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FATTY ACID PROFILE OF SARDINE FISH OIL AS A SOURCE OF OMEGA 3

Profil Asam Lemak Minyak Ikan Sardin Sebagai Sumber Omega 3

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ABSTRACT

Sardines, or lemurs fish, is a type of pelagic fish commonly found in the waters of the Bali Strait, with high oil content but low quality, although it still contains important elements for health such as omega 3. This research aims to identify the fatty acid profile of sardine fish oil as a source of omega 3 before and after purification. The method used in this research is divided into two parts: purification and testing of fatty acid profiles. The purification process is divided into 3 stages: degumming with citric acid, neutralizing sodium hydroxide (NaOH), and whitening using 5% magnesol XL. Fatty acid profile testing is divided into 3 stages: fatty acid extraction, methyl ester formation, and fatty acid identification. The results showed that the percentage of Saturated Fatty Acids (SFA) in crude oil and pure oil was 30.06% and 22.85%, respectively; for Monounsaturated Fatty Acids (MUFA), it was 11.84% and 8.83%, while multi-chain unsaturated fatty acids (PUFA) were 24.46% and 21.52%. The percentage of omega 3 in crude fish oil and pure oil is 20.89% and 14.87%, respectively; omega 6 is 3.35% and 6.53%; and omega 9 is 6.15% and 5.24%. These findings show that PUFA has a significant percentage after SFA, and the highest PUFA content is omega 3, especially Eicosapentaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). Thus, this research shows that pure fish oil has the potential to be further developed into products rich in omega 3.

Keywords: Fish Oil Purification, Omega 3, Sardine or Lemurs Fish Oil, The Fatty Acid Profile

ABSTRAK

Ikan sardin atau lemuru adalah jenis ikan pelagis yang umum ditemukan di perairan Selat Bali, dengan kandungan minyak yang tinggi tetapi kualitasnya rendah, meskipun masih mengandung elemen penting untuk kesehatan seperti omega 3. Tujuan penelitian ini adalah untuk mengidentifikasi profil asam lemak dari minyak ikan sardin sebagai sumber omega 3 sebelum dan sesudah pemurnian. Metode yang digunakan dalam penelitian ini terbagi menjadi dua bagian, yaitu pemurnian dan pengujian profil asam lemak. Proses pemurnian dibagi menjadi 3 tahap: *degumming* dengan asam sitrat, netralisasi menggunakan natrium hidroksida (NaOH), dan *bleaching* menggunakan magnesol XL 5%. Pengujian profil asam lemak terbagi

menjadi 3 tahap: ekstraksi asam lemak, pembentukan metil ester, dan identifikasi asam lemak. Hasil penelitian menunjukkan bahwa persentase asam lemak jenuh (SFA) pada minyak kasar dan minyak murni masing-masing adalah 30,06% dan 22,85%; untuk asam lemak tidak jenuh rantai tunggal (MUFA) adalah 11,84% dan 8,83%; sedangkan asam lemak tidak jenuh rantai banyak (PUFA) sebesar 24,46% dan 21,52%. Persentase omega 3 pada minyak ikan kasar dan minyak murni berturut-turut sebesar 20,89% dan 14,87%; omega 6 sebesar 3,35% dan 6,53%; serta omega 9 sebesar 6,15% dan 5,24%. Temuan ini menunjukkan bahwa PUFA memiliki persentase yang cukup signifikan setelah SFA, dan kandungan PUFA yang paling tinggi adalah omega 3, khususnya asam eikosapentaenoat (EPA) dan asam dokosaheksaenoat (DHA). Dengan demikian, penelitian ini menunjukkan bahwa minyak ikan murni memiliki potensi untuk dikembangkan lebih lanjut menjadi produk yang kaya omega 3.

Kata kunci: Minyak Ikan Sardin atau Lemuru, Omega 3, Pemurnian Minyak Ikan, Profil Asam Lemak

INTRODUCTION

Sardines or lemuru are one type of pelagic fish besides tuna, mackerel, and mackerel which are widely found in the waters of the Bali Strait because they are a source of income for fishermen (Wijaya & Priyono, 2019). Therefore, sardines or lemuru have economic and strategic value and can be used as a supplier of raw fish materials for the processing industry.

Sardines or lemuru are also a type of fish that contain a lot of oil and other nutrients, where 100 g of sardines contain calcium, potassium, magnesium, zinc, iron, taurine, and arginine (Eka et al., 2021; Kaihena & Ukratalo, 2022; Santos *et al.*, 2023). Sardines or lemuru can be further processed into processed products, but the by-products in the form of oil waste produced will also be a lot. This is supported by research by Suseno et al. (2014[b]) that as many as 20-30 tons of lemuru fish or sardines used in the fish processing industry will produce 15-20% oil. This waste has low quality but still has added value if various stages of purification are carried out.

