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Effect of Different Heating Temperatures on Albumin Concentration and Antioxidant Activity of *Rasbora argyrotaenia*

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Abstract

Rasbora argyrotaenia (*wader*) is a local freshwater fish rich in protein and bioactive compounds, including albumin and antioxidants. Heat treatment may induce protein denaturation and reduce the activity of these bioactive compounds. This study aimed to evaluate the effect of different heating temperatures on the albumin concentration and antioxidant activity of *R. argyrotaenia*. Samples were divided into three groups: fresh fish (control), heated at 70°C, and heated at 80°C. Albumin concentration was determined using UV–Vis spectrophotometry, whereas antioxidant activity was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay based on IC_{50} and Antioxidant Activity Index (AAI) values. The highest albumin concentration was observed in the fresh fish (14.144 ± 0.096 g/L), followed by samples heated at 70°C (12.576 ± 0.079 g/L) and 80°C (12.048 ± 0.072 g/L). The IC_{50} values increased from 31.571 ppm in the fresh extract to 45.250 ppm and 57.650 ppm after heating at 70°C and 80°C, respectively, while the corresponding AAI values decreased from 3.16 to 2.21 and 1.73. According to the AAI classification, the fresh extract and the extract heated at 70°C exhibited very strong antioxidant activity, whereas the extract heated at 80°C showed strong antioxidant activity. Overall, increasing heating temperature reduced both albumin concentration and antioxidant activity. Heat treatment at 70°C better preserved the functional quality of *R. argyrotaenia* than heating at 80°C. These findings highlight the importance of temperature control during thermal processing to maintain the nutritional and functional properties of *R. argyrotaenia*.

Keywords: albumin; antioxidant activity; DPPH; heating temperature; *Rasbora argyrotaenia*.

INTRODUCTION

Fish are an important source of high-quality protein and bioactive compounds that contribute not only to nutrition but also to disease prevention. Indonesia possesses one of the world's richest freshwater fish biodiversities (Mubarik et al., 2022), including *Rasbora argyrotaenia* , a native freshwater species widely consumed in Kalimantan (Suryani et al., 2019). *R. argyrotaenia* is recognized for its rich nutritional composition, containing high-quality protein, essential minerals, essential and non-essential amino acids, and biologically important fatty acids that contribute to its nutritional and functional value (Sogandi et al., 2020). Previous studies have reported that consumption of *R. argyrotaenia* increases vitamin D levels and reduces the expression of pro-inflammatory cytokines, including IL-1, IL-6, and IL-17, in patients with Systemic Lupus Erythematosus (SLE) (Partan et al., 2019). *R. argyrotaenia* has also shown potential for therapeutic applications, particularly in the management of malnutrition and anemia. Its extract has been reported to improve physical endurance in malnourished rats (Melatiasha et al.,2023) and contains essential minerals, including calcium (65.96 mg/L), zinc (1.8 mg/L), and cholecalciferol (2043.34 IU/mL), which may contribute to the management of calcium, zinc, and vitamin D_3 deficiencies (Partan et al., 2017).

The high protein content of *R. argyrotaenia* suggests its potential as a natural source of albumin. Among its bioactive components, albumin has attracted considerable attention because of its important physiological and antioxidant functions. Albumin is the most abundant plasma protein and plays a crucial role in maintaining oncotic pressure, transporting various endogenous and exogenous molecules, promoting wound healing, and exerting antioxidant activity through its free thiol groups, which are capable of scavenging reactive oxygen species (ROS) (Hutapea et al., 2023). Therefore, albumin serves not only as a nutritional protein but also

as a key component of the antioxidant defense system. Heat-induced changes in albumin concentration and molecular structure may reduce the antioxidant capacity of fish extracts by impairing the free thiol groups responsible for scavenging ROS (Nedić et al., 2023). In addition to albumin, the antioxidant defense system comprises endogenous antioxidants, such as glutathione, as well as exogenous antioxidants derived from dietary sources, including vitamin C, vitamin E, vitamin A, and flavonoids (Janciauskiene, 2020).

