Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 28(6): 2073 – 2082 (2024)
www.ejabf.journals.ekb.eg

Soluble Protein and Parvalbumin in Exported Yellowfin Tuna (*Thunnus albacares*) from Makassar, South Sulawesi, Indonesia

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ARTICLE INFO

Article History:
Received: Sept. 17, 2024
Accepted: Dec. 19, 2024
Online: Dec. 28, 2024

Keywords:
Allergen,
Parvalbumin,
SDS-PAGE,
Soluble protein,
Tuna

ABSTRACT

The yellowfin tuna (*Thunnus albacares*) is a highly valuable fish species and a significant export commodity for Indonesian fisheries. However, this fish contains an allergen protein, namely parvalbumin. The aim of this research was to analyze the parvalbumin content in the exported yellowfin tuna from Makassar. This research was conducted as a case study, with data collection performed using a survey approach. Samples were taken from a tuna exporting company in Makassar, which sources its yellowfin tuna raw materials from the districts of Bone, Bulukumba, and Palopo. Samples were collected at the time of receiving raw fish materials and processed products ready for export. The total water-soluble protein and its molecular weight profile were the parameters analyzed. The Bradford assay showed that the total water-soluble protein (%) in the raw tuna meat from Bone was 4.74 ± 0.17, from Bulukumba was 4.5 ± 0.05, and from Palopo was 5.06 ± 0.39. For the final processed product, the protein content from Bone was 4.92 ± 0.36, from Bulukumba was 4.86 ± 0.11, and from Palopo was 4.12 ± 0.14. The results from sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) visualization indicated that the molecular weight of yellowfin tuna parvalbumin was approximately 10kDa. The photocapture application confirmed a molecular weight range of 9–10kDa in both the raw materials and final products.

INTRODUCTION

Food allergies are immune responses triggered by allergens present in food ingredients **(Suseno *et al.*, 2016)**. Although it is possible to anticipate adverse reactions to food, certain individuals may nevertheless encounter unforeseen reactions. Food hypersensitivity is defined as the occurrence of reproducible reactions when consuming a certain quantity of trigger foods **(Walker, 2019)**. Proteins such as parvalbumin and other fish proteins like collagen, aldolase, and enolase typically cause fish allergies. Parvalbumin is involved in almost 95% of fish allergies **(Mukherjee *et al.*, 2023)**.

According to ***Stephen et al.* (2017)**, parvalbumin belongs to the water-soluble group and has a molecular weight ranging from 15 to 10 kDa. The yellowfin tuna and

other fish have identified parvalbumin as an allergen **(WHO/IUIS, 2022)**. Heat cannot remove parvalbumin, a heat-resistant protein that protease enzymes cannot easily digest **(Ruethers *et al.*, 2018)**, but washing can reduce its content by up to 95% **(Nugraha *et al.*, 2020)**. Fish with varying parvalbumin content exhibit higher levels of allergenicity **(Griesmeier *et al.*, 2010)**. Studies have demonstrated the presence of parvalbumin in tuna meat, suggesting its potential as an allergen **(Virgienie *et al.*, 2020)**. The parvalbumin content varies depending on the type of fish; the results of sodium dodecyl sulfate gel electrophoresis (SDS-PAGE) show that the parvalbumin content is different in salmon, trout, cod, carp, mackerel, and tuna **(Kuehn *et al.*, 2010)**. Clinical symptoms experienced after consuming fish protein or parvalbumin include urticaria, asthma, and anaphylactic reactions **(Perez-Gordo *et al.*, 2011)**.

Tuna is a leading commodity in Indonesia's fisheries sector that is in high demand on the international market. Tuna is a type of fish with high economic value and is the second largest foreign exchange earner for fisheries commodities **(Rahmansyah *et al.*, 2021)**. Tuna-skipjack tuna (TCT) generates 713.9 million USD in foreign exchange from Indonesian fisheries **(PDSPKP, 2021)**. Potential markets like Japan, the United States, and the European Union receive around 70 percent of Indonesia's tuna production, which accounts for 14.69% of the country's total fishery exports.

