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Determination of Albumin Level of Patin Fish from Kahayan River, Central Kalimantan by Biuret Method

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Abstract. Albumin plays important roles in the health of the body, such as stabilizing intravascular fluid pressure, facilitating molecular transport, and facilitating fluid movement in the body. The high cost of albumin, the limited availability of albumin sources, and the uncertainty surrounding its halal status underscore the imperative for this research. The objective of this study was to identify alternative sources of albumin, with a focus on exploring non-fish sources. Patin fish, a species endemic to Central Kalimantan, is a promising alternative due to its abundance and cost-effectiveness. The albumin content of steamed and dipped patin fish at 10, 15, 20, and 25 minutes was determined using the biuret method. The findings indicated that steaming yielded higher albumin content than dipping, with values of 4.41% and 3.88%, respectively. The optimal steaming duration was determined to be 15 minutes, while the optimal dipping duration was 20 minutes. These findings suggest that steaming may serve as a viable alternative source of fish albumin, offering a more economical option than snakehead fish.

Keywords : Albumin, Patin fish, Biuret method, Steaming, Dipping

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Introduction

Albumin belongs to a type of globular proteins that make up approximately 60% of the plasma in human blood. It is soluble in water, acids, bases, and dilute salt solutions. Albumin can be coagulated by heat and precipitated by saturated salts, such as ammonium sulfate. Its physiological functions include the maintenance of normal intravascular fluid pressure, facilitation of molecular transport, and regulation of fluid movement within the body [1]. In health sciences, albumin serves as an indicator of patient health status. Its levels can decline in patients with various medical conditions, including acute and chronic diseases, liver disease, kidney disease with proteinuria, bleeding, burns, and exudates [2,3]. Normal albumin levels in adults are approximately 3.5-5.2 g/dL. The clinical condition of low albumin levels in the body is referred to as hypoalbuminemia, a condition that can lead to various health complications, including fluid retention, metabolic imbalances, growth abnormalities, and muscle atrophy [4]. The necessity for albumin is amplified in postoperative patients to facilitate wound healing [3]. The management of albumin is typically accomplished by administering Human Serum Albumin (HSA), which is exorbitant and its efficacy is yet to be fully ascertained. Consequently, numerous studies have been conducted to identify cost-effective and readily available sources of albumin, to ensure its long-term availability. Several of food items, including beef, snakehead fish, and egg whites, have been reported to have high albumin content. Conversely, beans and some vegetables contain lower albumin levels [5].

The Central Kalimantan region has a landscape comprising rivers and peat swamps. These bodies of water serve as the habitat for a variety of endemic freshwater fish species found in Kalimantan, including Baung (*Hemibagrus nemurus*), Patin (*Pangasius hypopthalamus*), Toman (*Channa micropeltes*), Haruan (*Channa striata*), Jelawat (*Leptobarbus hoevenii*), and Tapah (*Wallago* sp.) [6]. It is well known that some freshwater fish contain an albumin protein called fish albumin. Among the fish species extensively studied for their albumin content, snakehead fish (*Channa striata*) have the highest albumin content. However, the cost of snakehead fish is often high, and their availability decreases. Another fish that is easily available and cheap in peaty waters is the patin fish. In 2023, patin production in Central Kalimantan reached 38,376.05 tonnes, ranking second largest in Indonesia after South Sumatra [7]. This finding indicates the feasibility of using patin fish as a functional ingredient beyond its conventional uses. Other advantages of Patin fish include low sodium, soft meat; easily digested, and contains calcium, iron, and other minerals. A comprehensive analysis reveals a nutritional composition of 68.6% protein, 5.8% fat, 3.5% ash, and 51.3% water, making it a notable source of nutrients for human consumption. The high protein content in patin fish can serve as a significant source of protein in daily food intake. Notably, patin fish fat is characterized by a significant proportion of unsaturated fatty acids, which have been linked to enhanced health benefits [8].

The method of preparation, whether through frying, baking, steaming, or dipping, has been demonstrated to decrease fish's nutritional value and albumin content [9]. Conversely, elevated cooking temperatures, such as those attained during frying or grilling, have markedly diminished fish's nutritional value compared with less extreme methods like steaming or dipping, which have been shown to maintain protein content [10]. This study aims to compare the albumin content of patin fish (*Pangasius sp*.) subjected to steaming and dipping methods. The study will also examine the effects of varying cooking times on the optimal processing technique for patin fish with high albumin content. The albumin content of patin fish post-treatment was analyzed using the Biuret method with a bovine serum albumin (BSA) standard solution.

