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Physicochemical properties, fatty acid composition and FTIR Spectra of Gabus (*Channa striata*) fish oil

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Abstract

This study aimed to characterize Gabus fish oil (GFO) with the scientific name of *Channa striata* through the determination of physicochemical properties, fatty acid composition, and FTIR spectra. Gabus fishes was obtained from Palagan (Central Java) and Bantul (Yogyakarta). Physicochemical properties of GFOs from Palagan and Bantul revealed acid values of 5.93±0.19 mg KOH/g and 2.39±0.05 mg KOH/g, saponification value of 163.39±1.96 mg KOH/g, 176.82±2.98 mg KOH/g, iodine value of 109.76±1.23 g I_2/100 g, 94.21±0.16 g I_2/100 g, peroxide value of 4.04±0.15 meq O_2/kg and 4.03±0.59 meq O_2/kg. Both oils showed significant differences in terms of physicochemical properties ($P<0.05$) except peroxide value having no significant difference ($P>0.05$). Fatty acid composition of GFO as determined by gas chromatography revealed that GFO contains a higher amount of unsaturated fatty acids such as linoleic acid and palmitoleic acid. Besides, GFO contains arachidonic acid which is act as a precursor for prostaglandin synthesis. FTIR spectrum of GFO is unique in terms of exact wavenumbers and peak intensities which supported that FTIR spectra are fingerprints that can be used for the characterization of edible fish oils.

1. Introduction

Consumption of fish and fish products has been linked to health benefits as reported in several epidemiologic studies and clinical trials. The abundance of omega 3 long-chain polyunsaturated fatty acid, including EPA and DHA, contained in fish are believed to be responsible for several biological activities offering human health benefits (Swanson *et al*., 2012). Clinical studies have reported that the consumption of omega-3 fatty acids contained in fish oil as dietary intervention could prevent cardiovascular disease (CVD) (Ander *et al*., 2003). Omega-3 fatty acids also have an immunomodulatory effect and are useful in treating inflammatory conditions such as rheumatic arthritis, inflammatory bowel disease and cystic fibrosis (Ruxton *et al*., 2004). EPA and DHA contained in fish oil are essential for proper fetal development and healthy ageing (Dunstan *et al*., 2007). The abundant levels of omega-3 long-chain polyunsaturated fatty acid (PUFA), the high degree of unsaturation of the fatty acid constituents, and the wide range of fatty acids contained in triacylglycerols are three characteristic features of fish oil that make it different from other oils (Hjaltason and Haraldsson, 2006).

Gabus fish or commonly known as snakehead or cork fish, with the scientific name of *Channa striata*, belongs to the family of Chanidae. It is one of the popular carnivorous freshwater fishes having high commodity values in the fish market (Bich *et al*., 2020). This fish is indigenous to many tropical and subtropical countries including Indonesia and Malaysia. Cork fish is a freshwater, air-breathing, and carnivorous fish, a valuable source of protein throughout the Asia Pacific region (Ikasari *et al*., 2020). The cultured snakehead has become an economically important freshwater fish in developing countries due to its beneficial effects on human health. High protein in this fish makes it potential to prevent stunting (Pasaribu *et al*., 2020). The high contents of albumin and omega-3 can accelerate the healing progress of a scratch wound as it is useful to form new tissue during the growth period (Ikasari *et al*., 2020). Baie and Sheikh (2000) had reported that Gabus

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fish enhances the synthesis of different glycosaminoglycans in healing wounds and increases the rate of wound contraction leading to a quicker healing process. Omega-3 polyunsaturated fatty acid contained in Gabus fish oil (GFO) can regulate prostaglandin synthesis and also influence the immune system (Durkin *et al*., 2021).

Gabus fish oil (GFO) is highly-priced in the markets, approximately 15-20 times more expensive than common vegetable oils. This is influenced by the cost of Gabus fish itself and its benefits. Thus, it is important to know the quality of fish oil to get the maximum benefit of GFO and it is also important to prevent adulteration from low-priced oils. FTIR spectroscopy in combination with chemometrics has been applied for analysis of GFO and GFO added with Patin fish oil, and this could be used as an authentication model for GFO (Putri *et al*., 2019). This study was aimed to characterize Gabus fish oil including its physico-chemical properties, fatty acid composition and FTIR spectral data.

2. Materials and methods

2.1 Materials

Gabus fishes were collected from two locations (Palagan and Bantul) having different characteristics in terms of environmental conditions in Yogyakarta, Indonesia. All chemicals and reagents used were of analytical grade.

