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Protein Concentrate From Tuna Head Waste Using Methanol-Acetone Solvent Extraction

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Abstract. The frigate tuna (Euthynnus affinis) is known for its high protein content in the head, making it suitable for the production of protein concentrate used in animal feed. This research aims to investigate the influence of adding a methanol-acetone mixture solvent, the duration of the extraction process, and the protein content in the resulting concentrate using the Kjeldahl method. The protein concentrate is produced through the maceration extraction method, where 30 grams of the frigate tuna head sample is mixed with a methanol-acetone solvent in various ratios (1:9; 3:7; 5:5; 7:3; 9:1) at a temperature of 50 °C and a stirring speed of 500 rpm, with extraction times ranging from 2 to 6 hours. Subsequently, filtration is performed, and the precipitate is dried using an oven at 100 °C for 30 minutes. The dried sample is then subjected to protein content testing using the Kjeldahl method. Research results indicate that both protein content and extraction yield values increase with the duration of the extraction process, while the water content decreases. The optimal result in maceration extraction is achieved with the methanol-acetone mixture (9:1) treatment and a 6-hour extraction time, yielding a protein content of 89.15 %, water content of 5.57 %, and an extraction yield of 23.86 %.Protein concentrate can be used as animal feed to increase protein needs. Animals that are given sufficient protein will fatten and make the animal healthy

Keywords: acetone, extraction, frigate tuna head, methanol, protein concentrate

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1. Introduction

Fish in the ocean contain different amounts of protein. for example milkfish has around 20-24% protein, catfish has around 12% protein, anchovies contain 18.83% protein and pufferfish have around 21.40% protein [1],[2],[3],[4]. Frigate tuna (Euthynnus affinis) is a type of small, elongated tuna characterized by a scaleless body and a rigid dorsal fin texture. Frigate tuna is highly sought after by consumers for use as food due to its excellent high protein content. The waters in Lamongan Regency are listed as a dominant fishery resource, and frigate tuna is a commonly found species in these waters.

According to the statistical data from the East Java Provincial Fisheries and Marine Affairs Office (DKP), the production of frigate tuna increased from 47,083.85 tons in 2019 to 52,747.57 tons in 2020.

The significant increase in production is not accompanied by information on waste processing. The utilization of waste from frigate tuna heads is also suboptimal as only 50% of the frigate tuna meat is consumed, with the rest being discarded as waste [5]. The waste from frigate tuna heads has a protein content of 60.56%, making it suitable for use as protein concentrate. The processing of frigate tuna into protein concentrate is carried out through the maceration method with the addition of an organic solvent.

According to previous research conducted by Manullang in 2018, the production of fish flour from catfish head material containing 27% protein through a boiling process resulted in a protein content of 23.25% [6]. Another study by Apriyana in 2014 reported that the production of fish flour from catfish head material yielded a protein content of 9.97% [7]. Research conducted by Widiyanto in 2018, focusing on the production of fish flour from catfish head material, found a protein content of 19.66% [8].

The duration and use of solvents significantly influence the final outcome of the protein concentrate product. Protein concentrate is a powdered product with a protein concentration above 20%. It is produced through the removal of fat and oil components, resulting in a high protein content. The longer the extraction time, the more fat and oil are extracted, leading to an increase in protein content [9]. It is expected that the addition of a methanol-acetone mixture solvent during the extraction process will result in a higher quantity of extract over time. The produced extract has qualities and characteristics that can be observed based on water and protein content, thereby affecting the quality of the protein concentrate as animal feed [10].

The objective of this research is to examine the influence of adding a methanol-acetone mixture solvent and the duration of the extraction process from frigate tuna head waste. Additionally, it aims to assess the protein content in the protein concentrate obtained from frigate tuna head waste using the Kjeldahl method.

2. Methods

2.1. Research Method

The materials used are waste from frigate tuna heads obtained from Siwalankerto Market, Surabaya, and a methanol-acetone solvent obtained from a chemical supply store located on Tidar Street, Surabaya.

