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Fatty Acid Profile and Differential Scanning Colorimetric (DSC) Characterization of Fish Oil Extracted From the Fresh Water *Catla catla* Fish

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ABSTRACT

The present investigation was undertaken with an objective to study the fatty acid profile and differential scanning calorimetric characterization of the fresh water Catla catla fish oil. The different physical properties of catla fish oil such as specific gravity, refractive index whilst the chemical properties such as iodine value, peroxide value saponification value and acid value were determined. Fatty acid profile of catla fish oil was determined by gas liquid chromatography. Catla fish oils gives a total of 18 fatty acid with variation of fatty acid concentration. The fatty acid composition of catla fish oil contained 55.57% of Total Saturated Fatty Acids (TSFA) and 41.13% of Total Unsaturated Fatty Acids (TUFA). The unsaturated fatty acids contain 19.53% of Monounsaturated Fatty Acids (MUFA) and 21.60 % of Polyunsaturated Fatty Acids (PUFA). Further, the catla fish oil was found to be good source of Docosahexaenoic (DHA) (7.36%) followed by the Linoleic acid (LA) (4.64%). However, the thermogram for catla fish oil by DSC sowed that the catla fish oil has both amorphous and crystalline portions. Glass transition (Tg) temperature occurred at -21.19°C while a crystallization temperature (Tc) was observed at about 21.71°C.

Key words: *Catla, fish oil, Characterization, Fatty acids.*

INTRODUCTION

India has a coastline of about 8129 km including Island territories. Inland fish production has shown remarkable increase from 0.218 million metric tonnes in 1950-51 to about 6.14 million metric tonnes in 2013-14. The total fish production during 2013-14 was 9.58 million metric tonnes with a contribution of 6.14 million metric tonnes (64 per cent) from inland sector and 3.44 million metric tonnes (36 per cent) from marine sector. Maharashtra contributes 4,67,460 tonnes of marine fish production and 1,35,220 tonnes of inland fish production[9].

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The Indian major carp culture mainly involves the three Indian major Carps (IMC) such as catla *(Catla catla)*, rohu *(Labeo rohita)* and mrigal *(Cirrhinus mrigala)* are widely cultivated in India and parts of Southeast Asia. The current annual production of the three IMC species is about 2 MMT[3].

Catla catla form a major component of Indian aquaculture, with a market share of over 90%, native to India, Bangladesh, Myanmar, Nepal, and Pakistan and introduced in many other countries as exotic species. Because of its high nutritive value, it is a highly priced food fish and of great demand in the market[17]. Fish and fishery products are important components of a healthy human diet. The fish products are rich in high-quality proteins, and provide high portions of valuable n-3 polyunsaturated fatty acids (PUFAs), liposoluble vitamins, and essential minerals[5].

Fish are a rich source of polyunsaturated fatty acids (PUFAs), namely, the n-3 and n-6 PUFAs, which are beneficial to human health. Fish meat and oils are good sources of unsaturated omega-3 fatty acids, eicosapentacenoic acid (EPA, 20:5n-3) and docasahexaenoic acid (DHA, 22:6n-3), as well as its precursor, alpha linolenic acid. Compared to beef and chicken, fish meat contains higher levels of n-3 PUFAs, which are known to be cardio-protective, anti-atherosclerotic, antithrombotic, and anti-arrythmitic and also play a role in reducing the cholesterol level, regulate prostaglandin synthesis and hence induce wound healing and stabilizing the electrical activity of the heart cells[10]. However, the conversion efficiency of pathway is very limited. Hence EPA and DHA must be obtained from the diet[6].

The chemical composition of the fresh catla fish contains 73.7g moisture, 19.5g protein, 2.4g fat, 1.5g ash, 2.9g carbohydrate, calcium 530 mg per 100 g and phosphorous 235 mg per 100g[8]. In fresh water major carps the essential amino acids contributed to 41 to 51% of the total amino acids. The fresh water catla fish is also contains marginal amount of polyunsaturated fatty acids (PUFA) and the omega 3 and 6 ratio (3/ 6) was ranging from 0.706 to 6.544 and catla showed the highest ratio. The most abundant fatty acid in all fishes was C16:0, ranging from 32.2% to 38.23%. The other major fatty acids detected were C18:0, C18:1 and C18:3[10].