2.2 Extraction method

Gabus fish oil was extracted from the viscera, head and bone of Gabus fish by direct pressing method. All of the parts were placed in an aluminium tray and were dried using a cabinet dryer for 24 hrs at 50^{o}C. The oils that appear after drying were collected and the residual was pressed directly with 100 kN force to extract the remaining oil. Collected oils were then centrifuged to separate sediment using 5000×*g* for 10 mins.

2.3 Acid value determination

Acid value (AV) is the number of milligrams of KOH required to neutralize the free fatty acids present in 1 g of oil or fat. AV was measured according to the AOAC official method (AOAC, 2000). The oil samples were placed in an Erlenmeyer flask and 10 mL of ethanol was added and mixed with 2 mL phenolphthalein. The mixture was titrated using 0.1 N KOH in ethanol with consistent shaking until the endpoint was detected with an observed colour change from white to pink.

2.4 Saponification value determination

The saponification value (SV) of an oil is defined as the number of milligrams of KOH required to neutralize the fatty acids resulting from the complete hydrolysis of 1 g of sample. Determination of SV was performed according to Akpan and Jimoh (2012). A weight of 1 g of samples was placed in an Erlenmeyer flask then 30 mL of KOH in ethanol were added. The Erlenmeyer was connected with an air condenser and boiled for 30 mins to complete the saponification. Few drops of phenolphthalein indicator were added to the warm solution and titrated with 0.5 N HCl until the endpoint colour from pink to colourless was observed. The same procedure was used for blank titration.

2.6 Peroxide value determination

The peroxide value (PV) is expressed as milliequivalents (meq) of radicals per kilogram of fat or oil. Determination of PV was carried out using the AOAC official method (AOAC, 2000). One gram of oil sample was dissolved in 30 mL chloroform: acetic acid (2:3) solution. A volume of 0.5 mL saturated KI solution was added to the mixture and left for 1 min in a dark place. Starch 1.5% as an indicator were added and titrated with 0.01 N sodium thiosulphate.

2.7 Iodine value determination

Iodine value (IV) is a measure of the total number of double bonds present in fats and oils. A 0.3 g of samples were weighed in an Erlenmeyer flask with a stopper. The samples were dissolved in 10 mL chloroform and 25.0 mL Wijs reagent (iodine chloride 1% in acetic acid glacial). The mixture was then placed in the dark for 30 mins. 10 mL of KI 15% solution and 15 mL of water were added, then titrated with sodium thiosulphate 0.1 N until the yellow colour almost disappeared. Few drops of 0.5% starch indicator were added and the titration continued until the blue colouration disappeared. The same procedure was used for the blank test (AOAC, 2000).

2.8 Fatty acid analysis using Gas Chromatography (GC)

Fatty acid composition of Gabus fish oil was performed using gas chromatography using flame ionization detector (GC-FID). Fatty acid was analysed as fatty acid methyl ester (FAME) according to Amit *et al*. (2020) with slight modification. Approximately 500 µL samples of GFO was added with 1.5 mL 2 M methanolic sodium methoxide at 60^{o}C for 5-10 mins with vigorous shaking. The mixture was cooled before the addition of 2 mL boron trifluoride (BF_3) then boiled again for 5-10 mins at 60^{o}C. After cooling down, the mixture was extracted using 1 mL heptane and 1 mL saturated NaCl. The vials were shaken and left for separation. The upper layer was transferred to GC vials and injected into DB-WAX column having a length of 30 m and a diameter of

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0.25 mm (Agilent Technologies 7890B, USA). Helium was used as carrier gas with a total flow rate of 1.6193 mL/min and pressure of 17.315 psi. The column oven temperature was programmed with the initial temperature of 50°C for 1 min as holding time, after that the temperature was increased to 230°C at a rate of 3°C/ min and maintained at 18 mins. The detector temperature was maintained at 280°C and injector temperature at 250°C with a split ratio of 50:1.

2.9 Measurement of FTIR spectra

FTIR spectra of GFO were measured using a Thermo Scientific iS10 spectrophotometer (Thermo Fisher Scientific, USA) equipped with deuterated triglycine sulphate (DTGS) detector and KBr/ Germanium as the beam splitter. FTIR spectra were scanned in the middle infrared region of 4000-650 cm^{-1} with a resolution of 8 cm^{-1} and a number of scanning of 32.

2.10 Statistical analysis

Values are presented as the mean±standard deviation of triplicate determination. Statistical analysis for physico-chemical values was carried out by independent t-test using SPSS software version 15.0 (SPSS inc., Chicago, IL, USA) and the significance was defined at $p<0.05$.

