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Chemical and Functional Properties of Myofibrillar Protein from Selected Species of Trash Fish

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Abstract

Trash fish is a captured fish that has low economic value. Trash fish has a reasonably high protein. However, myofibrillar protein from trash fish cannot form gel properly. Therefore, it is necessary to modify it by adding a gelling agent. This study aimed to determine myofibrillar protein's chemical and functional characteristics from trash fish as a food ingredient. The research method is in the form of laboratory experiments. The types of fish used as samples in this study were Chacunda gizzard-shad (*Anodontostoma chacunda* Hamilton, 1822), Orangefin ponyfish (*Leiognathus bindus* Valenciennes, 1835), and Sardines (*Sardinella fimbriata* Valenciennes, 1847). Myo fibril modification was carried out by adding 10 % carrageenan (wv^{-1}) to each trash fish sample. The results showed that the myofibrillar protein from Chacunda gizzard-shad had higher protein content (84.77 %), water holding capacity (524.78 %), and emulsion activity (8.85 m g^{-2}) than other samples. Chacunda gizzard-shad is characterized by more red meat than the others. This fish species also has the highest oil-holding capacity (620.35 %) and emulsion stability (4.58 h). The microstructure of the trash fish myofibrils is tighter and more hollow, so it has a better ability to absorb oil. Myofibrillar protein from another species, Orangefin ponyfish, has a relatively higher whiteness value (78.42) than other samples because white meat is dominant for this species. Modifying myofibrillar protein by adding agar and carrageenan can increase the hardness value of the sample due to the addition of agar. According to this study, the Chacunda gizzard-shad has the greatest potential to be an ingredient in high-value commercial food products.

Keywords: Chacunda gizzard-shad, Fish waste characterization, Fish proteins, Marine waste to food, Orangefin ponyfish, Rough fish, Sardine, Waste utilization

1. Introduction

In fishing operations, the fish catches contain not only high-economic value fish but also so-called trash fish (also known as rough fish or dirt fish) with lower economic value (Anam *et al*., 2021; Raissa *et al*., 2014). Uses of various marine waste, including trash fish, are broad – for feed and food production and a source for renewable energy production (Coppola *et al*., 2012; Rudovica *et al*., 2021; Susanto, *et al*., 2020). Several studies indicate trash fish utilization as a source for biogas production due to the scarcity of fossil fuels (Abdullah *et al*., 2020; Bücker *et al*., 2020; Burlakovs *et al*., 2022; Kafle and Kim, 2012; Setyobudi *et al*., 2021). However, technological difficulties must be overcome as a two-stage digester is recommended to avoid the non-homogeneity of trash fish (Adinurani *et al*., 2013, 2014; Hendroko *et al*., 2014). Facing the global problem of food shortage, trash fish should be considered a valuable raw material for food and feed production due to its considerable protein content, *i.e*., 15 % to 20 % (Erbay dan Yeşilsu, 2021).

Trash fish in Lamongan Regency, one of the marine sector fisheries in East Java, Indonesia, is dominated mainly by two species: Chacunda gizzard-shad (*Anodontostoma chacunda*, Hamilton, 1822) and Orange fin ponyfish (*Photopectoralis bindus*, Valenciennes, 1835). However, currently the utilization of these trash fish is minimal as they only are used for feed and salted fish production, even though they are suitable to be applied as

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food ingredients leading to higher economic value (Coppola *et al.*, 2012; Hasan *et al.*, 2021; Rudovica *et al.*, 2021).

Protein-rich fish muscles are divided into three types: sarcoplasmic, myofibrillar, and stroma (Dara *et al.*, 2021). Myofibrillar protein (65 % to 75%) prevails over the other component and is soluble in salt solution (Deng *et al.*, 2021). Myofibrillar protein consists of myosin, actin, tropomyosin, and actomyosin and influences fish products' quality and functional properties. Furthermore, myofibrillar protein has a role in the formation of gel and the process of flesh coagulation (Liu *et al.*, 2022).

Based on preliminary research, myofibrillar protein from trash fish cannot be transferred into a gel but needs modification by adding a gelling agent such as agar and carrageenan (Leha and Moniharapon, 2013). Agar is a complex of polysaccharides (having two main components, agarose and agaropectin) with rigid, fragile, and malleable gelling characteristics. At the same time, carrageenan is a hydrocolloid compound that consists of ester-sulfate content in a mixture with galactose and 3,6-anhydrogalacto polimer characterized by elastic gelling properties (du Preez *et al.*, 2020).

Agar and carrageenan can make interaction with proteins influencing viscosity and gel formation. Therefore, adding agar and carrageenan is expected to improve the gelation of myofibrillar protein from trash fish, resulting in its use as a raw material for surimi production (Li *et al.*, 2022). Surimi is an intermediate product and can be processed into products that need flesh elasticity. Generally, cod fish (a common name for various marine fish of the genus *Gadus* of the family *Gadidae*) is used as a raw material for surimi production (Millan *et al.*, 2021), but modified myofibrillar protein from trash fish can be assessed as a potential substitute for cod fish. This research investigates the chemical and functional characteristics of myofibrillar protein from trash fish, including its color, texture, and microstructure characteristics after the modification of the protein.

