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# Functional Properties of Collagen from Purple-spotted Bigeye (*Priacanthus tayenus* Richardson, 1846) Bone and Fins Extracted with Different Acids

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***Abstract*—This work aimed to evaluate the functional properties of collagen derived from the bone and fins of *Priacanthus tayenus* (Richardson, 1846) prepared with various organic acids. The extracted collagens yielded 0.83%, 1.43%, and 1.93% of acetic acid-extracted collagen (AEC), lactic acid-extracted collagen (LEC), and citric acid-extracted collagen (CEC), respectively, although no significant differences ($p > 0.05$). The high solubility was detected in all extracted collagen samples under low concentrations of sodium chloride (up to 20 g/L). Acetic and lactic acid-extracted collagens showed the highest solubility at pH 3 and pH 5 for citric acid-extracted collagen. The oil absorption capacity varied from 8.57 mL/mg to 15.94 mL/mg and was significantly the highest ($p < 0.05$) for the AEC sample. Although the highest water absorption capacity was noted in LEC (14.39 mL/mg) compared to AEC (12.44 mL/mg) and CEC (8.30 mL/mg), it is not significantly different ($p > 0.05$). All the extracted samples recorded higher values of foaming ability (from 78.33% to 88.33%) and stability (from 80% to 93.33%) at pH 4. Therefore, the emulsion characteristics comprising the emulsion ability and stability indexes were carried out under acidic pH conditions, and the results showed acetic-extracted collagen had the highest values compared to lactic and citric acid-extracted collagens. Taken together, *P. tayenus* collagens had good functional properties comparable to other collagen sources, supporting its further use in industrial processes.**

***Keywords*— *Priacanthus tayenus;* bone and fins; acid-extracted collagen; functional characteristics.**

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## I. INTRODUCTION

*Priacanthus tayenus* (Richardson, 1846), purple-spotted bigeye, is an economically important marine species for surimi/seafood processing [1]. It is called *lolong bara* in Malaysia, with big eyes as an identifying trait. It belongs to the family Priancanthidae, with a maximum length of around 35.0 cm [1]. Generally, a large number of fish by-products (around 60-75%) were produced during processing, including bone, fins, skin, head, and viscera [2], [3]. Fish by-products usually will be turned into low-value-added products such as silage, animal feed, fertilizer, and even fish snacks [4], [5]. Sometimes, underutilized or non-utilized by-products are discarded, resulting in environmental pollution, potential revenue losses, and increased financial burden due to disposal costs. An effective solution is needed to recover fish by-products, particularly in producing high-quality products such as collagen [6], [7], [8].

Collagen is extensively used in food and beverage, nutraceutical, biomedical and cosmetic factories [9], [10], [11]. Commercially, collagen from mammals such as bovine, porcine, and poultry is abundant due to its compatible characteristics [12]. However, bovine collagen is associated with health risks from transmissible spongiform encephalopathy (TSE), bovine spongiform encephalopathy

(BSE), foot-and-mouth disease (FMD), and while pigs-derived collagen is unacceptable for Muslims [9], [13]. In recent years, studies on fish collagen have increased due to its similar properties and possibly even better than collagens derived from terrestrial animals [14]. Collagens from fish species such as tuna, lizardfish, tiger grouper, parrotfish, pink ear emperor, tilapia, unicornfish, and barracuda have been successfully extracted and characterized [15], [16], [17], [18], [19], [20]. However, the study on the functional properties of fish collagens was much less explored. Therefore, this experiment aims to evaluate the functional characteristics of collagen from *P. tayenus* bone and fins prepared with different organic acids. The physical characteristics were also studied, including solubility in pH and sodium chloride (NaCl) solution, oil and water absorption capacities, foaming properties, and emulsion profiles.

## II. Materials and Method

### A. Materials

Collagens from the bone and fins of purple-spotted bigeye (*P. tayenus*) used in this study were prepared using various acids. Organic acids used in this work were obtained from Merck (Germany). Soybean oil (Vesoya, Malaysia) was obtained from a local shop. All the chemicals and reagents prepared in this research were of analytical grades.

