Vol. 15, No. 2 (2026): 499 - 509
http://dx.doi.org/10.23960/jtepl.v15i2.499-509

JURNAL TEKNIK PERTANIAN LAMPUNG

ISSN 2302-559X (print) / 2549-0818 (online)
Journal homepage : https://jurnal.fp.unila.ac.id/index.php/JTP

Development of Hypoallergenic "Pindang" Tongkol (*Euthynnus affinis*) Processed by Pressurized Heating

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Article History:

Received : 15 June 2025
Revised : 18 August 2025
Accepted : 30 August 2025

Keywords:

Allergy,
Autoclave,
Parvalbumin,
Pindang,
Tongkol.

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ABSTRACT

Tongkol (Euthynnus affinis) or Mackerel tuna consumption in Indonesia remains limited due to the presence of the allergenic protein parvalbumin. This study aimed to evaluate the chemical composition, physical properties, protein molecular weight profile, and allergenicity of pindang tongkol processed by pressurized heating. A Completely Randomized Design with three autoclave time treatments (15, 20, and 25 min) at 150 °C and 0.103 MPa was applied. Chemical composition and physical properties were analyzed, protein profiles were determined using SDS-PAGE, protein concentration by the Bradford method, and texture by Texture Profile Analysis (TPA). Allergenicity was assessed qualitatively by immunoblotting and quantitatively by ELISA. Pressurized heating altered the chemical composition and physical properties of tongkol. SDS-PAGE revealed protein band at 10–12 kDa, suspected to be parvalbumin, in all samples; however, immunoblotting detected parvalbumin only in the raw sample. Allergen content decreased by 71, 86, and 97% after 15, 20, and 25 min of heating, respectively, compared to raw fish. Pressurized heating during pindang processing effectively reduced allergenicity and may improve the safety of mackerel tongkol consumption for individuals with fish protein hypersensitivity.

1. INTRODUCTION

Fish is one of the primary protein sources consumed by the Indonesian population. Fish protein is highly digestible and rich in omega-3 fatty acids and essential vitamins (Schrama *et al*., 2022). Indonesia has substantial marine fish production, reaching 7,373,516 tons in 2023, with tuna contributing 738,529 tons to the total marine catch (KKP, 2025a). Despite this high production capacity, fish consumption in Indonesia remains relatively low compared to other Asian countries. National fish consumption was recorded at only 25.31 kg per capita in 2024 (KKP, 2025b). This relatively low consumption level may be attributed to limited public awareness of the nutritional benefits of fish, uneven distribution systems, and the presence of allergenic proteins in certain fish species (Djunaidah, 2017).

Mackerel tuna fish, locally called "tongkol", is one of the marine fish that causes many allergy cases in Indonesia. This is because tongkol contains parvalbumin of 0.3–0.7 mg/g with a molecular weight of 9–11 kDa (Kuehn *et al*., 2010). Parvalbumin is a protein that has a low molecular weight, namely 10–12 kDa (Sharp & Lopata, 2014; Kalic *et al*., 2020). Parvalbumin is the main protein that causes allergies in fish, so its content needs to be reduced or eliminated. Efforts to reduce the parvalbumin content include processing processes, one of which is through traditional fish processing through salt boiling (locally known as *pindang*). *Pindang* is a traditional and simple fish processing using a boiling process with salt (Pandit, 2016). This process is widely applied to fish because it is easy to do and fast, the cost is affordable, and improves the quality of the fish. However, the conventional boiling process has not been able to reduce the intensity of the protein band thought to be parvalbumin (Indrastuti, 2019). Therefore, other heating process conditions are needed that are more effective in reducing parvalbumin.

A more intensive heating process can be carried out at higher pressure using a pressurized heater or autoclave. Research by Arwani *et al*. (2022) proves that pressurized heating with an autoclave has proven effective in eliminating major allergens, such as tropomyosin, in white shrimp and mud crabs. The autoclaving process for 20 minutes (pressure 0.14 MPa at 121 °C) showed that there was a decrease in the intensity of the protein bands in crab (Yu *et al*., 2011). The heating process by autoclaving, results in protein degradation into smaller peptide fragments (De Jongh *et al*., 2015). The potential of the pressurized heating process to reduce allergens needs to be tested in the processing of *pindang* tongkol. However, to the best of our knowledge no study has investigated the effect of pressurized heating during *pindang* processing on parvalbumin reduction in tongkol fish. This research aims to analyze the chemical, physical characteristics, and level of allergenicity of tongkol fish processed using a pressurized heating process using an autoclave, as well as to obtain conditions for the pressurized heating process that are most effective in reducing the allergenicity of tongkol fish.