2. Materials and Methods

2.1. Fish samples and reagents

Trash fish species such as Chacunda gizzard-shad (*A. chacunda*), Orangefin ponyfish (*P. bindus*) and Sardine (*Sardinella fimbriata* Valenciennes, 1847) were selected for this study. Fresh fishes (on average 100 g in each sample) of these species were purchased at the central fish market in Lamongan Regency, East Java Province, Indonesia. Samples were kept in an ice box and placed in cold storage while transported to the Department of Agro-industry Technology, Faculty of Agricultural Technology, University of Jember, Indonesia. Upon arrival, samples were immediately kept in a freezer. Before analysis, amount of 2 500 g fish samples were thawed at room temperature, washed, filleted, and minced to uniformity by using a chopper.

Gelling agents (agar and *i*-carrageenan) were obtained from a chemical shop in Hiroshima, Japan. The gelling agent used was purchased from Sigma, with product code C1013. Reagents used in the research were NaCl, 0.1 M phosphate buffer solution (pH 7), HCl, NaOH, Lowry reagent, H_2SO_4, 4 % H_3BO_3, methanol, tris HCl buffer containing 0.1 % sodium dodecyl sulfate sucrose, urea, 2 % sodium dodecyl sulfate, 2 % 2-mercaptoethanol, 50 % glycerol, coomassie brilliant blue, staining solution, Na_2CO_3, $CuSO_4$, $KNaC_4H_4O_6{\cdot}4H_2O$ (sodium potassium tartrate), phenolic solution, glutaraldehyde, ethanol. All chemicals used were analytical grade from the manufacturer Merck, Germany.

2.2. Extraction of myofibrillar protein from trash fish

Myofibrillar protein samples were extracted from trash fish according to the method reported by (Dara *et al.*, 2021) with some modifications (Figure 1). First, fish mince was mixed with three volumes of 10 % NaCl in 0.1 M phosphate buffer (pH 7) and magnetic stirred (MYP11-2, Shanghai Meiyingpu Instrument Co., Ltd., China) at 4 °C for 3 min, followed by centrifugation (X1R, Thermo Fisher Technologies Co., Ltd., Waltham, MA, USA) at a speed of 4 000 rpm (1 rpm = 1/60 Hz) at 2 °C for 10 min. Obtained pellet decantation was repeated one time. Pellets were filtered through a filter cloth of four layers. Then the filtrate was added with three volumes of 10 % NaCl in 0.1M phosphate buffer (pH 7), followed by centrifugation at 4 000 rpm at 2 °C for 10 min. Finally, pellets obtained were wet myofibrillar protein. Next, the myofibrillar protein was added with 5 % sucrose (ww^{-1}) and dried by using freeze-drying. Samples were kept in an airtight chamber. All samples were prepared in triplicate.

The freezing process is carried out by adopting the method developed by Prosapio and Lopez-Quiroga (2020). The first stage of the product is frozen first at -50 ^{o}C. Then, the drying process (sublimation) is carried out by placing frozen products into a vacuum chamber. The pressure is maintained at about 0.036 psi (1 psi = 6.89 kPa) or about 0.0025 bar and the temperature is then raised in a controlled manner until it reaches about 100 °F (38 °C) so that the sublimation process occurs. In the mechanism of the freeze dryer, the water vapor produced is then sucked in and condensed so that it does not wet the product being dried. The drying process was carried out for 13 h.

2.3. Modification of myofibrillar protein from trash fish

Trash fish myofibrils could not form a gel. Then the myofibril modification was carried out using agar and carrageenan. First, myofibrillar protein samples from Chacunda gizzard-shad, Orangefin ponyfish, and Sardine were weighted 10 % (wv^{-1}) and added 1 % (wv^{-1}) of agar and carrageenan. Then, 20 mL of distilled water was added to the mixture. Afterward, the mixture was heated at 75 °C for 30 min and kept at room temperature until the solid gel was formed. Final solid gel samples were kept in the refrigerator until the analysis.