The fish oil produced is rich in essential fatty acids, which cannot be produced or synthesized by the body naturally. In terms of composition, unsaturated fats reach 75%, while saturated fatty acids reach 25%. Among the unsaturated fatty acids that are important for health is omega-3, which is found in various types of fish such as mackerel, milkfish, lemuru, layang, and tuna (Gunawan *et al.*, 2014). When sorted by omega-3 content, lemuru fish is in third place, after salmon and mackerel. Omega-3 fatty acids include EPA (Eicosapentanoic Acid), DHA (Docosahexanoic Acid), and Linoleate (Sarker, 2020). However, the dominant omega-3 fatty acids usually found in fish oil are EPA and DHA (Manduapessy, 2017).

Many studies have examined the fatty acid composition found in sardine oil. According to Pandiangan *et al.* (2023), sardine oil contains 1.3379% PUFA, with an omega-3 content of 0.5270%. Musbah et al. (2017) reported a PUFA level of 27.59%, including EPA of 12.20% and DHA of 7.39%. In addition, Dari *et al.* (2017) noted that the PUFA content in sardine oil reached 35.78%, with EPA measured at 15.18% and DHA at 15.65%. The omega-3 content in fish oil offers significant benefits, especially related to cardiometabolic conditions associated with mild chronic proinflammatory states, such as type 2 diabetes, hypertension, hypertriglyceridemia, and fatty liver disease (Gammone *et al.*, 2019). Sarker (2020) further emphasized that DHA and Linoleate can be very beneficial in improving cognitive abilities and intelligence. Based on the description above, it is necessary to test the fatty acid content profile of sardine fish oil as a source of omega 3 before and after purification. So that in the future, the hope is that the oil can be further developed in its application.

RESEARCH METHOD

Time And Location of Research

This research has been conducted for one month. For the sardine or lemuru fish oil samples used, namely fish oil from the flour industry in Bali, which was then purified at the Fish Oil Laboratory of the Department of Aquatic Product Technology, Bogor Agricultural University. The results of crude fish oil and pure oil were then tested for fatty acid profiles at the IPB Integrated Laboratory.

Tools and Materials

The tools used in the study were centrifuge (PLC Series), digital scales (Quattro), stirrer (Coming PC-420 D), electric stove, water bath, gas chromatography with flame ionization detector (FID), and glassware. Meanwhile, the materials used are sardine or lemuru fish oil, 95% alcohol, phenolphthalein (PP) (Merck), potassium hydroxide (KOH) (Merck), glacial acetic acid (Merck), chloroform (Merck), potassium iodide (KI) (Merck), distilled water, sodium thiosulfate ($Na_2S_2O_3$) (Merck), starch (Merck), trimethylpentane (isooctane) (Merck), p-anisidine (Sigma Aldrich), sodium hydroxide (NaOH) (Merck), and materials for testing fatty acid profiles (BF_3, NaCl, hexane, anhydrous Na_2SO_4, methyl ester, nitrogen, hydrogen).

Purification Stage

The purification process is divided into 3 stages, namely degumming, neutralization, and bleaching. The purification process is 100 mL of fish oil heated at a temperature of 50ºC; then continued with the degumming process by adding 2 mL of acid solution and then homogenized; then the neutralization stage by adding caustic soda (NaOH) then homogenizing and separating the oil from the impurity components; the bleaching stage is carried out by adding magnesol XL 5% (w/v) to the oil then homogenizing and separating the oil from the impurity components. The final result obtained is purified oil.

Fatty Acid Profile Testing Stage Based on Aoac (2012): 969.33

Fatty acid analysis refers to AOAC (2012), which consists of 3 stages, namely fatty acid extraction or soxhlet extraction, methyl ester formation (methylation), and identification of fatty acids by gas chromatography.

Data Analysis

Data analysis using qualitative descriptive data from fatty acid identification results.

RESULTS

Organic acids that have long chains and the number of carbon atoms ranges from four to twenty-four single carboxyl groups have non-polar hydrocarbon tails so that they are insoluble in water and appear like an oil layer (Lehninger, 1998). There are two groups of fatty acids, saturated and unsaturated. In crude oil, of the 10 types of saturated fatty acids analyzed, there are 2 types of saturated fatty acids among which have results <0.1%. While the results of the analysis of pure oil, of the 9 types of saturated fatty acids analyzed, there are 2 types of saturated fatty acids that have results <0.1% (Table 1).

