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FULL PAPER

Physicochemical characterization and fatty acid profiles of fish oil from milkfish (*Chanos chanos* F.)

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Abstract

Indonesian local fish including Milkfish (*Chanos chanos*) is a potential source of fish oil. It is well known that fish oil contains polyunsaturated fatty acids (PUFA) having health benefits. The objective of this study was to perform physicochemical characterisation and fatty acid profile of milkfish oil. This study used fish oil extracted from the head and flesh of milkfish using wet rendering. All samples were extracted at low temperature with pressing and are subjected to centrifugation. The result showed that milkfish flesh oil (MFO) and milkfish head oil (MHO) revealed significantly different parameters ($p<0.05$) in terms of physicochemical characteristics including acid value, peroxide value, iodine value, and saponification value. The acid value, peroxide, iodine and saponification values of MFO were 0.5 mg KOH/g, 6.8 meqO_2/kg, 95.3 g I_2/100 g and 183.9 mg KOH/g, respectively. The values for MHO were 0.7 mg KOH/g, 8.7 meqO_2/kg, 101.8 g I_2/100 g, and 200.7 mg KOH/g. The predominant fatty acids in MHO and MFO were palmitic, oleic and linoleic. MFO and MHO are found suitable to be consumed for beneficial health effects.

1. Introduction

Milkfish (*Chanos chanos* F.) or *bandeng* is a fish species that is distributed in tropical and subtropical Indo -Pacific oceans. Milkfish can grow and live in wide range of environmental conditions (Ali, 2017). Indonesia has been the second-largest producer of milkfish in Southeast Asia after the Philippines (Bayaga and Devega, 2005). Milkfish or *bandeng* is a popular raw material of Indonesian culinary food such as *bandeng* presto, *bandeng floss, bandeng nugget,* and *bandeng* meatball. Milkfish is considered to be a "fatty" fish because it contains a lot of fat in relation to its body weight. This fish is a potential candidate species with good production for fish oil.

Fish oils are a rich source of polyunsaturated fatty acid (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (Różyńska *et al*., 2016). Polyunsaturated long-chain fatty acids (PUFAs) are important substances for maintaining health and human growth development (Nazir *et al*., 2017). Moreover, fish oil is an excellent source of energy owing to its high content of methyl palmitate and methyl stearate as the predominant saturated fatty acid (SFA) and substantial amounts of mono-unsaturated fatty acids (MUFA) (Razak *et al*., 2001). This fish is a potential candidate species with good production potential for fish oil. It is considered the cheapest source of animal protein and as a source of omega-3 potential.

The extraction processes of fish oil can be classified into three groups, namely physical, biological and chemical (Adeoti, 2015). The most common method used for fish oil production involves three basic steps, including cooking at high temperatures (85-95^{o}C), pressing, and centrifuging (Bonilla and Hoyos, 2018) in which this method does not require chemicals during the process. Some modifications are applied such as the application of low temperature at 50^{o}C to avoid oxidation which damages the quality of fish oil. Pressing extraction involving the cooking of fish with a low

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temperature for a long time (24 hrs) can damage the structure of the cell which can press the oil from the cooked fish (Wulandari *et al*., 2017). However, high-temperature extraction leads to low quality of the product (Putri *et al*., 2020).

Bandeng can be produced as fish which the largest part of the body consumed was flesh and head as a by-product to determine the chemical composition of food material is important in nutrition perspective of human health (Chalamaiah *et al*., 2012). The physicochemical properties of edible fats and oils are important for their characterization (Rohman *et al*., 2019). These physicochemical parameters include acid value, peroxide value, iodine value and saponification value (Putri *et al*., 2020). The fatty acid composition of fish is affected by environmental factors, species and the production area (Bonilla and Hoyos, 2018). This study aimed to perform the physicochemical characteristics and fatty acid composition of flesh milkfish oil and head milkfish oil.

2. Materials and methods

2.1 Sample preparation and extraction

Milkfish was obtained from a local fish market in Juwana Pati, Central Java, Indonesia. The flesh and head of milkfish were cut into small pieces then placed into aluminium foil and subjected to a cabinet dryer at 50°C for 24 hrs. The dried samples were then subjected to pressing using manual hydraulic at 100 kN for 10-15 mins. The oils were then centrifugated at 5000 rpm for 10 mins to obtain pure oils with a clear appearance.