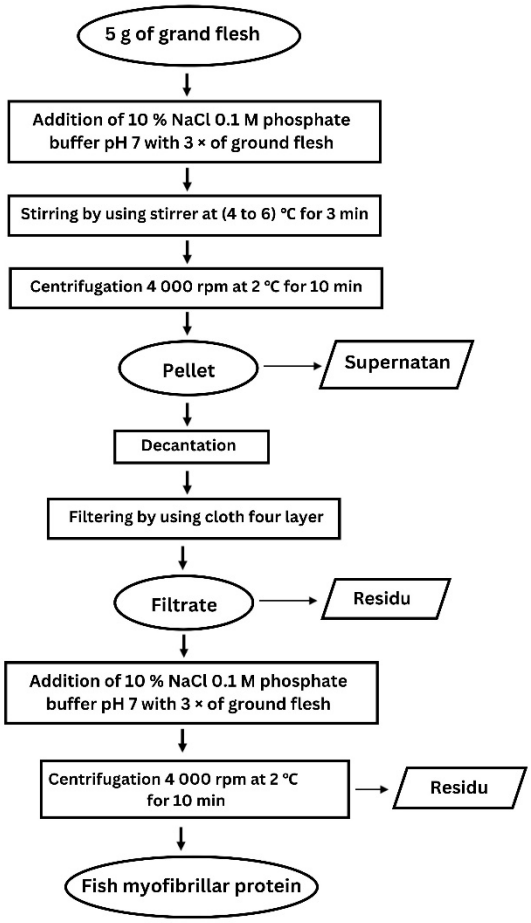


Fig. 1. The process of isolation of myofibrillar protein from trash fish

2.4. Analysis of chemical and functional properties

The chemical and functional properties of myofibrillar protein from trash fish *per se* and after modification were subjected to the detection of following parameters: protein content (AOAC, 2005); water holding capacity (WHC) (Zhang *et al*., 2015); oil holding capacity (OHC) (Nguyen *et al*., 2015); emulsionactivity and stability (Najafian and Babji, 2015); color (Seighalani *et al*., 2017); microstructure (Arfat and Benjakul, 2012; Shui *et al*., 2021); molecular weight (Fowler and Park, 2015); and texture (Petcharat and Benjakul, 2017).The resulting data of chemical and functional parameters were averaged over each variable and subjected to descriptive statistics (Adinurani, 2022).

2.4.1. Analysis of protein content (AOAC, 2005)

Amount of 0.1 g sample was weighed and then put into the Kjeldahl flask. Into the flask was added 2 mL of concentrated H_2SO_4 (Sulfuric acid 95 % to 97 % (Merck for analysis) and 0.9 g of selenium. The solution was then heated using digestion for 45 min. After cooling, the solution was added as much as 5 mL of distilled water and then distilled. The distillate is put into an Erlenmeyer, given 15 mL of 4 % boric acid solution and two drops of indicator. After that, the solution was titrated with 0.02 N HCl solution (HCl Brand, for analysis) to change the color to a slightly purplish-blue. Calculation on protein levels employed Equation (1):

$$\text{Protein (\%)} = \frac{(ts - tb) \times N\ HCl \times 6.25 \times MW\ Nitrogen}{\text{Sample weight} \times 1\ 000} \times 100\ \% \quad (1)$$

Description:
ts = titrated volume of sample HCl (mL), tb = titrated volume of blank HCl (mL)
N HCl = 0.0858, 6.25 = conversion factor from nitrogen to protein
Molecular weight (MW) nitrogen = 14.008 ($gmol^{-1}$)

2.4.2. Water holding capacity (*WHC*) (*Zhang et al. 2015*)

Analysis of water holding capacity (WHC) was carried out in the following way: The centrifuge tube was dried and then weighed (a gram). Next, a sample of 0.5 g (b gram) is put into a dry centrifugal tube whose weight is known. Into the centrifuge tube, 7× sample weight of distilled water was added. Each sample mixture was homogenized using a vortex (Vortex Mixer DLAB MX-S), and then separated using a centrifuge (Centrifuge HC-12A Microhematocrit) for 5 min (speed 2 000 rpm). Finally, the supernatant was poured, and the residue was weighed (c gram). After that, the calculation using Equation (2):

$$\text{WHC (\%)} = \frac{(c - a) - b}{a} \times 100\ \% \quad (2)$$

Description:
a = weight of empty centrifuge tube
b = sample weight
c = weight of precipitate at the end of centrifugation

2.4.3. Oil holding capacity analysis (*Nguyen et al., 2015*)

The sample's OHC (Oil Holding Capacity) analysis was carried out in the following way: The empty centrifuge tube was dried and weighed (a gram). Next, a 0.5 g (b-gram) sample is put into a centrifuge tube. The oil was added 7× the sample weight into the centrifuge tube. Next, the samples were homogenized using a vortex (Vortex Mixer DLAB MX-S). Then, the separation was performed using a centrifuge (Centrifuge HC-12A Microhematocrit) at 2 000 rpm for 5 min. Finally, the supernatant was poured, and the residue formed was weighed (c gram). After that, the OHC calculation was performed using Equation (3).

$$\text{OHC (\%)} = \frac{(c - a) - b}{b} \times 100\ \% \quad (3)$$

Discription:
a = weight of empty centrifuge tube
b = sample weight
c = weight of precipitate at the end of centrifugation

2.4.4. *Stability and emulsion power* (*Najafian and Babji, 2015*)

Emulsion power was measured as follows: 0.1 g of sample was added to 100 mL of 0.05M phosphate buffer pH 7, then stirred with a stirrer for 15 min. Furthermore, 25 mL of cooking oil was added to the sample and mixed using a blender (Philips Series 5000) for 3 min. The emulsion power of the samples was measured after mixing with a blender. Emulsion stability was measured by taking 1 mL with an interval of 10 min for sampling. The two samples were added with 5 mL of 0.1 % SDS and mixed with a vortex (Vortex Mixer DLAB MX-S). Measurements

were made using a spectrophotometer (UV Vis Spectrophotometer Shimadzu 1240) at a wavelength of 500 nm. The power and stability of the sample emulsion are calculated using Equation (4) and Equation (5):
Information:

$$EAI\ (m^2g^{-1}) = \frac{2 \times 2.303}{c \times (1 - \phi) \times 10^4} \times abs \times dilution \quad (4)$$