Table 1. Types of saturated fatty acids

Types of Saturated Fatty Acids	% w/w	
	Crude Oil	Pure Oil
Lauric Acid (C12:0)	0,11	0,06
Tridecanoic acid (C13:0)	0,06	-
Myristic acid (C14:0)	7,18	4,93

Pentadecanoic acid (C15:0)	0,60	0,42
Palmitic acid (C16:0)	16,70	13,11
Heptadecanoic acid (C17:0)	0,80	0,58
Stearic acid (C18:0)	3,92	3,20
Arachidic acid (C20:0)	0,37	0,30
Heneicosanoic acid (C21:0)	0,08	0,05
Behenic acid (C22:0)	0,24	0,20
SFA TOTAL	**30,06**	**22,85**

Based on the analysis results for crude oil, of the 5 types of monounsaturated fatty acids analyzed, there was 1 type of monounsaturated fatty acid among them that had a result of <0.1%. While the results of the analysis of pure oil, of the 4 types of monounsaturated fatty acids analyzed, there was 1 type of monounsaturated fatty acid that had a result of <0.1%. The data can be seen in Table 2.

Table 2. Types of monounsaturated fatty acids

Type of monounsaturated fatty acid	% w/w	
	Crude Oil	Pure Oil
Myristoleic acid (C14:1)	0,02	-
Palmitoleic acid (C16:1)	4,89	3,25
Cis-10-heptadecanoic acid (C17:1)	0,42	0,29
Elaidic acid (C18:1n9t)	-	0,05
Oleic acid (C18:1n9c)	6,09	5,24
Nervonic acid (C24:1)	0,42	-
TOTAL MUFA	**11,84**	**8,83**

Based on the analysis results for crude oil, of the 8 types of polyunsaturated fatty acids analyzed, there was 1 type of polyunsaturated fatty acid that had a result of <0.1%. While the results of the analysis of pure oil, of the 6 types of polyunsaturated fatty acids analyzed, there was no type of polyunsaturated fatty acid that had a result of <0.1%. The data can be seen in Table 3. The proportion of SFA is greater than PUFA, and MUFA is lower than both in crude oil and pure oil (see Table 4).

Table 3. Types of polyunsaturated fatty acids

Types of polyunsaturated fatty acids	% w/w	
	Crude Oil	Pure Oil
Linoleic acid (C18:2n9t)	0,06	-
Linoleic acid (C18:2n6c)	1,19	5,14
γ-linolenic acid (C18:3n6)	0,17	0,13
Cis-11,14-eicosedienoic acid (C20:2)	0,16	0,12
Cis-8,11,14-eicosetrienoic acid (C20:3n6)	0,21	-
Arachidonic acid (C20:4n6)	1,78	1,26
Cis-5,8,11,14,17-eicosapentaenoic acid (C20:5n3)	8,37	6,03
Cis-4,7,10,13,16,19-docosahexaenoic acid (C22:6n3)	12,52	8,84
PUFA TOTAL	**24,46**	**21,52**

Table 4. Proportion or ratio of fatty acids

Category	Crude Oil	Pure Oil
SFA	30,06	22,85
MUFA	11,84	8,83
PUFA	24,46	21,52
Σ Omega 3 (%)	20,89	14,87
Σ Omega 6 (%)	3,35	6,53
Σ Omega 9 (%)	6,15	5,24

DISCUSSION

Saturated Fatty Acid (SFA)

Saturated fatty acids are a type of fatty acid that does not have double bonds so it is susceptible to oxidation and the formation of free radicals. In crude oil and pure oil, the highest levels of saturated fatty acids are palmitic acid (C16: 0), followed by myristic acid (C14: 0), and then stearic acid (C18: 0). Parts of saturated fatty acids that are synthesized in cells are phospholipids, diacylglycerols, and ceramides (Korbecki & Bajdak-Rusinek, 2019). With the temperature treatment during the refining process, it will affect the percentage of palmitic fatty acid, but the percentage is still high compared to other saturated fatty acids, so it is easy to react (Isamu *et al.*, 2017). Agostoni *et al.* (2016) stated that although palmitic acid has a long-term negative impact on adults, palmitic acid also plays a very important role in cell membranes, secretion or excretion, and also in lipid transport in the body.

Total SFA in crude oil was 30.06% and pure oil was 22.85%. The results showed that there was a decrease in the total percentage of 7.21%. This shows that the purification carried out reduced the percentage of saturated fatty acids. The total SFA results are consistent with those produced by Suseno *et al.* (2014), that the SFA of crude sardine oil from Java Island has a total SFA percentage of 28.96%.

Monounsaturated Fatty Acid (MUFA)

Monounsaturated fatty acids are defined as a category of fatty acids characterized by one double bond at the carbon atom. Oleic acid (C18:1n9c) contains the highest concentration of monounsaturated fatty acids in both crude and virgin oils, followed by palmitoleic acid (C16:1). However, after the refining process, there was a decrease in the percentage of fatty acids. The total MUFA in crude oil was measured at 11.84%, while virgin oil showed a percentage of 8.83%. This shows a decrease of 3.01% in the total percentage. This finding is in line with a study conducted by Suseno *et al.* (2014[a]), which reported that the total MUFA of crude sardine oil sourced from Java Island was 16.73%.