2.2 Determination of acid value

Acid value (AV) was determined according to the AOAC official method (2000) with some modifications. Oil samples (for head, 1 g and flesh, 1 g) were accurately weighed into Erlenmeyer 250 mL and then added with 50 mL of neutralized ethanol 95% and 2 mL of phenolphthalein indicator solution 1%. The oil samples were then titrated with 0.1 N KOH-ethanolic until the appearance of the first permanent pink colour. The titration was titrated in three replicates. The permanent pink colour persisted for at least 30 s during titration. AV was calculated as:

$$\text{Acid value (mg KOH/g)} = \frac{\text{KOH volume (mL)} \times \text{KOH N} \times 5.61}{\text{Mass of samples (g)}}$$

2.3 Determination of peroxide value

Measurement of peroxide value (PV) can be used as an indication of peroxides contained in the analysed oil. PV was determined according to the AOAC official method (2000). A gram of each sample was accurately weighed into a 250 mL Erlenmeyer flask then 30 mL of acetic acid and chloroform (3:2) were added, and swirled to mix well. The mixture was added with 0.5 mL of saturated potassium iodide solution and allowed to stand for exactly 1 min in a dark room. After that, the mixture was added with 30 mL of distilled water and swirled to mix. A starch indicator (1 mL) was added then titrated with 0.1 N sodium thiosulfate until the blue colour disappeared. PV was calculated as:

$$\text{Peroxide value (meq O2/1000 g)} = \frac{\text{Na}_2\text{SO}_3 \text{ volume (mL)} \times \text{Na}_2\text{SO}_3 \text{ N} \times 1000}{\text{Mass of samples (g)}}$$

2.4 Determination of iodine value

The iodine value was determined according to the AOAC official method (2000). A 300 mg of oil samples were accurately weighed and placed in 250 mL Erlenmeyer, added with 25 mL chloroform followed by 20 mL of Wijs solution. The solution was allowed to react in a dark room for 30 mins. A 10 mL of 10% potassium iodide along with 50 mL of deionized water were added to each sample. The mixture was titrated using 0.1 N sodium thiosulfate until the yellow colour disappeared. Starch indicator (1 mL) was added and the titration was continued until the blue colour disappeared. IV was calculated as:

$$\text{Iodine value (g I2/100 g)} = \frac{(\text{Na}_2\text{SO}_3 \text{ volume of blank} - \text{Na}_2\text{SO}_3 \text{ volume of sample}) \times \text{Na}_2\text{SO}_3 \text{ N} \times 12.69}{\text{Mass of samples (g)}}$$

2.5 Saponification value

The saponification value (SV) was expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify 1 g of oil. Determination of SV was carried out according to the AOAC official method (2000). An approximate 1 g of oil was accurately dissolved with 50 mL KOH-ethanolic in an Erlenmeyer flask then mixed until homogeneous. The solution was heated at temperatures 80-85°C for 30 mins. After that, the solution was cooled and added with 1 mL phenolphthalein. The mixture was titrated with 0.5 N HCl until the pink colour has just disappeared. SV was calculated as:

$$\text{Saponification value (mg KOH/g)} = \frac{(\text{HCl volume of blank} - \text{HCl volume of sample}) \times \text{HCl N} \times 56.1}{\text{Mass of samples (g)}}$$

2.6 Fatty acid composition

For the determination of fatty acid profile, the milkfish oils were subjected to methylation or derivatization into fatty acid methyl ester (FAME) (Rohman and Riyanto, 2020). A 0.5 mL of oil sample was added to 1.5 mL of methanolic-sodium. The solution was mixed and boiled at 60°C for about 5-10 mins then cooled. A 2 mL of BF_3 was added and boiled again at 60°C in about 5-10 mins then cooled. The sample was extracted with 1.0 mL Heptane and 1.0 mL saturated NaCl. The top layer is carefully collected, and 1 µL sample solution is injected into GC-FID Agilent 7890B equipped with DB-WAX column using programmed

oven temperature of 50-230°C with a temperature increase rate of 3°C /min.

2.7 Statistical analysis

Physicochemical characteristics data were statistically subjected to independent sample T-Test using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) with a significance level of 95% ($p<0.05$ was considered as significant).

