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Effect Of Drying Methods (Freeze Drying And Oven Drying) On The Characteristics Of Fish Protein Hydrolysate From Tuna Meat Flakes

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Abstract - Tuna processing industry by-products in the form of tuna meat flakes still have potential for further utilization, one of which is as a raw material for protein hydrolysate production. This study aimed to analyze the effect of drying methods on the chemical characteristics, degree of hydrolysis, and yield of fish protein hydrolysate (FPH) produced from tuna meat flakes through enzymatic hydrolysis using bromelain enzyme. The observed parameters included proximate analysis (moisture, ash, protein, fat, and carbohydrate contents), degree of hydrolysis (DH), and yield. The results showed that the hydrolysis process using bromelain enzyme produced a degree of hydrolysis value of 44.11 ± 1.40%, with a final process pH of 6.3, indicating that the proteins in tuna meat flakes were successfully hydrolyzed into smaller peptide molecules. The drying method significantly affected the chemical characteristics and yield of the fish protein hydrolysate. The oven-drying method was more effective in reducing moisture content, whereas freeze-drying was better at preserving nutritional components, particularly protein content, which reached 54.74 ± 0.59%, and produced a yield of 5.40%, higher than that obtained with oven drying (4.37%). Overall, freeze drying is recommended as the optimal drying method for producing fish protein hydrolysate with better nutritional quality and functional potential.

Keywords: Fish protein hydrolysate, Tuna, Bromelain, Freeze drying, Oven drying

I. INTRODUCTION

Tuna (*Thunnus* sp.) is one of the most economically important pelagic fishery commodities in Indonesia, serving both as a protein source and as a leading export commodity. High national tuna production is closely linked to intensive processing activities, particularly in the fillet and loin industry. However, these processing operations generate large volumes of solid waste, which can account for 50–60% of the total raw material weight, consisting of heads, bones, skin, and meat flakes still adhering to bones [1]. If not optimally utilized, this waste can cause environmental problems while simultaneously leading to a loss of potential value-added opportunities from fishery resources.

One portion of this waste with great potential for utilization is tuna meat flakes. This fraction still contains a high amount of good-quality protein, making it suitable for conversion into value-added products. According to [2], tuna processing by-products still contain 47.2% protein, 14.2% fat, and 35.2% ash, indicating that these by-products remain viable as raw materials for food products. One approach that can be taken is through protein hydrolysis to produce fish protein hydrolysate (FPH). Protein hydrolysate is the result of breaking down complex proteins into smaller peptides and free amino acids that are more easily absorbed and possess certain functional properties [3]. This is because most hydrolysis-derived peptides have low molecular weights (<2,500 Da), contributing to high protein solubility of 90.3–98.2% and ease of digestion and absorption by the body [4].

In addition to high nutritional value, fish protein hydrolysate is also known for its characteristic sensory properties, including a savory (umami) taste derived from free amino acids such as glutamate and aspartate. These compounds play an important role in naturally enhancing the flavor of food products [5]. In recent years, the use of fish protein hydrolysate as a natural food additive has been increasingly developed, particularly as a flavor enhancer. This aligns with growing consumer awareness of the use of natural ingredients and the reduction of synthetic additives such as monosodium glutamate (MSG), which in excessive consumption has been reported to potentially cause metabolic and neurological disorders [5]. Furthermore, the utilization of fishery industry waste and by-products into protein hydrolysate supports the implementation of a circular economy through value addition, resource utilization efficiency, and reduction of potentially polluting waste [6].

The production of fish protein hydrolysate is generally carried out through enzymatic hydrolysis using protease enzymes such as bromelain, papain, and Alcalase. Bromelain has been widely used in the production of protein hydrolysate from fish by-products because it can effectively hydrolyze proteins into smaller peptides and produce good bioactivity [7]. Papain is also an effective proteolytic enzyme in producing fish protein hydrolysate with a high degree of hydrolysis and good functional characteristics [8]. Meanwhile, Alcalase is one of the most widely used commercial protease enzymes in the production of protein hydrolysate from fishery by-products due to its high hydrolysis efficiency and ability to produce bioactive peptides [9]. Enzymatic methods are more widely used because they are selective, produce products of higher quality, and minimize the formation of undesirable compounds compared to chemical hydrolysis methods. Additionally, fish protein hydrolysate has been reported to have potential as functional food because it contains bioactive peptides with antioxidant properties and various other health benefits [10]. Bromelain was chosen because it is a protease derived from pineapple, is readily available, and relatively inexpensive. The use of bromelain on fishery by-products has also been reported to produce hydrolysate with high protein content and good antioxidant activity, thus potentially supporting the sustainable utilization of fishery waste [11]. Furthermore, bromelain demonstrates high hydrolysis effectiveness on fish proteins, including fishery by-products such as viscera and residual meat, making it widely used in the development of fishery-based protein hydrolysate [8].