2.2. Procedures

In the production of modified protein concentrate (Siagian, 2019), waste from frigate tuna heads is washed, cut into small pieces, and dried in an oven for 120 minutes at a temperature of 100°C. It is then ground and sieved to a size of 40 mesh, and 30 grams are weighed [11]. Extraction is performed using the maceration method with a 1:3 ratio between the material and a methanol-acetone mixture solvent according to the specified variables (1:9; 3:7; 5:5; 7:3; and 9:1), totaling 90 ml, over variable time periods (2; 3; 4; 5; and 6 hours), with stirring at 500 rpm at a temperature of 50°C. The mixture is then filtered to separate the liquid and sediment. The sediment is dried in an oven at 100°C for 30 minutes. Subsequently, grinding and sieving are performed with a 40-mesh size. The resulting protein concentrate is then analyzed for protein content using the Kjeldahl method.

Different solvent ratios will get different yield results, the more polar the solution in the container will increase the yield obtained [12]. The longer the extraction time will increase the amount of yield obtained, this is because the contact time between the solvent and the material is longer [13]. The recommended temperature in the extraction process is 50°C, because too high a temperature will damage the material and will cause the solvent to evaporate more quickly. The best results are at an extraction temperature of 50°C [14].

Protein content analysis based on SNI No. 01-2354.4-2006 using the Kjeldahl method consists of three stages: destruction, distillation, and titration. In the modified protein content analysis [15].

(a) Destruction

100 mg of the sample is placed in a 100 ml Kjeldahl flask, added with 4.875 g potassium sulfate, 0.075 g copper sulfate, 0.05 g selenium, and 15 ml concentrated sulfuric acid. The mixture is shaken until homogeneous and heated in an acid cabinet until the fumes cease for approximately 2 hours. Heating is continued until boiling, and the clear liquid is maintained for 30 minutes and allowed to cool.

(b) Distillation

The destruction result is distilled by slowly adding 50 ml of 40% NaOH, then adding distilled water up to 100 ml. I tis then heated to boiling until the liquid is mixed. The distillation result is collected in an Erlenmeyer flask containing 15 ml of 4% boric acid and 3-5 drops of methyl red indikator.

(c)Titration

The distillation result is titrated with 0,1 N hydrochloric acid. The titration endpoint is marked and completed by a color change to pink. Titrations are performed three times to obtain a constant concentration. The formula for determining the protein content is :

$$\frac{(V2-V1)\times N\ HCl\ x\ BE\ N\times FK}{Sample\ weight\ \ (mg)}\times 100\% \qquad (1)$$

where:

V1 = Volume of blank HCl,

V2 = Volume of sample HCl,

N HCl= Normality of HCl,

BE N = Nitrogen equivalence (14.007),

FK = Conversion Factor (6.25).

3. Results and Discussion

The research material, which is waste from frigate tuna heads, underwent analysis to determine its protein content using the Kjeldahl method, water content using the gravimetric method, and Cd and Pb levels using the AAS (Atomic Absorption Spectrophotometry) method at the Laboratory of the Institute for Industrial Research and Consultation, Ketintang, Surabaya. The analysis results of the frigate tuna head waste are as follows:

Table 1. Results of the analysis of frigate tuna head waste.

Material	**Type of Analysis**			
	Protein content	Water content	Pb metal	Cd metal
Frigate tuna head flour	60,56%	17,80%	0,35 mm/kg	0,13 mm/kg

From Table 1, it is compared with the SNI 01-2715-1996 regarding Grade II fish meal with a minimum protein content of 55% and a maximum water content of 12%. According to SNI 2729:2013, the permissible consumption of heavy metals Cd and Pb in animal feed has a maximum limit of 0.5 mg/kg for Cd and 0.4 mg/kg for Pb. This complies with the Indonesian National Standard, indicating that frigate tuna head flour can be utilized for protein concentrate production.

