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Determination of the Fatty Acid Composition of the *Dasyatis sephen* (F.) Liver Oil Captured from Cost of Jaffna Sri Lanka

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ABSTRACT

The liver oil from the cartilaginous fish was highly composed of poly- and highly unsaturated fatty acids. Extraction of oil from the *Dasyatis sephen* liver is simple and cheap. Therefore, the present study was conducted to investigate proximate Lipid Profile composition and physicochemical properties of *Dasyatis sephen* liver oil. Gas-Liquid Chromatography method

(GLC) was used to determine the Lipid Fatty acids profiles. The average Lipid level of *Dasyatis sephen* liver oil was found to be 69.54 % (w/w), greater than that from the *D. pastinaca* (58.27%) and *D. violacea* (57.33%). Crude liver lipid content was of were highly significant. The total SFA percentages was 44.2% and the predominant was C16 (palmitic acid) about 35.0%. Surprisingly, unsaturated fatty acids profiles of 20:5n-3 and 22:6n-3 exhibited as 0.5 % and 0.6 %. Physio-chemical properties such as moisture content, color, specific gravity, peroxide value, and fatty acid compositions were obtained under the tolerable standard. It demonstrated one of the locally available resources currently being wasted has the potential to use in the manufacturing of pharmaceutical and nutraceutical industries.

KEYWORDS Fish Liver Oil, Dasyatis sephen, Saturated fatty acids, Unsaturated fatty acids, Sri Lanka

INTRODUCTION

Lipid fraction extracted from tissues of oily fish and fishery by-products is known as Fish oil. It is one of the good sources of polyunsaturated fatty (PUFAs) Omega-3 (ω-3) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). This paper elaborates the study performed on the *Dasyatis sephen* liver oils from the dry fish processing industry were usually directly discarded into the sea.

Ray fish in Jaffna of Sri Lanka represent an interesting source of locally available quality fish. In this region, *Dasyatis sephenes* are caught for meat and dry fish production. Typically removal of the pectoral fins and discarding the remaining of the body eviscerates directly discarded into the sea. Nevertheless, it is well known that the Liver of *Dasyatis sephen* is rich in poly- and highly unsaturated fatty acids (PUFA and HUFA, respectively). It has been estimated that over 15000 kg of Ray fish is caught in Northern Sri Lanka each month (Department of Fisheries Report 2016).

Though Fish oil has numerous health benefits, only few can afford to take it because it is much expensive. Fluctuating market prices for Fish also have been an issue in fish oil production. Recently, the production of fish oil is becoming more demanding, as there is a sizable growing demand for high-quality fish oil in the world market.

Apart from its use as edible oil, it also has functional benefits in both pharmaceuticals and industries. A literature study done in the past has shown that little work has been done in terms of *Dasyatis sephen* liver oil and its fish by-products. Therefore, *Dasyatis sephen* liver oil may have used

as commercial raw materials for the manufacturing of pharmaceutical and nutraceutical products.

METHODOLOGY

Study Location

The study was carried out in the Gurunagar and Delft Islands Fisheries Harbors of Jaffna, Northern Provenience in Sri Lanka. All the Analytical tests were done in the Nutrition Laboratory and Meat Science Laboratory, Department of Animal Science, Food Science Laboratory, Department of Food Science, Faculty of Agriculture, University of Peradeniya, and in the Beuro Venters, Colombo, Sri Lanka.

(Dorsal View)
Plate 2.1: Sting Ray (*Dasyatis sephen*)

Sample Collection and Preparation

Three samples of *Dasyatis sephen* Fish (Forsskal, 1175) were collected from each Fisheries Harbors and Dry Fish Cottages of Jaffna, Northern Provenience in Sri Lanka. Samples were transported in sealed freezer containers to laboratory at 0 ˚C. They were stored at (-20 ˚C) in the deep freezer for analytical purposes. Each sample was taken out to thaw at room temperature to measure its lengths and weights as a whole. Samples' livers, offal (gut & tail), and carcass were eviscerated, and stored at -20 ˚C in sealed poly bags separately for further analysis.