EAI = Emulsifying activity index (m^2g^{-1})
c = protein concentration (gmL^{-1})
ϕ = oil volume fraction ($mL\ mL^{-1}$) of the emulsion
abs = absorbance
dilution = solution (SDS + emulsion)

$$ESI\ (time) = \frac{T \times \Delta t}{\Delta t} \quad (5)$$

Explanation:
ESI = Emulsifying Stability Index
T = absorbance for 0 hour
Δt = time reduction calculation Δ
T = absorbance reduction for 0 hour with the calculated absorbance

2.4.5. Color analysis (S*eighalani et al., 2017*)

Myofibril protein color analysis was performed using Konica Minolta's CR-10 Plus. Brightness (L*), redness/greenness (a*), and yellowness/blueness (b*) will be measured in the instrument. Color analysis is calculated using two formulas. First, the degree of whiteness will be calculated using Equation (6).

$$Whiteness = 100 - [(100 - L^*)^2 + a^{*2} + b^{*2}]^{1/2} \quad (6)$$

The second is to determine the color angle of the sample expressed in Hue. To calculate the degree of color using Equation (7):

$$H = \arctan \frac{b^*}{a^*} \quad (7)$$

Where,
H = 180 - tan -1 b/a (if a is positive and b is positive)
H = 180 + tan -1 b/a (if a is negative and b is positive)
H = 180 - tan -1 b/a (if a is negative and b is negative)

2.4.6. *Miofibril protein microstructure* (*Arfat and Benjakul. 2012; Shui et al., 2021*)

The protein microstructure of trash fish myofibrils was determined using a Scanning Electron Microscope (SEM) (Arfat and Benjakul, 2012) with several modifications (Shui *et al*, 2021). Freeze-dried trash fish myofibril samples were placed on a tip and mounted on an SEM device to view its microstructure. The first modified myofibril sample was cut with a size of 0.5 mm × 0.5 mm × 0.5 mm and immersed in a 2.5 % (v v^{-1}) glutaraldehyde solution in 0.2 M phosphate buffer (pH 7.2) for 2 h. Next, samples were washed using distilled water. Samples that had been washed with distilled water were then immersed in ethanol with graded concentrations of 50 %, 70 %, 80 %, 90 %, 95 %, and 100 % (v v^{-1}) for 5 min each. The sample is plated with gold for a few minutes. Finally, the sample microstructure was viewed using Scanning Electron Microscopy (Hitachi TM 3000).

2.4.7. Molecular weight analysis (*SDS-Page*) (*Fowler and Park. 2015*)

The molecular weight of myofibril protein was analyzed by SDS-PAGE. Amount of 0.2 g of myofibril protein was dissolved in 3.75 mL of 20 mM Tris-HCl buffer pH 8 containing 8 M urea, 2 % SDS, and 2 % 2-mercaptoethanol. The sample added with buffer is heated at 99 ^oC for 2 min. After that, stirring was carried out using a shaker (Oregon orbital shaker I HSR-200) for more than 20 h at 30 ^oC to dissolve the sample. A volume of 200 µL of the dissolved sample was taken, and 50 mM of Tris-HCl pH 8 buffer was added containing 5 % SDS, 5 % 2-mercaptoethanol, and 50 % glycerol as much as 50 µL. Samples that had been added buffer were heated at 99 ℃ for 1 min. A sample of 2.5 µL and a standard of 5 µL were put into wells of 12.5 % polyacrylamide gel that had been installed in an electrophoresis apparatus (AE 6530 serial number 5009405) and flown with a constant current of 20 mA for 75 min. When finished, the gel was taken and added with coomassie brilliant blue R-250 0.125 g 100 mL^{-1} and rinsed using distilled water three times. Protein bands will appear on the gel and be compared with the standard molecular weight (XL-Lader Broad Range SP-2110).

2.4.8. Texture analysis (*Petcharat and Benjakul, 2017*)

The tested sample was formed into a cylinder with a diameter of 2.5 cm. Cylindrical samples can be analyzed using a Rheometer (Fudoh Rheometer, Model NRM-2002J). A needle with a round tip with a diameter of 7 mm was pressed perpendicularly to the sample's surface until a puncture was formed with a puncture depth of 1 cm. The results will appear on the graph and be plotted into the formula so that the Hardness, Cohesiveness, and Adhesiveness values by Equation (8), Equation (9), and Equation (10).

$$Hardness\ (N\ cm^{-2}) = force\ (g) \times 0.0098 \times cross\text{-}section\ area\ of\ probe \quad (8)$$

$$Cohesiveness = \frac{A_2\ (peak\ area\ of\ second\ time)}{A_1\ (peak\ area\ of\ first\ time)} \quad (9)$$

$$Adhesiveness = peak\ area\ drawn\ at\ Y\text{-}axis\ neg.\ direction;\ A3\ (gf.mm) \quad (10)$$

2.5. Data analysis

The data were analyzed using simple statistics ($\bar{x} \pm \sigma$) and continued with the Least Significant Difference test (α = 5 %) to determine the better physicochemical properties of myofibrils (Adinurani, 2016).