After the hydrolysis process, the drying step is one of the important stages that determines the final quality of the resulting protein hydrolysate. Different drying methods can affect protein stability, moisture content, solubility, and the functional properties of the product. Two commonly used drying methods are freeze drying and oven drying [12]. Freeze drying is a low-temperature drying method capable of maintaining protein structure and bioactive compounds because the process occurs without direct heating, thus better preserving product quality [13]. In contrast, oven drying uses higher temperatures, is relatively simpler and more economical, but may cause changes in physicochemical properties and protein denaturation [12].

Although various studies have been conducted on the production of fish protein hydrolysate, research on the effect of drying methods on the characteristics of protein hydrolysate from tuna meat flakes remains limited. Yet, this information is crucial to determine the optimal processing method for producing a product with the best quality, both in terms of nutrition and functional properties. Based on this, the present study aims to analyze the effect of drying methods (*freeze drying and oven drying*) on the characteristics of fish protein hydrolysate produced from tuna meat flakes. The results of this study are expected to provide scientific information supporting the development of technologies for converting fishery waste into value-added products, as well as serving as a basis for developing fish protein hydrolysate as a natural food additive in the food industry.

II. MATERIALS AND METHODS

2.1 Materials

The main raw material used in this study was tuna meat flakes obtained from PT Benteng Laut Sejahtera, Bitung, North Sulawesi. Materials used in the production of fish protein hydrolysate (FPH) included bromelain enzyme (Nusaherbs brand) with an enzyme activity of 50,000 u/g as a hydrolysis catalyst, and distilled water as a solvent. Chemical reagents used for analysis included sodium hydroxide (NaOH) (Merck®, pro analysis/p.a.), hydrochloric acid (HCl) (Merck®, pro analysis/p.a.), sulfuric acid (H_2SO_4) (Merck®, pro analysis/p.a.), trichloroacetic acid/TCA (Merck®, pro analysis/p.a.), buffer solution (Merck®, pro analysis/p.a.), and other supporting materials used as required for laboratory analysis.

2.2 Equipment

Equipment used in this study included a freeze dryer (Cryodos 50Nr Telstar, Spain), a drying oven (Shellab, USA), a centrifuge (Hettich Universal 320R, Germany), a water bath (Memmert, Germany), a pH meter (Suntex SP2100, Taiwan), an analytical balance (A&D GR-202, Japan), and standard laboratory glassware such as graduated cylinders, Erlenmeyer flasks, and beaker glasses (Pyrex/Duran, USA/Germany). Analytical instruments included a UV-Vis spectrophotometer (Thermo Scientific, USA) and proximate analysis equipment according to the respective test parameters.

2.3 Methods

Fish protein hydrolysate was produced using enzymatic hydrolysis, referring to previous research with several modifications [14]. Tuna meat flakes were first cleaned and washed under running water, then minced to homogeneity to increase substrate surface area. The minced material was then mixed with distilled water at a ratio of 1:1 (w/v). The mixture was subsequently adjusted to pH 6.5–7.0 using 0.1 N NaOH or 0.1 N HCl, which represents the optimum condition for bromelain enzyme activity [7]. The mixture was then heated to 55°C using a water bath. Bromelain enzyme was added to the mixture at a concentration of 0.4% (w/w) relative to the substrate. Hydrolysis was carried out for 4 hours in a water bath with continuous agitation. After hydrolysis was complete, the enzyme was inactivated by heating at 90°C for 15 minutes to stop the hydrolysis reaction. The mixture was then centrifuged at 10,000 rpm for 15 minutes to separate the supernatant from the residue. The resulting supernatant was used as liquid fish protein hydrolysate. The liquid FPH was then divided into two portions for different drying treatments: freeze drying at −33.9°C and 0.073 mBar vacuum for 40 hours until a dry powder was obtained, and oven drying at 60°C for 24 hours until a dry powder was obtained.