### B. Preparation of Acids-extracted Collagen

Extraction of collagen from the combined bones and fins was conducted using a modified method by Jaziri et al. [16], and all process was carried out at 4°C (Fig. 1). A total of 100 g prepared samples was immersed in an alkaline solution with a solid-liquid ratio of 1/10 (g/mL), and the solutions were changed twice for 6 h with continuously stirring. After immersion was complete, the pre-treated collagens were then neutralized by adding distilled water. Next, all neutralized samples were subjected to demineralization using EDTA-2Na solution (0.5 M, pH 7.4) with a solid-liquid ratio of 1/10 (g/mL) for two days. The treated samples were then extracted using three organic acids with the same molarity and solid-liquid ratio of respectively 0.5 M and 1/15 (g/mL) for three days. When the extraction ended, all samples were filtered using a double layer of cheesecloth. The solubilized samples were precipitated with a 2.5 M of sodium chloride solution. After that, the precipitates were centrifugally separated at 11,000 g for 20 min, and the collected pellets (collagens) were then added with acids that were previously used for extraction. The samples were then dialyzed in around 20 volumes of the same acids with a lower molarity (0.1 M) for one day, continued by cooled distilled water for two days. After dialysis, liquid collagens were dried using a freeze-dryer machine (Labconco, US). The lyophilized samples were named acetic acid-extracted collagen (AEC), lactic acid-extracted collagen (LEC), and citric acid-extracted collagen (CEC). All collagen samples were then kept at -20°C. For yield determination, we used a formula previously developed based on the initial weight of prepared samples with the weight of lyophilized collagen [16]:

$$\text{Yield (\%)} = \frac{\text{Dry weight of collagen}}{\text{Initial weight of sample}} \times 100 \quad (1)$$

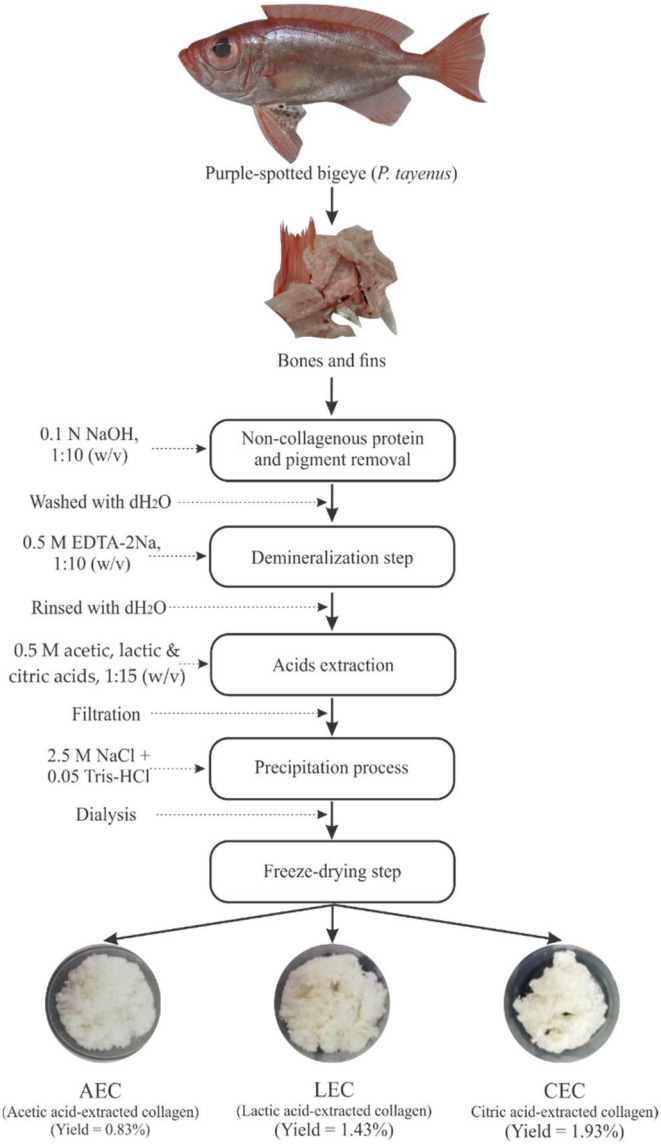


Fig. 1 Extraction and yield of collagens from *P. tayenus* bone and fins.