Experimental

Materials and Equipment. The patin fish used in this study were procured from Kahayan River Harbour, Palangka Raya City, Central Kalimantan. The following chemicals were utilized: acetate buffer, diethyl ether, sodium sulfite, potassium sodium tartrate, sodium hydroxide, copper (II) sulfate hydrate, BSA, and distilled water. The research equipment included an analytical balance, a centrifuge, a stopwatch, a shaker incubator, a thermometer, and a Barcov BRQ-UV series UV-Vis spectrophotometer.

Patin Fish Steaming. The patin fish was meticulously prepared by first removing the head, scales, entrails, and fins. Subsequently, the fish was meticulously divided into four equal segments and thoroughly washed. A container of water at a tem-

perature of approximately 80°C was prepared for the steaming process, with the patin fish being steamed for 10, 15, 20, and 25 minutes, respectively. The fish pieces and the accumulated water were removed and left for five minutes to facilitate the separation of the bones and skin from the meat. The meat obtained was then ground using a mortar [9].

Patin Fish Dipping. The preparation of the fish prior to piping was carried out using steps similar to those before. Each piece of fish was individually wrapped in aluminum foil and subsequently placed into a container of water at a temperature of approximately 80°C. The fish pieces were then subjected to pressure for various durations, ranging from 10 to 25 minutes. After the dipping phase, the fish pieces were meticulously sorted, and the meat was extracted and ground together with the puddle water [9].

Extraction of Patin Fish Albumin. Pureed patin fish meat, weighing 10 grams, was added to 25 mL of acetate buffer, pH 5. The mixture was then shaken for 10 minutes at an agitation speed of 250 rpm. Subsequently, the mixture centrifuge at 10,000 rpm for 20 minutes. The resulting clear layer, containing the protein, was then separated from the solid residue by centrifugation. Subsequently, 2 mL of a 25% sodium sulfite solution and 2 mL of diethyl ether were added to the protein layer. The mixture was then subjected to a second round of centrifugation at 10,000 rpm for 20 minutes. The upper layer, containing the ether and other proteins, was then separated. The remaining solution, the lower layer, was obtained as the albumin fraction [11].

Albumin Analysis Using Biuret. The biuret reagent was prepared by mixing 0.15 g of cupric sulfate hydrate, 0.6 g of sodium tartrate, 30 mL of 10% sodium hydroxide, and adding distilled water to the limit of 100 mL volumetric flask. Subsequently, 1 mL of albumin serum extracted from raw and cooked meat was added with 4 mL of distilled water, followed by homogenization. 2.5 mL of biuret was added, and the mixture was left to stand for 30 minutes at room temperature. The albumin levels were subsequently determined using a UV-Vis spectrophotometer at an absorption maximum of 540 nm. The resulting value was then plotted on the standard curve for BSA [12].

Result and Discussion

The determination of albumin extract content by steaming and dipping, as well as time variations, was carried out from the plotting results of the extract's absorbance with the prepared BSA standard curve that had been prepared. The regression equation obtained from the BSA standard curve was y = 0.5035x + 0.0731, with a value of R^2 = 0.9966. This equation demonstrates a strong correlation between the measured values of the extracted albumin and the known concentrations of albumin (see Figure 1).

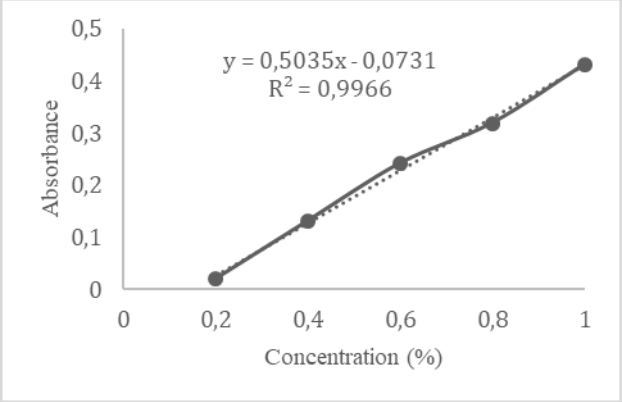

Figure 1. Standard curve of BSA

The extraction of albumin employing acetate buffer solution (pH 5), sodium sulfite, and diethyl ether demonstrated efficacy. The results of the extraction of patin fish albumin by steaming and dipping show differences in volume and albumin concentration. The steaming technique enables direct interaction between the water vapor and the fish meat, as previously reported by Chasanah & Raditya [13]. This approach yielded a greater volume of extract and facilitated the dissolution of a higher proportion of albumin than the dipping technique (see Figures 2 and 3). Additionally, the use of aluminum foil for the fish packaging prevention of moisture interaction with the fish meat, leading to a lower concentration of dissolved albumin. However, the use of aluminum foil as a wrapping material for fish meat can also result in the application of elevated heat levels, which have the potential to cause damage to the albumin [14, 15].