2.4 Degree of Hydrolysis Analysis

The degree of hydrolysis (DH) of the protein hydrolysate was determined by measuring the free amino acid content following the method described by [15]. Approximately 20 mg of protein hydrolysate was added to 20 mL of 10% TCA solution (w/v), then allowed to stand for 30 minutes. The sample was then centrifuged at 7,800 rpm for 15 minutes. The resulting supernatant was analyzed for nitrogen content using the Kjeldahl method (AOAC, 2005) with a conversion factor of 6.25. The degree of hydrolysis value was then calculated using the following formula:

$$DH\ (\%) = (N\text{-}TCA / N\text{-}Total) \times 100\%$$

2.5 Proximate Analysis

Proximate analysis was conducted to determine the chemical composition of the fish protein hydrolysate, including moisture, ash, protein, fat, and carbohydrate contents. Moisture content was determined by the oven method following Indonesian National Standard 01-2354.2-2015 on chemical testing of moisture content in fishery products. Ash content was determined based on Indonesian National Standard 2354.1:2010 on chemical testing method part 1, namely the determination of ash and acid-insoluble ash in fishery products. Protein content analysis was performed using the total Kjeldahl method in accordance with Indonesian National Standard 01-2354.4-2006 on protein content determination in fishery products, while total fat content was analyzed based on the method in Indonesian National Standard 2354.3:2017 on chemical testing method part 3 for total fat content in fishery products. Carbohydrate content was determined by the by-difference method, calculated as the remainder after subtracting moisture, ash, protein, and fat contents from 100%.

2.6 Experimental Design

This study used a Completely Randomized Design (CRD) with one factor, namely the drying method, consisting of two treatments: freeze drying and oven drying, each with two replications. Data were statistically analyzed using an Independent

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Samples t-test at a 95% confidence level ($\alpha = 0.05$) to determine differences between treatments. All statistical analyses were performed using IBM SPSS Statistics 27.

III. RESULTS AND DISCUSSION

3.1 Degree of Hydrolysis

The degree of hydrolysis (DH) is the primary parameter used to describe the extent of peptide bond cleavage during protein hydrolysis by an enzyme. The DH value represents the percentage of peptide bonds cleaved relative to the total peptide bonds available in the protein substrate, making it an important indicator for assessing the success of the hydrolysis process and the quality of the resulting protein hydrolysate. A higher DH value indicates that more protein has been degraded into short-chain peptides and free amino acids, which generally correlates with improved functional properties and bioactivity of the product [16].

The analysis results showed that the tuna fish protein hydrolysate produced had a degree of hydrolysis value of 44.11 ± 1.40%, with a final process pH of 6.3. This value indicates that the hydrolysis process using bromelain enzyme was effective in breaking down the proteins of tuna meat flakes into peptides with smaller molecular sizes. According to [17] protein hydrolysates with high DH values generally produce short-chain peptides with better solubility, high bioavailability, and greater potential for biological activity compared to intact proteins. Thus, the DH value obtained in this study is classified as high and demonstrates the success of the optimal hydrolysis process.

The high DH value in this study is thought to be influenced by several factors, including the effectiveness of the bromelain enzyme, pH conditions close to the optimum, and hydrolysis temperature suitable for proteolytic enzyme activity. Bromelain is known to have optimum activity at neutral to slightly acidic pH ranges and at approximately 55°C, so the process conditions used in this study supported maximum peptide bond cleavage [14]. Additionally, according to [18], the more suitable the process conditions are to the enzyme's optimum conditions, the higher the hydrolysis efficiency and resulting DH. Therefore, the DH value of 44.11% indicates that the hydrolysis process successfully produced fish protein hydrolysate with good potential functional quality for development as a value-added food ingredient.

3.2 Yield

Yield is one of the important parameters in fish protein hydrolysate production because it describes the efficiency of the processing in converting raw materials into the final product. The higher the yield value, the greater the amount of product obtained from the initial raw material, thus the process is considered more efficient both technically and economically. The results showed that the yield of fish protein hydrolysate (FPH) in the freeze drying treatment was 5.40%, higher than that of the oven drying treatment at 4.37%. This difference indicates that the drying method affects the quantity of the final product, where the freeze drying method was able to achieve higher solid retention compared to oven drying.

The higher yield in the freeze drying treatment is related to the low-temperature drying mechanism through the sublimation process, which is capable of retaining more dissolved solid components, including peptides, soluble proteins, and bioactive compounds, resulting in less mass loss during the drying process. In contrast, in oven drying, heat exposure during the drying process can cause loss of volatile components and partial degradation of organic compounds, resulting in a lower final product weight. According to [19], the yield of fish protein hydrolysate is strongly influenced by the type of raw material, the efficiency of enzymatic hydrolysis, and the drying method used. Additionally, [20] reported that the drying process is one of the important stages that affects the stability of bioactive components and the retention of biomolecules in fish protein hydrolysate, which can directly affect the final product quantity or yield.

