

Full Length Research Paper

Quality and fatty acid profile of the oil extracted from fish waste (head, intestine and liver) (*Euthynnus affinis*)

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Accepted 23 May, 2011

Marine lipid contains long-chain n-3 (omega-3) polyunsaturated (PUFA), particularly eicosapentaenoic acid (EPA) (C20:5 n-3) and docosahexaenoic acid (DHA) (C22:6 n-3). Consumptions of these PUFAs have been perceived to be important in human nutrition, health and disease prevention. Tuna, the important industrial fish, discharged substantial amount of wastes. These wastes include the head, liver and intestine. Considerable amount of lipid can be extracted from these wastes. The yield and chemical quality of the oils were determined. All the extracted oils were less than 8%, of which the highest was in head (7.01%). Among different lipid sources (head, intestine and liver), the free fatty acid and peroxide value significantly increased ($P < 0.05$) from head to liver. The highest iodine value was found in head lipid. The predominant fatty acids in tuna wastes were palmitic (C16:0; 27.63 - 32.74%), stearic (C18:0; 8.82- 13.62%), oleic (C18:1c; 9.16- 11.95%) and docosahexaenoic acid (DHA; C22:6; 14.18- 15.70%). Differential scanning calorimetry results for tuna waste lipid samples indicated that higher unsaturation in lipid sample showed lower cooling and melting temperature. The n-3 / n-6 ratio of the respective head, liver and intestine lipid samples showed value higher than 1. Due to n-3 fatty acid compound and n-3 / n-6 ratio, lipid from tuna head may be a valuable source for human consumption.

Key words: n-3 Fatty acid, fish waste, fish lipid, n-3 / n-6 ratio, EPA, DHA.

INTRODUCTION

Fish are cold-blooded animals with scaly streamlined bodies. Fish play essential roles in the world as, animal feed, fertilizer and food. They are special in the human food chain due to their nutritive components such as protein, vitamins A, B and D, minerals namely calcium, phosphorous, iodine and lipids (Ackman, 1994). The lipid

composition in fish is quite different from land animal lipids and vegetable oils due to the large quantity of two distinct n-3 fatty acids including eicosapentaenoic acid (EPA, C20:5, n-3) and docosahexaenoic acid (DHA, C22:6, n-3) that cannot be synthesized by human body (Jittrepotch et al., 2006). The highly unsaturated fatty acids (HUFAs); eicosapentaenoic (C20:5n-3) and docosahexaenoic (C22:6n-3) consumption is linked to the development of brain and nervous tissue in infants and visual function and reduces incidence of coronary heart disease (Shahidi and Miraliakbari, 2004; Innis, 2004; Uauy et al., 2001). Fish lipid is mainly stored in fish body in the subcutaneous tissue, belly flap, mesenteric tissue, head, muscle tissue and liver (Ackman, 1994). Most of the respected parts are going to be eliminated as a waste. Tuna is magnificent that it has high level of lipids which can be extracted from discarded heads, intestine,

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Abbreviations: EPA, Eicosapentaenoic acid; DHA, docosahexaenoic acid; PVe, peroxide value; IV, iodine value; FID, flame ionization detector; FAME, fatty acid methyl ester; FFA, free fatty acid; SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; DSC, differential scanning calorimetry.

and liver. So, the possibility of fish waste application for lipid production as a cheaper source than fish muscle to extract fish lipid could potentially generate significant revenue for fish processing industry and environment. Analysis of the extracted oil from the respective wastes (heads, intestine and liver) reflects the prominent presence of the important essential polyunsaturated fatty acids (PUFAs).

MATERIALS AND METHODS

Sample collection and preparation

A total of 120 (60 kg) tuna (*Euthynnus affinis*) were purchased from a local wet market in Pasar Borong, Serdang Malaysia. This species belongs to Perciformes order and Scombridae family. The mean weight and length of *E. affinis* were 500.00 ± 35.10 g, 25 ± 1.51 cm, respectively. The fish were kept on ice until processed in the laboratory. The heads were cut, separated and kept in freezer at the temperature of $-25 \pm 1^\circ\text{C}$ in sealed nylon plastic bag with nitrogen head space. Similar treatments were given to the intestine and liver.

Proximate composition

The moisture content was determined according to the 984.25 protocol Association of the Official Analytical Chemists (AOAC, 2005). The ash content was determined incinerating about 0.5 g of sample in a muffle furnace (Amalgam, Sheffield England) at 550°C for about 12h. The crude protein was determined by the Kjeldahl method. It was calculated using a nitrogen conversion factor of $\text{N} \times 6.25$ (Al-Gaby, 1998). Data were expressed as percent of wet weight. The crude lipid content was determined through the Bligh-Dryer method with slight modification by Kim et al. (1991).