2. RESEARCH MATERIALS AND METHODS

2.1. Material

The main ingredients used in this research were tongkol fish obtained from Traditional Fishery Products Processing (PHPT), Muara Angke, North Jakarta, distilled water, and salt. Apart from that, chemicals such as Tris 0.1 M, Glycine 0.5 M, materials for SDS-PAGE electrophoresis, primary monoclonal antiparvalbumin antibody (PARV-19; Sigma-Aldrich, USA), and Parvalbumin ELISA kit (3M^{TM} Fish Protein Elisa Wells, 3M, Australia) were also used.

2.2. Research Stages

This research was generally divided into 3 stages; namely: (1) characterization of the chemical composition and physical properties of raw tongkol and *pindang* tongkol; (2) salt boiling proccess using pressurized heating using an autoclave; and (3) characterization of the allergenicity of *pindang* tongkol products. At each stage, proximate analysis was carried out on SNI 01-2891-1992 on Food and Beverage Testing Methods (BSN, 1992), soluble protein content testing using the Bradford method (Bradford, 1976), allergenicity level analysis using the Enzyme-Linked Immunosorbent Assay (ELISA) method (Chen & Hsieh, 2014), molecular weight analysis using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Rivera *et al*., 2018), and analysis of allergen protein bands using the immunoblotting method (Wijaya *et al*., 2020). Other tests carried out on *pindang* tongkol were salt content in accordance with SNI 01-2359-1991 regarding how to test salt (BSN, 1991) and texture testing with Texture Profile Analysis (TPA) TA-XT2i (Indiarto *et al*., 2012).

2.2.1. Preparation of Raw Materials and *Pindang* Processing with Pressurized Heating

Fresh tongkol fish were selected with a uniform size (1 kg contains 6–7 fish), and brought to the laboratory using a cooler box with a temperature of 4.4 ˚C. The fresh fish was then cleaned, the entrails and gills removed, washed with clean water, and drained. Next, 1 L of water for every 1 kg of fish was prepared, then 250 g of salt (25% of the water) were added (Pandit, 2016), and stirred until homogeneous. The fish was then put into an autoclave together with a salt solution, and cooked at a temperature of 150 °C and a pressurized of 0.103 MPa, for a heating treatment of 15 min (code PP15), 20 min (code PP20), and 25 min (code PP25) (Kim *et al*., 2011; Carballeda-Sangiao *et al*., 2016). After heating, the *pindang* tongkol was drained and cooled.

2.2.2. Physicochemical Analysis of Fresh Tongkol Fish and *Pindang* Tongkol

Changes in the nutritional value of fresh tongkol and *pindang* tongkol were evaluated through proximate tests in accordance with SNI 01-2891-1992 concerning Methods for Testing Food and Drinks (BSN, 1992). The analysis of salt content in *pindang* tongkol was analyzed in accordance with SNI 01-2359-1991 concerning Salt Testing Methods (BSN, 1991). The salt content in the sample was calculated using Equation (1).

$$\text{Salt Content} = \frac{[(Vol\ AgNO_3\ x\ NAgNO_3) - (Vol\ NH_4CNS\ x\ N\ NH_4CNS)]\ x\ 58{,}44\ x\ 100}{Sample\ weight\ x\ 1000} \quad (1)$$

The physical properties of the texture of *pindang* tongkol were analyzed using TPA, which refers to Indiarto *et al.* (2012). Samples taken measuring 3×3×3 cm^3 were pressed with a cylindrical probe (diameter 6 mm) twice. The probe speed was set to 5 mm/s and the sample was pressed to 30% of its initial height. The parameters observed include hardness, springiness, cohesiveness, and chewiness. Data obtained from TA-XT2i texture analyzer software.

2.2.3. Protein Extraction

Analysis of the protein band profile of fresh tongkol and *pindang* tongkol began with a protein extraction process that refers to Sletten *et al.* (2010) and Liu *et al.* (2011). A sample of 6 g was added to 30 mL of buffer 0.1 mol Tris/0.5 mmol Glycine pH 8.78 (4 °C) overnight at a temperature of 4 °C. The samples were centrifuged at 10,000 rpm for 30 min at 4 °C. The supernatant obtained was then filtered using a filter cloth and stored at -20 °C.