### C. Solubility in Different pH and NaCl Concentration

The solubility of *P. tayenus* collagens was adopted from the methods of Moniruzzaman et al. [21] and Jaziri et al. [22] with slight modifications. Both solubility tests were determined at a wavelength of 750 nm under a spectrophotometer. The relative solubility in all the collagen samples was measured based on the protein component in the supernatant and detected with the protocol developed by Lowry et al. [23]. A standard used in this study was the bovine serum albumin (BSA), and the following formula was applied to determine relative solubility:

$$\text{Relative solubility (\%)} = \frac{\text{Current concentration of protein}}{\text{The highest concentration of protein}} \times 100 \quad (2)$$

### D. Water/Oil Absorption Capacities

Water absorption capacity (WAC) and oil absorption capacity (OAC) were obtained according to the protocol of Chen et al. [24] with a slight alteration. Both WAC and OAC were measured using this equation:

$$\text{WAC/ OAC (\%)} = \frac{\text{Volume of supernatant}}{\text{Initial volume of water or oil before centrifuge}} \times 100 \quad (3)$$

### E. Foaming Ability and Stability

Foaming ability (FA) and foaming stability (FS) of *P. tayenus* extracted collagens were carried out as described by Akram and Zhang [25] with slight modification. The calculation of FA and FS were as follows:

$$\text{FA (\%)} = \frac{\text{Volume of collagen solution after homegenized}}{\text{Initial volume of collagen solution}} \times 100 \quad (4)$$

$$\text{FS (\%)} = \frac{\text{Volume of collagen solution after homegenized for 60 mins}}{\text{Initial volume of collagen solution}} \times 100 \quad (5)$$

### F. Emulsion Test

Emulsion test consists of emulsion ability index (EAI) and emulsion stability index (ESI). EAI and ESI of *P. tayenus* collagens were executed as Chen et al. [24] with some modifications. Both emulsion test was determined using the following equation:

$$\text{EAI} = \frac{2 \times 2.303 \times A0}{0.25 \times \text{collagen weight (g)}} \quad (6)$$

$$\text{ESI} = \frac{A0 \times \Delta t}{\Delta A} \quad (7)$$

### G. Statistical Analysis

Analysis of variance (ANOVA) was used to analyze data and the differences of means were done by Duncan multiple range test. All data analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, New York). Average comparisons were accepted at confidence of $p < 0.05$.

## III. Results and Discussion

### A. Yield of Acids-extracted Collagen

The extracted collagens from *P. tayenus* bone and fins prepared with various acids treatment were shown in Fig 1. The yields of extracted collagens were 0.83%, 1.43% and 1.93 % of AEC, LEC, and CEC, respectively. Although there were differences on the obtained yields, but no significant differences ($p > 0.05$). Comparatively, the yields of collagens extracted from the scales of miiuy croaker (0.64%) [26] and tilapia (0.77%) [18] were lower than our results; however, bigeye tuna skin collagen (13.5 g/100g) [15] and sturgeon fish skin collagen (9.98 g/100g) [27] yielded the greatest. It could be suggested that the fish by-products source used is of greater importance in producing collagen. Besides that, the various organic acids used, and extraction procedures employed during the production of fish collagen from *P. tayenus* bone and fins might be affected the yield of all fish collagens [15]. These acid-extracted collagens were then subjected to studies of functional properties composed of solubility in different treatments, water, and oil absorption capacities, foaming ability and stability, and emulsion ability and emulsion stability indexes.