3. Results and Discussion

3.1. Chemical and functional properties

3.1.1. Myofibrillar protein content

The highest myofibrillar protein content was detected for Chacunda gizzard-shad (84.77 %), but lower for Orangefin ponyfish (65.08 %), with an average of 66.44 % for investigated Sardine (Table 1). According to Tahergorabi *et al*. (2011), myofibrillar protein content can reach 70 % to 80 % of the total protein content, depending

on the fish species. Moreover, the amount of red and white flesh in a fish affects myofibrillar protein content. Chacunda gizzard-shad contains a greater amount of red flesh than the other fish. Dale *et al.* (2021) claimed that myofibrillar protein content for red flesh is higher by 62.40 %, while for white flesh lower by 59.20 %. Furthermore, the freeze-drying process affects maintaining myofibrillar protein content, preventing denaturation. In his study, Novian (2005) stated that dried myofibrillar protein content from gold band goat-fish (*Upeneus moluccensis* Bleeker, 1855) could increase by after freeze-drying 38 % than if another drying method is applied.

3.1.2. Water Holding Capacity

Water Holding Capacity (WHC) determines the loss of water during transport, storage, processing, and cooking. The type of material generally influences Water Holding Capacity (Damat *et al.*, 2021). The WHC means the ability of proteins to trap water and hold it performing as an advantage property for the food system. According to Table 1, the highest value of WHC is attributed to Chacunda gizzard-shad (524.78 %).

Najafian and Babji (2015) showed in their study claimed that WHC is affected by total protein concentration, ionic strength, temperature, and other components such as the content of hydrophilic polysaccharides, fats, and salt. WHC also might be influenced by the duration of heating and protein storage conditions. For example, surimi with a weak gel structure reflects low water holding capacity (de Castro *et al*., 2017). Also, the drying method affects the WHC as freeze-drying can lead to better outcomes because freeze-dried surimi powder has the highest WHC than spray-dried one (Shaviklo, 2015).

3.1.3. Oil holding capacity

The oil holding capacity (OHC) is the amount of oil trapped in the protein matrix at certain conditions. For example, Table 1 reveals the highest myofibrillar protein OHC from trash fish investigated for Sardine (620.35 %), followed by Chacunda gizzard-shad (513.26 %) and Orangefin ponyfish (455.30 %), respectively.

Table 1. Physico-chemical properties of trash fish protein myofibrils

	Protein content (%)	WHC (%)	OHC (%)	EA (m^2g^{-1})	ESI(h)	Whiteness
MT	66.44±0.95a	511.13±2.44b	620.35±2.80c	8.61±0.88b	4.58±1.22c	75.17±0.47a
MO	65.08±2.61a	352.22±0.61a	455.30±3.79a	6.19±0.16a	1.53±0.17a	78.42±0.38b
MC	84.77±0.39b	524.78±3.45c	513.26±1.99b	8.85±0.28c	3.76±1.27b	75.24±0.20a

Note: Numbers followed by the same letter in the same column are not significantly different based on the Difference test (α=5%)

MT – Sardine; MO – Orangefin ponyfish; and MC – Chacunda gizzard-shad

The highest value of OHC is attributed to Sardine because its structure contains bigger pores and, therefore, can better trap oil (Phung *et al.* 2020). Furthermore, Ulloa *et al*. (2017) indicated that the protein structure is the decisive factor in oil absorption as more lipophilic structures have more nonpolar protein branches contributing to the increased oil absorption capacity.

3.1.4. Emulsion activity and stability

Emulsion activity can be defined as the ability of the material to assist in establishing an emulsion, whereas emulsion stability can be defined as the ability of a material to maintain an emulsion formed. These parameters are affected by total protein content and solubility. The emulsion activity and stability of a myofibrillar protein from trash fish are reflected in Table 1.

The highest emulsion activity value is detected for samples derived from Chacunda gizzard-shad (8.85 m^2 g^{-1}), and it is dependent on total myofibrillar protein content. Also, Najafian and Babji (2015) stated that increasing protein concentration levels could increase emulsion activity and stability. In addition, these parameters are influenced by pH, ionic strength, and heat treatment (de Castro *et al*., 2017).

The highest relative emulsion stability for myofibrillar protein from trash fish is attributed to Sardine (4.58 h), followed by Chacunda gizzard-shad (3.76 h), and Orangefin ponyfish (1.53 h). Gao *et al*. (2018) indicated that the emulsion activity of proteins is inversely proportional to emulsion stability that is caused by the rupture of the protein membrane. Protein solubility will determine the resulting gel strength. Protein heating conditions cause denaturation and increase fragility, rupturing the protein membrane. According to Li *et al*. (2022), increasing the protein concentration can also improve the stability of the emulsion.