Heating is an essential processing step that is almost always applied before fish consumption. Previous studies have reported that heating at temperatures ranging from 60°C to 80°C begins to affect protein stability (Borzova et al., 2016), including albumin, through denaturation and aggregation processes. Nevertheless, this temperature range remains commonly employed in fish processing (Fitrawan et al., 2023). Therefore, temperatures of 70°C and 80°C were selected in the present study to evaluate changes in albumin concentration and antioxidant activity during thermal processing. Increasing temperature may also accelerate the degradation of antioxidant compounds, thereby reducing the free radical scavenging capacity of fish extracts. The extent of these changes depends on both processing temperature and heating duration, highlighting the importance of identifying appropriate processing conditions to preserve fish quality.

Despite the well-documented nutritional composition and biological benefits of *R. argyrotaenia*, evidence regarding the simultaneous effects of controlled heating temperatures on albumin concentration and antioxidant activity remains limited. Although previous studies have investigated the effects of cooking on the protein composition of several fish species, comparable information for *R. argyrotaenia* is still scarce. Therefore, this study aimed to evaluate the effects of controlled heating temperatures on albumin concentration and antioxidant activity of *R. argyrotaenia* and to identify the processing temperature that best preserves its functional properties.

MATERIALS AND METHODS

Materials

The primary material used in this study was *R. argyrotaenia*, obtained from local fishermen along the Kahayan River, Palangka Raya, Central Kalimantan, Indonesia. Additional materials included distilled water, physiological saline (0.9% NaCl), 96% ethanol, bovine serum albumin (BSA) solution, Bromocresol Green (BCG) reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH) reagent, and ascorbic acid.



Figure 1. *Rasbora argyrotaenia*.



Figure 2. Homogenized *R. argyrotaenia* sample.

Procedures

Preparation of R. argyrotaenia Extract

Fresh *R. argyrotaenia* samples were randomly assigned to three treatment groups: a control group without heat treatment, a group heated at 70°C, and a group heated at 80°C. The fish were cleaned and homogenized using a blender. A total of 400 g of homogenized fish was mixed with 800 mL of sterile distilled water at a 1:2 (w/v) ratio until a homogeneous mixture was obtained. The homogenate was then filtered through sterile muslin cloth to separate the filtrate from the solid residue. Aliquots of 100 mL of the filtrate were transferred into heat-resistant containers and heated in a water bath at either 70°C or 80°C for 25 min, whereas the control group received no heat treatment. Subsequently, all samples were centrifuged at 2,000 rpm for 15 min. The resulting supernatants were collected and used for albumin concentration and antioxidant activity analyses. Each treatment was performed in triplicate.

Albumin Concentration Analysis

Albumin concentration was determined at the Biochemistry Laboratory, Faculty of Mathematics and

Natural Sciences, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia, using the Bromocresol Green (BCG) method. Briefly, 1 mL of the sample supernatant was mixed with 4 mL of BCG reagent and incubated at room temperature for 10 min. The absorbance was measured at 628 nm using a UV–Vis spectrophotometer. Albumin concentration was determined from a bovine serum albumin (BSA) standard curve prepared at different concentrations and calculated using the corresponding linear regression equation. Each sample was analyzed in triplicate.

Antioxidant Activity Assay

The antioxidant activity of *R. argyrotaenia* extract was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The filtrate was diluted to obtain sample concentrations of 2, 4, 6, 8, and 10 ppm. Briefly, 2 mL of each sample concentration was mixed with 2 mL of 0.1 mM DPPH solution, followed by the addition of 96% ethanol to a final volume of 10 mL. The mixture was homogenized using a vortex mixer and incubated at room temperature in the dark for 30 min. Absorbance was measured at 517 nm using a UV–Vis spectrophotometer. Ascorbic acid (vitamin C) was used as the positive control, whereas the negative control consisted of 2 mL of 0.1 mM DPPH solution mixed with 8 mL of 96% ethanol without the addition of the sample. The percentage of DPPH radical scavenging activity (% inhibition) was calculated using the following equation, and the IC_{50} value was determined from the linear regression of sample concentration against percentage inhibition.

$$\%\ Inhibition = \frac{Absorbance\ blank - \ Absorbance\ samplel}{Absorbance\ blank}\ x\ 100\%$$

The relationship between sample concentration (x) and percentage inhibition (y) was evaluated using linear regression analysis (y = bx + a). The IC_{50} value was obtained by substituting y = 50 into the regression equation, where y represents the percentage inhibition and x represents the sample concentration. Lower IC_{50} values indicate greater antioxidant activity. The Antioxidant Activity Index (AAI) was subsequently calculated using the following equation:

$$AAI = \frac{DPPH\ concentration\ (ppm)}{IC50\ of\ the\ sampel\ (ppm)}$$

The antioxidant activity was classified according to the Antioxidant Activity Index (AAI), as presented in Table 1.