2.2.4. Soluble Protein Analysis by the Bradford Method

Analysis of dissolved proteins refers to Bradford (1976). 5 µL of fresh tongkol and *pindang* tongkol protein extract solution was taken, then 250 µL of Bradford reagent was added. The microplate was shaken in the ELISA reader for 5 seconds before reading the absorbance at a wavelength of 595 nm. Bovine serum albumin (BSA) solution was used as a standard.

2.2.5. Analysis of Changes in Molecular Weight Profiles of Protein Extracts using SDS-PAGE

SDS-PAGE testing is used to separate proteins based on their molecular weight. Analysis begins with the preparation of separating protein (15 µL) which is mixed with sample buffer (1:1 v/v) and heated at 90 °C for 5 min. Next, the proteins were injected into the gel (15 µL) and separated at 100 V for 90 min using running buffer. The gel was stained with Coomassie Brilliant Blue solution for 15–20 min, then destaining was carried out until protein bands were visible. Molecular weight and protein intensity were analyzed using Gel Analyzer 23.1.1. and calculated by Equation (2).

$$\text{Protein band intensity (area/mg)} = \frac{The\ area\ under\ the\ gel\ analyzer\ result\ curve}{Protein\ concentration\ \left(\frac{mg}{mL}\right)\ x\ Injection\ volume\ (mL)} \quad (2)$$

2.2.6. Allergen Band Profiling by Immunoblotting

Immunoblotting analysis is a qualitative allergenicity test using specific PARV-19 monoclonal antibodies. The immunoblotting procedure refers to Wijaya *et al.* (2020) modified. Unstained SDS-PAGE gels were transferred to polyvinylidene fluoride (PVDF) membranes by blotting at 90 V for 90 minutes. The membrane was fixed with 50% methanol for 2 minutes, blocked with 5% skim milk in phosphate buffered saline with Tween20 (PBST) for 1 hour, then washed 3 times with PBST for 5 minutes. The membrane was incubated with Monoclonal Anti-Parvalbumin antibodies (1:3000) and goat anti-mouse IgG-HRP (1:30,000) for 1 hour while shaking, then washed again 3 times with PBST. Visualization was carried out using a chromogenic substrate until color appeared, and the reaction was stopped by washing the membrane using distilled water. Positive results are indicated by a dark purplish protein band.

2.2.7. Analysis of Reducing Allergenicity Levels with ELISA

Allergenicity testing was carried out using the 3M™ Fish Protein ELISA Kit, sandwich ELISA method which refers to Chen & Hsieh (2014). Samples weighing 0.5 g were added with 4.5 mL of 1X extraction buffer solution, then incubated in a water bath shaker at a temperature of 50–60 °C for 25 min. After that, the sample was centrifuged at 5000–7000 rpm for 20–30 s. A total of 100 µL of supernatant was mixed with 900 µL of 1X diluent solution, then vortexed until homogeneous. A total of 100 µL of this solution was added to the ELISA well, incubated on an orbital shaker (400 rpm, 20–25 °C) for 30 min, and washed with 1X washing solution four times. Next, 100 µL of 1X parvalbumin HRP conjugate solution was added, incubated on an orbital shaker for 10 min in the dark, and washed again four times. Then, 100 µL of Chromogenic Substrate (TMB) solution was added, incubated for 10 min in the dark, then 100 µL of stop solution was added, and the absorbance was measured at 450 nm for 30 min.

2.3. Data Analysis Methods

In this study, a Completely Randomized Design (CRD) was used with treatment varying the duration of autoclave time of 15, 20, and 25 minutes at a temperature of 150 ˚C, and a pressure of 0.103 MPa. Each treatment was carried out in duplicate and repeated in duplicate. Data analysis was carried out using Microsoft Excel, as well as analysis of variance (ANOVA) using SPSS IBM 27 and Duncan's advanced test to determine treatments that were significantly different at the 5% level.