Thus, pertaining to the above discussion, in the present investigation, the attempt was made to extract the oil from the catla fish and the fatty acid profile and differential scanning calorimeter characterization of fish oil was carried out.

MATERIALS AND METHODS

Materials used and methods adopted for the present investigations are presented under suitable headings and subheadings.

Fresh Catla fish

Fresh water catla fish were procured from the Parbhani local market. The catla fish were brought to laboratory of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani and used to carry out the present study.

Chemicals

Chemicals used in this investigation were of analytical grade. They were obtained from Department of Food Chemistry and Nutrition, College of Food Technology, VNMKV, Parbhani.

Extraction and characterization of Catla Fish oil

Catla fish oil was extracted from the fish flour by using the soxhelt apparatus with petroleum ether as solvent and the extracted oil was used to study the different physical and chemical characteristics of fish oil. Methods used for determining the physicochemical characteristics are summarized under the subheadings.

Colour measurement

Colour (L*, a*, b* values) of the catla fish oil was determined by using Hunter Colour Flex Meter. L* is known as the lightness and extends from 0 (black) to 100 (white). The other two coordinates a* and b* represent redness (+ a) to greenness (- a) and yellowness (+ b) to blueness (-b), respectively were recorded. Three measurements were taken and their means were reported[15].

Refractive index

The Refractive Index was measured with an Abbe's refractometer equipped with a thermo stated circulator at 25^0C as outlined by Pearson[16].

Specific gravity

The specific gravity was measured at room temperature using 25 ml specific gravity bottle and calculated by using following formula.

Sp Gravity of oil= Density of oil /density of water

Density

The density of oil was measured by mass over volume measurement**.**

Density = mass of oil/ volume of oil

Viscosity

A Brookfield Viscometer Model DV-E was used to measure the viscosity of catla fish oil[4]. Viscosity was determined to at constant speed of 100rpm and at constant temperature with a spindle number S-62 and it was expressed in terms of centipoise per second (cP.).

Acid value, Peroxide value, Iodine value and Saponification value

Free fatty acid percentage (as oleic), peroxide value, iodine value and saponification value were determined by using the methodology given by AOAC[2].

Fatty acid profile of Catla fish oil by GC

Fatty acid profile of catla fish oil was done by gas liquid chromatography. Fatty acid methyl ester (FAME) was prepared according to the method of Morrison and Smith[13]. A catla fish oil (10–20 mg) was saponified for 1hr with 1 ml of methanolic KOH (0.7 N) at 60 ºC, followed by neutralisation with 1 ml of methanolic HCl (0.7 N). The resulting free fatty acids were extracted in hexane and evaporated to dryness. The fatty acids were methylated using boron trifluoride (14% in methanol) and 0.2 ml benzene. The FAME was extracted in hexane, washed with water and evaporated to dryness. Fatty acid analysis was performed using a gas–liquid chromatograph (Shimadzu, GC-14B, Shimadzu Corporation, Japan) (Plate 6) fitted with a fused silica capillary column (BP 21: 30 m length, 0.30mm i.d., 0.50 µm film thickness). The GC was equipped with a flame ionization detector, Clarity Lite 420 integrator and at isothermal conditions. The column temperature was set at 220ºC, the injector temperature at 230ºC and the detector temperature at 240ºC. Nitrogen gas was used as the carrier gas with a flow rate of 1 ml/min. Individual fatty acids in the catla fish oil were identified by comparison with the retention times of standard fatty acid methyl esters.

Tocopherol content

Tocopherol composition of the catla fish oil was analyzed using an Agilent HPLC series 1200 (Agilent, Waldbronn, Germany) with Chem Station software. The separation was with a ACE 5 SIL normal phase column (150mm, 4.6mm i.d.) and quantification was with tocopherol standards (Merck, Darmstadt, Germany) as method described by Panfili *et al*[14].

Differential Scanning Calorimetry (DSC) of catla fish oil

Thermal property of catla fish oil was characterized using a Differential Scanning Calorimetry as per the method given by Wetten *et al*[18]. The DSC 204 F1 Phoenix (Netzsch, Germany). Nitrogen, at the rate of 200 ml/min, was used as purge gas; 11mg of catla fish oil was sealed in aluminium pan and heated from 20°C up to 200°C at the rate of 10°C/min, followed by a cooling cycle back to 30°C at the same rate.