Determination of peroxide, iodine and free fatty acid contents

To determine the peroxide value (PV), iodine value (IV) and free fatty acid (FFA) content the methods from American Oil Chemists' Society AOCs (1997) were used.

Determination of fatty acid composition

About 50 μl of oil solubilised in 950 μl of hexane was esterified using sodium methoxide catalyst based on Christie (1993) method. The fatty acid composition was analysed by gas chromatograph (Hewlett Packard 6890) equipped with a flame ionization detector (FID) and a fused silica capillary BPX-70 column (60 m \times 0.32 mm i.d., 0.25 μm film thickness) from SGE (Melbourne, Australia). The oven temperature was set at 115°C , raised to 180°C at rate of $8^\circ\text{C}/\text{min}$ and held for 10 min and finally raised to 240°C at rate of $8^\circ\text{C}/\text{min}$ 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 fatty acid methyl ester (FAME) with the standard 37 component FAME mixture (Ariffin et al., 2009).

Thermal behaviour determination

To determine the thermal properties of the lipid, a Perkin-Elmer Diamond differential scanning calorimeter (Perkin-Elmer Corp.,

Shelton, CT, USA) was used.

Statistical analysis

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

RESULTS AND DISCUSSION

Proximate composition

Moisture, protein, ash and lipid contents are usually used as indicators of nutritional value of fish (Stansby, 1962). According to Dempson et al. (2004), the higher content of water in fish is associated with lower protein and lipid content. The moisture, protein and ash content of different waste samples are shown in Table 1. The amount of moisture content in *E. affinis* waste samples were from 68.79 to 76.74%. The highest percentage of moisture in tuna waste samples was found in the liver. The lowest protein value of tuna waste samples was noticed in the liver whereas the highest value was found in the head. Ash content varied from 1.10 to 4.77% in tuna. Maximum ash was recovered in the head of tuna. The one way analysis of variance showed significant difference ($P < 0.05$) between the ash content of head and the intestine and liver. The moisture, protein and ash content of *E. affinis* wastes were in agreement to the report by Hale (1984) and Mukundan et al. (1979) except their head ash content which showed higher value. The higher ash content could be due to boney head of tuna fish.

Yield of fish lipid

The lipid contents of head, intestine and liver of *E. affinis* are shown in Table 1. Comparatively, the head expresses the highest lipid content followed by intestine and liver. Based on Ackman (1994) classification, the lipid content of the respective tissues located between 4 and 8% by weight was categorized as medium fat fish product. This includes head (7.01%), intestine (4.46%) and liver (3.70%). In this study, tuna waste lipids content is within the range of cod offal (4.30%) and sardine head (5.67%) (Shahidi et al., 1991; Khoddami et al., 2009). The waste of tuna is opted as being a good source of lipid.

Free fatty acid, peroxide and iodine value

The free fatty acid (FFA) value is one of the most important factors to check the lipid quality. The lower FFA content showed higher quality and lower further

Table 1. Proximate composition and chemical characteristic of *E. affinis* wastes lipid ^A.

Component	Head	Intestine	Liver
Moisture content%	68.79±0.51 ^a	74.89±0.48 ^b	76.74±0.49 ^b
Protein content%	19.30±0.31 ^a	19.01±0.14 ^a	18.06±0.68 ^a
Ash content%	4.77±0.37 ^b	1.34±0.80 ^a	1.10±0.11 ^a
Lipid(%)	7.01±0.40 ^b	4.46±0.57 ^a	3.70±0.20 ^a
FFA (% palmitic acid)	4.08±0.03 ^a	6.06±0.07 ^b	7.28±0.03 ^c
PV (meq O ₂ kg ⁻¹)	7.31±0.66 ^a	11.71±0.24 ^b	15.68±0.17 ^c
IV (g I ₂ 100g ⁻¹)	149.41±0.32 ^c	146.13± 0.32 ^b	143.15± 0.53 ^a

^AEach value in the table represents the mean ± standard deviation. Means within each row with different letter are significantly different (P < 0.05). Free fatty acid (FFA), peroxide value (PV) and iodine value (IV).

oxidation. The maximum limit for FFA content is reported to be 7% (Bimbo, 1998). The lipid samples from *E. affinis* liver exceeded this limit. The FFA content of *E. affinis* waste lipids are shown in Table 1.