3. RESULTS AND DISCUSSION

3.1. Proximate Data on Fresh Tongkol Fish Meat and *Pindang* Tongkol

Based on the results of the proximate test, tongkol experienced changes in nutritional value due to the heating process. Changes in the nutritional value of fresh tongkol meat and *pindang* tongkol resulting from the treatment can be seen in Table 1. In the PP15, PP20, and PP25 treatments there was a significant decrease in water content. This is because the use of salt in the boiling process can reduce the water content. Salt has a higher osmotic pressure, so it will absorb the water contained in the fish so that equilibrium occurs (Koesoemawardani *et al*., 2020). Treatments PP20 and PP25 experienced a significant increase in ash content when compared to the control. This is related to the water content and use of salt. The decrease in water activity (a_w) caused by the use of salt which functions as a dehydration agent will increase the ash content in the product (Hafez *et al*., 2019). Apart from that, the use of high temperatures also causes water content to decrease so that it is correlated with an increase in ash content (Ramanda *et al*., 2023).

Table 1. Proximate data on fresh tongkol and *pindang* tongkol

Parameter	Sample				
	FT	PT (control)	PP15	PP20	PP25
Water content (% wb)	73.43±018^{d}	66.43±0.4^{b}	67.04±0.24^{c}	66.37±0.07^{b}	64.96±0.01^{a}
Ash content (% db)	6.36±1.13^{a}	10.59±0.20^{b}	11.24±0.10^{bc}	12.13±0.02^{c}	12.07±0.08^{c}
Protein content (% db)	89.08±2.14^{d}	84.88±0.33^{c}	77.1±1.16^{b}	76.14±0.07^{b}	72.49±1.30^{a}
Fat content (% db)	2.97±0.07^{c}	2.17±0.09^{a}	2.38±0.12^{b}	2.39±0.24^{b}	2.28±0.20^{b}
Carbohydrate content (% db)	1.60±1.08^{a}	2.35±0.04^{a}	9.28±1.39^{b}	9.34±0.15^{b}	13.15±1.42^{c}

Note: Values followed by the same letter indicate there is no significant difference based on DMRT (p <0.05). Values are expressed as the mean ± SD of 2 replicates and 2 repetitions of analysis. FT = fresh tongkol meat; PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.

The protein content in the PP15 and PP20 treatments was significantly different from the PP25 treatment. The heating process can cause proteins that have low molecular weights and water-soluble proteins to move into the cooking water (Tian *et al*., 2018). In addition, a salt concentration of 25% can cause a decrease in protein content and yield when compared to a salt concentration of 20% (Barat *et al*., 2006). However, protein can still be digested and utilized as a source of amino acids and peptides (Damodaran *et al*., 2017; Liu *et al*., 2023).

The fat content in *pindang* tongkol decreased at PP15, PP20, and PP25, when compared to fresh meat (FT). This is because the process of making *pindang* uses high temperature and pressure, so that the fat in the fish is damaged (Hidayat *et al*., 2020).

3.2. Salt Level of *Pindang* Tongkol

The salting method used in this research is the wet salting method, namely by dissolving 25% salt in 1 L of distilled water to produce a saturated salt solution. Based on Figure 1, the salt content of *pindang* tongkol is not significantly different between control samples and samples with pressurized heating for 15 minutes, 20 minutes and 25 minutes. According to Tsai *et al*. (2022), the use of high pressures of 300, 400, and 600 MPa had no effect on increasing the salt content in fish. The use of high pressure can destroy noncovalent bonds between molecules which causes a decrease in water bonds and causes loss of water and salt (Ros-Polski *et al*., 2015).

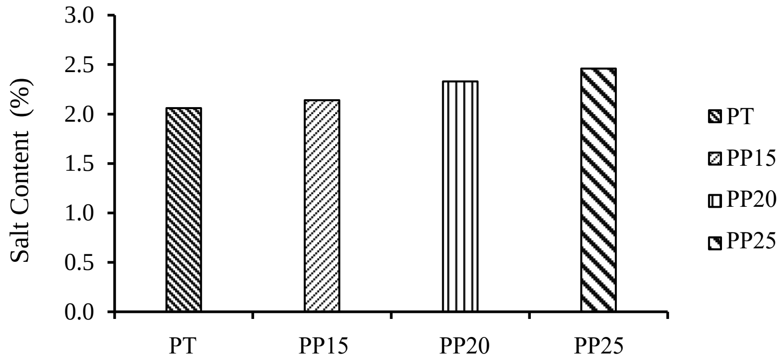


Figure 1. Salt content in samples of *pindang* tongkol. [Note: Values followed by the same letter indicate there is no significant difference based on DMRT ($p < 0.05$). PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.]

