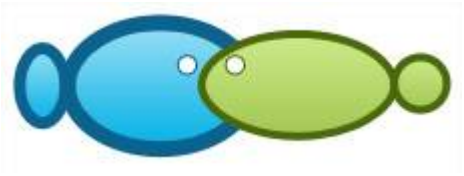

The use of striped catfish (*Pangasianodon hypophthalmus*) abdominal fat as a raw material for highly nutritional fish oil

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Abstract. The abdominal fat is a by-product of the processing of striped catfish (*Pangasianodon hypophthalmus*) as fish fillets and smoked fish which is usually thrown away even though it has potential as a raw material for fish oil. This study aimed to evaluate the use of abdominal fat of striped catfish as a raw material for fish oil enriched with fish protein concentrate and *Chlorella* powder so that it becomes highly nutritious fish oil. This study used a 1-factor experimental method with 4 treatment levels, namely the ratio of raw materials for fish oil enriched with striped catfish protein concentrate and *Chlorella* powder. The high nutritional fish oil raw material mix consisted of striped catfish oil, *Chlorella* powder, and striped catfish protein concentrate with the following percentages; 40%, 20%, 40% (SP1), 35%, 30%, and 35% (SP2), 40%, 0%, and 60% (SP3), 40%, 60%, and 0% (SP4) respectively. The coating material consisted of 25% dextrin (DT), 5% Twin 80 (TW), and 70% water. Content analysis of highly nutritious fish oil included protein, fat, essential amino acid, and essential fatty acid. The results indicated that the SP1 formulation was the best formula based on protein content (17.02%) and fat content (12.18%), followed by SP2, SP3, and SP4. This data was also supported by essential amino acid profiles (threonine, methionine, valine, phenylalanine, isoleucine, leucine, and lysine), and essential fatty acid profiles (oleic, linoleic, linolenic, arachidonic, eicosatrienoic, docosahexaenoic, and eicosadienoic).
Key Words: *Chlorella* powder, essential amino acids, essential fatty acids, fish oil, food supplement, striped catfish protein concentrate.

Introduction. Striped catfish (*Pangasianodon hypothalmus*) fillet processing activities produce about 45% by-product waste, in the form of fish bones, meat still attached to the bones (swallowing meat), stomach contents consisting of fat and offal or digestive organs (Leksono et al 2014). The processing activity of smoked striped catfish produces by-product waste in the form of stomach contents (Syahrul & Dewita 2016). The stomach content consists of fat and offal (the remaining digestive organs including stomach contents, abdominal fat, bones, skin, and trimming) of which 5% is not been utilized optimally (Sembiring et al 2018).

At the Fish Processing Center, Koto Mesjid, Kampar Regency, Indonesia, this smoked fish industry produced by-products as stomach contents of 1-1.5 tons per working day through the year. The stomach contents consist of abdominal fat (9.14%) and stomach contents (9.71%). These by-products are generally not utilized and are simply thrown away, even though these by-products have the potential to be used as raw material for fish oil and at the same time as a source of raw materials for the manufacture of health supplements (Syahrul & Dewita 2016; Sembiring et al 2018; Ayu et al 2019).

Fish oil is known to be rich in two important omega-3 fatty acids called eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The benefits of fish oil appear to come from its omega-3 fatty acid content. This oil-rich fish species includes mackerel (*Rastrelliger brachysoma*), herring (*Clupea harengus*), tuna (*Thunnus obesus*), and salmon (*Salmo salar*). Omega-3 fatty acids reduce pain and swelling and prevent easy blood clotting. Several fish oil products are FDA-approved as prescription drugs for lowering triglyceride levels. Fish oil is also available as a supplement. Fish oil

supplements are sometimes used for heart health and mental health, but there is no clear evidence to support most of these uses (Miller et al 2016; Nhut et al 2019 Sakurai et al 2021).

Health supplements are non-drug food products designed to supplement the nutrients needed for body health, such as vitamins, minerals, fiber, amino acids, and fatty acids, when the nutritional content of the food we consume does not meet the nutritional adequacy rate. Therefore, health supplements at this time have become a necessity for the community to maintain their health in order to stay fit. However, the price of health supplements circulating in the market has not been reached by the wider community. On the other hand, sources of raw material for health supplements based on fish oil are very abundant because they are not utilized, so they have the potential to become an environmental problem (Tran-Tu et al 2017; Lestari et al 2020).