In comparison between different parts of *E. affinis* waste lipid, head oil has the lowest FFA content than intestine and liver. This higher content of FFA in the intestine and liver can be associated with bacterial and enzyme activity from microorganisms or biological tissues (Ashie et al., 1996).

Peroxide value (PV) of the waste lipids showed significant difference (P < 0.05) between head, liver and intestine. The peroxide values of *E. affinis* waste lipids are shown in Table 1. The lowest PV is related to head lipid (7.31 meq O₂ / kg) and the highest was related to the liver lipid (15.68 meq O₂ / kg). According to Young (1986), the PV of crude fish lipid was between 3 to 20 meqO₂ / kg. In this research, the PV of waste lipid samples from *E. affinis* did not exceed the later ranges. The maximum limit of PV of crude fish lipid is 8 meqO₂ / kg to be acceptable for human consumption (Boranet al., 2006). The head lipid of *E. affinis* did not reach 8 meqO₂ / kg in contrast with intestine and liver lipid samples.

According to Endo et al. (2005), fish lipid represents a wide range of iodine value (IV) from 55 to 188 g I₂ / 100 g. The IV of *E. affinis* waste lipids are shown in Table 1. The IV of *E. affinis* waste lipid ranged from 143.15 to 149.41 gI₂ / 100 g, which showed the highest for head and the lowest for liver. The IV of the waste lipid samples in this research showed lower amount than fish flesh lipid such as sardine (156.2 gI₂ / 100 g) and tuna (162.0 gI₂ / 100g) flesh (Endo et al., 2005). This difference can be owed to different capture seasons, sex, maturity and different fish species and kind of sample.

Fatty acid profile

The fatty acid (FA) compositions of the lipid extracted from head (Figure 1), intestine and liver of the *E. affinis* are shown in Table 2. The tuna (*E. affinis*) waste lipid samples were primarily comprised of four major FA, namely palmitic acid C16:0, stearic acid C18:0, oleic acid

C18:1c and docosahexaenoic acid C22:6n-3.

Palmitic acid was the highest among the saturated fatty acids (SFAs) with the range of 27.63 to 32.74% followed by stearic acid ranged from 8.82 to 13.62% of the total FA. The most abundant monounsaturated fatty acids (MUFAs) in these fish waste lipid samples were oleic acid. Greatest proportion of PUFAs was for DHA made up from 14.18 to 15.70% of total FA followed by arachidonic acid (C20:4n-6). The identified arachidonic acid was ranged from 2.20 to 6.66%. The PUFAs, of extracted lipid accounted for more than 23%, while the MUFAs made up about 20.82 to 24.20% of the total fatty acids. The SFAs content ranged from 47.02 to 55.20%.

One way analysis of variance (ANOVA) of data of tuna wastes lipid showed that, the highest significant change (P < 0.05) in fatty acids was in liver which has the highest SFAs content (55.20%) and lowest PUFAs (23.98%) than head (47.02% SFA and 28.77% PUFA) and intestine (51.00% SFA and 27.43% PUFA). Zuraini et al. (2006) study on fatty acid profile of Malaysian *Channa* spp. fish stated higher content of SFAs (45.57%), followed by PUFAs (32.54%) and MUFAs(15.02%). Generally, the fatty acid profile found in the present study for tuna wastes was in agreement with the result reported by Mbarki et al. (2008) for SFA (52.56%), PUFA (28.62%) and MUFA (10.67%) on bonito fish muscle fatty acid profile.

Fatty acid composition of tuna wastes showed high content of palmitic acid, oleic acid and DHA. These findings are in agreement with those reported for fatty fish by García-Arias et al. (2003) and some lean fish obtained by Osman et al. (2001). In other research on fatty acid composition of some Malaysian fresh water fish, high content of palmitic, palmitoleic acid, oleic acid and docosahexaenoic acid was reported by Suriah et al. (1995). The distinctive difference of the saturated, monounsaturated and polyunsaturated fatty acids content in tuna wastes lipid and other fish lipids may be attributed to the seasonal changes and environmental effect of tropical fish species and also in the post-spawning period (Colin et al., 1993; Suriah et al., 1995; Bandararra et al., 1997; Gamez-Meza et al., 1999).