3. Results and discussion

3.1 Physico-chemical properties

Gabus fish oil (GFO) was extracted by direct pressing without using any solvent. It was found that Gabus fish from Palagan and Bantul contained 4.30% and 4.88% of oils respectively. Fishes containing lower than 5% by weight of fat are called lean fish. Lean fish store their fat in the form of triacylglycerol in the liver and it contains fat-soluble vitamins like vitamin A and D. Low-fat fish usually have whiter flesh as a result of higher water content (Osman *et al*., 2001). Fat content in fish was affected by a few things such as species, season, geographical regions, age, and maturity (Aliyu-Paiko *et al*., 2012).

Table 1 shows the acid value (AV), saponification value (SV), peroxide value (PV) and iodine value (IV) of GFO from Palagan and Bantul. The chemical properties of GFO showed a significant difference ($p<0.05$) except PV. The difference was influenced by the geographical regions where the fish is growing up and the feed given. AVs of GFO from Palagan and Bantul were 5.93±0.19 mg KOH/g and 2.39±0.05 mg KOH/g respectively. AV is the measurement of free fatty acids present in oils or fats. The low AV obtained indicated that GFO was acceptable for edible applications. SVs of GFO were 163.39±1.96 mg KOH/g and 176.82±2.98 mg KOH/g. SV is an indicator of the molecular weight of chain lengths of the constituent fatty acid (Olakunle and Umar, 2018). The value of around 195 indicates that oil contains a mainly high molecular mass of fatty acid and is edible for consumption (Boukandoul *et al*., 2018). The iodine value (IV) gives a measure of the degree of unsaturation in the triacylglycerol of fats and oils. IVs of GFO was found to be 109.76±1.23 g I_2/100 g and 94.21±0.16 g I_2/100 g. These results are in line with the IVs reported by Molla (2016) (110.85±0.18 g I_2/100 g) and Paul *et al*. (2013) (87.34±0.64 g I_2/100 g). These values were higher than those in vegetable oils like palm oil (50.1-54.9 g I_2/100 g) (Chong, 2012). The high level of IV indicated that GFO had a higher amount of unsaturated fatty acid. This characteristic can be used for the identification and differentiation of oils and fats. Peroxide values (PV) determine the content of hydroperoxides in the oils and are used as a measurement of rancidity occurs by autoxidation. The PVs of GFO were 4.04±0.15 meq O_2/kg and 4.03±0.59 meq O_2/kg. This value is lower than the previous study by Paul *et al*. (2013) with a PV of 42.24±2.32 meq O_2/ kg. It means that GFO used in this study was still in a fresh condition and the oxidation product was still low.

Table 1. Chemical properties of Gabus fish oil from Palagan and Bantul

Parameters	Gabus fish oil	
	Palagan	Bantul
Acid value (mg KOH/g)	5.93±0.19[a]	2.39±0.05[b]
Saponification value (mg KOH/g)	163.39±1.96[a]	176.82±2.98[b]
Iodine value (g I_2/100 g)	109.76±1.23[a]	94.21±0.16[b]
Peroxide value (meq O_2/kg)	4.04±0.15[a]	4.03±0.59[a]

Values are presented as mean±SD, n = 3. Values with different superscript are significantly different $P\leq0.05$.

3.2 Fatty acid composition

Table 2 shows the fatty acid compositions of GFO from Palagan and Bantul obtained by GC. The highest fatty acid contained in GFO was linoleic acid (C18:2) accounting for 36.85% for GFO from Palagan and 37.37% for GFO from Bantul. The important polyunsaturated fatty acids of EPA in GFO were to be 0.21% and 0.15%. This value is lower than that in marine fish oil such as sardine fish oil with a value of 4.35% (Osman *et al*., 2001). Freshwater fish usually consist of more omega-6 polyunsaturated fatty acid, whereas marine fish are rich in omega-3 especially DHA and EPA (Rahman *et al*., 1995). Arachidonic acid contained in GFO Palagan and Bantul was 0.79% and 0.70% respectively. Arachidonic acid is a precursor of prostaglandin and thromboxane biosynthesis, and this

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Table 2. Fatty acid compositions of Gabus fish oil from Palagan and Bantul

Fatty acids	Fatty acid compositions	
	GFO from Palagan	GFO from Palagan
C12:0 (lauric acid)	0.17	0.14
C14:0 (myristic acid)	2.25	1.99
C15:1 (pentadecanoic acid)	0.67	0.39
C16:1 (palmitoleic acid)	23.20	24.08
C18:1 (oleic acid)	5.39	5.53
C18:2 (linoleic acid)	36.85	37.37
C18:3, n-6 (γ-linoleic acid)	22.58	22.76
C18:3, n-3 (linolenic acid)	2.12	3.37
C20:0 (arachidic acid)	0.31	0.44
C20:1 (gadoleic acid)	0.31	0.27
C20:2 (*cis*-11,14-Eicosadienoic acid)	1.47	*Not detected*
C20:3, n-6 (*cis*-8,11,14-Eicosatrienoic acid)	0.56	0.46
C20:3, n-3	1.14	1.00
C20:4, n-6 (arachidonic acid)	0.79	0.70
C20:5, n-3 (EPA)	0.21	0.15
C22:0 (Docosanoic acid)	0.32	0.29
C22:2, n-6 (*cis*-13,16-Docosadienoic acid)	0.52	0.26
C24:0 (lignoceric acid)	0.63	0.56

fatty acid influenced the ability of GFO as a medicine to reduce pain and inflammation (Chedoloh *et al*., 2011).