### B. Solubility Profile

The solubility of the AEC, LEC, and CEC samples at different pH are shown in Fig 2A. The highest solubility for AEC and LEC was noted at pH 5 and CEC at pH 3. Collagen has high solubility at pH between 2 and 5, with a relative solubility rate exceeding 80% [21]. According to Li et al. [26], the lowest solubility of collagens from the scales of miiuy croaker occurs at neutral pH (pH 7) and slightly alkaline pH (pH 8 and 9). Meanwhile, Nurkhoeriyati et al. [28] reported the lowest solubility of duck meat at pH 5-6. In the present study, all acid-soluble collagens showed decreasing relative solubility below 60% at pH 7 and 9. The isoelectric point (pI) for all collagen forms was identified at pH 7 and pH 9. This could be due to the high hydrophobic interactions among the molecules in collagen, thus causing the relative solubility rate low [15]. Meanwhile, the relative solubility (%) at pH 11 was also high. This finding could be due to the effect of repulsion on collagen molecules treated at pH above that of the pI. Additionally, in very acidic solutions (pH 1), collagen is likely to undergo molecular destruction and thus, resulting in impaired solubility [27].

Solubility at different NaCl concentrations of all acid-soluble collagens is depicted in Fig 2B. At low sodium chloride concentrations (0 to 20 g/L), all collagens showed a very high percentage of relative solubility. This is because salt ions bind weakly to the surface of the charged protein group without disrupting any hydration loop of the collagen domain [21]. At NaCl concentration of 30 g/L, all acid-soluble collagens showed a sharp decrease in solubility. This occurs because of salting out during the precipitation process; as a consequence, the increase in hydrophobic interactions and aggregation, competing with the protein for water, thereby causing protein to precipitate [15]. Our NaCl solubility pattern results were similar to Jaziri et al. [16] in acid-soluble collagen obtained from the lizardfish scale.

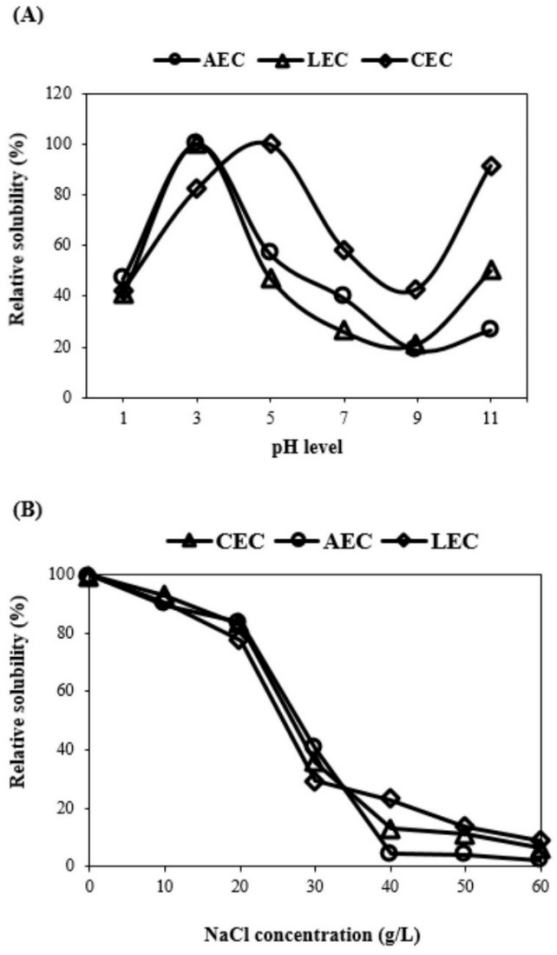


Fig. 2 (A) Solubility at various pH tests and (B) Solubility at different NaCl concentrations of collagens extracted from P. *tayenus* bone and fins.

### C. Water and Oil Absorption Capacities

Water absorption capacity (WAC) is the amount of water collagen absorbed. This property is based on the direct interaction of collagen molecules with water and solvents [25]. WAC of extracted collagens with different acids is depicted in Fig 3. AEC had the highest WAC of 14.39 mL/mg, followed by LES and CEC collagens of 12.44 mL/mg and 8.30 mL/mg, respectively: although not significantly different ($p > 0.05$). The differences noted in WAC of each extracted collagen could be due to the differences in solubility, particle size, and micro-morphology of collagen as suggested by Chen et al. [24]. The high WAC value of extracted collagen is important in controlling moisture in food products [29].