Tuna export products are typically processed into loins, which undergo a comprehensive handling and processing procedure from raw materials to final products. The thickest part of the tuna, the frozen tuna loin, is maintained at a product center temperature of -18°C **(Abdullah *et al.*, 2021)**. Processing can alter the structure and chemical properties of proteins, such as parvalbumin, leading to denaturation, aggregation, and binding to fat. These changes can affect the protein's allergenicity, especially for individuals with sensitivities to processed proteins. Tuna meat contains parvalbumin, an allergenic protein that can trigger allergic reactions in humans who consume the fish. Therefore, this study aimed to analyze the content of water-soluble proteins and parvalbumin as allergenic proteins in yellowfin tuna to ensure food safety for exported fishery products.

MATERIALS AND METHODS

One of the tuna processing companies in Makassar, which exports tuna to America, Europe, and Japan, provided yellowfin tuna samples. The company sources its tuna from three districts in South Sulawesi: Bone, Bulukumba, and Palopo. Sampling was conducted both when raw materials were received and when the processed tuna products were ready for export.

1. Extraction of soluble protein (Kobayashi *et al.*, 2016)

The tuna meat was prepared in the form of loins. Ten grams of the sample were crushed and mixed with a 0.01 M Tris-HCl buffer solution at pH 7.4 in a 1:4 ratio. The

mixture was then homogenized using a laboratory homogenizer. After homogenization, the sample was centrifuged at 4ºC at 8000 rpm for 30 minutes. The supernatant obtained from the centrifugation process was used as the sample extract.

Determination of extract total protein content (Bradford, 1976)

Determination of protein content in the extract of tuna fish meat was carried out following the Bradford method using bovine serum albumin (BSA) as a standard. Bradford reagent solution was made by mixing 10mg of coomassie brilliant blue (CBB) into 50ml of 95% ethanol and 10ml of 85% phosphoric acid. The solution was then added with distilled water to a volume of 100ml. The standard solution was made by dissolving 50mg of BSA with 25ml of distilled water to obtain a concentration of 2mg/ ml. The standard solution was then diluted to obtain concentrations of 0.75, 0.5, 0.25, 0.125, and 0.1mg/ ml. Protein content was then determined with the Bradford method by mixing 5ml of Bradford reagent into 2ml of sample, 2ml of standards with various concentrations, and 2ml of Tris HCl (blank). The mixture solution was incubated at room temperature for 5 minutes. The absorbance of the solution was measured using a spectrophotometer at 595nm wavelength.

2. SDS-PAGE of protein extract (Laemmli, 1970)

The protein profile of the extracted protein was analyzed using SDS-PAGE with a 15% separating gel, prepared by mixing 1.75mL of aquadest, 3.75mL of 30% acrylamide, 1.85mL of 1.5 M Tris-HCl (pH 8.8), 75µL of 10% SDS, 75µL of 10% APS, and 7.5µL of TEMED. The prepared gel solution was then introduced into the SDS-PAGE apparatus using a micropipette. The stacking gel solution was allowed to solidify for approximately 30 minutes. A 10µg sample was mixed with a sample buffer in a 1:1 ratio, vortexed to homogenize, and then spun down. The sample was heated in a thermostat set at 95°C for 10 minutes. Approximately 20µL of the sample was loaded into the polyacrylamide gels. Electrophoresis was conducted at 400mA and 85V for 15 minutes, followed by 400mA and 170V for 60 minutes. The electrophoresis was stopped when the marker color reached approximately 0.5cm from the bottom of the gel. Afterward, the gel was stained with a solution containing 0.05% Coomassie Brilliant Blue (CBB), 40% methanol, 10% acetic acid, and 50% aquadest for 90 minutes. The gel was then destained in two steps: the first step with a solution of 40% methanol, 10% acetic acid, and 50% aquadest for 30 minutes, followed by the second destaining step with 20% methanol, 10% acetic acid, and 70% aquadest for 30 minutes. Finally, the gel was immersed in 5% acetic acid solution for 15 minutes. After scanning the gel, the protein bands were quantified using Photocapt software.

Data analysis

Data obtained were analyzed descriptively and were presented in the form of figures.

RESULTS

1. Concentration of extracted protein

The total protein contained in the yellowfin tuna meat extract was determined using the method of **Bradford (1976)**. A standard curve was prepared using BSA which produced a regression equation of y = 0.0176x + 0.1684 and an R^2 value of 0.7594. This equation was used to calculate the total protein content in the tuna meat extract.