One of the quality requirements for fish *pindang* products is the salt content. According to SNI 2717:2017 (BSN 2017), wet salting fish *pindang* has a maximum salt content limit of 10%. Thus, the salt content of *pindang* tongkol as a result of this research (around 2.1–4.34%), can meet the quality requirements for salt content according to SNI 2717:2017, namely not exceeding 10% (BSN, 2017).

3.3. Physical Characteristics of *Pindang Tongkol*

Texture profile analysis (TPA) is an instrumental method used to measure the texture profile of various materials, especially in the food industry (Guiné, 2022). Data on the texture profile of *pindang* tongkol is presented in Table 2. Hardness is the maximum force detected in the first compression in units of kg, g, or N (Sun *et al*., 2018). Samples PP20 and PP25 differ significantly from PP15. However, the longer the heating time, the hardness of the tongkol fish will tend to decrease. This is due to protein degradation so that the hardness of the fish will decrease (Zhang *et al*., 2013). The springiness, cohesiveness, and chewiness parameters were not significantly different between all treatments. This is because the water content decreases which causes a complete loss of turgor pressure and a decrease in the number of multimolecular water layers so that springiness and cohesiveness tend to increase. However, the pressurized heating process caused protein degradation which weakened the protein gel network so that chewiness decreased (Wang *et al*., 2025).

Table 2. Texture profile of *pindang* tongkol resulting from pressurized heating

Parameter	Heating Treatment			
	PT	PP15	PP20	PP25
Hardness (g)	2316.2±245.5^{b}	3099.6±24.61^{b}	2297.15±306.3^{a}	1633.9±187.5^{a}
Springiness	0.7297±0.066^{a}	0.6939±0.020^{a}	0.71242±0.001^{a}	0.7245±0.008^{a}
Cohesiveness	0.627±0.145^{a}	0.6395±0.639^{a}	0.639±0.008^{a}	0.6703±0.027^{a}
Chewiness	1088.69±453.5^{a}	1219.47±105.5^{a}	1045.77±140.3^{a}	796±132.2^{a}

Note: Values followed by the same letter indicate there is no significant difference based on DMRT ($p < 0.05$). Values are expressed as the mean ± SD of 2 replicates and 2 repetitions of analysis. PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with press pressurized ure heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.

3.4. Changes in the Molecular Weight Profile of *Pindang* Tongkol Extract

SDS-PAGE is a method used to separate proteins based on their molecular size (Bouatra *et al*., 2013). The processing process is responsible for protein degradation during cooking which causes changes in protein band density (Zhang *et al*., 2013). The protein profile in the protein extracts of fresh tongkol and *pindang* tongkol is presented in Figure 2. The number of protein bands in fresh tongkol meat (FT) is greater than in the pindang product, namely 16 protein bands. The number of protein bands for control *pindang* tongkol (PT) was reduced compared to FT, namely to 10. In the PP15 sample there were 9 protein bands, while the PP20 sample had the same number of protein bands as PP25, namely 12.