3.1.5. Protein color

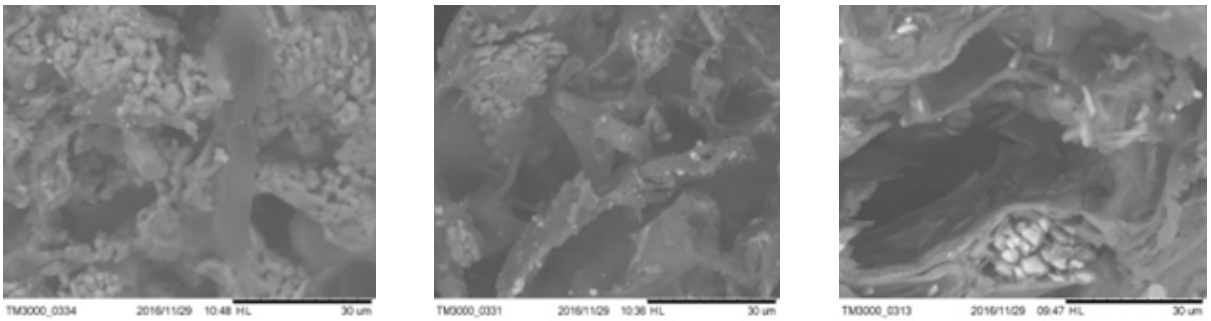
The color of myofibrillar protein is stated by whiteness value (WV). Whiteness, an important indicator, is used to measure the character of meat (Wu *et al*., 2022). The WV of myofibrillar protein depends on the fish species (Park *et al*., 2012). According to Table 1, the WV for myofibrillar protein was highest for Orangefin ponyfish (78.42), followed by similar results for Chacunda gizzard-shad (75.24) and Sardine (75.17). The pigment content in flesh causes the difference in color – Orangefin ponyfish has dominant white flesh than red flesh while Chacunda gizzard-shad fish has dominant red flesh.

According to Cropotova *et al*. (2021), demersal (benthic) fish is characterized by lower myoglobin content than pelagic fish. For example, Orangefin ponyfish is demersal fish and has lower myoglobin content than Chacunda gizzard-shad, that is pelagic fish, and, therefore, the first one is whiter. Dara *et al*. (2021) explained that demersal fish gold band goat-fish (*Upeneus moluccensis* Bleeker, 1855) has the highest brightness level (78.7 ± 1.0) compared to big-eyed pelagic fish (*Selar crumenophthalmus* Bloch, 1793) amount 67.2± 0.7. In addition, the autoxidation of myoglobin to metmyoglobin

(Mannino *et al*., 2020) may cause a pigment change to less bright or brownish during fish storage (Harnkarnsujarit *et al*., 2015; Niu *et al*., 2022).

3.1.6. Microstructure of protein

The microstructure of myofibrillar protein varies by fish species (Figure 2): for Sardine, it is more tightly with small pores compared with Orangefin ponyfish and Chacunda gizzard-shad. The microstructure of Chacunda gizzard-shad has larger pores. As it was mentioned previously, the microstructure of myofibrillar protein affects the oil-holding ability of proteins. Similar and compact protein microstructure can improve the gelation properties of surimi from silver carp (Gao *et al*., 2021). In addition, protein microstructure is affected by the duration of fish storage at freeze conditions (Xu *et al.*, 2019).



MT MO MC

Fig. 2. Microstructure of myofibrillar protein from trash fish at a magnification of 2 000× (MT – average for Sardine; MO – Orangefin ponyfish; and MC – Chacunda gizzard-shad)

3.1.7. Molecular weight of protein

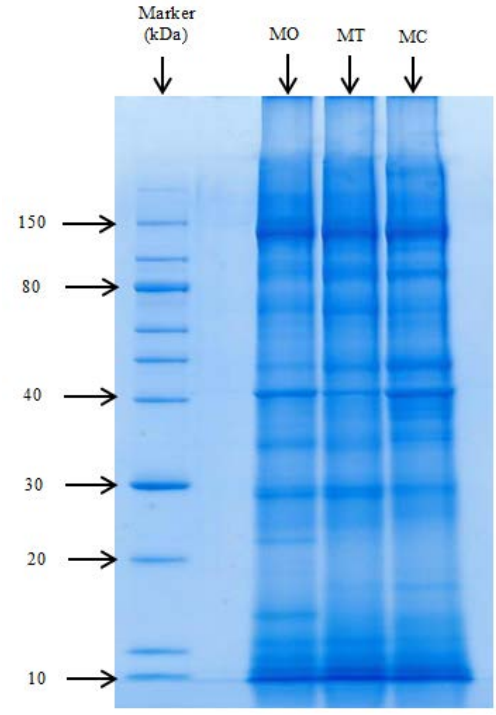


Fig. 3. Molecular weight (kDa) for fish myofibrillar protein from trash fish (MT – average for Sardine; MO – Orangefin ponyfish; and MC – Chacunda gizzard-shad; volume of marker = 5 µL and volume of a sample = 2.5 µL)

According to Figure 3, the myofibrillar protein of the fish has two major bands: 150 kDa and 40 kDa. In addition, the myofibril protein bands in Sardine fish (100, 60 to 80, 50), Orangefin ponyfish (100, 60 to 80, 50, 30 to 40, 15 to 20), and Chacunda gizzard-shad fish (100, 50, 30 to 40, 15 to 20) identified. Protein with a molecular weight of 140 kDa is C protein (a type of cod fish muscle myofibrillar protein), while protein with a molecular weight of 165 kDa is M protein (Brenner, 2019).