Table 1. Classification of Antioxidant Activity Based on the Antioxidant Activity Index (AAI).

Antioxidant Activity	AAI Value
Weak	< 0.5
Moderate	0,5 < AAI < 1.0
Strong	1.0 < AAI < 2.0
Very strong	> 2.0

Source: adapted from Scherer and Godoy (2009).

RESULTS AND DISCUSSION

Albumin Concentration of *R. argyrotaenia*

The albumin concentration of *R. argyrotaenia* was determined to evaluate the effect of different heating temperatures on its albumin content. The results are presented in Table 2.

Table 2. Albumin Concentration of *R. argyrotaenia* under Different Heating Temperatures.

Sample	Absorbance (Mean ± SD)	Albumin Concentration (g/L)
Fresh fish	1.032 ± 0.004	14.144 ± 0.096
Heated at 70°C	0.934 ± 0.003	12.576 ± 0.079
Heated at 80°C	0.901 ± 0.004	12.048 ± 0.072

As shown in Table 2, increasing heating temperature resulted in a progressive decrease in both absorbance and albumin concentration. The highest albumin concentration was observed in the fresh fish (control), whereas the lowest concentration was detected in samples heated at 80°C.

Antioxidant Activity of *R. argyrotaenia* Extract

Table 3. Antioxidant Activity of R. argyrotaenia Extract and Ascorbic Acid Determined by the DPPH Assay.

Sample	Concentration (ppm)	Absorbance	Inhibition (%)	IC_{50} (ppm)	AAI
Ascorbic acid	2	0.361	30.04	3.805	26.28
	4	0.238	53.88		
	6	0.149	71.12		
	8	0.040	92.25		
	10	0.028	94.57		
Fresh *R. argyrotaenia* extract	2	0.473	8.33	31.571	3.16
	4	0.440	14.73		
	6	0.434	15.89		
	8	0.430	16.67		
	10	0.408	20.93		
R. argyrotaenia extract heated at 70°C	2	0.510	1.16	45.250	2.21
	4	0.503	2.52		
	6	0.492	4.65		
	8	0.481	6.78		
	10	0.462	10.47		
R. argyrotaenia extract heated at 80°C	2	0.587	-13.76	57.650	1.73
	4	0.545	-5.62		
	6	0.527	-2.13		
	8	0.512	0.78		
	10	0.497	3.68		

The antioxidant activity of *R. argyrotaenia* extracts determined by the DPPH assay is presented in Table 3. Ascorbic acid, used as the positive control, exhibited substantially higher antioxidant activity than all *R. argyrotaenia* extracts, as indicated by the lowest IC_{50} value (3.805 ppm) and the highest AAI (26.28). Among the fish extracts, the fresh extract showed the highest antioxidant activity, with an IC_{50} value of 31.571 ppm and an AAI of 3.16. Increasing heating temperature resulted in progressively higher IC_{50} values and lower AAI values, indicating a reduction in antioxidant activity. The extract heated at 80°C exhibited the lowest antioxidant activity, with an IC_{50} value of 57.650 ppm and an AAI of 1.73. According to the AAI classification, ascorbic acid and the fresh *R. argyrotaenia* extract were classified as having very strong antioxidant activity, whereas extracts heated at 70°C and 80°C remained within the strong antioxidant category.

Discussion

The results demonstrated that increasing heating temperature led to a progressive reduction in both albumin concentration and antioxidant activity of *R. argyrotaenia* extract. This trend was evidenced by decreased albumin concentrations and increased IC_{50} values following heat treatment. Conversely, the Antioxidant Activity Index (AAI) declined with increasing heating temperature, indicating a reduction in antioxidant capacity.