Hepatosomatic Index (HIS)

The liver weight to body weight ration was calculated.

Extraction of *Dasyatis sephen* Liver Crude Oil

Frozen livers were weighted and thawed at room temperature at 27 °C. The livers were washed and cleaned. Then they were sliced to obtain steaks. The liver steaks were minced thoroughly using the electric homogenizer. The Homogenized samples were weighted.

The modified Bligh and Dyer method (1959) was used to extract the crude oil in the liver samples. 50 g liver samples were homogenized for two minutes using an electric homogenizer with 100 mL of methanol and 50 mL of chloroform. Then, again 50-mL of chloroform was added to homogenize for another 30 Seconds. The homogenized mixture was diluted with 50 mL of distilled water.

Mixture samples were filtered by a Whatman No.42 filter paper lined with Buncher funnel under vacuum suction. 20 mL of chloroform used to rinse the residues, and the filtered portion was transferred to a separatory funnel. The chloroform layer containing liver oil (bottom layer) was separated by removing it. Whatman No.1 filter paper lined with Buncher funnel under vacuum suction. It was used to Filter Finely. 20 mL of chloroform used to rinse the residues. To remove moisture, the filtrate was passed through 3 g of anhydrous sodium sulfate.

Extraction of Fish oil from the Chloroform layer was carried out by transferring the filtrate into a dry pre-weighed round-bottom flask of rotary vacuum evaporator. The chloroform was removed using a rotary vacuum evaporator at 40 °C. Extracted crude liver oil was weighed and treated with 0.02 BTH to keep moisture free. The prepared crude liver oil was stored at (-20 °C) for further analysis.

Crude Oil Recovery Percentage

Given below equation was used to calculate the Crude oil content in the Liver sample. Equation: Percentage of lipid in the fish liver

$$\% \text{ Lipid} = \frac{W_2}{W_s} \text{ X } 100$$

W_S= Weight of the sample (g)
W_2= Weight of Lipid extracted after evaporation (g)

Determination of fatty acid composition in Crude oil

Gas-Liquid Chromatography (GLC) method was used to determined Fatty acids profiles, according to Buchgraber et al. (2000).

Preparation of fatty acid methyl ester (FAME)

0.074 g of Sodium methoxide (CH_3NaO) (0.5M) was weighed accurately and dissolved in 2 mL of methanol. 100 mg of methylheptadecanoate acid (1mg/mL) was dissolved in 100 ml of hexane.

100 mg sample was weighed into a 15 mL screw-capped methylation tube, and 1 mg/mL of internal standard (Methylheptadecanoate), 2mL of 0.5M sodium methoxide, and 300 UL of dichloromethane were added. Meanwhile, the mixture was kept in a heat block at 50°C for 30min. It was allowed to cool to room temperature. Drop by drop 5mL of distilled water was added. After that, 100 UL of glacial acetic was added.

The tube with contents was centrifuged at the speed of 1500rpm for 10 minutes at 5°C. The top hexane layer was separated and added in to a 2ml GC Vial. Again 500 UL of hexane was added and centrifuged at the speed of 1500rpm for 10 minutes at 5°C. The top hexane layer was separated and added to the same GC vial. Vials were sealed with Para film and frozen immediately at (-20°C) until GC analysis.

Gas-Liquid chromatography analysis of the sample

The prepared FAME samples were analyzed by injecting 1 µL into GLC (Shimadzu, 14-B, Japan), equipped with a Flame Ionization Detector (FID). A fused silica capillary column (100 m, 0.25 mm id and 0.20 µm film thickness) attached with Chromotopac data processor (Model-CR6A, Shimadzu, Japan). The split ratio was 100:1. Temperatures of the Injector and detector were maintained at 260°C. Helium was used as carrier gas at a flow rate of 20 mL/sec. The initial column oven temperature was maintained at 140°C for 5 min and increased to 220°C at the rate of 4°C/min, then maintained at that temperature for 10 min. Fatty acids were identified by comparison of their retention time with authentic standards (SUPELCO 37 Component FAME Mix, Sigma Aldrich) (Kuksis *et al.*, 1967). The amount of each fatty acid were expressed as a percentage (%) of the sum of all fatty acids in the sample.

