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Fatty Acid Profile of the Oil Extracted from Fish Waste (Head, Intestine and Liver) (*Sardinella lemuru*)

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Abstract: Fish lipid contains long-chain n-3 (omega-3) PUFA, particularly EPA (C20:5 n-3) and DHA (C22:6 n-3). Consumptions of these PUFAs have been perceived to be important in human nutrition, health and disease prevention. World fish lipid request continue to increase. Fish lipid that contributes to the nutritional needs is currently being extracted from liver or muscle of cod, herring, mackerel and sardine. Sardine, the important industrial fish, discharged considerable amount of wastes. These wastes include the head, liver and intestine. Substantial amount of lipid can be extracted from these wastes. All the extracted oils were less than 6 % of which the highest was in liver (5.80 %). The predominant fatty acids in sardine wastes were palmitic (C16:0; 27.80- 35.56 %), stearic (C18:0; 5.90- 9.30 %), oleic (C18:1c; 15.47- 21.79 %) and docosahexaenoic acid (DHA; C22:6; 11.87- 15.95 %). The n3 / n6 ratio of the respective head, liver and intestine lipid samples showed the value higher than 1. Due to n-3 fatty acid compound and n-3 / n-6 ratio, lipid from sardine waste may be a valuable source for human consumption.

Key words: N-3 fatty acid · Fish waste · Fish lipid · N-3 / n-6 ratio · EPA · Docosahexaenoic acid

INTRODUCTION

Recently, fish and shellfish catch improved from 40 to 133 million metric tons and this improvement was paralleled to the need for processed fish. Production of fish waste reciprocates with increase in tonnage of fish caught [1]. Fish products for human consumptions include fresh and frozen, whole and fish fillet [2]. Most of the discards composed of head, intestine, skin, bones and etc. These wastes have high content of nutritive compounds like protein which is the substrate of fish meal production [3]. The other important nutritive compound is the essential fatty acids. Some of the essential acids beneficial to human health are the long-chain n-3 polyunsaturated fatty acids (PUFAs), including, C20:5 n-3 and C22:6 n-3. They are recognized as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), respectively. N-3 PUFAs consumption is linked to the development of brain and nervous tissue in infants and visual function and reduces incidence of coronary heart disease [4-6].With growing public awareness of the clinical benefits of EPA and DHA and population growth, there is a dramatic need to locate a compatible source. Sardine is magnificent that it has high level of lipids which contains the important eicosapentaenoic acid (C20:5 n-3) and docosahexaenoic acid (C22:6 n-3). Sardine processing virtually eliminates heads, intestine and liver. Analysis of the extracted oil from these respective wastes (heads, intestine and liver) reflects the prominent presence of the important essential PUFAs.

MATERIALS AND METHODS

Sample Collection and Preparation: A total of 315 (30 kg) sardine (*S. lemuru)* were purchased from a local wet market in Pasar Borong, Serdang Malaysia. The average weight and length of each fish was 95±1.80 g and 22±0.25 cm, respectively. The fish were kept on ice until processed in the lab. The heads were cut, separated and kept in the freezer (-25°C). Similar treatments were given to the intestine and liver.

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Lipid Extraction: The procedure for the lipid extraction was based on modified Kinsella method. About 50 g of fish wastes were selected randomly and homogenized in a warring blender for 2 min with a mixture of 50 ml chloroform and 100 ml methanol. One volume of chloroform (50 ml) and one volume of distilled water (50 ml) were added to the mixture and blended for 30 sec, respectively. The homogenate was filtered through a Whatman No.1 filter paper on a No.3 Buchner funnel with a slight suction and the filterate collected and transferred to a separatory funnel to allow for phase separation. The lower fraction was collected and filtered. It was then transferred to a rotary evaporator for evaporation. The sample was then collected for the fatty acid analysis [7].

Determination of Fatty Acid Composition: 50 µL of oil solubilised in 950 µL of hexane was esterified using sodium methoxide catalyze based on Christie method [8]. The fatty acid composition was analysed by GC (Hewlett Packard 6890) equipped with a flame ionization detector (FID) and a fused silica capillary BPX-70 column (60 m x 0.32 mm i.d., 0.25µm film thickness) from SGE (Melbourne, Australia). The oven temperature was set at 115°C, raised to 180°C at rate of 8°C/min and held for 10 min and finally raised to 240°C at rate of 8°C/min and held for 10 min. The sample size was 1 µL and flashed through with carrier gas (helium) at rate of 1.6 ml/min. Identifications of the methyl esters were made by comparison of retention times of FAME with the standard 37 component FAME mixture [9].

Statistical Analysis: Values are presented as the mean±standard deviation of triplicate determinations. Statistical analysis was carried out by one-way analysis of variance (ANOVA, with Tukey test) using SPSS software (Version 14.0 software, SPSS Inc., Chicago, IL, USA) and significance was defined at $p < 0.05$.