On the other hand, oil absorption capacity (OAC) is the amount of oil absorbed per mg of lyophilized collagen. Oil absorption is associated with proteins' physical entrapment of oil or fat[30]. The OAC for proteins correlates with non-polar amino acid residues. The hydrophobic interaction between non-polar amino acid molecules with the oil hydrocarbon chains determines the absorption capacity value[24]. In the present study, all acid-soluble collagens had a significant difference ($p < 0.05$) in OAC values, with AEC collagen exhibiting a greater absorption capacity (15.94 mL/mg) compared to LEC (12.36 mL/mg) and CEC (8.57 mL/mg) solubilized samples, as illustrated in Fig 3. The results suggest that acetic acid-soluble collagen has more non-polar amino acid residues than lactic and citric-soluble collagens [24]. The OAC of AEC collagen may be regarded as an essential trait in food ingredients [15].

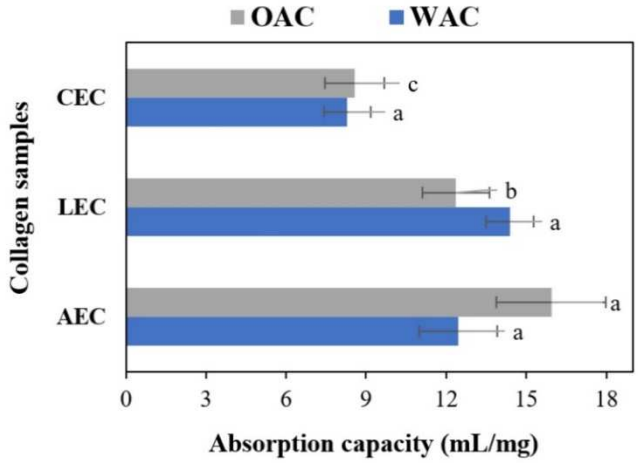


Fig. 3 Water absorption capacity (WAC) and oil absorption capacity (OAC) of collagens from *P. tayenus* bone and fins.

### D. Foaming Properties

Foaming properties tested in this study consisted of foaming ability (FA) and foaming stability (FS), which are important characteristics of collagen. Foaming capacity is closely associated with their film-forming ability at the air-water interface [31]. The foaming properties of collagen are measured through the ability and stability of foaming at different pH levels, i.e., pH 4, pH 7 and pH 10. The percentages of foaming properties for all acid-extracted collagens isolated from *P. tayenus* bone and fins are depicted in Fig 4A and B. The FA of AEC at pH 4, 7 and 10 was 81.67%, 70.00%, and 83.33%, respectively. For LEC, the FA at pH 4, 7 and 9 was 78.33%, 76.67% and 78.33%, respectively, while the FA of 88.33%, 80.00% and 83.33% was recorded in CEC treated at pH 4, 7 and 10, respectively. The FA in the present study was higher compared to the FA of brown bullhead (*Amiurus nebulosus*) skin collagen extracted from acid (14%) and pepsin (4%) [31], but was comparable to the acid extracted squid skin collagen (83%). For FS, at pH 4, 7 and 9 of AEC valued 81.67%, 70% and 10%, respectively. The LEC had 80%, 76.67% and 78.88 of FS found in pH 4, 7 and 9, respectively, whilst the FS of CEC was 93.33%, 81.67% and 86.67% observed at pH 4, pH 7 and pH 10, respectively. Overall, the FS of *P. tayenus* collagens were relatively high, especially in CEC, and all foaming properties of extracted collagens relatively decreased at pH 7. This might be due to the low solubility and weak repulsive forces between collagen molecules, which are not strong enough to prevent aggregation. When collagen molecules form aggregates, the interaction between proteins and water required for foaming is weak [24]. Similar results were obtained in studies of red stingray skin collagen and chicken sternal cartilage collagen [25][27].