RESULTS AND DISCUSSION

Hepatosomatic Index (HSI) of Dasyatis sephen liver

The average body weights were found to be 2301.05g, 2594.17g, and 2847.84g average lengths were 137.67cm, 153.49cm and 175.74cm. The average liver weight were 253.69g, 289.52g and 313.98 g respectively. Calculated HSI value was 9.05 %. Moreover, the average lengths of *Dasyatis sephen* were measured to be. The HSI value of *Dasyatis sephen* fish is comparatively greater than the findings of Özyılmaz and Öksüz, 2015 which is 8.25 %. This may be due to the increased liver weight and the maturity level of fish.

Table 4.1: The average length (cm), Total weight (g), Liver weight (g) and Hepatosomatic index (HSI) of the *Dasyatis sephen*.

Fish species	Length (cm)	Total weight (g)	Liver weight (g)	HSI (%)
Stingray-$_1$	137.67	2301.05	253.69	9.07
Stingray-$_2$	153.49	2594.17	289.52	8.96
Stingray-$_3$	175.74	2847.84	311.92	9.13

Mean±SD values were presented (n = 3)

Dasyatis sephen Liver Crude Oil Recovery Percentage

The average liver crude oil recovery of the *asyatis sephen* was 69.54 %. All of the *Dasyatis sephen* had a large amount of lipids in their liver. However, the levels of liver lipid not varied greatly from one species to another (Özyılmaz and Öksüz, 2015).

Plate: 4.1 Fish oil Colour

Fatty acid Profile of *Dasyatis sephen* crude liver oil

The predominant SFA fatty acid in the stingray was C16 (palmitic acid), which had a value of 10.2%. Clearly, the average level of C16 in the *Dasyatis sephen* liver oil was significantly higher than the level of the other *Dasyatis sephen* liver oils. The total levels of PUFA in all the fish liver oils seems similar.

The total SFA percentages of *Dasyatis sephen* fish in this study was 44.2%. The Total SFA percentages of *Dasyatis sephen* liver oils from *Dasyatis sephen* that were previously reported were 34.97%, respectively (Navarro-Garcia *et al*., 2010). The percentages of total SFA in *Dasyatis sephen* liver oil were not in agreement with the finding from the current study.

Table 3.1. Fatty acid composition of stingray fish liver oil

Carbon Chain	Stingray
C12:0	4.1
C13:0	0.7
C14:00	0.3
C16:0	35.0
C17:0	0.4
C18:0	2.5
C20:0	1.2
ΣSFA	44.2
C16:1	10.2
C18:1	1.3
ΣMUFA	11.5
C18:2	0.3
C20:4	1.4
Σn6	1.7
C20:5n3	0.5
C22:6n3	0.6
Σn3	1.1
ΣPUFA	2.8
n3/n6	0.64
DHA/EPA	0.83

CONCLUSIONS

Dasyatis sephen liver was obtained during dry fish processing Cottages of Jaffna, Northern Province, Sri Lanka. Liver oil extracted through the Bligh and Dyer method gave the total recovery percentage of 69.54 % with high quality.

The total SFA percentages was 44.2%, and with predominant C16 (palmitic acid) about 35.0%. Unsaturated fatty acids profiles of 20:5n-3 and 22:6n-3 exhibited as 0.5 % and 0.6 %. Physio-chemical properties such as moisture content, color, specific gravity, peroxide value, and fatty acid compositions were obtained under the tolerable standard. Oil extracted from *Dasyatis sephen* liver eviscerates from the dry fish industry could be a potential source for pharmaceutical and nutraceutical industries.

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