The variation of n-3 PUFA of *E. affinis* wastes lipid and

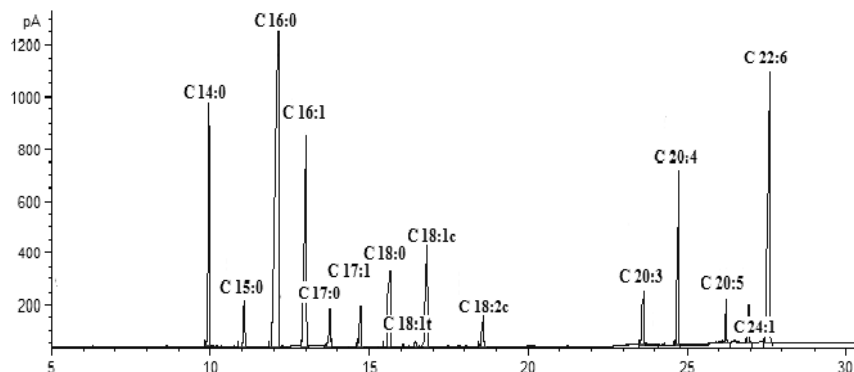


Figure 1. Chromatogram of the fatty acid composition of *E. affinis* head.

Table 2. Fatty acid content of *E. affinis* waste lipid (g/100g)^A.

Fatty acids	Head	Intestine	Liver
14:0	7.49±0.09 ^b	6.34±0.42 ^b	3.76±0.42 ^a
15:0	1.53±0.03 ^a	1.31±0.04 ^a	3.73±0.17 ^b
16:0	27.63±0.09 ^a	30.86±0.76 ^b	32.74±0.66 ^b
16:1	10.07±0.33 ^c	7.70±0.17 ^b	4.03±0.14 ^a
17:0	1.55±0.02 ^a	1.39±0.04 ^a	1.35±0.13 ^a
17:1	1.64±0.04 ^{ab}	1.69±0.05 ^b	1.38±0.10 ^a
18:0	8.82±0.46 ^a	11.10±0.72 ^a	13.62±0.51 ^b
18:1 n-9t	0.40±0.23 ^a	0.72±0.11 ^a	0.45±0.12 ^a
18:1 n-9c	10.22±0.48 ^a	9.16±0.43 ^a	11.95±0.06 ^b
18:2 n-6c	2.49±0.06 ^b	1.00±0.00 ^a	1.35±0.15 ^a
20:3 n-6	2.44±0.04 ^a	3.73±0.13 ^{ab}	4.55±0.53 ^b
20:4 n-6	6.66±0.01 ^b	5.68±0.45 ^b	2.20±0.46 ^a
24:1	1.87±0.02 ^a	2.30±0.21 ^{ab}	3.01±0.28 ^b
20:5 n-3	1.48±0.05 ^a	2.71±0.08 ^b	1.70±0.40 ^a
22:6 n-3	15.70±0.62 ^a	14.31±0.95 ^a	14.18±0.93 ^a
Σ SFA	47.02	51.00	55.20
Σ MUFA	24.20	21.57	20.82
Σ PUFA	28.77	27.43	23.98
Σ n-6 FA	11.59	10.41	8.10
Σ n-3 FA	17.18	17.02	15.88
n-6/n-3	0.67	0.61	0.51

^AEach value in the table represents the mean ± standard deviation. Means within each row with different letter are significantly different ($P < 0.05$). Saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA)

other fish shown in Table 3, indicate that the n-3 PUFA content of tuna waste lipid was within the range of some other fish such as sardine head, sardine liver, black siakap, tilapia, African cat fish, (Khoddami et al., 2009; Suriah, 1995).

Compare to some other researches on *Sardinia pilchardus* with C22:6n-3 levels of 11.30% (Beltran and Moral, 1991; Leonardis and Macciola, 2004), *Clupea pilchardus* with C22:6n-3 levels of 16.92% (Castrillon et al., 1997), *Sardinella lemuru* liver with C22:6n-3 levels of

12.97% (Khoddami et al., 2009) and salmon by-product 12.9% (Wu and Bechtel, 2008), tuna waste lipid in this study showed high level of C22:6n-3.

The arachidonic fatty acid (C20:4n-6) plays an important role on growth and is a precursor of prostaglandin and thromboxane (Suriah et al., 1995). Furthermore, C20:4n-6 show a brilliant role in growth. The 20:4n-6 content of tuna waste lipid was in high level. The high level of C20:4n-6 in the lipid samples of tuna waste is most probably due to the lower oxygen solubility

Table 3. n-3 PUFA of *E. affinis* waste lipid and some other fish species (g/100 g).