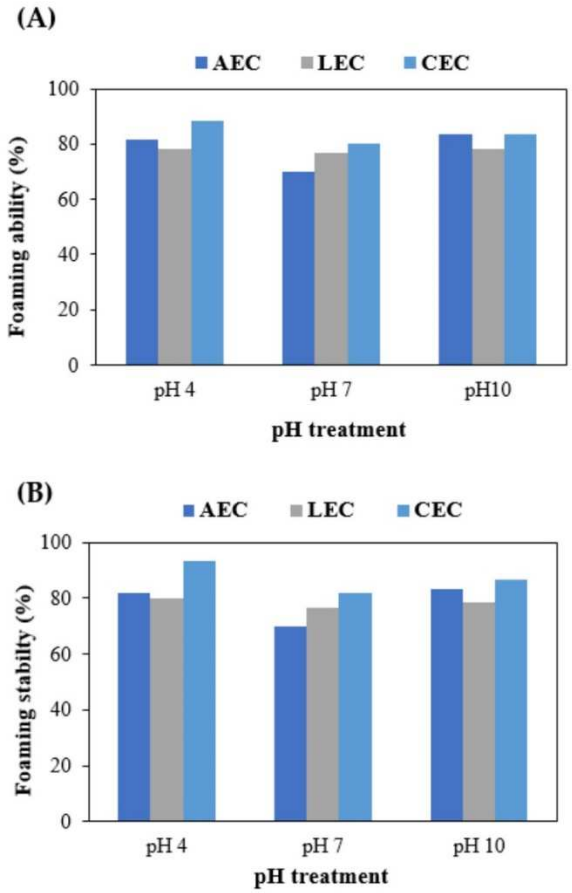


Fig. 4 (A) Foaming ability (FA) and (B) Foaming stability (FS) of collagens from *P. tayenus* bone and fins.

### E. Emulsion characteristics

The emulsion is defined as an interface of water with oil (water-oil interface). Generally, emulsion properties are divided into two parameters, viz. EAI and ESI. EAI determines the amount of oil that can be emulsified per unit of protein, whilst ESI determines the rate of emulsion resistance at a treated time [32]. In the present study, all

collagens from *P. tayenus* bone and fins were treated under acidic conditions at pH 4. Fig 5. shows the EAI for the AEC, LEC, and CEC samples at different pH conditions. AEC showed greatest EAI (115.27 $m^2/g$) compared to LEC (66.82 $m^2/g$) and CEC (49.74 $m^2/g$). It may be due to the high surface hydrophobicity of collagen prepared by aiding various acids, increasing emulsifying activity. AEC and the other two collagens may have the potential to be absorbed in the oil and aqueous phase [33]. The ESI values of the various extracted collagens are also presented in Fig 5. The results showed that AEC has a greater ESI (58.50 min) than those observed in LEC (46.67 min) and CEC (40.90 min). From these results, collagen from *P. tayenus* bone and fins, particularly extracted with acetic acid, may be used as an emulsifying agent.

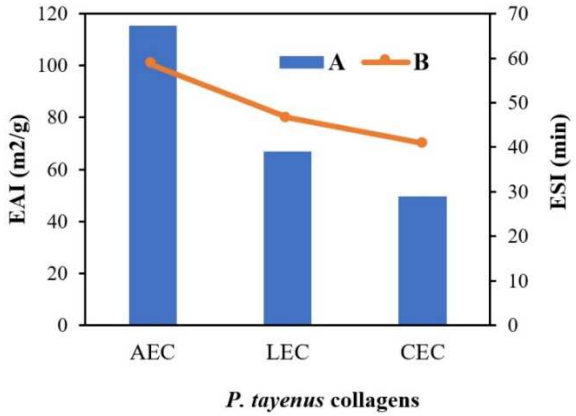


Fig. 5 A. Emulsion ability index (EAI) and B. Emulsion stability index (ESI) of collagens from *P. tayenus* bone and fins.

## IV. Conclusion

Functional properties for collagens of the purple-spotted bigeye (*P. tayenus*) bone and fins extracted with different acids were evaluated. All the collagen samples exhibited high solubility at low concentrations of sodium chloride (0-20 g/L). Solubility at different pH treatments indicates highest solubility at pH 3 for the AEC and LEC samples, and at pH 5 for the CEC sample. For oil and water absorption capacities, foaming and emulsion properties, the *P. tayenus* collagens had great functionality and agreed with other previous investigations of collagen from other sources, such as fish and chicken. This study may contribute to further developments of alternative collagen from fish sources and may be used as a basic understanding for further applications, especially from a food and pharmacy point of view.

## Acknowledgment

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