

Extraction and characterization of fish protein concentrate from Tilapia (*Oreochromis niloticus*)

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Abstract

The utilization of tilapia fish as a source of protein stock is not only to empower its abundance in aquaculture production but also as enrichment material for low protein food products such as food products for children under five years. However, the physicochemical characterization of amino acids and fatty acids remains limited. This study aimed to characterize the physicochemical composition, amino acids, and fatty acids profile of fish protein concentrate (FPC) extracted from cultivated tilapia using isopropyl alcohol. Following deboning, fish were minced and subsequently immersed in isopropyl alcohol (1:3 w/v) for 24 hrs, sieved, dried, and ground to yield FPC powder. Then, the moisture content, protein, fatty acids, whiteness index, bulk density, odour, water absorption, oil absorption, and amino acids profile of FPC powder were analysed. The results showed the current tilapia FPC as category C, containing 56.93±0.31% of protein, 7.40±0.01% of lipid, 65.84±0.10% of whiteness index, 2.40±0.14 mL/g of water absorption, 0.52±0.01 g/mL of bulk density and 1.03±0.24 of oil absorption. Tilapia FPC had 11 essential amino acids (58.81%) and 9 nonessential amino acids (41.19%). The tilapia FPC also contained 8 saturated fatty acids, 7 monounsaturated fatty acids, and 12 polyunsaturated fatty acids. Essential amino acids and unsaturated fatty acids content of the tilapia FPC shows promising potential in improving nutritional values of food for under five years old children, with potential benefits ranging from emotional intelligence, brain, and mental development.

1. Introduction

Tilapia is Indonesia's most popular cultivated fish species besides carp, silver catfish, catfish, and giant gourami (Tomasoa and Azhari, 2019). In 2017, the Ministry of Marine Affairs and Fisheries Republic of Indonesia reported that tilapia cultivation was the second rank, with total national production reaching 1.2 million tons/year. North Sulawesi recorded tilapia production reaching 91,571.39 thousand tons/year, with the most significant number being Manado and Minahasa, Indonesia (KKP, 2021). One of the areas in North Sulawesi that has a high potential for tilapia cultivation is the Sangihe Islands. The total production of

aquaculture commodities reaches 295 tons/year (KKP, 2018). The popularity of tilapia aquaculture is mainly related to its superior characteristics, including disease resistance, environmental tolerance, ability to consume various types of feed, fast growth, and reproductive capacity (Gupta and Acosta, 2004; Soelistyowati *et al.*, 2010) and more importantly its protein content, which is quite high (Chakraborty *et al.*, 2011). Therefore, domestically cultivated tilapia can become a source of new raw materials for fish protein concentrate (FPC) preparations imported from outside Indonesia.

The primary purpose of making FPC preparations is as a fortification material or to enrich low-protein food

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products such as food products for children under 5 years (Rieuwpassa et al., 2018) to accept low-protein food products in terms of nutrition and quality. Research on FPC using various types of fish has been reported to have been shown to increase protein levels in different kinds of processed foods, for example, the addition of tilapia FPC in biscuits and snacks (Dewita et al., 2011; Afriani et al., 2016), catfish FPC in wonton crackers (Salampessy and Siregar, 2012) and FPC from skipjack eggs on biscuits for infants/toddlers or complementary foods for breast milk (Rieuwpassa et al., 2019). Even so, the physicochemical characterization, amino acids, and fatty acids of FPC are still limited to a few fishery commodities such as carp egg (*Cyprinus Carpio* L) (Faizah, 2020), sunglir fish (Rieuwpassa and Cahyono, 2019), tuna (*Thunnus* sp.) (Monicarani, 2017), skipjack roe (Rieuwpassa et al., 2013), and sand sea cucumber (*Holothuria scabra*) (Karnila et al., 2011).