RESULTS AND DISCUSSION

The results obtained during the course of investigation are depicted and discussed under different headings.

Physico-chemical properties of the catla fish oil

Physical properties of catla fish oil comprises of melting point, refractive index and the specific gravity, whilst the chemical properties are iodine value, saponification value, and acid value etc. The results pertaining the same are tabulated in the Table 1.

Table 1: Physico-chemical properties of the catla fish oil

Physico-chemical properties of catla fish oil		
Sr. No.	**Parameters**	**Results**
1	% yield of oil from fish flour	5.89
2	Specific gravity	0.902
3	Refractive index	1.664
4	Iodine value (mg I/g oil)	162.33
5	Acid value (mg KOH/g)	4.55
6	Peroxide value (meq O2/kg)	7.4
7	Saponification value (mg KOH/g)	194
8	Free Fatty acid (%)	0.8

*Each value is an average of three determinations

It is revealed from the Table 1 that the yield of the fish oil extracted from the fish flour was determined to be 5.98%. The catla fish oil had the specific gravity 0.902. Refractive index is used mainly to measure the change in unsaturation as the fat or oil is hydrogenated. The refractive index of oils depends on their molecular weight, fatty acids chain length, degree of unsaturation and degree of conjugation. The catla fish oil showed a refractive index of 1.664.

Iodine values give an estimation of the amount of unsaturated fatty acids in the triglyceride molecules of fat and oil. Iodine value for the selected catla fish oil was found to be 162.33mg I/g oil. In general the higher degree of unsaturation i.e. the higher iodine value, the greater is the liability of the oil or fat to become rancid by oxidation. Therefore the *Catla catla* fish species oil had higher tendency to become rancid by oxidation than the oils having lower iodine value.

Acid value is commonly related by means of movement of lipase initiated from biological tissue or microorganisms, Acid and peroxide values are used to measure the deterioration in the sensory properties of oil. The reported suitable limit for acid value is 7-8 mg KOH. From the above Table 1 the acid value was noted 4.55mg KOH.

Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil. Further, from the above results it was found that the saponification values of *Catla catla* fish oil was 194. The higher saponification values indicate the presence of low proportion of lower fatty acids. Result also indicated that *Catla catla* fishes contained high proportion of higher chain fatty acids.

Peroxide value and free fatty acids content in the fish oil was found 7.4meq O_2/kg and 0.8% respectively. Peroxide value is also the measure of the unsaturation of the oils. The results are in accordance with the results reported by Manirujjaman *et al*[11].

Fatty acid profile of the catla fish oil

The lipids after extraction from the fish flour subjected to gas chromatography by preparing the fatty acids methyl esters to determine the fatty acid profile and tocopherol content. Detailed fatty acids are listed in Table 2 and graphically shown in Figure 1 and the tocopherol content of the catla fish oil is shown in the Figure 2.

Table 2: Fatty acid profile of the catla fish oil

Fatty acid profile			
Name of fatty acids	**Chemical configuration**	**Retention Time (Min.)**	**Area (%)**
Saturated fatty acids			
a. Lauric acid	C_{12}:0	16.265	0.65
b. Myristic acid	C_{14}:0	19.377	8.27
c. Palmitic acid	C_{16}:0	22.702	36.61
d. Heptadecanoic acid	C_{17}:0	24.272	3.04
e. Stearic acid	C_{18}:0	25.849	6.00
f. Arachidic acid	C_{20}:0	28.858	0.66
g. Behenic acid	C_{22}:0	31.763	0.34
Unsaturated fatty acids			
I. Monounsaturated fatty acids			
a. Palmitoleic acid	C_{16}:1	23.876	3.98
b. Oleic acid	C_{18}:1n9	26.867	14.47
c. Eicosenoic acid	C_{20}:1	30.003	1.08
II. Polyunsaturated fatty acids			
a. Linoleic acid	C_{18}:2**n6**	28.329	4.64
b. Gamma linolenic acid	C_{18}:3**n6**	29.778	0.73
c. Alpha linolenic acid	C_{18}:3**n3**	30.311	3.24
d. cis 11,14-Ecosadienoic acid	C_{20}:2	31.215	0.45
e. cis 8,11,14-Ecosatrienoic acid	C_{20}:3	32.340	0.29
f. Arachidanoic acid	C_{20}:4	33.246	0.75
g. Eicosapentanoic acid	C_{20}:3	35.230	4.14
h. Docosahexanoic acid	C_{22}:6	40.193	7.36
Tocopherol (mg/100g of catla fish oil)		12.624	30.43