The by-product of abdominal fat has many potential benefits for the health of the human body because it is rich in unsaturated fatty acids, such as omega-3, omega-6, and omega-9 fatty acids. It was recorded that the omega-9 fatty acid content in striped catfish was 26.22%. So that it can be used as a raw material for the manufacture of fish oil supplements (Domiszewski et al 2011; Sugata et al 2016; Hua et al 2019). This problem has prompted several researchers to look for innovations that can overcome it, by utilizing the by-product of abdominal fat of striped catfish. To complete the nutrition, fish oil supplements need to be fortified with protein-rich raw material sources, namely microalgae *Chlorella* sp. and striped catfish protein concentrate. Fish protein concentrate is a product for human consumption that is made from fish meat by reducing most of its fat and water content, so that a high protein content is obtained. According to Dewita et al (2020), striped catfish which is processed into fish protein concentrate contains 79.6% protein.

Chlorella is the most cultivated eukaryotic algae. It grows in fresh, brackish and seawater and is widely used as a dietary food and dietary supplement, as well as in the pharmaceutical and cosmetic industries. *Chlorella* has potential as a food source; it contains proteins, carotenes, some immunostimulants, polysaccharides, vitamins and minerals. In general, *Chlorella* is used as a raw material for the production of nutritional supplements and drugs because it contains various nutrients such as amino acids, peptides, proteins, vitamins, sugars, nucleic acids, enzymes, and lots of fibers (Fisher et al 2016; Halima et al 2019; Sarker et al 2020). This study aimed to evaluate the use of abdominal fat of striped catfish as a raw material for highly nutritional fish oil by blending it with *Chlorella* powder and striped catfish protein concentrate.

Material and Method

Raw material. This research was conducted from January 2021 to September 2022. The main raw materials used were solid waste from filet processing and smoking of striped catfish at the Fish Processing Center, Kampar, Indonesia. The solid waste, approximately 300 kg, was obtained in the form of abdominal fat (belly), swallow meat (flesh remaining on the bone), bones, and offal. *Chlorella* sp. is the result of cultivation in the Planktonology Laboratory, Faculty of Fisheries and Marine Sciences, University of Riau. The medium used was the Dahril solution (Halima et al 2019; Dahril et al 2020). Fish protein concentrate was prepared from the flesh of striped catfish cultivated in Kampar Regency, Riau Province.

Experimental design. This research was conducted with a one-factor perfect randomized design, namely, fish oil formulation, consisting of 4 levels (Table 1) namely SP1 (fish oil 40%, *Chlorella* powder 20%, and fish protein concentrate 40%), SP2 (fish oil 35%, *Chlorella* powder 30%, and fish protein concentrate 35%), SP3 (fish oil 40%, and fish protein concentrate 60%) and SP4 (fish oil 40% and *Chlorella* powder 60%).

Extraction and refining of striped catfish oil. Extraction of fish oil from abdominal fat as the smoked striped catfish processing waste was carried out by referring to the modified Damongilala (2008) method. Abdomen fat was separated from the stomach

contents of the striped catfish, washed and drained, then chopped into small pieces, and heated using a hotplate for 5 hours at 70ºC. Abdominal fat after heating was filtered using a filter cloth to obtain a yield in the form of liquid (crude oils). The crude striped catfish oil obtained was then stored in a dark bottle.

Table 1

Microencapsulated formulations of fish oil, *Chlorella*, and fish protein concentrate to become highly nutritional fish oil from striped catfish

Treatment formula	*Fish oil (%)*	*Chlorella powder (%)*	*Fish protein concentrate (%)*
SP1	40	20	40
SP2	35	30	35
SP3	40	0	60
SP4	40	60	0

Purification of striped catfish oil referred to modified Hastarini et al (2013), and Dewita et al (2020). Coarse-striped catfish oil was placed in a container coupled with a vacuum filter. The oil was heated to 60°C, then bentonite clay and activated charcoal were added with a concentration of 2% each by weight of oil. After that, the oil was stirred at 500 rpm while heated to 70°C for 30 minutes. Then the oil was filtered using a vacuum filter and the weight of the resulting oil was weighed as a pure striped catfish oil yield.