3.3 Proximate Characteristics of Fish Protein Hydrolysate

Proximate characteristics are one of the main parameters used to evaluate the nutritional quality and stability of a food product, including fish protein hydrolysate (FPH). Proximate analysis includes moisture, ash, protein, fat, and carbohydrate contents, which collectively describe the chemical composition of the final product and serve as important indicators in determining the quality of dry food materials. In protein hydrolysate products, proximate composition is not only influenced by the quality of the raw material and the hydrolysis process, but is also greatly affected by the applied drying stages [16]. Different drying methods can cause changes in moisture content, nutrient concentration, and the stability of bioactive components due to differences in temperature, pressure, and water removal mechanisms during the process [7]. In this study, the drying methods compared were oven drying and freeze drying, each with different process characteristics. The results of the proximate analysis of FPH by drying method are presented in Table 1.

Table 1. Proximate Characteristics of Fish Protein Hydrolysate from Tuna Meat Flakes Based on Drying Method

Parameter	Oven Drying (%)	Freeze Drying (%)
Moisture Content	9.66 ± 0.06	12.13 ± 0.46
Ash Content	6.28 ± 0.07	6.64 ± 0.22
Protein Content	53.87 ± 0.19	54.74 ± 0.59
Fat Content	1.27 ± 0.08	1.64 ± 0.02
Carbohydrate	28.93 ± 0.13	24.85 ± 1.25

3.4 Moisture Content

Moisture content is an important parameter in fish protein hydrolysate as it directly affects product stability, shelf life, and microbiological safety during storage. The analysis results showed that the moisture content of the fish protein hydrolysate (FPH) dried using the oven drying method was 9.66 ± 0.06%, lower than that of the freeze drying treatment at 12.13 ± 0.46%. Based on the Independent Samples t-test results, a significance value of $p<0.05$ was obtained, indicating that the drying method had a significant effect on the moisture content of FPH. This difference shows that the drying method plays an important role in determining the efficiency of water removal in the final product.

The lower moisture content in the oven drying treatment is presumably due to the use of direct heat that accelerates the evaporation of free water from the material matrix. During the drying process, the increase in temperature causes increased mobility of water molecules, so that water transfer from the internal parts of the material to the surface occurs more rapidly. According to [7], the use of high temperatures during the drying process significantly increases the efficiency of free water removal, resulting in a product with lower final moisture content. These results are consistent with the findings of [12], who reported that fish protein hydrolysate dried using an oven had lower moisture content compared to freeze drying because heat drying is more effective in reducing the free water content in the material.

In contrast, the higher moisture content in the freeze drying treatment is related to the drying mechanism through sublimation, namely the phase change of water from ice to vapor under vacuum conditions and low temperature. Although this method is very effective in maintaining protein structure and heat-sensitive compounds, some bound water may remain trapped in the material matrix if the secondary drying process is not yet optimal [21]. Nevertheless, freeze drying is known to be superior in maintaining the pore structure of the material and improving the rehydration capacity of the product, so it is often recommended for protein products that require better nutritional stability and functional properties [22].

3.5 Ash Content

Ash content is a parameter that describes the total amount of inorganic minerals contained in a food material and is often used as an indicator of mineral content in fish protein hydrolysate. The analysis results showed that the ash content of fish protein hydrolysate (FPH) in the freeze drying treatment was 6.64 ± 0.22%, slightly higher than that of the oven drying treatment at 6.28 ± 0.07%. This indicates that the mineral content of the product is relatively stable with respect to differences in the drying method used.

In general, mineral content in food materials has high thermal stability and is therefore not easily degraded during processing, including during drying. These results are consistent with the research of [12], who reported that the drying method

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did not have a significant effect on the ash content of fish protein hydrolysate because minerals are relatively resistant to temperature changes. In addition to being affected by the drying method, ash content can also be influenced by the type of raw material, initial preparation processes, and the degree of separation of the liquid fraction during the hydrolysate production process. According to [23], the mineral content in fish protein hydrolysate is greatly influenced by the characteristics of the raw material and the efficiency of the separation process during the centrifugation and filtration stages.

