Physico-Chemical Characteristics of Fish Protein Concentrate Wild Tilapia (*Oreochromis niloticus*) Using 90% Ethanol Solvent

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Abstract- Wild tilapia is a pest in the ponds of milkfish and shrimp cultivators and has not been widely used by pond farmers so that small wild tilapia is just thrown away. Utilization of wild tilapia meat as a raw material for protein preparations such as protein concentrate is the right target so as to optimize the potential of wild tilapia. Protein concentrates can be extracted using organic solvents such as ethanol. The aim of this study was to extract protein concentrate from wild tilapia using ethanol as a solvent and to analyze its physico-chemical properties. The stages of this research consisted of fat extraction with 90% ethanol and analysis of its physico-chemical properties. The data are discussed descriptively. The FPC yield obtained was around 19.11% with a protein content of 86.51%, a fat content of 0.55%, whiteness of 86.9% and a moisture content of 10.14%. These results indicate that the FPC of wild tilapia which is extracted for fat using 90% ethanol solvent is almost classified as FPC type A because the FPC water content of wild tilapia is slightly higher. The results of this study are considered not optimal so it is necessary to modify the fat extraction method to obtain better FPC quality.

Keywords- extraction, ethanol, tilapia, protein

I. INTRODUCTION

Tilapia live in groups and have territory or territory that can live in fresh or brackish waters. This fish can adapt to various habitats so it is considered a fish that has a high distribution rate in the world. Wild tilapia fish are classified as omnivorous fish so they can live in different environments because they do not depend on certain food sources. The types of food are algae, organic particles, and small invertebrates. Wild tilapia has been designated as one of the invasive alien species (exotic species) that can have the greatest negative impact on native fish habitats.

According to [19] the high nutritional content in fish is very useful for health. Consumption of fish continuously is also proven to be able to inhibit the adverse effects of heart disease. According to nutritionists, consuming 30 g of fish a day can reduce the risk of death from heart disease by up to 50%. Tilapia fish (*Oreochromis mossambicus*) has the potential to be developed because it is a cheap source of animal protein so that tilapia fish is very efficient to be used as PFC.

Proximate Composition of Wild Tilapia [19]	
Proteins	15.87 %
Fat	0.59 %
Water Content	80.87 %
Ash Content	0.34 %

Fish protein concentrate (FPC) is a food ingredient for human consumption, with the amount of protein produced being more than its initial state because it is concentrated [22]. FPC has high protein content with high digestibility [5]. According to [6] FPC is divided into three types, namely type A (minimum protein content of 67.5% and maximum fat content of 0.75%), type B (minimum protein content of 67.5% and maximum fat content of 3%) which is fish meal which does not have a specific odor, taste and color but when added to food ingredients mostly leave the taste of fish. and type C (minimum protein content of 67.5% and maximum fat content of 10%) is fish meal that is made hygienically. According to [17]. Fish protein concentrate is a product produced by removing fat and water to produce a high protein concentrate.

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Concentrate production is generally carried out by separating meat, crushing and washing with water, reducing fat with organic solvents at a predetermined temperature, drying and flouring. Extracting fish protein concentrate can use organic solvents, for example iso-propanol, methanol, ethanol with different time and temperature variations to remove fat and water so that high protein levels are obtained. The use of alcohol solvents can produce good quality FPC, although extraction with alcohol has a weakness, namely that there is still a solvent aroma in the resulting FPC. The process to remove water and fat can be done by pressing, drying or extraction. There are several factors that must be considered to obtain high-quality FPC, including the type of fish, extraction methods, processing stages, raw materials and extraction time. Isopropyl alcohol and ethanol are commercial solvents that are often used in the FPC extraction process. It has the disadvantage that there is still a solvent aroma in the resulting FPC.

The manufacture of fish protein concentrate is an innovation in developing a form of protein that is easy to apply to low protein food products. Fish protein concentrate Mostly applied to high-carbohydrate foods.