RESULTS AND DISCUSSION

Yield of Fish Lipid: The lipid contents of head, intestine and liver of *S. lemuru* and three other fish are shown in Table 1. Comparatively, the liver expresses the highest lipid content followed by head and intestine. Based on Ackman (1994) classification, the lipid content of the respective tissues located between 4-8% by weight which was categorized as medium fat fish product [10]. This includes head (5.67 %), intestine (5.08 %) and liver (5.80 %). In this study, sardine waste lipid content is within the range of cod offal and herring wastes lipid [11-13]. The waste of sardine is opted as being a good source of lipid.

Table1: Lipid content of *Sardinella lemuru* waste and some other fish waste[A]

Fish species	Lipid (g/ 100g)
Sardinella lemuru head (Sardine)	5.67±0.21
Sardinella lemuru intestine (Sardine)	5.08±0.25
Sardinella lemuru liver (Sardine)	5.80±0.30
G.morhua (cod offal)[11]	4.3
Clupea harengus(herring waste)[12]	9.6
Tenualosa ilisha (herring waste)[13]	8.5

[A]values for sardine waste lipid are means ± S.D.

Table2: Fatty acid profile of *Sardinella lemuru* waste lipid [A].

Fatty acids	Head	Intestine	Liver
C14:0	6.75±0.88^{b}	1.90±0.00^{a}	3.35±0.55^{a}
C15:0	1.61±0.16^{a}	ND	1.16±0.23^{a}
C16:0	27.80±0.78^{a}	33.59±0.16^{ab}	35.56±1.78^{b}
C16:1	6.54±0.39^{b}	5.90±0.07^{ab}	4.10±0.51^{a}
C17:0	1.19±0.07^{a}	1.31±0.06^{a}	1.20±0.27^{a}
C17:1	1.88±0.01^{b}	2.17±0.04^{c}	1.45±0.07^{a}
C18:0	9.07±0.26^{b}	5.90±0.95^{a}	9.30±0.40^{b}
C18:1 n 9t	0.39±0.03^{ab}	0.98±0.27^{b}	0.26±0.07^{a}
C18:1 n 9c	15.47±0.04^{a}	21.79±0.53^{b}	16.93±1.05^{a}
C18:2 n 6c	2.19±0.08^{b}	4.36±0.06^{c}	1.28±0.04^{a}
C20:3 n 6	1.82±0.12^{a}	2.10±0.10^{a}	2.11±0.43^{a}
C20:4 n 6	5.59±0.34^{b}	4.84±0.15^{b}	3.55±0.07^{a}
C24:1	1.94±0.06^{b}	1.46±0.16^{a}	4.12±0.02^{c}
C20:5 n 3	1.84±0.15^{a}	1.73±0.10^{a}	2.76±0.14^{b}
C22:6 n 3	15.95±1.17^{b}	11.87±0.10^{a}	12.97±0.73^{ab}
Σ SFA	47.22	42.70	50.57
Σ MUFA	26.22	32.30	26.86
Σ PUFA	26.39	24.90	22.67
Σ n-6 FA	9.60	11.30	6.94
Σ n-3 FA	17.79	13.60	15.73

[A]values are means and S.D; Means with the same letter within the raw were not significantly difference at *P?0.05* level; ND: Not detected.

Fatty Acid Profile: The FA compositions of the lipid extracted from head, intestine and liver of the *S. lemuru* are shown in Figures 1 and Table 2. With reference to Figures 1 and Table 2 the fatty acids of the lipid of *S.lemuru* waste are generally composed of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid. GC chromatograms illustrate that all the *S.lemuru* waste samples have the common fatty acids such as palmitic, stearic, oleic, arachidonic, eicosapentaenoic acid and docosahexaenoic acids.

The total saturated fatty acids (SFA) content of these wastes ranged between 42.70 to 50.57 % which was highest in liver lipid and lowest in head lipid.