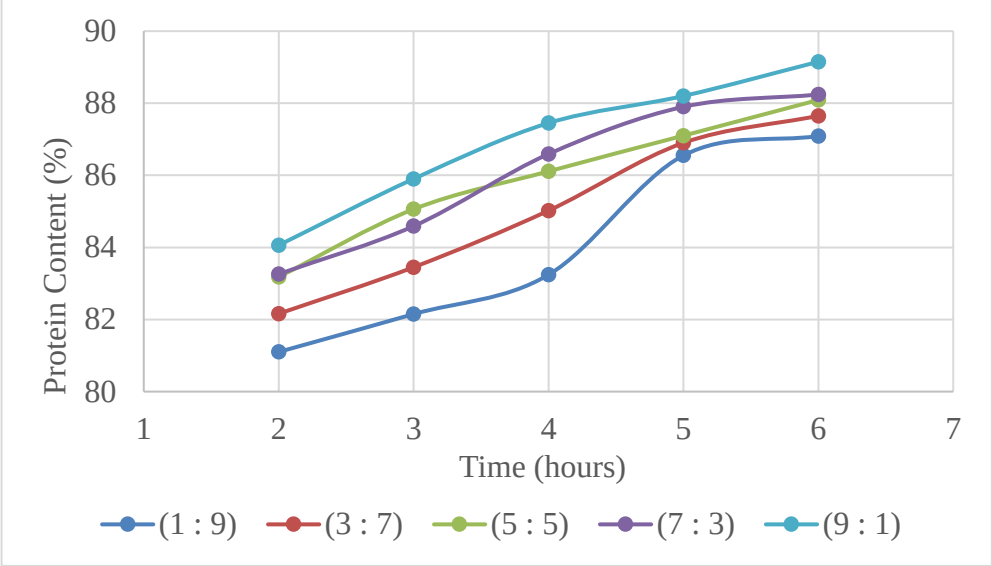

Figure 1. Relationship between Extraction Time and Protein Content

Based on Fig.1, the graph shows the relationship between extraction time and protein content, with protein content ranging from 81.1% to 89.15%. The highest protein content was achieved with the methanol-acetone mixture (9:1), reaching 89.15%. This aligns with SNI 3148.3 2009, which specifies a minimum protein content of 30% for layer chicken feed ingredients. The high protein content is attributed to the prolonged contact time between the material and the solvent, allowing compounds like fats and oils to dissolve in the solvent, while a significant amount of protein precipitates in the material. According to Heriansyah, the ability of each solvent to dissolve fats and oils is influenced by the polarity of the solvent. Solvents with high polarity (high dielectric constant) can dissolve more solutes. Methanol, with a dielectric constant of (33), is more effective in binding fat and oil compounds compared to acetone, which has a dielectric constant of (21) [16].