Fish name	n-3 PUFA
Tuna head	17.18
Tuna intestine	17.02
Tuna liver	15.88
Sardine head	17.79
Sardine liver	15.73
Black siakap	6.80
Tilapia	7.09
African cat fish	1.19

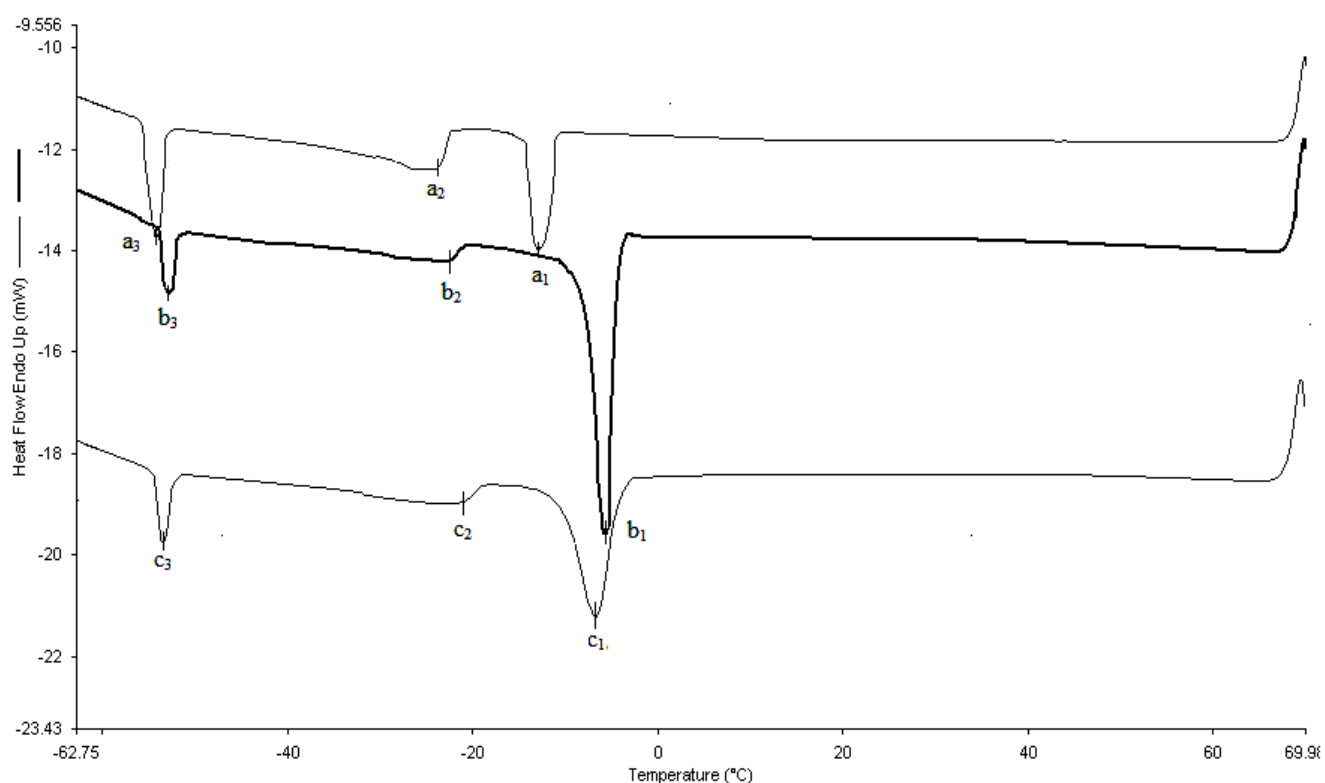


Figure 2. DSC cooling curves of head (a) Liver (b) and intestine (c) lipid of *E. affinis*

in warmer water (Suriyah et al., 1995).

The n-6/n-3 ratio of *E. affinis* waste lipid samples ranged from 0.51 to 0.67 (Table 2). These findings are in agreement with those obtained from fatty fish (Jamilah et al., 2008). The n-6/n-3 ratio is a good index for comparing relative nutritional value of fish lipid for different species (Piggott and Tucker, 1990; Jamilah et al., 2008). Osman et al. (2001) pointed out that n-6/n-3 ratio of 0.24 to 0.66 for some selected Malaysian marine fish. Optimal balance for these ratios in human body is 1:1 (Simopoulos, 1989), while World Health Organisation (WHO) recommends n-6/n-3 ratio of not more than 5.0 in total human diet

(Vujkovic et al., 1999). Many researches indicated that risk of heart attack and many common disorders can significantly increase with the reduction of the intake of seafood or fish lipid rich in EPA and DHA.