So far, the characterization of physicochemical, amino acids and fatty acids in tilapia FPC has not been widely carried out. Even though information on the initial characteristics of FPC preparations is crucial to know for the efficiency and effectiveness of its application in various food preparations (Rieuwpassa et al., 2019), as a domestic prima donna commodity, tilapia has vast potential as a raw material for FPC. As one of the outermost regions in northern Sulawesi and the Indonesian fisheries economic development centre, the Sangihe Islands have begun to encourage the use of tilapia, which has been unable to compete with fish caught from the sea. Therefore, this study aimed to utilize tilapia cultivated in the Sangihe islands as raw material for making FPC and characterize the chemical, physical, amino acids, and fatty acids of cultivated tilapia FPC.

2. Materials and methods

2.1 Materials

The main ingredient used is black tilapia (*Oreochromis niloticus*) collected from fish farmers in Utourano Village, North Tabukan District, Sangihe Islands, North Sulawesi, Indonesia. The tilapia used were 7 fish with a weight of 142-145 g/head. Other materials used are isopropyl alcohol (IPA) (Merck), Whatman filter paper no. 42, and aluminium foil. The tools used include cutting boards, knives, glass beakers, scales, spoons, measuring cups, funnels, electric ovens, Erlenmeyer, blenders, sieves, oven, whiteness meter, gas chromatograph (GC), high-performance liquid chromatography (HPLC).

2.2 Fish protein concentrate extraction

Fresh tilapia was weeded and cleaned of dirt and

blood. Then, the fish was filleted for the meat. The fish meat was separated from the skin and bones. Next, the meat was ground using a blender. Fine meat was weighed to determine the initial weight to calculate the amount of solvent used. The extraction method followed the instructions by Rieuwpassa et al. (2018), modified. The ratio of ground meat and the solvent was 1:3 w/v. The refined meat used was 955 g, and the IPA solvent used was 2865 mL. The fine meat was put in a 5 L glass beaker, and then the solvent was added. After that, it was stirred until homogeneous and left for 8 hrs at room temperature (21-26°C). After soaking, it was filtered using filter paper. The filtered precipitate was dried in an oven at 45°C for 16 hrs. After drying, it was ground using a blender and sifted.

2.3 Proximate analysis

Tilapia FPC flour was analysed proximately, including water content, fat content, and protein content, based on AOAC (1988).

2.4 Physical analysis

Tilapia FPC flour was physically analysed, including bulk density (Wirakartakusumah et al., 1992), water absorption, oil absorption (Beuchat, 1997), and degree of whiteness using the Kett Electric Laboratory C-100-3 Whiteness meter.

2.5 Amino acid analysis

The amino acid composition was detected using HPLC (Shimadzu) according to Foh et al. (2011). The solid and liquid samples were 0.5 g and 0.5 mL, respectively. Amino acid standard solutions were prepared in standard series 0, 1, 5, 10, 25, and 50 using 25 mL of distilled water. The reagents used included orthophthaldehyde (OPA) reagent, trisodium citrate pH 3.25 (as the mobile phase), HCl 6 N, HCl 1N, and HCl 0.01 N. Furthermore, amino acid detection was carried out using HPLC with a Shim-pack VP ODS column 5 mm 150 × 4.6 mm, column oven CTO 10 ASVP, detector RF 20 Fluorescence detector, run time 30 mins, Wavelength detector 450 nm, flow rate 1 mL/min, injection volume 10 µL. The results were processed using the Lab. solution ver. Software. 5.6.1 for Windows. The chemical score (CS) was computed to study the nutritional value of tilapia fish protein concentrate. The score is related to the essential amino acids (EAA) profile in a standard protein as described by Joint WHO/FAO/UNU (2007). The chemical score was calculated using the equation (Riyadi et al., 2019):

$$\text{Chemical Score} = \frac{\text{EAA in sample protein (mg/g)}}{\text{EAA in standard protein (mg/g)}}$$