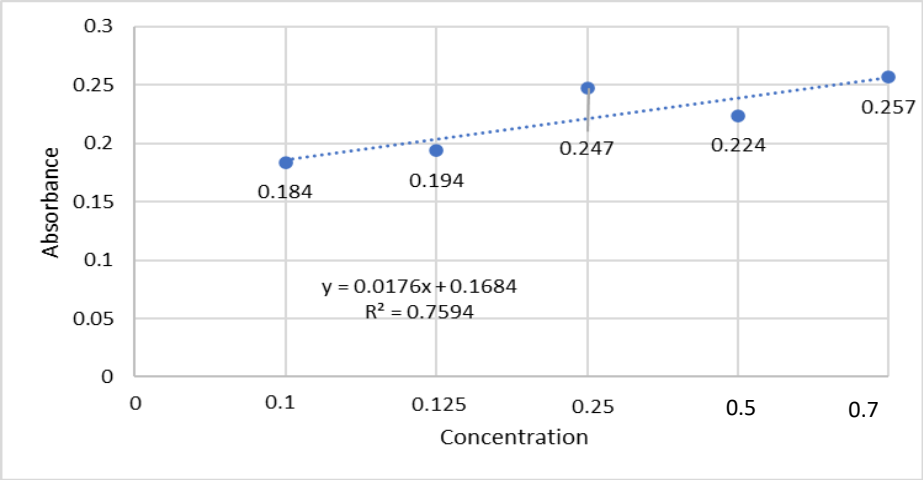

Fig. 1. BSA standard curve. Source: Primary data

The water-soluble or protein concentration in tuna fish meat with raw material and final product treatments showed no significant effect on soluble protein values. The results demonstrated insignificant differences.

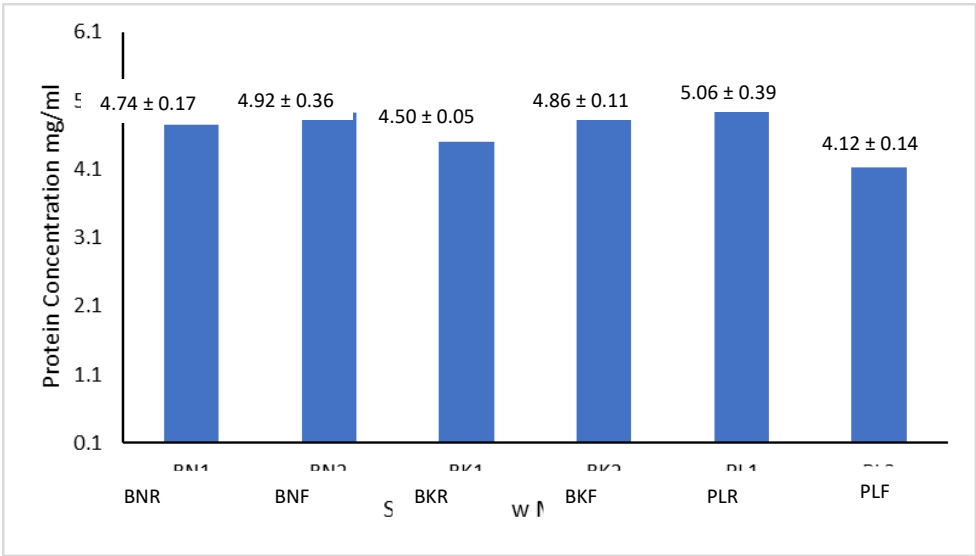

Fig. 2. Water soluble protein concentration of protein in the raw materials and processed products of yellowfin tuna.

Protein concentration in raw materials and final products. BNR = Raw materials from Bone, BNF = Finish products from BNR, BKR = Raw materials from Bulukumba, BKF = Finish products from BKR, PLR = Raw materials from Palopo, PLF = Finish products from PLR.

The water-soluble protein test did not reveal a significant difference between the raw materials and export-ready products. This lack of variation can be attributed to the fact that the raw materials used in the study all come from the same species, the yellowfin tuna. Additionally, the tuna from the Bone, Bulukumba, and Palopo regions had similar weights, ranging from 20 to 30 kg per fish. This weight range is indicative of the maturity level of the gonads in the yellowfin tuna. Furthermore, all the tuna originated from fishing areas within Bone Bay, where the tuna share the same habitat and diet, contributing to similar protein profiles across the different regions.