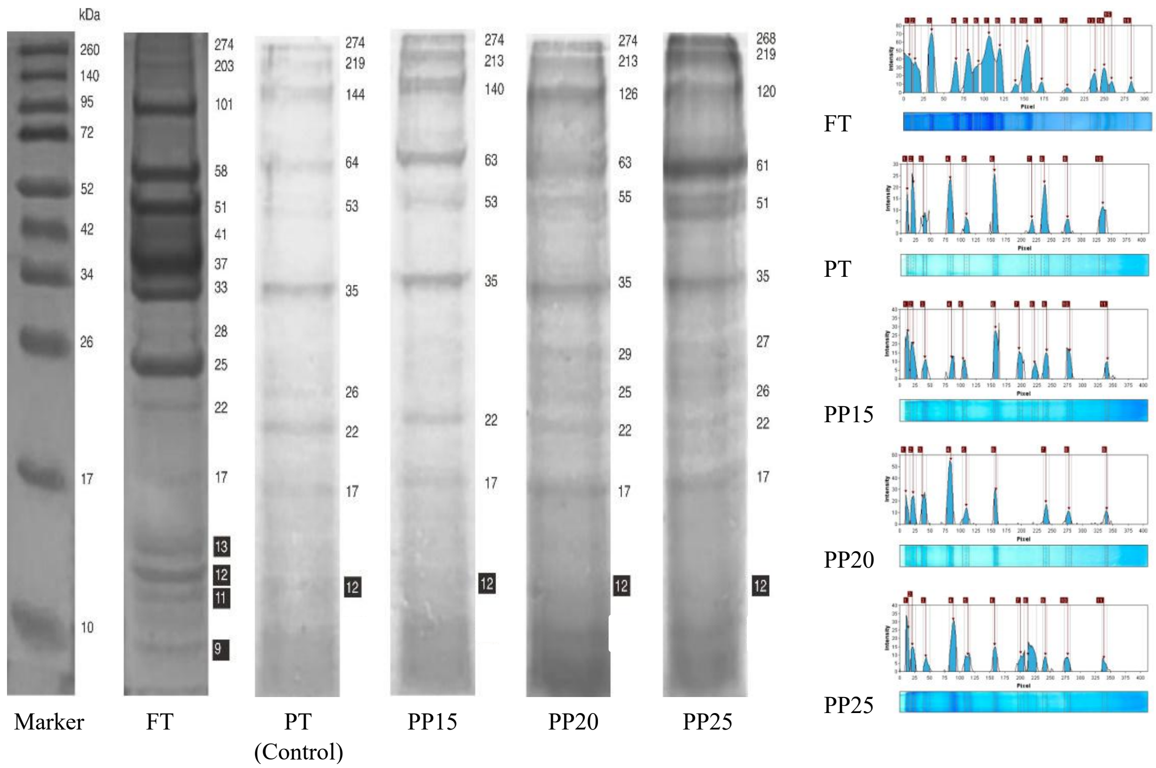


Figure 2. Molecular weight profile of tongkol protein extract and products resulting from boiling and pressurized heating. Note: FT = fresh tongkol meat; PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.

The increase in the number of protein bands that have a small molecular weight is caused by the pressurized heating process, which is thought to cause the protein to denature into smaller fragments (Jiang *et al*., 2018). In all samples there is a protein that has a molecular weight of 12 kDa. This shows that the sample still contains parvalbumin, which has a low and very stable molecular weight, namely 10–12 kDa (Sharp & Lopata, 2014). Even though each sample contains what is thought to be parvalbumin, the intensity varies. The protein band intensity of samples PT, PP15, PP20, and PP25 was thinner or even undetectable when compared to FT samples. Apart from the protein band which is thought to be parvalbumin, based on SDS-PAGE analysis other protein bands which are thought to be tropomyosin and collagen were also obtained. The decrease in the intensity of the protein bands in fresh tongkol meat and its *pindang* products is presented in Figure 3.

3.5. Allergen Band Profile Based on Immunoblotting

Immunoblotting or known as western blotting is a qualitative method for detecting and analyzing certain proteins in complex mixtures. This process includes separating proteins based on size using gel electrophoresis, followed by transfer of the proteins to a membrane, and qualitative detection with specific antibodies (Lappalainen *et al*., 2013). The immunoblotting results of fresh tongkol meat and *pindang* tongkol fish are presented in Figure 4.

Figure 4 shows that the pressurized heating process has an effect on the reduction of parvalbumin. In samples PT, PP15, PP20, and PP25, it was seen that parvalbumin was not detected in the immunoblotting results. On the other hand, in FT, there is a protein band that proves the presence of parvalbumin. This is in line with the data from the ELISA test, that in the PT, PP15, PP20 and PP25 treatments the level of allergenicity has decreased by > 70%.

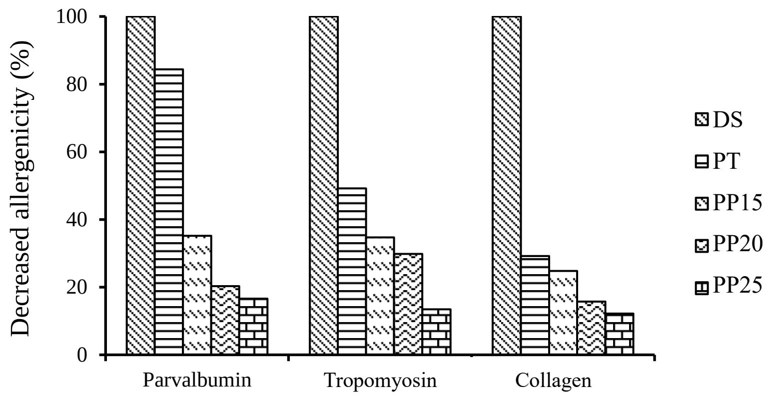


Figure 3. Decreased allergenicity as indicated by the intensity of protein bands in *pindang* tongkol samples compared to fresh tongkol meat. [Note: FT = fresh tongkol meat; PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.]