Orangefin ponyfish and Chacunda gizzard-shad samples do not have the dominant band with a molecular weight of 60 kDa to 80 kDa. Troponin H has a molecular weight of 70 kDa to 78 kDa (Mukund and Subramaniam, 2020). The same samples also do not have a dominant band with a molecular weight of 30 kDa to 40 kDa, which can indicate myosin regulatory light chain at 24 kDa to 30 kDa, glutathione-S-transferase two at 32 kDa to 35 kDa, tropomyosin at 35 kDa to 37 kDa, and actin at 43 kDa (Mukund and Subramaniam, 2020). Moreover, Orangefin ponyfish and Chacunda gizzard-shad samples also have the dominant band with molecular weights of 15 kDa to 20 kDa. According to Yang *et al*. (2019), molecular weight in a range from 15 kDa to 20 kDa is attributed to troponin C (18 kDa), myosin essential light chain (18 kDa), and flight in (20 kDa).

3.1.8. Modification and comparison of myofibrillar protein

The myofibrillar protein from Orangefin ponyfish, Chacunda gizzard-shad, and Sardine cannot make a gel which might be caused by fish protein denaturation during the storage frozen before its isolation. It has been detected that storage frozen can negatively affect the protein content of surimi from threadfin bream (Setyawan *et al*., 2017) and may induce degradation of gel strength (Zhang *et al*., 2020). According to these results, protein modification is needed to improve gelling ability. It is expected that adding agar and carrageenan improves the gelation of myofibrillar protein from trash fish.

3.1.9. Protein color after modification

The color of modified myofibrillar protein from trash fish is described by the hue value (Binatha, 2016) that is compared to the hue of myofibrillar protein from cod fish (Table 2).

Table 2. Comparison of modified fish myofibrillar protein with cod fish myofibrillar protein

Modified fish myofibrillar protein	Parameters			
	Hue Value	Hardness (Nm^{-2})	Cohesiveness (gfs^{-1})	Adhesiveness (gfmm)
CC: Carrageenan+MC	98.68	0.0005	0.35	879
CO: Carrageenan+MO	114.51	0.0006	0.62	963
CT: Carrageenan+MT	98.40	0.0004	0.58	570
AC: Agar+MC	103.40	0.0204	0.42	5.538
AO: Agar+MO	100.70	0.0197	0.37	5.303
AT: Agar+MT	93.79	0.0217	0.41	4.562
*)MCd: Cod fish myofibrillar protein	121.93	0.0713	0.26	116

*) Pamujiati *et al*., 2020

Generally, the hue of modified myofibrillar protein from trash fish was lower than for protein from cod fish. However, Wei *et al*. (2012) stated that the hue value between 90 and 125 is attributed to yellow color, and, according to the table of hue conversion, all of the modified myofibrillar protein samples and cod fish myofibrillar protein have the same color – yellow that can be an advantage for surimi production from trash fish.

3.1.10. Texture after modification

The texture of modified myofibrillar protein has various results (Table 2). The texture is described by hardness, cohesiveness, and adhesiveness. Hardness is generally related to the texture of a product (Damat *et al.*, 2020). Hardness is defined as the necessary force to achieve deformation. Cohesiveness is defined as the ability of a material to receive stress. Adhesiveness means the need for force to overcome the attractive force between the surface of the material and the surface of the other material at their contact surface (Tee and Siow, 2017). Myofibrillar protein from cod has the highest hardness value (0.0713 Nm^{-2}), whereas CT has the lowest detected hardness value (0.0004 Nm^{-2}), which is affected by total protein content. Protein content in cod fish is 16 % to 19 % (Oliveira *et al*., 2012) – almost twice higher that in Orangefin ponyfish (8.80 %), Chacunda gizzard-shadfish (9.30 %), and Sardine (10.10 %).

CO has the highest detected cohesiveness (0.62 gf s^{-1}), whereas MCd has the lowest (0.26 gf s^{-1}). On the other hand, AC has the highest adhesiveness (5.538 gf mm), whereas MCd has the lowest adhesiveness (116 gf mm). Moreover, gel texture depends on the fish species used for the preparation of surimi and the concentration of salt used for the protein solubilization. Also, temperature, time of heat treatment, and moisture content may play a role (de Oliveira *et al*., 2017).

The addition of agar to myofibrillar protein increased hardness value remarkably, while carrageenan did not. Belova and Dyshlyuk (2016) stated that the gel from agar is characterized as rigid, fragile, and malleable, while the gel from carrageenan is predominantly elastic. Also, the cohesiveness values after adding carrageenan are higher than for the samples made with agar. According to Gao *et al*. (2016), combining soy protein isolate and carrageenan can increase the gel strength of salt-soluble meat protein caused by the molecular interaction of both. Adding carrageenan and protein isolate from legumes can improve the gel strength but may decrease the whiteness (Bashir *et al.*, 2017). On the other hand, the gel from carrageenan is more elastic. Therefore, adding agar resulted in a lower hardness value for myofibrillar protein from fish than at modification.