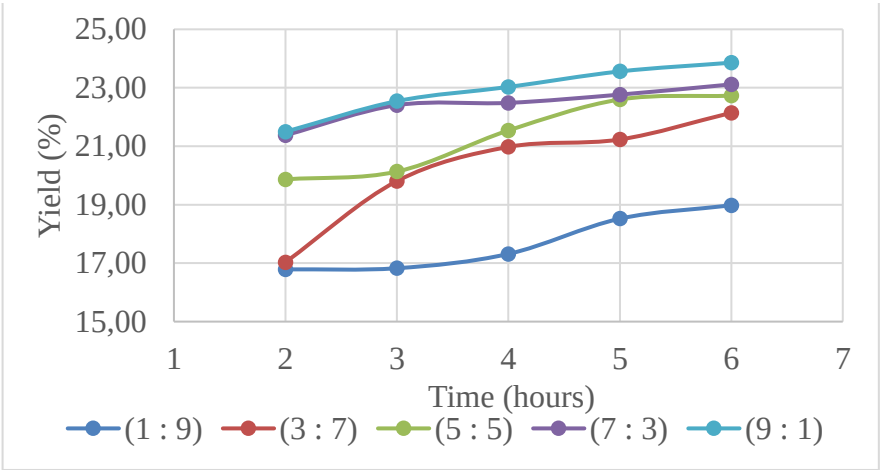

Figure 2. Relationship between Extraction Time and Yield

Based on Fig.2, the graph depicts the relationship between extraction time and yield, with the yield ranging from 16.79% to 23.86%. The highest yield was obtained with the methanol-acetone mixture (9:1), reaching 23.86%. A yield value is considered good if it exceeds 10%. The elevated yield value is attributed to the prolonged contact time, allowing more material to be extracted in the solvent. Methanol, having a higher boiling point (64°C) than acetone (56°C), does not evaporate quickly during the extraction process. This enables it to bind more fats and oils in the material compared to acetone. Stirring during extraction aims to generate a higher yield since the contact between the material and the

solvent occurs more frequently. According to Ningsih (2015), if the material is soaked for too long, more components such as fats that dissolve in the solvent will increase. The longer the extraction time, the higher the yield value obtained. Methanol, as one of the polar solvents, enhances the yield obtained because it can dissolve all organic compounds, contributing to the higher yield value [17].

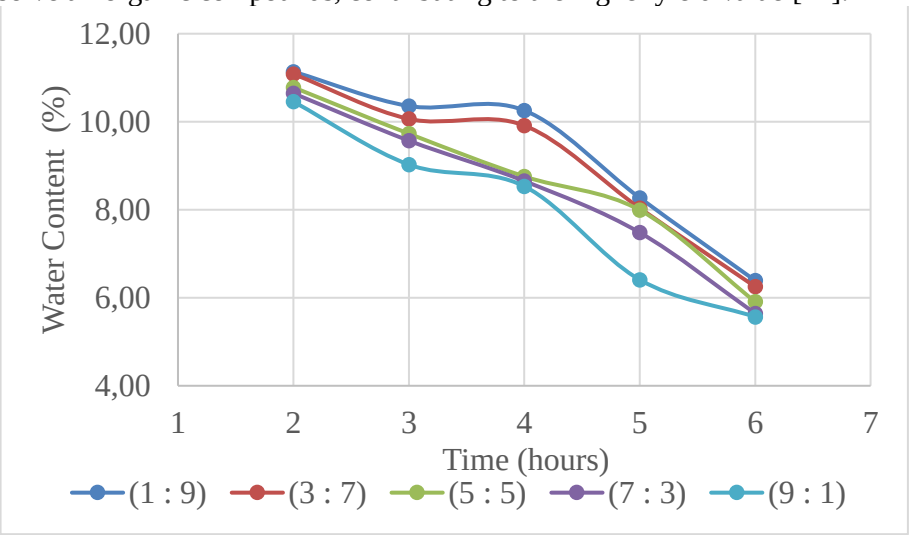

Figure 3. Relationship between Extraction Time and Water Content

Based on Fig.3, the graph shows the relationship between extraction time and water content, with the water content ranging from 5.57% to 11.14%. The optimal water content was achieved with the methanol-acetone mixture (9:1), which is 5.57%. This aligns with SNI 3148.3 2009, which specifies a maximum water content of 14% for layer chicken feed ingredients. The low water content is attributed to the prolonged extraction time, resulting in the evaporation of a significant amount of water vapor and causing a decrease in the material's water content. According to Manfaati (2019), the water content tends to decrease with the duration of the extraction process, This is because the pores in the material open, and the air density becomes loose, facilitating the evaporation of water from the material [18]. According liur (2020) suggests that water content is a determining factor in the shelf life of food materials. Higher water content in feed materials leads to high moisture, promoting bacterial growth and causing the feed material to spoil easily [19],[20].

4. Conclusion

Based on the conducted research, it can be concluded that the extraction time and the mixture of solvents influence the quality of the protein concentrate product. The best results were obtained with a ratio of 9:1 and an extraction time of 6 hours, yielding a protein content of 89.15%, water content of 5.57%, and yield of 23.86%. The protein concentrate in this research can be used as animal feed to fatten livestock. It is hoped that for future research, the heavy metal levels in tuna heads will get a value of 0 so that animal feed will be healthier.

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