3. Results and discussion

The flesh and head of a milkfish were taken and the oils contained were extracted using wet pressing consisting of cooking, pressing and centrifugation (Rubio-Rodríguez *et al*., 2008). Milkfish flesh oil (MFO) had a more light-yellow colour compared to milkfish head oil (MHO) having a yellow-orange colour (Figure 1). Table 1 compiles the yield obtained during the preparation of MFO and MHO. The oils obtained were then subjected to physico-chemical characterization and fatty acid composition and the results were compiled in Table 2. The acidity of oil is an important quality parameter related to the presence of free fatty acid (FFA) and other non-lipid acid compounds. FFA is primarily produced by the hydrolysis reaction of triacylglycerol. The acid value (AV) was determined to express the acidity of studied fats and oils (Putri *et al*., 2020). AVs of the MFO and MHO were 0.522±0.025 mg KOH/g and 0.667±0.024 mg KOH/g, respectively. Based on the independent t-test, the p-value from both samples was 0.004 meaning that both samples were significantly different ($p<0.05$). According to the Food and Agricultural Organization (FAO, 2017) about the standard for fish oils, the acceptable AN (fish oil) ≤ 3 mg KOH/g, and both samples were in the range to be edible oils. AN depends on several factors including oil composition, extraction process and freshness of the raw material (Dominguez and Barbagallo, 2018).

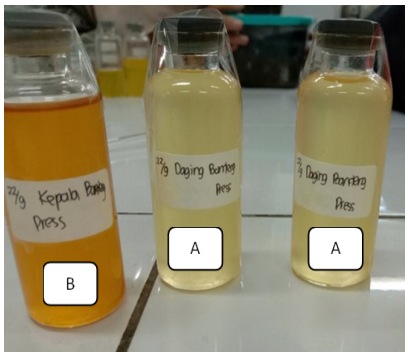

Figure 1. Milkfish flesh oil (A) and milkfish head oil (B) used during this study extracted using wet extraction processing with processing.

Peroxide value (PV) is the most important value to determine the degree of oil damage during oxidation. The oil damage can occur due to the oxidation process by oxygen from the air binding unsaturated fatty acid in the oils during heat processing (Lusas *et al*., 2012) or storage (Phung *et al*., 2020). The smaller PV means better-quality oils. PV is expressed as milligram equivalents of peroxide oxygen in a kilogram of oil and PV was used as a measurement of rancidity (Ndidiamaka and Ifeanyi, 2018). In this study, PV obtained for both samples was < 10, which is 6.830±0.095 meqO_2/kg (MFO) and 8.683±0.124 meqO_2/kg (MHO). Based on the independent t-test, PVs of MFO and MHO were significantly different with a p-value of 0.000 ($p<0.05$). The American Standard for Testing Materials (ASTM) and World Health Organization (WHO) stipulated that the permitted maximum PV was not more than 10 meqO_2/kg of the oils. Thus, both samples were suitable for consumption since PVs of studied oils were < 10 (Bako *et al*., 2017). FAO set up PVs for fish oil in which the acceptable PV was ≤ 5 meqO_2/kg (FAO, 2017). In this study, peroxide values were high because the oil is not refined. Peroxide or hydroperoxide is intermediate species which is unstable species that can react with KI quickly (Mahboubifar *et al*., 2016).

Table 1. The yield of milkfish oils obtained during the extraction of milkfish

No.	Sample	Wet sample (g)	Mass of oil (g)	Yield (%)
1	Milkfish flesh	4082.85	286.54	18.3
2	Milkfish head	1917.16	113.67	22.1

Table 2. Physicochemical properties of milkfish oil

Physicochemical properties	Sample	
	MFO	MHO
Acid value (mg KOH/g)	0.522±0.025	0.667±0.024
Peroxide value (meq O_2/kg)	6.830±0.095	8.683±0.124
Iodine value (g I_2/100 g)	95.297±0.742	101.812±1.464
Saponification value (mg KOH/g)	183.902±1.872	200.699±1.714

MFO = Milkfish flesh oil; MHO = milkfish head oil.

