stellaris skins gelatin*, tilapia skins gelatin, and commercial gelatin.

Amino acids	Residues/100 residues		
	A. stellaris skin gelatin	Dog shark skin gelatin*	Tuna skin gelatin*
Serine	4.42	3.61	4.36
Glutamamine/glutamic acid	8.98	7.69	7.26
Phenylalanine	3.03	1.58	1.37

Isoleucine	1.24	1.58	1.09
Valine	3.25	2.52	2.95
Alanine	9.15	10.9	12.2
Arginine	10.57	5.29	4.76
Glycine	29.60	32.8	33.2
Lysine	3.50	2.77	2.57
Aspartate acid/asparagine	4.88	3.64	4.08
Leucine	2.62	2.17	2.04
Tyrosine	0.83	0.08	0.26
Proline	12.53	9.90	10.1
Threonine	4.00	2.08	2.33
Histidine	1.38	0.86	0.88

*) Shyni et al. [2014]

4. Conclusion

The yield, pH, moisture, protein, fat, gel strength, gelling, and melting points of *A. stellaris* skins gelatin are significantly affected by the concentration of phosphoric acids. Only viscosity and ash content are not significantly affected. The amino acid composition of *A. stellaris* gelatin was rich in glycine, proline, alanine, and glutamic acid.

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