Chlorella powder preparation. Cultivation of *Chlorella* was carried out for 3 weeks, then harvested using a plankton filter to obtain *Chlorella* slurry. Then the *Chlorella* slurry was dried in a cabinet dryer at a temperature of 40-50^{o}C for 3-4 hours. The collected *Chlorella* powder was used as a raw material for supplements.

Fish protein concentrate preparation. Fresh striped catfish, weighing 1-1.5 kg per fish, were filleted and skin removed. The fish fillets were cut into small pieces and finely ground using a meat grinder with the addition of 0.5% salt. Furthermore, this mashed meat was steamed for 30 minutes and then pressed to release some of the water. Then a 0.5 N $NaHCO_3$ solution was added to the steamed mashed meat until an isoelectric pH was reached, stirred thoroughly to form a paste. The paste was then extracted by immersing it in isopropyl alcohol (1:3) for 10 hours until a precipitate or residue formed. Then the residue was dried at 40-50^{o}C for 15 hours in a drying cabinet.

Preparation of highly nutritious fish oil. Highly nutritious fish oil was formulated as in Table 1 and processed by microencapsulation using a spray drier (Faldt & Bergenstahl 1995). As a coating material we used dextrin (25%), twin 80 (5%), and water (70%). The formula referred to Dewita et al (2020) and Wresdiyati et al (2010).

Protein content analysis. The protein analysis step consists of three stages: digestion, distillation and titration (AOAC 1980). Protein levels were measured using the micro-Kjeldahl method. A quantity of 1 g sample was weighed into a 100 mL Kjeldahl flask and 0.25 g of selenium and 3 mL of concentrated H_2SO_4 were added. Samples were destroyed at 410^{o}C for approximately 1 hour until the solution became clear and then cooled. After cooling, 50 mL of distilled water and 20 mL of NaOH 40% were added to the Kjeldahl flask and distillation was carried out at a temperature of 100°C. The distillation results were collected and placed in a 125 mL Erlenmeyer flask containing a mixture of 10 mL of boric acid H_3BO_3 and 2 drops of bromkerosol green indicator and pink methyl red. Distillation was terminated when the amount of distillate reached 40 mL and turned bluish green. The distillate was then titrated with 0.1 N HCl until the color changed to pink. The titrant volume was read and recorded. A blank solution was analyzed in the same way as the sample. In this method the total nitrogen content was measured and the protein content was calculated by % crude protein = % N x protein conversion factor (6.25).

Fat content analysis. Analysis of fat content was based on AOAC (2005). Place 5 g (W1) of the sample on the filter paper on both ends of the wrapper wrapped with absorbent cotton, put it in a grease cuff, put the wrapped sample in a constant weight (W2) grease bottle, connect the Soxhlet tube and seal it. The grease boot was placed in a Soxhlet tube extraction chamber, rinsed with a benzene grease solvent, and then refluxed for 6 hours. The grease solvent in the grease bottle was distilled until all the grease solvent was evaporated. During distillation, the solvent was placed in the extraction chamber, removed so that it did not return to the fat flask, the fat flask was dried in an oven at 105°C, and then the flask was cooled. Return the desiccator to constant weight (W3) and calculate the fat content.

Fatty acid analysis. The analytical method used was based on the principle of converting fatty acids into their derivatives, namely methyl esters, so that they can be detected chromatographically (AOAC 1999). Gas chromatography (GC) has a working principle of separation between gas and liquid thin films based on different types of materials. Analysis by gas chromatography is based on the partitioning of the components. Fat content = W3 – W2 x 100 W1 of a liquid between the mobile phase in the form of gas and the stationary phase in the form of a non-volatile solid or liquid attached to an inert support material. The components to be separated must be volatile at the temperature at which the separation is carried out, so the operating temperature is usually higher than room temperature and derivatization is usually carried out for samples that are difficult to volatilize. In the case of fatty acid analysis, first, the fat or oil samples are hydrolyzed into fatty acids, then transformed into their ester form which is more volatile. The transformation was carried out by means of methylation to obtain fatty acid methyl esters (FAME). Furthermore, FAME was analyzed by means of gas chromatography. The gas chromatography tool used was Shimadzu GC 2010+ device.