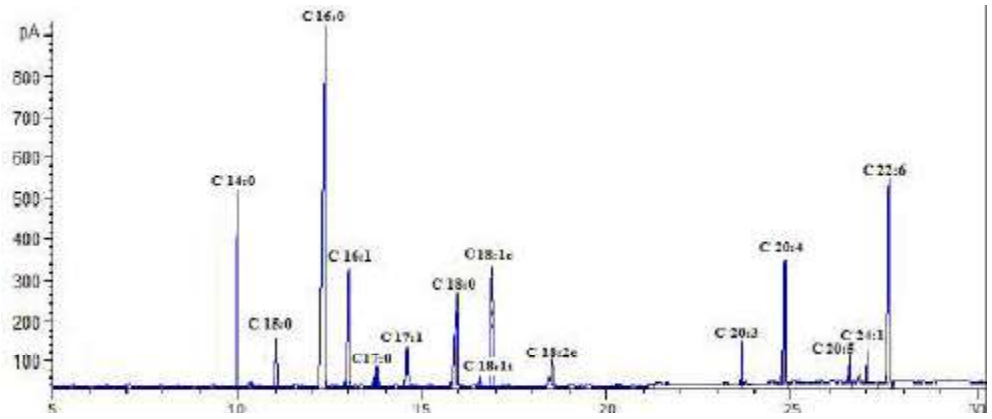

Fig. 1: Chromatogram of the fatty acid composition of S.lemuru head lipid

The predominant SFAs in all samples were palmitic and stearic acid. The highest levels of C16:0 (35.56%) and C18:0 (9.30%) were determined in liver lipid.

The total mono unsaturated fatty acid (MUFA) of head, intestine and liver lipid of sardine wastes were determined to be 26.22%, 32.30% and 26.86%, respectively. The main MUFA identified was C18:1c. In *S.lemuru* waste lipid, oleic acid constituted 15.47%, 21.79% and 16.93% in head, intestine and liver respectively.

The total PUFA of head, intestine and liver lipid of sardine wastes were determined to be 26.39%, 24.90% and 22.67%, respectively. The PUFA of the extracted waste lipid predominantly compose of C22:6 n-3 followed by C20:4 n-6. The C22:6 n-3 fatty acids in sardine wastes lipid was 15.95 %, 11.87% and 12.97% for head, intestine and liver respectively. The identified C20:4 n-6 fatty acid was ranged from 3.55% to 5.59%.

The composition is similarly showed by other fish lipids, such as salmon and rainbow trout [14, 15]. Comparatively the results which expressed the different proportion of MUFAs and PUFAs with other fish lipids may be due to the environmental effect of tropical fish species [16, 17]. The distinctive difference of the SFAs, MUFAs and PUFAs content in sardine wastes lipid and other fish lipids may be attributed to the seasonal changes and the changes in plankton species in their diet and also in the post-spawning period [18, 19].

The variation of n-3 PUFA of *S. lemuru* wastes lipid and other fish shown in Table 3, indicate that the n-3 PUFA content of sardine waste lipid was within the range of some other fish such as black siakap, red tilapia, african cat fish, European pilchard and spotlined sardine [17, 20, 21].

Table3: N-3 PUFAof *Sardinella lemuru* waste lipid and some other fish species

Fish name	
Sardine head	17.79 recent study
Sardine intestine	13.60 recent study
Sardine liver	15.73 recent study
European pilchard[20]	23.21
Spotlined sardine[21]	28.80
Black siakap[17]	6.80
Tilapia[17]	7.09
African cat fish[17]	1.19

Table4: N3 / n6 ratio of *Sardinella lemuru* waste lipid and some other fish species

Fish name	
Sardine head	1.85 recent study
Sardine intestine	1.20 recent study
Sardine liver	2.27 recent study
Indian mackerel[26]	1.67
Menhaden[26]	2.03
Striped sea cat fish[26]	1.78
Tilapia[17]	1.26

Compare to some other researches on *Sardina pilchardus* with C22:6 n-3 levels of 11.30 % [20, 22], *Clupea pilchardus* with C22:6 n-3 levels of 16.92 % [23] and salmon by-product 12.9 % [24], sardine waste lipid in this study showed high level of C22:6 n-3.

The C20:4 n-6 fatty acid plays an important role on growth and is a precursor of prostaglandin and thromboxane [17]. Furthermore, C20:4 n-6 show a brilliant role in growth. The 20:4 n-6 content of sardine waste lipid was in high level. The high level of C20:4 n-6 in the lipid samples of sardine waste is most probably due to the lower oxygen solubility in warmer water [17].

The two important classes of essential fatty acids which are not interconvertible are n-3 and n-6 FAs. The ratio between n-3 and n-6 is a very useful index for comparing the nutritional value of fish lipid due to their human health effects on coronary heart disease, cancer and autoimmune diseases. It may be used for nutrient intake during human evolution as a 1:1 ratio [25]. The ratio in sardine waste lipid was in good proportion than some other fish species shown in Table 4. Therefore, it needs to be more focused on lipid extraction from fish waste.

CONCLUSION

Regarding the suitable amount of lipid content, amount of n-3 fatty acids and n-3/ n-6 ratio, the waste of sardine could be used as a decent substitute source to extract the fish lipid. The main advantage of fish lipid from fish waste is that it is much cheaper compared with the fish lipid extracted from flesh. This lipid considered as the highly attention source for human consumption as well as industrial use. In this sense, the financial benefits can be obtained and environmental pollution is certainly decreased.

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