II. RESEARCH METHOD

a. Research Object and Location

The object of this research is to make fish protein concentrate from wild tilapia as a pest that is not utilized by pond farmers. Fish protein concentrate concentration was carried out at the THP Laboratory, Faculty of Marine Sciences and Fisheries, Hasanuddin University. Chemical analysis was carried out at the Biochemistry Laboratory, Department of Processing Technology of Prikanan Products, Pangkep State Agricultural Polytechnic. Physics analysis was carried out at the Integrated Laboratory of PKP Hasanuddin University.

b. Research methods

The solvent used in this study was 90% ethanol and the material used was wild tilapia. Referring to research [8] which was modified with the treatment of a solvent: ingredients ratio of 6:1 and a long fat extraction time of 20 minutes, 40 & 60 minutes (data not shown).

c. Research procedure

- ***Sample preparation***

Wild tilapia is obtained from unutilized farmers' crops, then repaired by fillet and skinless method, fish meat that has been skinless then the white meat and red meat are separated then the white meat is crushed using a grinder. Pureed wild tilapia meat is washed in water and ice with a ratio of 1:4 then squeezed using a filter cloth, washing is done 3 times. It is then packaged and frozen in preparation for fat extraction.

- ***Extraction of wild tilapia***

Fat extraction was carried out using 90% ethanol solvent. After going through the freezing and glazing process, the wild tilapia is squeezed and the fat is extracted. The ratio of ethanol solvent and material is 6:1 with a fat extraction time of 20 minutes. The process of extracting wild tilapia fat is carried out by stirring and changing the solvent 3 times to prevent saturation. Then it is filtered and pressed to get the precipitate. The FPC precipitate was dried in an electric oven at 65ºC for 18 hours. The results of the drying are powdered or mashed and sifted through a 100 mesh sieve. Furthermore, FPC wild tilapia flour is ready to be analyzed.

d. Testing Procedure

FPC Chemical Parameters

- ***Water content analysis***

Analysis of the water content was carried out in accordance with SNI 2354.2: 2015 [3] using the vacuum oven method at the temperature to be used 95°C-100°C then the cup was put in the oven for at least 2 hours. After that, the empty cup was transferred to the desiccator for 30 minutes until it reached room temperature and the weight of the empty cup (A) was weighed. Then weigh ± 2 g of the prepared test sample in the cup (B), then put the filled cup in the vacuum oven at 95°C-100°C, with an air pressure of not more than 100 mmHg for 5 o'clock. Then the cup was transferred using a clamp into the desiccator for ± 30 minutes and then weighed (C). Calculation of water content as follows:

$$\textbf{Water content} = \frac{\boldsymbol{B - C}}{\boldsymbol{B - A}} \boldsymbol{x}\ \mathbf{100\%}$$

Information:
A : empty cup weight expressed in g
B : cup weight + initial sample, expressed in g
C : cup weight + dry sample, expressed in g

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- ***Ash content analysis***

Ash content analysis was carried out according to SNI 2354.1:2010 [2]. The cup to be used in the oven first for 30 minutes at a temperature of 100-105 ºC, then cooled in a desiccator to remove moisture and weighed (A). The sample is weighed as much as 2 g in a cup that has been dried (B), then burned over the burner flame until it is not smoking and followed by ashing in the furnace with a temperature of 550-600 ºC until complete ashing (occasionally the furnace door is opened slightly so that oxygen enters). The sample that has been ashed is cooled in a desiccator and weighed (C), the combustion stage in the furnace is repeated until a constant weight is obtained. Ash content is calculated by the formula:

$$\textbf{Ash content}(\%) = \frac{\boldsymbol{C - A}}{\boldsymbol{B - A}}\ \boldsymbol{x}\ \mathbf{100}\%$$

Information:
A : Weight of empty porcelain ash cup (g)
B : Weight of porcelain ash cup with sample (g)
C : Weight of porcelain ash cup with sample after drying (g)