2.6 Fatty acid analysis

Tilapia FPC sample 2 g was dissolved in hexane (p.a.), stirred for 30 mins, and let stand for 4 hrs, then filtered (AOAC, 1988). The 5-20 mL of filtrate took and was added 0.5 M of KOH and 5 mL of methanol. Then the filtrate was incubated at 80°C for 15 mins. After the filtrate was cooled, 1 mL of distilled water and 5 mL of hexane were added and shook for 1 min. The solution was centrifuged at 3000 rpm for 10 mins. Then the supernatant was taken as much as 1-3 mL, transferred, and dried with nitrogen gas. After that, 1 mL of BF₃/methanol solution was added and heated at 100°C. After the filtrate was cooled, 1 mL of distilled water and 2 mL of pentane (p.a.) were added, shook for 1 min, and centrifuged at 3000 rpm for 10 mins, and the supernatant was taken. Then the residue was dissolved with 10 µL of n-hexane. Fatty acids were detected by Gas Chromatography (GC). Samples that have been filtered on a 0.45 m PFTE membrane and degassing were injected into the GC. After that, 0, 0.1, 0.5, 1, 2.5, and 5 µg/mL of standard solutions were prepared. After the sample and standard were ready, it was injected into GC Shimadzu GC-2010AF with capillary Column Restek FAMEWAX 30 m, ID 0.25 mm df 0.1 m with a helium carrier, FID detector, with a 25-minute duration. The results will come out in the form of peak chromatography automatically by the GC.

2.7 Organoleptic

The organoleptic odour of fish protein concentrate (FPC) uses an odour scoring test where the highest score is 5, and the lowest is 1 (Rieuwpassa et al., 2013). The scores given are 1 = very strong fish odour; 2 = strong fish odour; 3 = weakfish odour; 4 = very weak fish odour; and 5 = no fish odour. The tilapia FPC sample was prepared in a container and put in an organoleptic chamber. The panellists will give subjective values according to their respective smells. The number of panellists was 30 participants and were classified as untrained.

3. Results

3.1 Physico-chemical

The chemical composition of FPC was sufficient as a starting information thus FPC can be used as a dry protein preparation material to enrich food products, especially food for children under five years. The physical properties of FPC are helpful for information on the physical changes of food products if added or fortified with FPC. The results of testing the physical and chemical properties of tilapia FPC are shown in Table 1.

3.2 Amino acid compositions

The quality of fish protein concentrate (FPC) was determined by the composition of its constituent amino acids, primarily essential amino acids (EAA). Fish protein concentrate comprises more than 50-80% protein, thus the type and quantity of amino acids are significant to know. The influence of solvent, temperature and extraction time also impacts the damage to the amino acids that make up FPC. The amino acid composition of tilapia FPC is shown in Table 2.

3.3 Fatty acid compositions

In addition to containing protein, fish also contains fatty acids, especially unsaturated fatty acids such as DHA and EPA. Several previous research results show that FPC still leaves a small amount of fat content (Rieuwpassa et al., 2013; Rieuwpassa et al., 2018; Rieuwpassa et al., 2019). However, it has not identified the fatty acid content of the FPC extract. The fatty acid composition of tilapia FPC can be seen in Table 3.

4. Discussion

Protein and lipid are the parameters that determine the quality of FPC. Fish Concentrate Protein (FPC) with protein content > 65% and fat < 0.75% is a category A FPC group. The results showed that the protein content was still below the quality of category A FPC, and the fat was still high. The study results showed that tilapia FPC was still classified as category C FPC (Protein < 60%

Table 1. Physico-chemical of tilapia fish protein concentrate (FPC)

Parameter	Total	Reference Tilapia FPC (Foh et al., 2011)
Chemical		
Protein (%)	56.93±0.31	82.39±0.55%
Lipid (%)	7.40±0.01	1.81±0.12 %
Water (%)	9.44±0.04	3.81±0.18 %
Physical		
Whiteness (%)	65.84±0.10	71.85±4.56
Water absorption (mL/g)	2.40±0.14	2.47±0.57
Oil absorption (mL/g)	1.03±0.24	2.43±0.21
Bulk density (g/mL)	0.52±0.01	0.34±0.02
Odour organoleptic	3.00±0.56 (weakfish odour)	