Amino acid analysis. Amino acid analysis (AOAC 1999) was carried out using high-performance liquid chromatography (HPLC). Before use, the HPLC device and syringe must be rinsed first with the eluent to be used for 2-3 hours and distilled water. Amino acid analysis using HPLC consists of 4 stages, namely: a step for making protein hydrolyzate; stages of drying; stages of derivatization; stages of injection as well as analysis of amino acids.

Preparing protein hydrolyzate. A sample of 30 mg was weighed and crushed. The crushed sample was acid hydrolyzed using 1 mL of 6 N HCl which was then heated in an oven at 110°C for 24 hours. Heating in the oven was carried out to remove gas or air present in the sample and to speed up the hydrolysis reaction.

Drying stage. Samples that had been hydrolyzed at room temperature were transferred into a 50 mL evaporator flask, rinsed with 2 mL of 0.01 N HCl and the rinse liquid was put into the evaporator flask. This process was repeated up to 2-3 times. The sample was then dried using a freeze dryer in a vacuum to convert cysteine into cystine, 10-20 mL of water was added to the sample and dried with a freeze dryer. This process was repeated up to 2-3 times.

Derivatization stage. A volume of 30 µL of the derivatization solution was added to the drying product. The derivatization solution was made from a mixture of OPA (*o*-phtalaldehyde) stock solution and potassium borate buffer solution pH 10.4 with a ratio of 1:2. The OPA stock solution was prepared by mixing 50 mg of OPA crystal into 4 mL of methanol and 0.025 mL of mercaptoethanol. This mixture was shaken slowly and then 0.050 mL of brij-30 30 solutions was added and followed by adding 1 M borate buffer to achieve the desired pH of 10.4. This OPA reagent stock solution was stored in a dark-colored bottle at 4^{o}C until used. The derivatization process was carried out so that the detector easily detects the compounds present in the sample. Further dilution was carried out by adding 20 mL of acetonitrile 60 or 1 M sodium acetate buffer, then left for 20 minutes. The solution was then filtered using Whatman filter paper.

Injection into HPLC. The filter results were taken as much as 5 µL to be injected into the HPLC. The concentration of amino acids present in the material was determined by preparing a standard chromatogram using ready-to-use amino acids that have undergone the same treatment as the sample. The content of amino acids in 100 g of material can be calculated by the formula: amino acids = Area of sample area x C x fp x BM x 100 area of standard area sample weight µg. Note: C = standard concentration of amino acids (g mol^{-1}); fp = dilution factor; BM = molecular weight of each amino acid (g mol^{-1}). The condition of the HPLC apparatus during the amino acid analysis was carried out as follows: temperature at 27°C room temperature; HPLC column type = ultra techspere Column C-18; eluent flow rate = 1-minute; pressure = 3000 psi; mobile phase = Na-acetate buffer and methanol 95; Detector = fluorescence; and wavelength = 254 nm.

Result and Discussion

Nutritional content of highly nutritional fish oil. In this study the nutritional content of highly nutritious fish oil was measured based on its protein content. The best formulations were SP1 (17.02%) and SP2 (15.09%), and then followed by SP3 (10.82%), and SP4 (9.93%) (Table 2). When viewed from the fat content, the best formula sequentially was SP3, and followed by SP4, SP2, and SP1 (Table 2).

Table 2

The nutritional value of highly nutritional fish oil enriched with chlorella, and protein concentrate of striped catfish

Nutritional content (%)	*Formulation*			
	SP1	*SP2*	*SP3*	*SP4*
Protein	17.02	15.09	10.82	9.93
Fat	12.18	12.24	13.21	13.19

The high protein content is thought to be due to the fact that the SP1 and SP2 formulations contain protein source raw materials, namely *Chlorella*, and fish protein concentrate. While other formulations (SP3 and SP4) were dominated by fat content. *Chlorella* is used as a raw material for making nutritional supplements because it contains chemicals such as amino acids, peptides, proteins, vitamins, sugars, and nucleic acids (Fisher et al 2016; Syahrul & Dewita 2016; Sarker et al 2020).