*Each value is an average of three determinations

It is observed from Table 2 and Figure 1 that there was considerable difference among concentration of fatty acid profile of the catla fish oil. The fatty acid composition also influenced by variety of factors such as species, maturation period, size, age of fish, seasonal conditions and geographical location[7]. Catla fish oils give a total of 18 fatty acid with variation of fatty acid concentration which is based on Gas chromatography obtained. The results shown that local catla fishes are made up of long chain fatty acid with a minimum carbon chain length of 12 to 22 carbons which is considered as typical characteristic of fish oil.

The fatty acid profile analysis of catla fish oil (Table 2 and Figure 1) indicates that the presence of major fatty acids such as Palmitic acid (36.61%), Oleic acid (14.47%), Myristic acid (8.27%), Stearic acid (6.00 %), Docosahexaenoic acid (7.36%), Linoleic acid (4.64 %), Eicosapentaenoic acid (4.14%), Palmitoleic acid (3.98 %), alpha linolenic acid (3.24 %),Heptadecanoic acid (3.04 %), Eicosenoic acid (1.08 %), Gamma linolenic acid (0.73 %)and Arachidic acid (0.66 %). The total tocopherol content was observed 30.43mg per 100g of catla fish oil.

It is observed from present finding that among the saturated fatty acids the C16:0 i.e. palmitic acid (36.61%) concentration was found to be higher as compared to the other saturated fatty acid followed by the C14:0 i.e. Myristic acid (8.27%). Saturated fatty acid C16:0, these are the major constituent of muscle lipid of the most fishes. The finding of the present experiment more or less similar to the results declared by Mohamed and Alsabahi[12], who reported a higher concentration of C16:0 in the muscle tissue of *Lateniloticus* fish.

In mono-unsaturated fatty acids, C18:1 fatty acid concentration (14.47%) was found to be highest. The second major fatty acid found in the MUFA was palmitoleic acid having concentration 3.98%.The present findings regarding the C18:1 fatty acid is almost comparable with the concentration reported by Ackman *et al*[1].

The Polyunsaturated fatty acid content in fish oil ranged between C18 to C22 in chain length. Further, it was found that the catla fish oil contains higher concentration of the Docosahexaenoic (7.36%) followed by the Linoleic acid (4.64%). This results obtained are in close agreement with the results reported by the Jitender *et al*[10]. They reported the fatty acid profile of some Indian fresh water and marine water fishes. The recent study regarding the nutritional properties of catla fish flour oil suggested that, fish oil have higher nutritional value due to the presence of unsaturated fatty acids.