Table 2. Amino acid compositions of tilapia fish protein concentrate (FPC)

No	Amino Acid	Quantity (g/100 g)		Chemical Score of EAA (mg/g protein)	
		Tilapia FPC	Reference (Foh et al., 2011)	Tilapia FPC	EAA requirements for under five years old children
Essential amino acid (EAA)		58.81%	55.12%		
1	Threonine	2.28	4.32	24.72	31* / 27**
2	Valine	5.07	4.58	54.34	43* / 32**
3	Methionine	1.60	3.14	17.16	25**
4	Isoleucine	2.74	4.07	29.36	32*
5	Leucine	3.88	7.92	41.00	66* / 55**
6	Tyrosine	2.61	3.26	27.74	47**
7	Phenylalanine	1.91	3.93	20.14	–
8	Histidine	3.76	2.37	39.91	20*
9	Lysine	5.21	9.27	55.30	57* / 51**
10	Arginine	3.46	5.86	37.00	–
11	Tryptophan	0.75	0.32	8.07	8.5* / 7**
Non-essential amino acid (NAA)		41.19%	44.88%		
12	Asparagine	0.46	–		
13	Serine	2.18	3.85		
14	Glutamic Acid	7.10	14.87		
15	Proline	1.82	2.51		
16	Glycine	2.33	4.05		
17	Alanine	2.92	5.37		
18	Aspartic Acid	5.54	8.75		
19	Glutamine	0.39	–		
20	Cysteine	0.56	0.53		

*WHO/FAO/UNU (2007), **Dewita et al. (2017)

Table 3. Fatty acid compositions of tilapia fish protein concentrate (FPC)

	Fatty Acid	Total (mg/g)
Saturated Fatty Acid (SFA)	C6:0 Caproic acid	0.37
	C8:0 Caprylic acid	0.14
	C10:0 Capric acid	0.65
	C12:0 Lauric acid	9.53
	C14:0 Myristic acid	5.94
	C20:0 Arachidic acid	0.22
	C16:0 Palmitic acid	7.40
	C18:0 Stearic acid	3.19
Monounsaturated Fatty Acid (MUFA)	C16:1 n-7 Palmitoleic acid	2.46
	C14:1 Myristoleic acid	1.09
	C18:1 n-7 Vaccenic acid	0.14
	C18:1 n-9 Elaidic acid	0.07
	C18:1 n-9 Oleic acid	14.97
	C20:1 n-9 Gondoic acid	0.59
	C22:1 n-9 Erucic acid	0.07
Polyunsaturated Fatty Acid (PUFA)	C18:4 n-3 Stearidonic acid	0.66
	C18:3 n-3 α -Linolenic acid (ALA)	1.71
	C20:4 n-3 Eicosatetraenoic acid	0.51
	C20:5 n-3 Eicosapentaenoic acid (EPA)	2.02
	C22:6 n-3 Docosahexaenoic acid (DHA)	0.74
	C18:2 n-6 Linoleic acid	3.67
	C18:2 n-6 Linolelaidic acid	0.22
	C18:3 n-6 γ -Linolenic acid	0.18
	C20:2 n-6 Eicosadienoic acid	0.19
	C20:3 n-6 Dihomo- γ -linolenic acid	0.16
	C20:4 n-6 Arachidonic acid (ARA)	0.81
C22:5 n-6 Docosapentaenoic acid	0.22	