Highly nutritional fish oil amino acid profile. In this work, the highly nutritional fish oil formulations contain essential amino acids threonine, methionine, valine, phenilalanine, isoleucine, leucine and lysine (Table 3). The formulae of SP1 and SP2 meet the (essential amino acid) intake standards per day (NRC 1989; Gwin et al 2021). Similar results were reported by some other authors, namely Sembiring et al (2018), Dewita et al (2020), and Amri et al (2021) who reported that striped catfish abdominal fat contains essential amino acids threonine, methionine, valine, phenylalanine, isoleucine, leucine and lysine.

Table 3

The content of essential amino acids in highly nutritional fish oil which is enriched with Chlorella, and striped catfish protein concentrate (mg kg^{-1})

Essential amino acids	*SP1*	*SP2*	*SP3*	*SP4*	*Standard**
Threonine	2.41	2.04	0.64	0.16	1.8
Methionine	1.66	1.58	0.33	0.12	1.44
Valine	2.96	3.42	1.09	0.26	2.7
Phenylalanine	2.43	2.45	0.63	0.36	1.8
Isoleucine	3.23	3.74	0.85	0.41	2.7
Leucine	4.45	3.62	1.47	0.35	3.06
Lysine	3.26	3.64	1.28	0.18	2.7

* DFCAI (2019).

Essential amino acids are amino acids that cannot be synthesized by humans or vertebrates body. The human body does not have a metabolic pathway to synthesize these amino acids, they must be obtained from the diet (Hou et al 2015; Hou & Wu 2018). It is generally accepted that there are nine essential amino acids, phenylalanine, valine, tryptophan, threonine, isoleucine, methionine, histidine, leucine, and lysine, depending on an individual's metabolic status. From a nutritional point of view, we can get all 9 essential amino acids from a complete protein. By definition, a complete protein contains all the essential amino acids. Complete protein is usually obtained from animal foods, with the exception of soy. Essential amino acids are also available from incomplete proteins, usually plant foods (Hoffman & Falvo 2004; Le et al 2016).

Methionine serves to assist the elimination of toxins from the liver, accelerate liver regeneration, and lower blood cholesterol levels, while leucine helps break down muscle proteins, promoting bone healing, according to Wresdiyati et al (2010). The roles of each of the necessary amino acids included in fish oil supplements are distinct. Isoleucine is necessary for the body's creation and storage of protein, the synthesis of haemoglobin, the operation of the thymus and pituitary glands, healthy growth, the maintenance of the nitrogen balance in the body, and the synthesis of other non-essential compounds. Amino acids are necessary for the synthesis of haemoglobin and the regulation of blood sugar levels. To elevate hormone levels, elevate mood, and act as an aphrodisiac, phenylalanine is required (Gu et al 2019). According to Gwin et al (2021) and Hou & Wu (2018), valine promotes growth, is good for the nervous and digestive systems, helps with nervous, muscle, mental, emotional, and sleeplessness disorders, enhances muscular coordination, repairs damaged tissue, and maintains nitrogen balance in the body.

The neurotransmitter norepinephrine is produced by the human body using phenylalanine. This neurotransmitter performs a variety of significant tasks, one of which is facilitating communication between the brain and the body's nerve cells (Flydal & Martinez 2013). Melatonin and serotonin are produced with the aid of tryptophan. Serotonin is known to assist control mood, pain, sleep, and appetite, and melatonin aids in regulating the sleep-wake cycle. Niacin, a vitamin B3 required for DNA synthesis and energy metabolism, can also be produced by the liver using tryptophan (Carhart-Harris & Nutt 2017). Threonine is a crucial amino acid involved in protein synthesis, ESC proliferation and differentiation, lipid metabolism, intestinal health, and other processes (Finlay & Cantrell 2011).

The majority of people obtain histidine through food. It's used for development, tissue healing, and the production of blood cells. It aids in nerve cell defense. The body uses it to produce histamine (Brosnan & Brosnan 2020). Leucine is a necessary amino acid for the production of proteins. Leucine's carbon skeleton can also be utilised, like that of other amino acids, to produce ATP. Leucine can, however, also control a number of biological functions, including protein synthesis, tissue regeneration, and metabolism (Elhiti & Stasolla 2009). Lysine is crucial for healthy development and is involved in the creation of carnitine, a nutrient that converts fatty acids into energy and lowers cholesterol (Xie et al 2021).