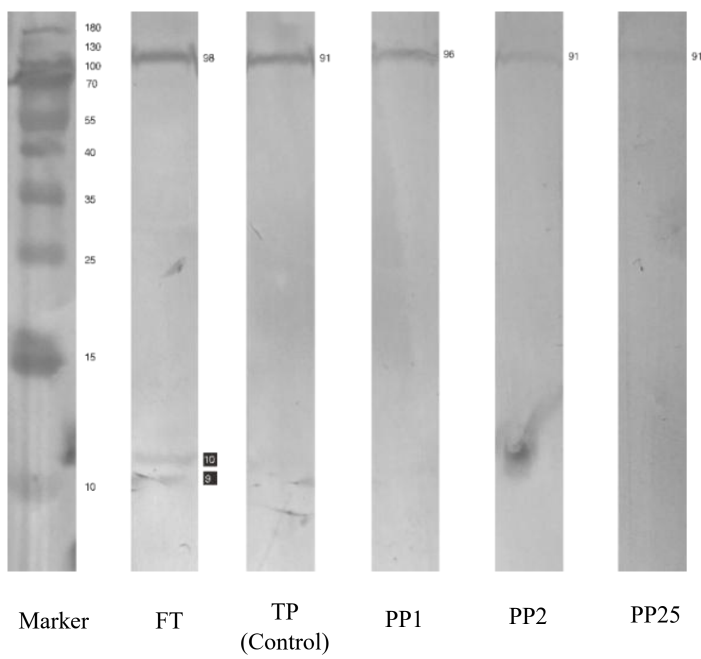


Figure 4. Immunoblotting results of fresh tongkol and *pindang* tongkol meat show allergens with a molecular weight of 10–13 kDa. [Note: M = marker; FT = fresh tongkol meat; PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min].

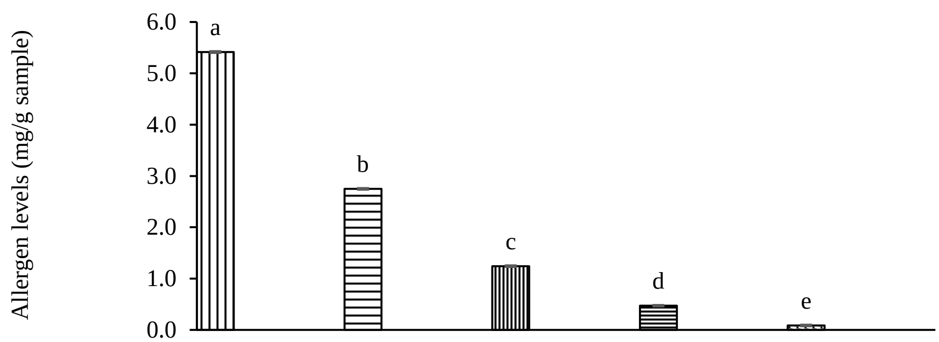
Pressurized heating at high temperatures with an autoclave can reduce parvalbumin significantly due to its effect on the secondary structure of parvalbumin. One example of an application of high pressure is the process of canning tongkol fish, which uses high temperatures in packaged conditions (Somkuti *et al*., 2012). The canning process shows that the parvalbumin is denatured due to the destruction of the epitope. According to Wulan *et al*. (2024), tiger prawn powder extract that is processed by heating using an autoclave no longer contains allergenic proteins. Fish allergenicity is thought to change with parvalbumin degradation or polymerization which can reduce or increase the number of IgE epitopes (Misnan *et al*., 2005). The autoclave processing process has been proven to be very effective in reducing the tropomyosin content in white shrimp and mangrove crabs, when compared to the boiling and frying process (Arwani *et al*., 2022).