and fat content > 3%) (FAO, 1975). It is suspected that the process of extracting fat from fish meat has not been maximized by Isopropyl Alcohol (IPA) solvent. In addition, it is suspected that it is related to the extraction technique, which is not repeated thus the solvent is saturated. Saturation is caused by reaching a balance point between solvent and solute (Ramdja et al., 2009; Cikita et al., 2016; Chairunnisa et al., 2019). The separation of fat from fish meat is due to the polarity of the solvent (Tirtajaya et al., 2008; Hewavitharana et al., 2020). According to Asfar et al. (2014), changing the solvent every hour can help optimally extract fat in the FPC extraction process. The water content shown by tilapia FPC was comparable to Rieuwpassa and Cahyono (2019), which resulted in 9.34% water content of FPC sunglir fish dried in an oven at 45°C for 8 hrs.

Odour values and whiteness are physical properties that FPC must meet. The whiteness and odour test of FPC is important because it affects the appearance and odour of food products fortified with FPC. According to Rieuwpassa et al. (2013), the FPC extraction process aims to reduce fat content and can also change the colour to become whiter. The more fat extracted, the higher the white grade value. Furthermore, the decrease in fat content also affects the odour value. If the fat content is still high, it is easier for oxidation to occur, which causes a rancid odour. Rawdkuen et al. (2009) and Rieuwpassa et al. (2013) explained that FPC extraction using a solvent removes fat and materials such as pigment and blood as well as constituents of a fishy/rancid odour.

Tilapia Fish Protein Concentrate (FPC) was also tested for oil absorption, water absorption and bulk density. Water and oil absorption is a parameter of the test carried out to determine the ability of FPC to absorb water and fat mL/g. In addition, the ability to absorb water can also provide an initial profile of protein components, especially the ratio of polar and non-polar amino acids in protein concentrates (Nehete et al., 2013). The results showed that the water absorption ability of FPC tilapia was almost comparable, while the oil absorption capacity was lower than the research of Foh et al. (2011). The high protein and lipid content in Foh et al. (2011) study was due to different extraction techniques. Foh et al. (2011) used enzymes in the extraction process, which have better fat-degrading abilities, while this study used IPA solvents with slow fat-degradation capabilities. Water content in the study of Foh et al. (2011) was lower, due to different drying methods. Foh et al. (2011) used a freeze-drying system, while this study used a drying oven. In the study by Foh et al. (2011), levels of whiteness and higher oil absorption, due to the high-fat content of the FPC produced in this study. However, the results of this study

indicate that although the physicochemical value of FPC in Table 1 shows a lower value than the study of Foh et al. (2011), the FPC method and product in this study can be used by small and medium industries with limited tools and materials (such as in the Sangihe Islands, Indonesia).

Previous studies have found the water absorption capacity of 2.47 mL/g and an oil absorption of 1.12 mL/g of tilapia FPC (Rieuwpassa et al., 2020). Bulk density measurement aims to determine the ability of FPC powder to cover a certain volume of space (WHO, 2012). The results showed that 1 g of tilapia FPC could cover 0.52 spaces/volume.

The results showed that tilapia FPC contained 20 amino acids consisting of 11 essential amino acids and 9 non-essential amino acids. This amount is more than the results of the study of Foh et al. (2011), they only obtained 18 amino acids from tilapia FPC, while Shamloo et al. (2012) only obtained 17 amino acids from a hydrolysis protein of red tilapia. The percentage of essential amino acids is higher when compared to the results of the study by Foh et al. (2011), particularly the amino acids valine, histidine, and tryptophan. Valine plays a role in mental development, muscle coordination, and calmness. In addition, valine is beneficial for muscle growth, tissue repair, and energy (Pillai and Kurpad, 2012). Histidine and arginine are needed by children under five years of age, histidine is non-essential in adults but is essential in baby food (Gibbs, 2020). Tryptophan helps produce serotonin, which plays a role in improving sleep quality, easing anxiety and depression, increasing emotional intelligence, and also helping to manage pain tolerance (Friedman, 2018).