The albumin concentration in the raw meat extract of patin fish was 4.75%. However, following the steaming process, a decline in albumin extract concentration was observed, reaching 3.28%, 4.14%, 3.67%, and 3.56% at 10, 15, 20, and 25 minutes, respectively. The percent loss of albumin

was 30.9%, 12.8%, 22.7%, and 25.1%, respectively. In contrast, the concentration of albumin extract decreased to 2.98%, 3.66%, 3.88%, and 3.56% after dipping 10, 15, 20, and 25 minutes, respectively. The respective percent loss of albumin was 37.3%, 22.9%, 18.3%, and 25.1%. The albumin levels were most reduced by steaming for 15 minutes (4.14%) and dipping for 20 minutes (3.88%). The findings indicated that the steaming technique was more effective in extracting albumin in patin fish compared to dipping. It is also worth mentioning that the albumin level in patin fish is not much different from that in raw snakehead fish, which is around 6.2% [16].

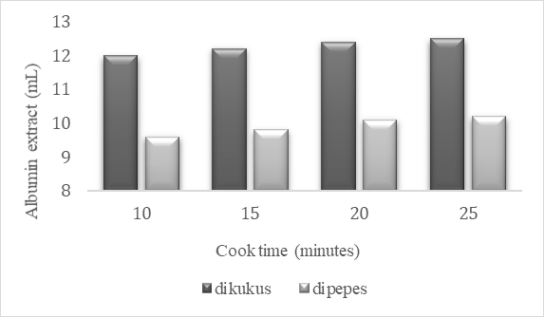

Figure 2. Volume of albumin extracts

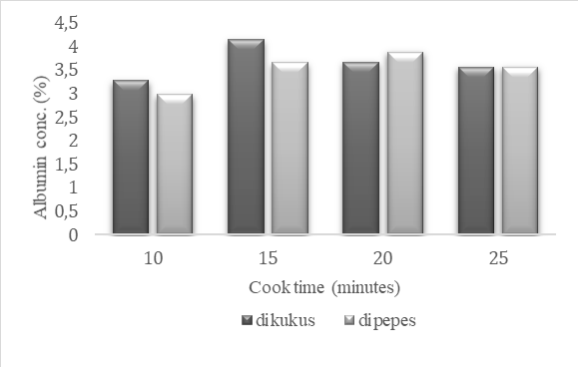

Figure 3. Albumin concentration after cooked

Albumin is a simple protein consisting of a single polypeptide chain with a molecular weight of 66.4 kD and composed of 585 amino acids. Beyond its role in repairing damage to body cells, albumin is also imperative for cell growth and maintenance during the critical stages of growth and development in children. A deficiency of albumin in toddlers can impede cell development in the brain, resulting in malnutrition and stunting. Albumin is typically sourced from blood, specifically from human, pig, and cow plasma (bovine serum albumin). However, concerns regarding its halal status persist. An alternative source of albumin is fish albumin, which is extracted from various types of fish using water solvents to maintain its original structure and functional properties so that it is not denatured during extraction [17]. Denatured proteins undergo secondary, tertiary, and quaternary structural modifications without breaking peptide bonds and changes in the amino acid sequence of their structure. These structural modifications generally induce irreversible changes in the physicochemical properties of proteins, such as the loss of solubility and biological activity [18].

The steaming and dipping techniques were selected because they ensure that the water does not contact the fish directly, minimizing the loss of nutritional value compared to boiling, frying and grilling. These methods also prevent excessive heat from interacting with the fish protein, thereby preventing protein denaturation. The water vapor formed during steaming has been found to dissolve only the fat and albumin in the fish. At temperatures of 80°C, albumin begins to clump, and the protein becomes less rigid, facilitating digestion. Conversely, temperatures exceeding 100°C lead to more rapid coagulation, resulting in a harder and more dense protein structure [19, 20]. The cooking duration also impacts the decline in albumin levels, as fluctuating temperatures can lead to its degradation [21,22]. The proteolysis process, the decomposition of protein and fat by enzymes contained in meat at low pH, is another potential cause of the decrease in albumin levels. The initial stage of this process involves the breakdown of protein into larger macromolecules, leading to increased protein dehydratio [23,24].

Conclusion

The extraction of albumin from patin fish was successfully conducted using acetate buffer pH 5, sodium sulfite, and diethyl ether. The Biuret method was used to determine the albumin content, and the results showed that steaming for 15 minutes and dipping for 20 minutes had the highest albumin content of 4.41% and 3.88%, respectively. These findings indicate that these specific cooking times produce the least albumin loss. This study findings are considered as an alternative source of albumin from Central Kalimantan endemic fish that can be extracted or consumed directly for people with hypoalbuminemia.

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Author Contributions

The research was designed and supervised by Yuliana, who also wrote the article, while three other students (Dimas Pramudita, Wenika Simbolon, Marsya Imelya) carried out the laboratory experiments.

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