These unsaturated fatty acids have double bonds that can be quickly broken down and produce more stable saturated fatty acids when reacting with other ingredients. When fish oil is exposed to light, oxygen, and temperature during the refining process, the unsaturated double bonds it contains also become saturated or the transformation of unsaturated double bonds into saturated bonds occurs (Dari *et al.*, 2017; Untari *et al.*, 2020). According to Jamaluddin et al. (2018), oleic acid or also known as omega-9 fatty acids has the ability to help increase High-Density Lipoprotein (HDL) cholesterol levels and reduce Low-Density Lipoprotein (LDL) cholesterol levels.

Polyunsaturated Fatty Acid (PUFA)

There are more than one or many double bonds on carbon atoms that form the definition of polyunsaturated fatty acid groups. The highest types of unsaturated fatty acids in crude oil and pure oil are found in DHA and EPA. Homayooni *et al.* (2014) found and reported something similar that the PUFA content of sardines was dominated by DHA at 17.4% and

EPA at 15.3%. Further researchers reported that the EPA and DHA content in sardine oil from flouring by-products were 15% and 6-11%, respectively (Suseno *et al*., 2013; Suseno *et al*., 2014[c]). Furthermore, the EPA content was 16.540% and DHA was 0.744% (Andhikawati *et al*., 2020); EPA of 11.22% and DHA of 10.53% (Putri *et al.*, 2022)

Omega-3, one of the most important unsaturated fatty acids, is known to help prevent various health conditions such as cancer and high blood pressure. The omega-3 group in fish oil is EPA and DHA, which can inhibit the activation of oxidant-sensitive transcription factors (Martorell *et al.*, 2014).

Fatty Acid Ratio

The percentage of SFA is higher than PUFA and the percentage of MUFA is lowest for crude oil and pure oil. These results are not in line with the research of Andriyani et al. (2017) that the proportion of PUFA is greater than SFA. Based on the research results of Rahayu *et al*. (2014) that lemuru fish obtained from Muara Angke had a total SFA of 28.34%, MUFA of 7.33%, and PUFA of 20.08%. These results indicate that the total SFA is higher followed by PUFA and MUFA. Meanwhile, according to the research results of Dari *et al*. (2017), the higher the level of purification of sardine fish oil, the lower the SFA, MUFA, and PUFA values. Loppies *et al.* (2021) stated that another factor that causes changes in fatty acid content besides oxidation or hydrolysis is an increase in a component that has an impact on decreasing other components. For example, when the SFA value is high, the MUFA and PUFA values will decrease. This is also in line with the results of the study that the SFA content is higher than PUFA and MUFA.

It is further explained that the composition of fatty acids is different for each fish depending on the feed (Manjusri *et al*., 2014), season and environment (Hong *et al*., 2015), and is a group of wild fish or farmed fish (O'Neill *et al*., 2015). The presence of fatty acids is indeed very important for the body's metabolic system. The factors that cause the fatty acid content of each fish to differ, such as species, temperature, habitat and type of food (Insani *et al*., 2017). According to Suroso (2013), the number of double bonds causes an increase in reactivity to oxygen and has an impact on the oxidation of fish oil.

The PUFA group that cannot be synthesized by the body, namely EPA and DHA, functions to reduce the death rate from heart attacks by up to 70%. According to Sarker (2020), lemuru fish contains 26.8% omega 3. Based on the results of the study (Andhikawati *et al.*, 2020), the total omega 3 content in lemuru fish oil is 17.284% which gradually decreases over 30 days to 4.72%. Based on the results of the study by Dari *et al*. (2017), sardine fish oil with one level of purification has a higher omega 3 content (32.24%) followed by omega 9 (4.41%) and omega 6 (4.07%). The functions of omega-3 include lowering triglyceride levels, increasing HDL, stopping platelet aggregation and reducing inflammation, oxidative stress (imbalance of free radical molecules and antioxidants), improving endothelial function (Fuentes *et al.*, 2018). According to FAO (2010), the recommended consumption of omega-6 ranges from 3% -8% of energy; the recommended consumption of omega-3 ranges from 0.5% -2.5% of energy. Meanwhile, the recommended consumption ratio of omega-6 and omega-3 in fish oil is 5%:1-10%:1%, which can effectively reduce the risk of various diseases including heart disease, diabetes, and cancer.

CONCLUSION

PUFA has a fairly large percentage after SFA and the highest PUFA content is omega 3, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). So this study shows that pure fish oil has the potential to be further applied into a product rich in omega 3.

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