Apart from the processing process, the addition of salt (NaCl) in making *pindang* also plays a role in reducing the parvalbumin content because it can cause aggregation or precipitation of proteins. The research results of Sletten *et al*. (2010) showed that the addition of NaCl to salted fish caused a significant decrease in parvalbumin band intensity when compared to fish that were not given the addition of NaCl.

3.6. Changes in Allergenicity Based on ELISA

ELISA is a highly sensitive and specific quantitative method used to detect and quantify target antigens in biological samples (Lappalainen *et al*., 2013). This method can be used to determine changes in the allergenicity content of food ingredients. Food processing techniques can cause a decrease, no change, or even an increase in antigenicity and allergenicity (Jiang & Rao, 2021). Allergen levels indicating a decrease in allergenicity in fresh tongkol meat and *pindang* tongkol fish are presented in Figure 5.

The level of allergenicity of tongkol has decreased significantly. The control sample without pressurized heating (PT) had an allergen content of 2.10 mg/g protein with a reduction in allergenicity of 49.2%. The highest reduction in allergenicity of samples with pressurized heating was PP25, namely 98.4% with an allergen content of 0.06 mg/g protein, while the lowest reduction was PP15, namely 77%, all of which were more significant reductions compared to conventional heating in control samples. The longer the pressurized heating time on the sample, the more the level of allergenicity can be reduced.



	FT	PT	PP15	PP20	PP25
Allergen content (mg/g sample)	5.41	2.75	1.24	0.47	0.08
Allergen decrease (%)	0.00	49.2	77.0	91.2	98.4

Figure 5. Allergen levels in fresh tongkol meat and *pindang* tongkol. [Note: Numbers followed by the same letter indicate that there is no significant difference according to DMRT ($p < 0.05$). Values are presented as the mean ± SD of 2 experimental repeats and 2 analytical repeats. FT = fresh tongkol meat; PT = *pindang* tongkol without pressurized heating (control); PP15 = *pindang* tongkol with pressurized heating for 15 min; PP20 = *pindang* tongkol with pressurized heating for 20 min; and PP25 = *pindang* tongkol with pressurized heating for 25 min.]

Heating can cause the protein structure to open and re-formation of disulfide bonds which will form aggregates. The formation of these aggregates can lock epitopes to the inside of the molecule, thereby reducing allergen sensitivity (Tian *et al*., 2018). According to Wulan *et al*. (2024), food ingredients that have undergone pressurized heating in an autoclave for 5 minutes can experience a decrease in their allergenicity. The use of pressurized heating with an autoclave can reduce the allergen content in tongkol, which occurs due to the influence of the secondary structure of parvalbumin (Somkuti *et al*., 2012). Apart from being influenced by heating, the decrease in allergen levels can be caused by the salt content in *pindang* tongkol. Salt can affect protein properties in the form of disrupting protein stability or causing protein denaturation, solubility and biological properties (Shao *et al*., 2022).

4. CONCLUSION

Pressurized heating significantly altered the chemical composition and physical characteristics of *pindang* tongkol. Increasing heating time reduced texture profile including hardness, while springiness, cohesiveness, and chewiness were not significantly affected. The parvalbumin protein band was detected only in fresh tongkol meat, indicating effective reduction after processing. Pressurized heating at 150 °C and 0.103 MPa for 15, 20, and 25 min reduced allergen content by up to 98%, with the 25-minute treatment (PP25) showing the greatest reduction. These findings demonstrate that pressurized heating combined with salting can serve as a promising strategy to develop hypoallergenic tongkol fish products. This approach may enhance product safety for individuals with fish protein hypersensitivity and provide added value for the fish processing industry.

ACKNOWLEGMENT

The author would like to express his deepest gratitude to the Directorate General of Higher Education, Research and Technology, Ministry of Education, Culture, Research and Technology for the research funding provided under contract number 18814/IT3.D10/PT.01.03/P/B/2023 for Prof. Dr. Ir. Nurheni Sri Palupi, M.Si.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
RCS		✓	✓		✓	✓			✓		✓			
NW				✓				✓		✓		✓		
NSP	✓	✓		✓			✓	✓		✓		✓	✓	✓

C: Conceptualization	Fo: Formal Analysis	O: Writing - Original Draft	P: Project Administration
M: Methodology	I: Investigation	E: Writing - Review & Editing	Fu: Funding Acquisition
So: Software	R: Resources	Vi: Visualization	
Va: Validation	D: Data Curation	Su: Supervision	

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