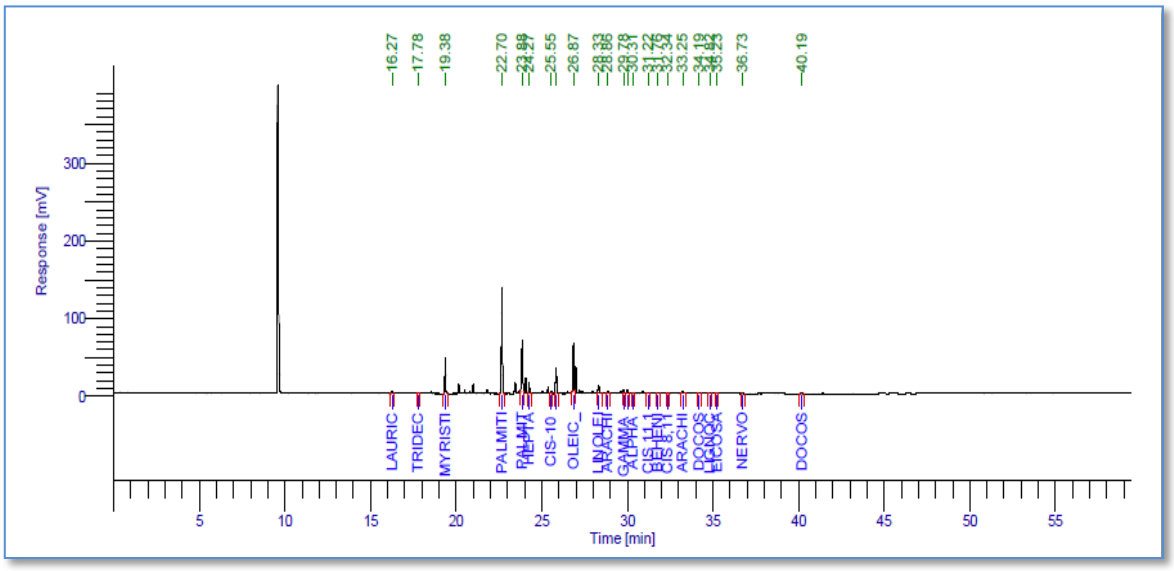

Fig. 1: Fatty acids chromatogram of catla fish oil

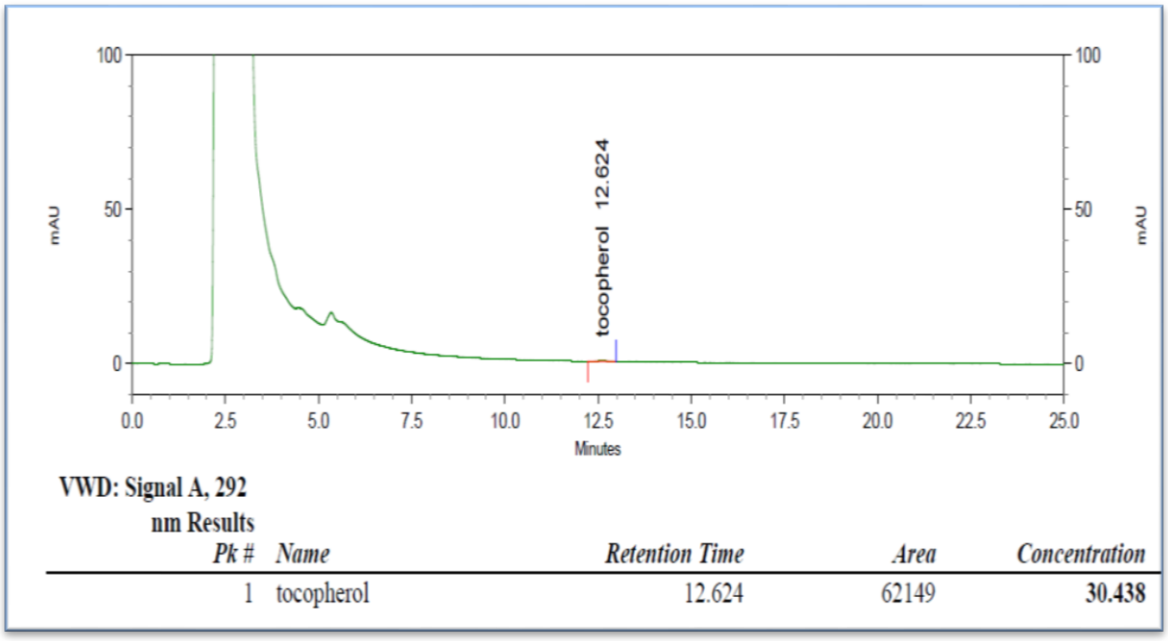

Fig. 2: Chromatogram of tocopherol content of catla fish oil

Overview of fatty acid composition of catla fish oil

Different amounts of the saturated fatty acids, unsaturated fatty acids, polyunsaturated fatty acids, n-3, n-6 were determined and their relation are summarized in the following Table 3.

Table 3: Overview of fatty acid composition of catla fish oil

Overview of fatty acid composition	
Fatty acids	**Contribution**
Total Saturated Fatty Acids (TSFA)	55.57%
Total Unsaturated Fatty Acids (TUFA)	41.13 %
Monounsaturated Fatty Acids (MUFA)	19.53 %
Polyunsaturated Fatty Acids (PUFA)	21.60 %
Trans Fatty Acids	< 0.01 %
PUFA/TSFA	0.38
n-6	5.37 %
n-3	14.74 %
n-6/n-3	0.364
TSFA/TUFA	1.35

The overview of fatty acid composition of catla fish oil contained 55.57% of Total Saturated Fatty Acids (TSFA) and 41.13% of Total Unsaturated Fatty Acids (TUFA). The unsaturated fatty acids contain 19.53% of Monounsaturated Fatty Acids (MUFA) and 21.60 % of Polyunsaturated Fatty Acids (PUFA).