3.1.11. Microstructure after modification

Figure 4 shows modified AC (agar with protein from Chacunda gizzard-shad) has a more tightly porous structure than other samples. In contrast, modifying AO (agar with protein from Orangefin ponyfish) leads to bigger pores and sprawls. Modified AT (agar with Sardine) has much smaller pores. A third of each sample creates a three-dimensional network with various densities. All myofibrillar protein trash fish modified by agar samples have different microstructures than MCd (cod fish myofibrillar protein). AC and AT have tightly porous structures, while MCd has bigger pores; AO tends to create a three-dimensional network, while MCd does not. The same thing happened in AT.

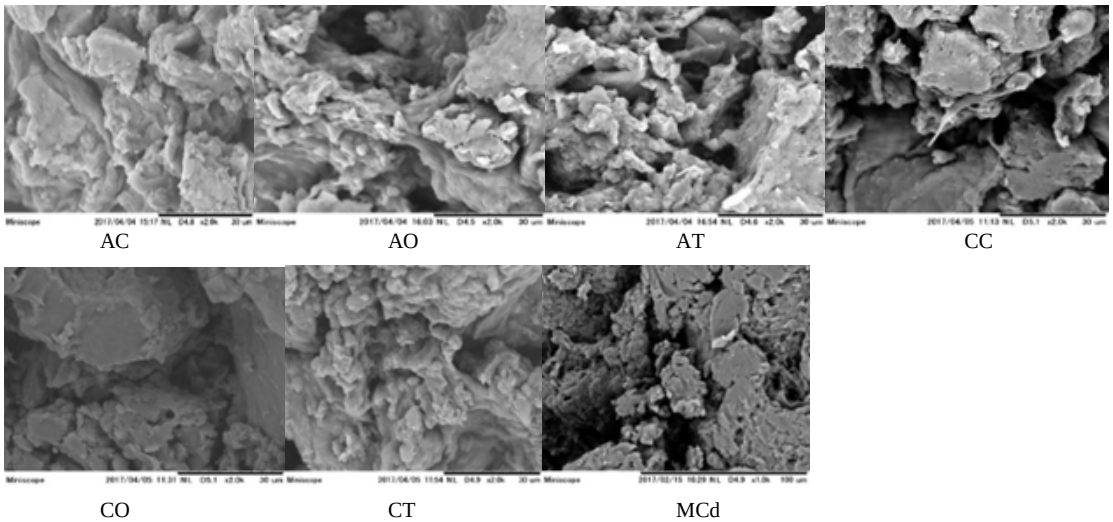


Figure 4. The microstructure of modified fish myofibrillar protein at a magnification of 2 000×, where AC means agar+MC; AO – agar+MO; AT – agar+MT; CC – carrageenan+MC; CO – carrageenan+MO; CT – carrageenan+MT; MCd – cod fish myofibrillar protein

According to the protein content, AC has the highest protein content (84.77 %), which affects the three-dimensional network formation. In general, the microstructure of fish myofibrillar protein changed by adding agar compared with the native microstructure of fish myofibrillar protein (Figure 4). Fish myofibrillar protein modified by adding carrageenan has a different microstructure. Modified CC (carrageenan with protein from Chacunda gizzard-shad) has a microstructure like a slab and large pores. The microstructure of CO (carrageenan with protein from Orangefin ponyfish) is tight and a little porous, and the surface tends to be a little surging. Modified CT (carrageenan with Sardine protein) has a porous structure and rough surface like a three-dimensional network. All myofibrillar protein samples modified by adding carrageenan have similarities with the microstructure of MCd. The microstructure of modified CC and CO has almost the same microstructure as MCd, but the microstructure of modified CT tends to create a three-dimensional network as for MCd. After adding carrageenan, the microstructure of fish myofibrillar protein tended to have a finer porous structure and create a three-dimensional network than the native microstructure of fish myofibrillar protein (Figure 4). The results are supported by the study of Liu *et al*., (2023) who stated that the increase in carrageenan concentration up to 0.75 % may result in a finer gel matrix and also improve the compactness of protein gel network. Myofibrillar protein microstructure and texture are interrelated (Cheng *et al*., 2014). Based on the results of this study, it can be concluded that the Chacunda gizzard-shad trash fish (*A. chacunda*) can be used as a raw material for various rich food products in protein and have high economic value.

4. Conclusions

The study revealed that the protein content of trash fish Chacunda gizzard-shad was higher (84.77 %) than for other samples. For this species, characteristics of myofibrillar protein were higher water-holding capacity (524.78 %) and more significant emulsion activity (8.85 m g^{-2}) than other samples. Sardine had a higher ability of oil holding capacity (620.35 %) and emulsion stability (4.58 h) than other fish myofibrillar protein samples, but Orangefin ponyfish had the highest whiteness value (78.42). Assessing the molecular weight of myofibrillar protein samples of trash fish, the dominant bands were at 150 kDa and 40 kDa, meaning that the protein band has a higher concentration than other bands.

Modifying fish myofibrillar protein by adding agar and carrageenan led to increased hardness values (up to 0.0217 N m^{-2}), although the value was still lower than for cod fish myofibrillar protein (0.0713 Nm^{-2}). In addition, the hue value indicated that the color of all modified myofibrillar protein was the same – yellow. Based on the results of this study, it can be concluded that the Chacunda gizzard-shad trash fish (*A. chacunda*) can be used as a raw material for various rich food products in protein and have high economic value.

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