- ***Analysis of protein levels***

Analysis of protein content was carried out according to SNI 01.3254.4:2006 [1]. Analysis of protein content used is the semi-micro Kjeldahl method. The method of determination includes the stages of destruction, distillation and titration. The destruction stage is carried out to change the protein in the material into ammonium sulfate salt. In the distillation step, this salt is reacted with a base and the ammonia is evaporated to be absorbed in a boric acid solution. The amount of nitrogen contained can be determined by titration with HCl. Initially, 2 g of the material was weighed in a Kjeldahl flask and then 1.9 ± 0.1 g K_2O_4, 40 ± 10 mg HgO, 2.0 ± H_2SO_4 were added. Furthermore, with the addition of boiling stones, the solution is boiled 1-1.5 hours until the liquid becomes clear. After the solution was cooled and diluted with distilled water, the sample was distilled by adding 8-10 ml of NaOH-$Na_2S_2O_3$ solution. The distillation results are collected in an Erlenmeyer containing 5 ml of H_3BO_3 and 2-4 drops of indicator (methyl red and alcohol with a ratio of 2:1). The distillate obtained was then titrated with 0.1 N HCl solution until the color changed from green to gray. The result obtained is a total of N, which is then expressed in a conversion factor of 5.6. Protein content calculated based on the calculation formula:

$$\textbf{Protein content}(\%) = \frac{(\boldsymbol{V_A - V_B})\ \boldsymbol{x\ HCl\ x\ N\ HCl\ x}\ \mathbf{14,007}\ \boldsymbol{x}\ \mathbf{5,6}\ \boldsymbol{x}\ \mathbf{100}}{\boldsymbol{W\ x}\ \mathbf{1000}}$$

V_A : ml HCl for sample titration
V_B : ml HCl for blank titration
N : Normality of standard HCl used.
14.007 : Atomic weight of nitrogen.
5,6 : Protein conversion factor for fish [11]
W : Sample weight (g)
Protein content is expressed in units of g/100 g sample (%).

- ***Analysis of fat content***

Fat content analysis was carried out by a Soxhlet method in accordance with SNI 2354-3:2017 [4]. Carefully weigh 2 g (A) of the sample in a 250 mL beaker. Add 20 mL of concentrated p.a HCl and 30 mL of water and a few boiling stones. Then cover the beaker with a watch glass and simmer for 15 minutes. Rinse the watch glass with hot water. Then prepare a funnel and coarse filter paper. Strain hot and rinse with hot water until the pH is neutral or equal to the pH of the rinse water. Then dry the filter paper and its contents in an oven at a temperature of 100°C for 15 minutes. Take the weight of an empty round bottom flask (B g) and then insert the filter paper into the fat sleeve. Put 50 mL (or according to Soxhlet's volume) of diethyl ether into a round bottom flask. Then insert the fat sleeve into the Soxhlet extractor and install the Soxhlet circuit correctly. Extract the fat with an extraction cycle of about 5 minutes/cycle for 4 hours. Then vaporize the solvent in a round bottom flask to dryness. Put the round bottom flask containing fat into the oven at 105°C for ± 2 hours to remove the remaining solvent and moisture. Cool the round bottom flask containing fat in a desiccator for 30 minutes. Weigh the round bottom flask containing fat (C g) to a constant weight. Calculate the fat content using a formula:

$$\textbf{Fat content}\ (\%) = \frac{(\boldsymbol{C - B})}{\boldsymbol{A}}\ \boldsymbol{x}\ \mathbf{100}$$

A : sample weight (g)
B : weight of empty round bottom flask (g)
C : weight of round bottom flask and extracted fat (g).

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FPC Physics Parameters

- ***FPC white degree***

Analysis of color test for tilapia fish flour FPC was carried out in accordance with [10] ; [13].

The color measurement of the sample was measured with a digital colometer test (T-135), to measure the value of L (brightness), a (red-green mixed chromatic color) and b (blue-yellow mixed chromatic color) by attaching the colometer sensor to the sample. The results of the data L, a and b obtained are then calculated using the white degree formula as follows [12]:

$$\boldsymbol{W}(\%) = \mathbf{100} - \sqrt{(\mathbf{100} - \boldsymbol{L})^{\mathbf{2}} + \boldsymbol{a}^{\mathbf{2}} + \boldsymbol{b}^{\mathbf{2}}}$$

Information :
W : degree of whiteness
L : brightness
a : red if marked + and green if marked –
b : yellow if marked + and blue if marked –

- ***Water absorption [24]***

A sample of 1 g was put into a centrifuge tube and then added with 10 mL of distilled water, then stirred with a spatula and allowed to stand at room temperature for 30 minutes. After that it was centrifuged at 3,000 rpm for 30 minutes. The volume of unabsorbed water was then measured with a measuring cup.