The Trans Fatty Acids in fish oil was less than 0.01%. The n-6/n-3 ratio of fish oil was 0.364. The concentration of n-6 and n-3 fatty acids was found to be 5.37% and 14.74% respectively. The ratio of Total Saturated Fatty Acids to Total Unsaturated Fatty Acids was 1.35.

Differential Scanning Colorimetric analysis of catla fish oil

Differential scanning calorimetry (DSC) measures the energy absorbed (endotherm) or produced (exotherm) as a function of time or temperature. The result of the DSC analysis of catla fish oil is presented in the Figure 3.

The glass transition temperature (Tg) is an important characteristic of noncrystalline and semicrystalline materials, but Tg is a particularly significant property of many common polymers. At a temperature below Tg, amorphous and semicrystalline polymers tend to be hard and brittle because the polymer chains are locked in a tangled, coiled position. Above Tg, the polymeric chains are able to more easily rotate and slip past each other, and the polymer becomes softer and more ductile. Generally the glass transition point depends on the processing of the material, as well as that material's natural characteristics such as structure, bonding, and molecular weight. Since it takes energy to break these bonds, the glass transition appears on a DSC curve as an endothermic process. In DSC, the Tg can be found by a permanent decrease in baseline heat flow, and Tg is usually taken as the inflection point in the curve.

The crystallization temperature (Tc) is another important transition that occurs in some polymeric materials. At the crystallization temperature, the polymer loses its random chain arrangement, intermolecular bonds form, and the polymer molecules become more ordered. Formation of bonds during crystallization is an exothermic process, so an increase in heat flow (a peak on the DSC curve) accompanies the crystallization process. Generally, the Tc is found by finding the onset point of the crystallization curve. Note that many amorphous polymers never undergo crystallization. The thermogram for catla fish oil sowed that the catla fish oil has both amorphous and crystalline portions (Figure 3). Glass transition (Tg) temperature occurred at -21.19°C while a crystallization temperature (Tc) was observed at about 21.71°C. One exothermic peak and one endothermic peak are exhibited by the sample corresponding to its glass transition and melting respectively.

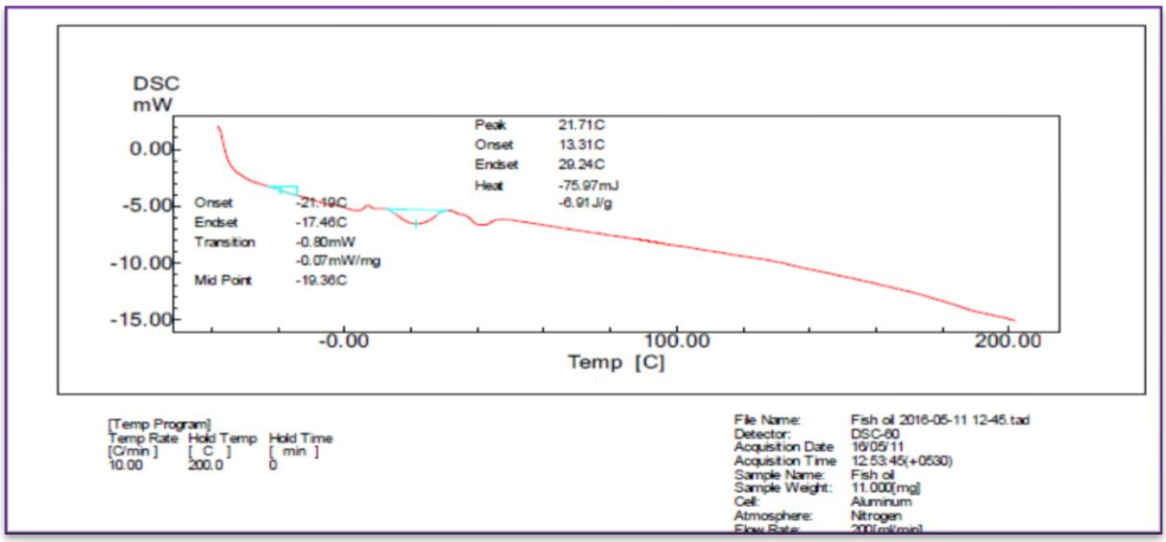

Fig. 4: Differential Scanning Colorimetric analysis of catla fish oil

CONCLUSION

From the above study it can be concluded that the *Catla catla* fish oil contains higher concentration of good quality lipids and shows the amorphous and crystalline portions.