Essential fatty acid profile of highly nutritional fish oil. The results of this study indicated that the composition of the essential fatty acid profile of highly nutritional fish oil consisted of oleic, linoleic, linolenic, arachidonic, eicosatrienoic, docosahexaenoic, and eicosadienoic (Table 4). Similar results were reported by some other authors, namely Damongilala (2008), Durmuş (2019), Putri et al (2021), and Sokamte et al (2020) who mentioned the physicochemical and fatty acid profile of fish oil from various aquatic organisms. Fish fat contains the essential fatty acids oleic, linoleic, linolenic, arachidonic, eicosatrienoic, docosahexaenoic, and eicosadienoic. The fatty acid composition of marine organisms ranged from 27.68 to 36.59% saturated fatty acids, 8.99 to 35.84% monounsaturated fatty acids, and 10.69 to 39.57% polyunsaturated fatty acids (Durmuş 2019).

From Table 4 it can be seen that the content of essential fatty acids, especially the content of oleic and linoleic fatty acids, in the fish oil supplement formulation SP1 is

higher than the other formulations. The synthesis of unsaturated fatty acids with two or more double bonds, such as linolenic acid (omega 3) and linoleic acid (omega 6), is limited in the body. Therefore, these two fatty acids are essential for the body. Omega-9 fatty acids, on the other hand, can be synthesized by the body, so we don't have to worry too much about our body experiencing a deficiency of these omega-9 fatty acids.

Table 4

Essential fatty acid content (mg kg^{-1}) nutritional fish oil enriched with *Chlorella*, and striped catfish protein concentrate

No	*Essential fatty acid*	*SP1*	*SP2*	*SP3*	*SP4*
1	Oleic	42.87	39.88	39.77	38.23
2	Linoleic	12.43	11.04	11.27	10.65
3	Linolenic	1.09	0.84	0.3	0.89
4	Arachidonic	0.49	0.46	0.35	0.30
5	Eicosatrienoic	0.44	0.42	0.41	0.40
6	Docosahexaenoic	0.34	0.25	0.14	0.09
7	Eicosadienoic	0.45	0.35	0.31	0.30

Ayu et al (2019) reported that striped catfish oil is rich in omega-9 fatty acids, which is 26.22%. Oleic acid is a fatty acid belonging to the MUFA (monounsaturated fatty acid) group which has an 18:1 D9 structure with the molecular formula $CH_3(CH_2)7C = C(CH_2)$ 7COOH, and is an omega-9 group because it has a double bond at the 9 ends of the chain. The same thing was also reported by other researchers (Putri et al 2021) who reported the same type of fatty acids in tilapia oil, namely oleic acid, linoleic acid, linolenic acid, arachidonic acid, eicosatrienoic acid, docosahexaenoic acid eicosadienoic acid.

Essential fatty acids are a particular class of fatty acids that the body requires for biological activities but cannot create on its own, necessitating dietary consumption. Depending on the species from which it came and seasonal fluctuations, fish oil has different fatty acid compositions. Fish oil is largely made from pelagic fatty fish species, which are a significant source of long-chain polyunsaturated fatty acids, notably omega-3 fatty acids, in the modern human diet. In many value-added food items or health food capsules, fish oil is used as a beneficial additive and ingredient and frequently sells for a high price. They lower blood cholesterol levels and reduce the risk of heart disease (Miller et al 2016; Sakurai et al 2021).

Conclusions. Based on the protein content, the best formulations were SP1 (17.02%) and SP2 (15.09%). The content of essential amino acids in the SP1 and SP2 fish oil supplement formulations met the standard intake of essential amino acid per day, especially judging from the content of threonine, methionine, leucine, and lysine. The content of essential fatty acids in fish oil supplement formulation SP1 (oleic, linoleic, linolenic, arachidonic, eicosatrienoic, docosahexaenoic, and eicosadienoic) is higher than in other formulations, especially the content of oleic and linoleic fatty acids. Abdominal fat of striped catfish can be recommended as a raw material for highly nutritional fish oil by blending it with *Chlorella* powder and striped catfish protein concentrate.

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Conflict of interest. The authors declare that there is no conflict of interest.

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