$$\boldsymbol{WAC} = \frac{(\mathbf{W1} + \mathbf{AW}) - (\mathbf{W2} + \mathbf{UAW})\ \boldsymbol{ml}}{\boldsymbol{weight\ of\ sample}\ (\boldsymbol{g})}$$

WAC : water absorption capacity (ml/g)
AW : absorbed water (ml)
UAW : unabsorbed water (ml)
W1 : initial weight (g)
W2 : final weight (g)

- ***Oil absorption capacity*** [8]

A total of 1 g of sample and 10 mL of vegetable oil were put into a centrifuge tube, then stirred with a spatula for 1 minute. After being allowed to stand for 30 minutes, the tube was centrifuged at 3,000 rpm for 30 minutes. The volume of unabsorbed oil was measured with a measuring cup.

$$\boldsymbol{OAC} = \frac{(\mathbf{W1} + \mathbf{AO}) - (\mathbf{W2} + \mathbf{UAO})\boldsymbol{ml}}{\boldsymbol{weight\ of\ sample}\ (\boldsymbol{g})}$$

OAC : oil absorption capacity (ml/g)
AO : absorbed oil (ml)
UAO : unabsorbed oil (ml)
W1 : initial weight (g)
W2 : final weight (g)

e. Data analysis

Data is processed using Microsoft Excel and discussed descriptively and supplemented by relevant literature.

III. RESULT AND DISCUSSION

a. Yield

The FPC yield is obtained from the ratio between the weight of the FPC flour produced and the weight of the raw material (pulled wild tilapia meat) multiplied by 100%. Yield has an effect on the value of production economically and the amount of raw materials for a production process [15]. the total yield of FPC resulting from the extraction of 200 g of minced wild tilapia meat yields a yield of 19.11%. This result is higher than the results of a study [16] which obtained a protein concentrate yield of locally cultivated tilapia of 16.53%. FPC yield contains more protein than other components such as fat [16]. Each FPC yield is affected by drying temperature,

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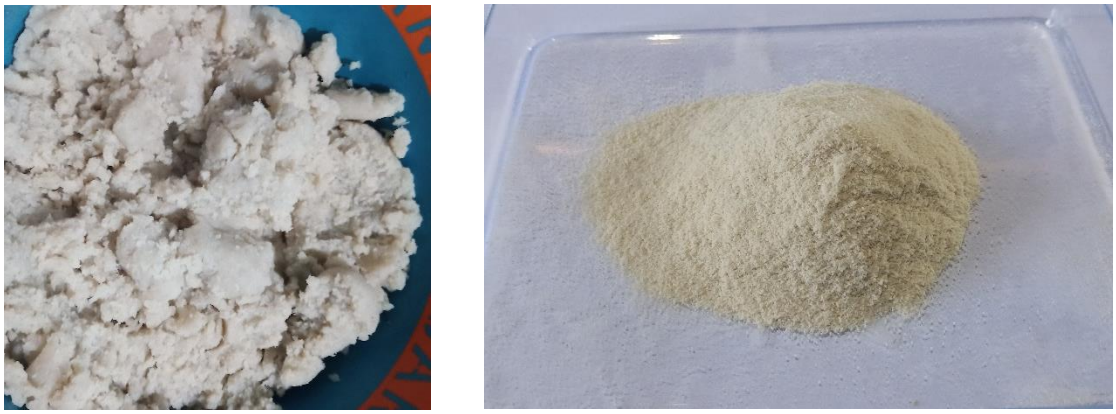
drying equipment, drying time, solvent used, FPC manufacturing technique and type of fish used. Pureed wild tilapia meat and FPC tilapia flour can be seen in Figure 1.



(a) (b)

Figure 1 : (a) Wild tilapia meat puree, and (b) Wild tilapia FPC flour

b. Characteristics of wild tilapia protein concentrate

The results showed that some of the FPC parameters from wild tilapia had standard quality according to FAO type A. The test results can be seen in Table 2.

Table 2. Results of the physico-chemical analysis of wild tilapia FPC.