The decrease in albumin concentration observed in the present study may be attributed to protein denaturation induced by heat treatment. Albumin is a globular protein whose three-dimensional structure is stabilized by hydrogen bonds, hydrophobic interactions, and disulfide bonds (Acharya & Chaudhuri, 2021). Increasing temperature can disrupt these stabilizing interactions, resulting in the unfolding of the albumin tertiary structure, followed by protein aggregation. Consequently, a portion of the albumin loses its solubility and is no longer present in the soluble form that can be detected during analysis. As a result, the measured albumin concentration decreases progressively with increasing heating temperature (Hutapea et al., 2023).

The findings of the present study are consistent with those reported by Borzova et al. (2016), who demonstrated that heating at temperatures between 60°C and 80°C accelerates albumin denaturation and aggregation, thereby reducing the fraction of soluble protein. Similar findings were reported by Fitrawan et al. (2023), who observed a significant decrease in albumin concentration in Indian scad (*Decapterus russelli*) following steaming at increasing temperatures. Furthermore, Utami et al. (2017) reported that heat treatment not only affects albumin stability but also alters the amino acid composition and reduces the overall protein quality of fish.

The reduction in antioxidant activity following heat treatment indicates that the bioactive compounds present in *R. argyrotaenia* extract are heat-sensitive. Antioxidant activity is primarily determined by the ability of bioactive compounds to donate electrons or hydrogen atoms to neutralize free radicals (Ahmad et al., 2025). Exposure to elevated temperatures may induce the

degradation of antioxidant compounds, oxidation of specific amino acids, and conformational changes in proteins that contribute to antioxidant activity, thereby reducing their ability to scavenge DPPH radicals (Tadesse & Emire, 2020). Consequently, a higher extract concentration is required to inhibit 50% of DPPH free radicals, as reflected by the increased IC_{50} values.

The AAI values obtained in the present study showed that ascorbic acid exhibited the highest antioxidant activity among all treatments. Nevertheless, the fresh *R. argyrotaenia* extract was still classified as having very strong antioxidant activity, whereas heat treatment reduced the AAI to the strong category in samples heated at 80°C. These findings indicate that thermal processing diminished the antioxidant potential of *R. argyrotaenia* extract, although a substantial level of antioxidant capacity was retained after heating.

The findings of the present study are consistent with those reported by Oluwaniyi et al. (2016), who demonstrated that cooking reduces the antioxidant activity of fish through the degradation of antioxidant compounds and increased lipid oxidation. Similarly, Chan et al. (2022) emphasized that temperature control during food processing is essential for preserving nutritional quality and bioactive compounds. Furthermore, Pruteanu et al., (2023) reported that antioxidants with low thermal stability undergo a significant loss of activity when exposed to excessive heat.

Overall, the findings of the present study indicate that heating temperature is a critical factor influencing the functional quality of *R. argyrotaenia*. Heating at 70°C preserved a greater proportion of albumin concentration and antioxidant activity than heating at 80°C. Therefore, controlling the processing temperature may represent a practical approach to preserving the nutritional value and functional properties of *R. argyrotaenia* as a locally available food resource.

CONCLUSIONS

The present study demonstrated that heating temperature is an important factor influencing the albumin concentration and antioxidant activity of *R. argyrotaenia*. Heat treatment at 70°C and 80°C progressively reduced albumin concentration and antioxidant activity, as evidenced by lower albumin concentrations, higher IC_{50} values, and lower Antioxidant Activity Index (AAI) values. Compared with heating at 80°C, heat treatment at 70°C better preserved the functional quality of *R. argyrotaenia* by maintaining higher albumin concentration and antioxidant activity. These findings highlight the importance of temperature control during thermal processing to preserve the nutritional and functional properties of *R. argyrotaenia* and provide a scientific basis for its development as a functional food derived from local freshwater fish resources. Future studies should investigate specific antioxidant compounds, amino acid profiles, and heat-induced protein structural changes to further elucidate the mechanisms responsible for the reduction in albumin concentration and antioxidant activity during thermal processing.

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