3.3 FTIR spectral data

The main chemical composition of oils and fats are triacylglycerols. Therefore, it becomes more difficult to detect a particular oil, especially when it is adulterated with another oil. FTIR spectroscopy can be used as a characterization method because FTIR spectra are considered a fingerprinting tool, in which no two oils have the same FTIR spectra both in number and peak intensity (Che Man *et al*., 2011). FTIR spectroscopy can be used to determine, characterize, and analyze both qualitative and quantitative analyses. FTIR spectroscopy method has been widely used in the analysis because it is fast, easy sample preparation, and is not destructive (Rohman, 2017). Figure 1 shows FTIR spectra of GFO from Palagan and Bantul and also as a comparison there was also presented FTIR spectra of palm oil.

All FTIR spectra in both samples and palm oil appear quite similar as indicated by the principal component analysis (PCA) profile (Syifa *et al*., 2020). GFO from two regions had similar spectra both in terms of peak number and intensity. However, GFO can be distinguished from palm oil in terms of the peak intensity in several wavenumber regions including the peaks at 3008 cm^{-1} (a), 1651 cm^{-1} (f), 1117 cm^{-1} (l), and 1097 cm^{-1} (m). Peak 1117 cm^{-1} and 1097 cm^{-1} are attributed to the absorption of the C-O bond from the ester in the triacylglycerol. Peak 3008 cm^{-1} and 1651 cm^{-1} are responsible for the degree of unsaturation of fatty acids in triacylglycerols (TAG) (Rohman *et al*., 2014).

4. Conclusion

Gabus fish (*Channa striata*) is freshwater fish that

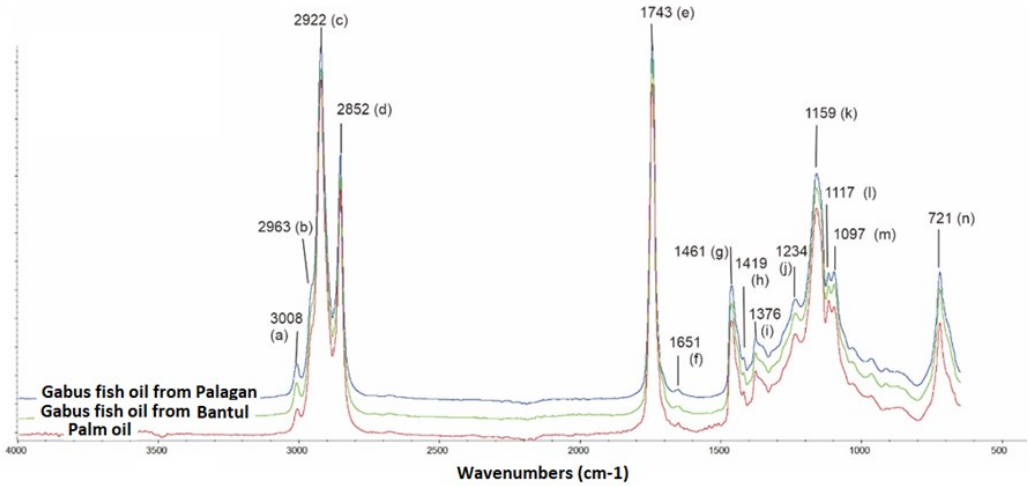

Figure 1. FTIR spectra of Gabus fish oil and palm oil scanned at mid-wavelength region 4000-650 cm^{-1}

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has become economically important in Indonesia due to its potential effect on human health. Gabus fish oil collected from Palagan and Bantul showed almost similar physicochemical properties, fatty acid composition and FTIR spectra. The importance of characterization analysis is to ensure the quality of GFO and to prevent adulteration. The fatty acid composition of GFO was measured by gas chromatography. The highest fatty acid composition in GFO was unsaturated fatty acid namely linoleic acid and palmitoleic acid. FTIR spectrum of GFO is unique and different from other edible oil such as palm oil.

Conflict of interest

The authors declare no conflict of interest.

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