WHO/FAO/UNU (2007) stated requirements amino acid in children 0-5 years, namely histidine (22 mg/kg/daily), isoleucine (36 mg/kg/daily), leucine (73 mg/kg/daily), lysine (64 mg/kg/daily), threonine (34 mg/kg/daily), tryptophan (9.5 mg/kg/daily), and valine (49 mg/kg/daily). The amino acid analysis results showed that the quality of Tilapia FPC had a good composition of essential amino acids. However, the FPC produced in this study is FPC category C. In addition, one of the indicators of FPC quality was determined by the amount of lysine because lysine is easily degraded due to processing (Rutherford and Moughan, 2012; Rieuwpassa et al., 2020). The amount of lysine is still relatively high, indicating that the extraction process does not result in a large amount of lysine loss.

The fatty acid analysis showed that tilapia FPC contained 27 fatty acids consisting of 8 saturated fatty acids, 7 monounsaturated fatty acids, and 12 polyunsaturated fatty acids. The total of fatty acids from

tilapia FPC was 57.92 mg/g, with SFA of 47.37% (27.44 mg/g), MUFA of 33.47% (19.39 mg/g) and 19.14% PUFA (11.09 mg/g). The composition of unsaturated fatty acids, which are more than saturated fatty acids, proves that fish has an excellent quality of fatty acids. This result is in line with several research results, which showed that fish has more unsaturated fatty acid composition than saturated fatty acids. Nadia, Huli, Rejeki *et al.* (2020) found 4 saturated fatty acids and 4 unsaturated fatty acids in tilapia juveniles. Nadia, Nadia, Rosmawati *et al.* (2020) found 4 saturated fatty acids and 4 unsaturated fatty acids in larasati tilapia baby fish. While Sari *et al.* (2018) found 6 unsaturated fatty acids and 3 saturated fatty acids in anchovies.

The omega-3 fatty acids such as α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) can add to the nutritional wealth of FPC, where EPA and DHA have an essential role in the development of infants and toddlers and can prevent degenerative diseases (Swanson *et al.*, 2012; Lauritzen *et al.*, 2016). Omega-3 has an essential role in reducing cholesterol in the blood by influencing the mechanism of lipoprotein production by the liver to lower bad fats such as Low-Density Lipoprotein (LDL) (Pizzini *et al.*, 2017). Therefore, foods that contain Omega-3 are very good for consumption.

In addition to omega-3, tilapia FPC contains omega-6 (linoleic acid) and omega-9 (oleic acid). Omega-6 is a precursor to the formation of arachidonic acid, which plays a role in compiling other compounds in the body and being a means of communication between cells (Turolo *et al.*, 2021). Omega-6 also has an essential role in helping omega-3 work. In addition, other benefits of omega-6 help bodybuilders prevent muscle rupture and increase muscle growth (Diana, 2013). FPC tilapia contains omega-9 which has an important role in lowering LDL, which causes the most coronary heart disease (Johnson and Bradford, 2014). According to Hariyadi and Triono (2006), oleic acid can lower LDL blood cholesterol and increase HDL (High-Density Lipoprotein) greater than omega-3 and omega-6. The content of essential amino acids and unsaturated fatty acids of tilapia FPC shows promising potential in increasing the nutritional value of foods, such as under five years old children food for emotional intelligence, brain, and mental development.

5. Conclusion

This finding confirmed that the tilapia FPC extracted once for 24 hrs using IPA solvent was classified as FPC category C. However, the tilapia FPC produced in this study had a complete amino acid and fatty acid composition, especially 11 essential amino acids,

including valine, histidine, lysine, and tryptophan and 19 unsaturated fatty acids, including α -linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid (omega-6), and oleic acid (omega-9) which plays an essential role in the development of the baby's brain during. This research supports the production of FPC for small and medium enterprises with limited tools and materials. This research must continue by determining the extraction treatment to produce FPC with A quality. It is necessary to research the utilization and fortification of FPC in processed food products.

Conflict of interest

The authors declare no conflict of interest.

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