2. Molecular weight profile of the water-soluble protein

The analysis of parvalbumin protein content using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) method shows the following visualization of bands in the parvalbumin protein:

Protein Parvalbumin of SDS-PAGE

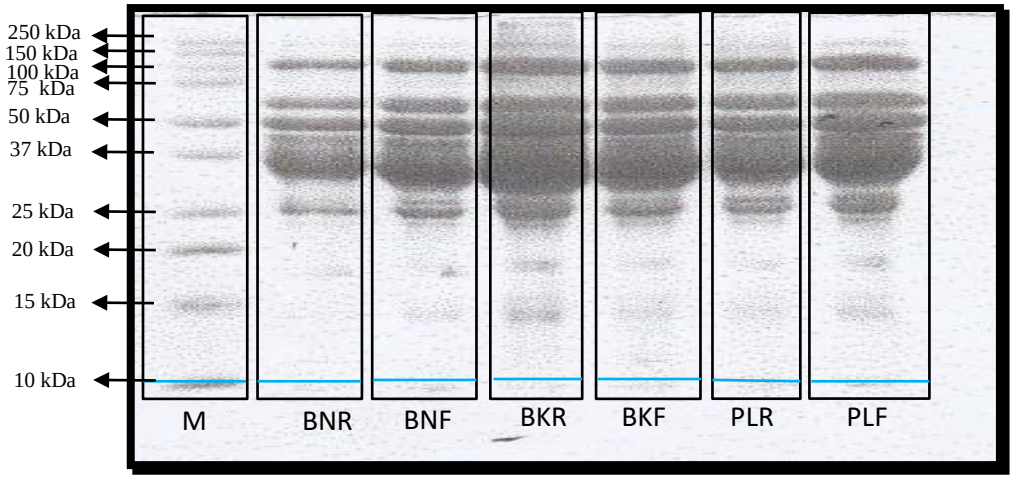

Fig. 3. Molecular weight profile of the water-soluble protein of yellowfin tuna light meat. Where, M = Marker 10 – 250 kDa. BNR = Raw materials from Bone, BNF = Finish products from BNR, BKR = Raw materials from Bulukumba, BKF = Finish products from BKR, PLR = Raw materials from Palopo, PLF = Finish products from PLR.

The water-soluble protein of the yellowfin tuna showed molecular weight ranging from 245–10kDa. The electrophoresis results also showed that the protein band varied in thickness where a thicker band indicates higher protein concentration. Fig. (3) shows that the parvalbumin in the raw materials and final product the yellowfin tuna has a molecular weight of 9.91–10.21kDa.

DISCUSSION

1. Water – soluble protein

The concentration of water-soluble protein in the yellowfin tuna meat in this study was found to be similar, despite the different districts of origin. This similarity can be attributed to several factors. One primary factor is the consistency in the species, as all the samples were from the yellowfin tuna, which share common biological characteristics. Additionally, the similarity in the fish's living habitat, the Bone Bay area, plays a crucial role. Fish residing in the same habitat tends to consume similar food sources, which affects their protein composition.

As noted by **Andhikawati *et al.* (2021)**, habitat conditions and diet are key factors influencing the chemical composition of fish meat. The similarity in the fish's food source within the same habitat leads to comparable protein content, regardless of the district of origin. Furthermore, the samples from all three districts—Bone, Bulukumba, and Palopo—had similar weight ranges of 20-30kg, indicating that they belong to the same cohort. Fish within a cohort, being of similar age and body size, also tend to have similar body compositions. According to **Collette *et al.* (1983)**, the yellowfin tuna reaches gonadal maturity at around 2.5–3 years old, with a weight range of 20–30kg, which aligns with the sample weights in this study.