REFERENCES

1. Ackman, R.G., McLeod, C., Rakshit, S. and Mishra, K.K., Lipid and fatty acids of five fresh water food fishes of India. *J. of Food Lipids*. **9:** 127-145 (2002).
2. A.O.A.C. Official Methods of Analysis of AOAC International (16th edition). A.O.A.C. *International Arlington*, VA, USA (1998).
3. Ayyapan, S. and Diwan, A.D., Fisheries research and development in India. *Fishing Chemistry*. **26(1):** 19-21 (2006).
4. Brookfield Engineering Laboratories Inclusive. Middlebore, USA, manual no. M/92-021-K1098. 34 (1999).
5. Fallah, A.A., Zeynali, F., Saei-Dehkordi, S.S., Rahnama, M. and Jafari, T., Seasonal bioaccumulation of toxic trace elements in economically valuable fish species from the Caspian Sea using GFAAS. *J Verbraucherschutz and Lebensmittelsicherheit*. **6:** 367-374 (2011b).
6. Gallagher, M., The nutrients and their metabolism. In: Mahan L.K., Escott Stump S. 12th edition Elsevier Inc., Missouri, USA. Krause's Food and Nutrition Therapy. 39-143 (2008).
7. Gonzalez, M.I., Alvarez, G.N. and Gonzalez, C.J., Near-infrared spectroscopy (NIRS) with a fibre-optic probe for the prediction of the amino acid composition in animal feeds. *Talanta*. **69:** 706- 710 (2006).
8. Gopalan, C. Sastri. and Subramanian, Nutritive value of Indian foods, published by National Institute of Nutrition, ICMR, Hyderabad. pp 55 (2011).
9. Handbook on Fisheries Statistics. Government of India Ministry of Agriculture Department of Animal Husbandry, Dairying and Fisheries Krishi Bhavan, New Delhi. 165-192 (2014).
10. Jitender, K.J., Pal, A.K., Devivaraprasad, A.R., Sahu, N.P., Venkateshwarlu, G. and Vardia, K., Fatty acids composition of some selected Indian fishes. *African J of Basic and Applied Science.* **4(5):** 155-160 (2012).
11. Manirujjaman, M., Khan, M.M.H., Meftah, U., Minarul, I., Matiar, R., Khatun, M., Shahangir, B. and Islam, M.A., Comparison of different nutritional parameters and oil properties of two fish species (*Catla catla* and *Cirrhinus cirrhosus*) from wild and farmed sources found in Bangladesh. *J. Food and Nutrition Research.* **2(1):** 47-50 (2014).

12. Mohamed, E.H.A. and Al Sabahi, G.N., Fatty acids content and profile of common commercial Nile fishes in Sudan. *International J Fish Aquaculture*. **3(6):** 99-104 (2011).
13. Morrison, W. and Smith, L., Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron luoridemethanol, *J. of Lipid Research.* **5:** 600–608 (1964).
14. Panfili, G. Fratianni, A. Irano, M., Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. J *Agric Food Chemistry*. **51:** 3940–3944 (2003).
15. Park, J.W. and Lin, T.M.J., Surimi: Manufacturing and evaluation. In Park, J. E.(Ed.*).* Surimi and Surimi Seafood. 33-106 (2005).
16. Pearson, D. A. Chemical analysis of foods (7^{th} edition). Edinburgh: Churchilll, Livingstone. Raton: Taylor and Francis Group. 422-511 (1976).
17. Vanitha, M. Dhanapal, K. and Vidya Sagar Reddy, G., Quality changes in fish burger from Catla (*Catla Catla*) during refrigerated storage. *J Food Science Technology*. **52(3):** 1766–1771 (2015).
18. Wetten, I.A. and Herwaarden, A.W., Splinter R and Ruth SM. Oil analysis by fast DSC *Procedia Engineering*, **87:** 280-283 (2014).