3.6 Protein Content

Protein content is the primary parameter in evaluating the quality of fish protein hydrolysate as it reflects the concentration of soluble protein obtained through the enzymatic hydrolysis process and retained during the drying stages. The analysis results showed that the protein content of fish protein hydrolysate (FPH) in the freeze drying treatment was 54.74 ± 0.59%, slightly higher than that of the oven drying treatment at 53.87 ± 0.19%. This indicates that both drying methods were still able to maintain the protein content of the final product well. Based on the Independent Samples t-test results, the protein content of fish protein hydrolysate dried using the freeze drying method was not significantly different ($p > 0.05$) compared to the oven drying method.

The slightly higher protein content in the freeze drying treatment is presumably related to the low-temperature drying mechanism that is capable of maintaining protein structural integrity and minimizing thermal denaturation during the drying process. According to [7], low-temperature drying methods tend to be more effective in retaining protein content because they do not cause significant damage to peptide bonds or changes in the secondary structure of proteins. Conversely, although the oven drying treatment produced slightly lower protein content, the values still show that heat drying did not cause significant protein loss. The slight decrease is thought to be caused by partial denaturation due to heat exposure, which can alter the protein structure without removing total nitrogen altogether. The research results of [24] showed that the freeze drying method was able to maintain protein structural stability better than heat drying, especially for myofibrillar proteins that are sensitive to high temperatures. Thus, both drying methods are still suitable for use in FPH production; however, freeze drying is more recommended when the primary objective is to maximize protein quality retention.

3.7 Fat Content

Fat content is one of the important parameters in the characterization of fish protein hydrolysate as it affects oxidative stability, sensory quality, and shelf life of the product. The analysis results showed that the fat content of fish protein hydrolysate (FPH) in the freeze drying treatment was 1.64 ± 0.02%, higher than that of the oven drying treatment at 1.27 ± 0.08%. This indicates that the drying method affected the stability of lipids during the production process of the protein hydrolysate.

According to [7], low-temperature drying is more effective in retaining heat-sensitive components, including lipid fractions, because of the minimal direct heat exposure during the process. These results are consistent with the research of [12], who reported that fish protein hydrolysate dried using freeze drying showed better fat retention compared to oven drying due to reduced thermal degradation during drying. The lower fat content in the oven drying treatment is presumably caused by lipid oxidation resulting from heat exposure during the drying process. This process can cause degradation of unsaturated fatty acids into volatile compounds, thereby reducing the measured fat content. Research by [24] showed that drying using high temperatures can increase protein and lipid oxidation in fish products, which impacts the reduction of nutritional quality and product stability. Therefore, although oven drying is more process-efficient, freeze drying is considered superior in maintaining the fat fraction and nutritional quality of FPH, especially when the product is directed toward functional food applications with high nutritional value.

3.8 Carbohydrate Content

Carbohydrate content in fish protein hydrolysate (FPH) in this study was calculated using the by-difference method, based on the remainder after subtracting the sum of moisture, ash, protein, and fat contents from 100%. Therefore, the carbohydrate value is greatly influenced by changes in other proximate components. The analysis results showed that the carbohydrate content of FPH in the oven drying treatment was 28.93%, higher than that of the freeze drying treatment at 24.85%.

The higher carbohydrate value in the oven drying treatment is presumably related to a concentration effect due to lower moisture content compared to freeze drying. Additionally, because the protein and fat contents in the oven drying treatment were slightly lower than in freeze drying, mathematically the carbohydrate value calculated by the by-difference method becomes higher. According to [18], the indirect method of calculating carbohydrates is greatly influenced by the accuracy of other proximate parameters, so small changes in one component can significantly affect the final carbohydrate value.

The lower carbohydrate content in the freeze drying treatment indicates that this method is better able to retain main components such as protein and fat, so proportionally the carbohydrate value becomes lower. Research by [24] showed that low-temperature drying methods tend to better maintain the integrity of nutritional components compared to heat drying, so changes in the relative composition between components can occur in the final product. Thus, the difference in carbohydrate content in this study more reflects changes in the proportion of nutritional components due to the drying method, rather than a direct change in carbohydrate content.

IV. CONCLUSION

Based on the research results, it can be concluded that the drying method affects the chemical characteristics and yield of fish protein hydrolysate (FPH) produced from tuna meat flakes through enzymatic hydrolysis using bromelain. The oven drying method demonstrated better performance in reducing moisture content, while freeze drying was more effective in preserving nutritional components and producing a higher yield. The hydrolysis process also demonstrated a high success rate, as indicated by the high degree of hydrolysis value, which indicates that the raw material protein has been optimally degraded into smaller-sized peptides.

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