The water-soluble protein content in fish meat varies by species, typically comprising around 10% of the total fish protein **(Laksono *et al.*, 2024)**. In pelagic fish such as mackerel, tuna, and marlin, water-soluble proteins constitute about 45% of the total protein **(Hamaguchi *et al*., 2007)**. For instance, marlin has about 44% water-soluble protein in its dorsal meat **(Wahyuni *et al.*, 1998)**, and other tuna species, like albacore, skipjack, and yellowfin tuna, have comparable levels of water-soluble proteins—47, 42, and 45%, respectively **(Sánchez-Zapata *et al.*, 2011)**. Thus, despite regional differences, the water-soluble protein content in yellowfin tuna was consistent across the samples in this study, likely due to shared environmental and biological factors.

2. Parvalbumin

The handling of yellowfin tuna meat for export, which includes processes such as receiving raw materials, preparing loins, injecting carbon monoxide (CO), freezing, and cold storing, does not affect the parvalbumin protein. This finding is in line with the study of **Tsai *et al.* (2023)**, which stated that cold storage does not alter the immunoreactivity to the parvalbumin allergen. Moreover, non-thermal processing technologies, such as ultraviolet radiation, hydrostatic pressure, and Maillard reactions, have been shown to significantly reduce fish allergenicity. However, conventional heat processing has a limited effect on parvalbumin allergenicity **(Sujatmiko *et al.*, 2023)**. Parvalbumin, a protein present in yellowfin tuna, is resistant to heat, meaning that it retains its ability to trigger allergic reactions, even after cooking or heating. The structural components of

parvalbumin, with a characteristic molecular weight of approximately 10kDa, remain unchanged even when exposed to high temperatures **(Arif & Hasnain, 2010)**.

The results of this study indicate that the protein bands for parvalbumin are visible at 10kDa in both raw materials and final products, regardless of their district of origin (Bone, Bulukumba, or Palopo). However, the protein band is faint, suggesting the presence of parvalbumin in low amounts. Parvalbumin is mainly found in the red muscle of tuna **(Bossuyt *et al.*, 2005)**, with the highest concentrations located in the dorsal red meat section. This aligns with the molecular weight findings, which show a 10 kDa band that is positive for parvalbumin antiserum, confirming its identification **(Kobayashi *et al.*, 2016)**.

The biochemical, structural, and functional properties of parvalbumin contribute to its role in allergic reactions, as noted by **Arif and Hasnain (2010)**. Processing methods, such as washing, can reduce the allergenic content of parvalbumin in fish **(Nila *et al.*, 2020)**, while boiling and other methods may alter protein band separations and the molecular weight of proteins **(Mahardika *et al.*, 2023)**. The allergenicity of a food ingredient can either decrease, increase, or remain unchanged depending on the processing methods **(Golkar *et al.*, 2019)**.

Parvalbumin content is known to vary by fish species. **Kuehn *et al.* (2010)** reported species-specific ranges of parvalbumin content in raw fish, with tuna containing about 0.03mg of parvalbumin per gram of muscle. This is much lower than other fish species such as herring, which contains 4.75mg of parvalbumin per gram. In cooked fish extracts, parvalbumin content can range from 0.2% in tuna to 16% in herring. The molecular weight of parvalbumin in the yellowfin tuna observed in this study (10 kDa) is consistent with previous findings **(Kobayashi *et al.*, 2016)**. Studies also show that large migratory fish, such as the yellowfin tuna, swordfish, and bigeye tuna, have low levels of parvalbumin **(Lee *et al.*, 2012)**, reducing the likelihood of allergic reactions in humans. As **Klueber *et al.* (2019)** stated, tuna's low parvalbumin content makes it less likely to cause allergies in individuals with fish allergies.

CONCLUSION

The water-soluble protein content in the meat of yellowfin tuna was found to be relatively consistent, regardless of whether the meat was in its raw material or finished product form, or the district of origin of the raw materials. This consistency suggests that factors such as the type of meat and geographic source had minimal impact on the water-soluble protein levels.

However, the parvalbumin content in the yellowfin tuna from the three different districts (Bone, Bulukumba, and Palopo) was similarly low. The parvalbumin protein was identified at a molecular weight of approximately 10kDa, which is characteristic of this

allergenic protein. The low concentration of parvalbumin in the tuna further reduces the likelihood of allergic reactions in individuals sensitive to fish allergens. This low level of parvalbumin supports the notion that the yellowfin tuna may be a safer option for individuals with fish allergies, as its allergenic potential is significantly minimized.

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