Thermal behavior

The two physical events which are used to characterize thermal behaviour of lipid samples are melting and crystallization, require for intake or release of the thermal enthalpy. Differential scanning calorimetry (DSC) is very

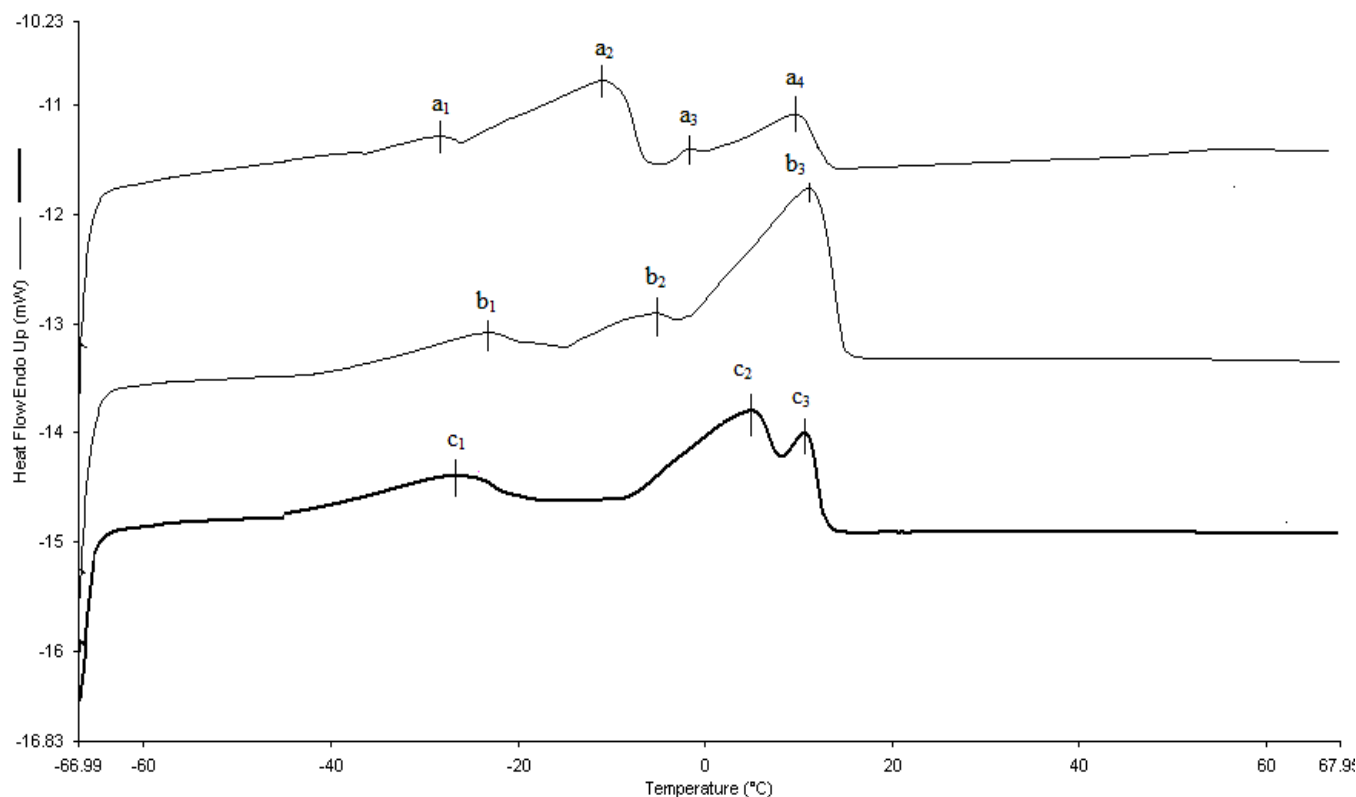


Figure 3. DSC melting curves of head (a), Liver (b) and intestine (c) lipid of *E. affinis*.

suitable to verify these physical properties of lipid samples. The cooling and melting point of tuna head were -54.94 and 14.67°C (Figures 2 and 3). These results for melting and cooling points of intestine and liver were 15.51 to 53.86°C and 14.46 to 54.05°C , respectively. Based on the analysis of DSC results for all different extracted oils, there is clear that tuna waste lipids have a high degree of unsaturated fatty acids.

CONCLUSION

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

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