Chemical Parameters	***Amount***	***FAO standard [6]]***		
		Type C	***Type B***	***Type A***
Protein %	86.51	Min. 67.5	Min. 67.5	Min. 67.5
Fat %	0.55	Max. 10.0	Max. 3.0	Max. 0.75
Water %	10.14	Max. 10.0	Max. 10.0	Max. 10.0
Ash %	0.70			
Physical Parameters				
White degree %	86.9			
Oil Absorbency g/g	2.39			
Water Absorption g/g	2.19			

The FPC protein content of wild tilapia extracted from fat using 90% ethanol is classified as high and according to FAO type A standards. In addition, the technique of changing the solvent every 20 minutes three times helps remove fat from fish meat. The protein content of wild tilapia in this study was around 85.51%, which was very high compared to the results of [16] research, which produced a FPC of locally cultivated tilapia of 61.13% and research [18] which produced a FPC of tilapia with a protein content of 79.10%. The FPC fat content of wild tilapia is low and according to the FAO type A standard with a range of 0.55%, which is much lower than the results of a study [16], resulting in a local cultured tilapia fat content of 7.11%. FPC quality is also determined by the degree of whiteness or degree of whiteness. The results showed that the FPC of wild tilapia meat had a whiteness degree of 86.9%, still higher than skipjack tuna egg protein concentrate [15] and protein concentrate from locally cultivated tilapia [16]. The low level of whiteness of FPC flour is caused by the fat which tends to have a yellowish color and the presence of carotenoid pigments in the fat after it is extracted [23].

Analysis or other characteristics tested to determine the quality of wild tilapia protein concentrate are moisture content, ash content, oil absorption and water absorption. The results of testing the water content in this study were slightly higher than the FAO standard, around 10.14%, due to the less than optimal temperature and drying time. The moisture content of a material determines the shelf life of the material. The FPC of water and ash content of wild tilapia in this study was lower than the water content (4.24%) and ash content (1.95%) of the FPC of white carp Cirrhinus mrigala eggs [25]. According to [26], the average water content of fish protein hydrolysis ranges from 0.1-10.29% while the average ash content of fish protein hydrolysis ranges from 1.76-25.94%. The ability to hold water from outside and inside in food is known as water absorption. This ability is useful to determine the ability of food to bind free and bound water in food ingredients so as to facilitate absorption in the body. The FPC of wild tilapia meat has lower water absorption ability than fish egg protein concentrate [15] and higher than snapper egg protein concentrate [21]. The ability to bind water by fish protein concentrate is caused by the presence of amino acids which have polar properties. [17] explained that the absorption of oil is the ability of the interaction of a substance to oil. The results showed that 2.39 g of FPC from wild tilapia meat could absorb 1 g of oil. The absorption ability of this oil is still lower than the results of a study [14] which obtained that Hake fish protein meal was able to absorb 4.67 g/g oil.

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The FPC of wild tilapia produced in the study as a whole almost met FAO type A standards, namely a minimum protein content of 67.5%, a maximum fat content of 0.75% and a moisture content of 10.0%. According to FAO, FPC is divided into three types, namely type A (minimum protein content of 67.5% and maximum fat content of 0.75%), type B (minimum protein content of 67.5% and maximum fat content of 3%), which is fish meal. does not have the specifications of smell, taste and color but when added to food most of it leaves the taste of fish. and type C (minimum protein content of 67.5% and maximum fat content of 10%)

IV. CONCLUSIONS AND SUGGESTIONS

a. Conclusion

Based on the results of the study, it can be concluded that the FPC extracted from wild tilapia meat with a ratio of 90% ethanol solvent and ingredients 6:1 with a fat extraction time of 20 minutes is almost classified as the FAO standard FPC type A overall with a protein chemical composition of 86.51 %, 0.55% fat, and 10.14% water. The physical characteristics include 86.9% whiteness. FPC wild tilapia can be used as a protein preparation and can be used as a substitution, fortification and addition to products that are low in protein.

b. Suggestion

The research results were not optimal so it was necessary to modify the extraction method, for example the length of time for multilevel fat extraction, the type of solvent, and the use of a dryer, to obtain FPC with better quality. In addition, FPCs need to be applied to food products that are high in carbohydrates but low in protein.

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