Iodine value (IV) is a measure of overall unsaturation degree, defined as the number of grams of iodine absorbed by 100 g of fats or oils (Norziah *et al*., 2009). IV determines the stability of oils to oxidation (Asuquo *et al*., 2012). High IV shows that the oils contain a higher degree of unsaturation and have good qualities (Babalola and Apata, 2011). In this study, IV for MFO was 95.297±0.742 g I_2/100 g and 101.812±1.464 g I_2/100 g for MHO. Statistic test revealed that IVs for both oils were significantly different with a p-value of 0.005 ($p<0.05$). Based on ASTM, the allowable IV was 82-88 g I_2/100 g (Bako *et al*., 2017). Rai *et al*. (2010) reported that the acceptable fish oils were oils with typical IVs of 95-118 g I_2/100 g.

Saponification value (SV) is an index of the average molecular mass of fatty acid in the oil samples. SV is the

number of milligrams of potassium hydroxide required to neutralize the fatty acid resulted from complete hydrolysis of 1 g of oil samples (Bako *et al*., 2017). The high SV indicates that the oil samples had a lower molecular weight of fatty acid (Nazir *et al.*, 2017). The SVs obtained were 183.902±1.872 mg KOH/g (MFO) and 200.699±1.714 mg KOH/g (MHO). SVs in both samples revealed significantly different based on independent sample t-test with a p-value of 0.001 ($p < 0.05$). According to ASTM, the SV in fish oil is in the range of 175-201 mg KOH/g (Bako *et al*., 2017). All SVs of the sample in this study were within ASTM standard.

The fatty acid compositions of MFO and MHO were shown in Table 3. Palmitic, oleic, and linoleic acids are the three fatty acids that predominate in both oil samples. The results obtained were different to those reported by Bayaga and Devega (2005), Agustini *et al.* (2011), and Maulana *et al*. (2020). Bayaga and Devega (2005) reported that the most abundant fatty acids in milkfish oil were lauric, oleic and palmitic acids which together composed about 50% of the total fatty acids. Many factors may contribute to these differences such as place of origin. Other factors contributing to these differences include the harvesting seasons, fish food, extraction process which may influence the fat and lipid of the milkfish (Kumar *et al*., 2014). The fatty acid profile affects the shelf-life, flavour and stability of the oil. The ratio of oleic to linoleic acid is a measure of oil stability and it is a critical factor in determining oil quality. Shelf-life and flavour are determined by how quickly the oxidative rancidity occurs (Babalola and Apata, 2011).

Table 3. Fatty acid profiles of milkfish flesh oil (MFO) and milkfish head oil (MHO)

Fatty Acid	% fatty acids				
	MFO	MHO	A	B	C
Lauric acid	0.84	0.62	23.12	0.63	-
Myristic acid	3.52	4.27	9.9	1.42	-
Myristoleic acid	0.18	0.33	0	2.31	-
Pentadecanoic acid	0.92	2.15	0.17	-	1.86
cis-10-Pentadecenoic acid	0.16	0.16	-	-	-
Palmitic acid	29.01	28.33	17.69	22.2	27.2
Palmitoleic acid	6.28	7.42	0.72	4.23	3.91
Heptadecanoic acid	0.54	0.87	0.14	0.32	0
cis-10-heptadecenoic Acid	0.67	1.24	0.11	-	1.09
Stearic acid	6.38	5.41	2.85	16.2	9.05
Oleic acid	23.29	19.88	13.95	20.1	23.8
Linoleic acid	12.9	11.36	1.55	14.8	10.8
Linolenic acid	1.68	2.22	2.78	2.48	6.31
Arachidic acid	0.24	0.23	0.2	0.17	-
Cis-11- Eicosenoic acid	2.35	2.14	1.16	0.69	1.55
Cis-11,14-Eicosadienoic Acid	1.44	1.31	0.53	-	-
Cis-8,11,14-Eicosatrienoic Acid	1.35	1.26	0.45	-	0.81

A = Bayaga and Devega (2005), B = Agustini *et al*. (2011) and C = Maulana *et al*. (2020)

4. Conclusion

Milkfish oil, extracted from milkfish (a common food commodity for the Indonesian community), has potential application in functional food oils. Physicochemical properties (acid value, peroxide, iodine and saponification values) of milkfish flesh oil (MFO) and milkfish head oil (MHO) were significantly different based on an independent t-test with a p-value <0.05. The acid value, peroxide, iodine value, and saponification values were acceptable with standards for fish oil. Fatty acid profiles showed that milkfish oil contained main fatty acids namely palmitic, oleic and linoleic acid.

Conflict of interest

The authors declare no conflict of interest.

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