CHAPTER VII

Cholesterols, Triglyceride and Vitamin Contents of Fish Species

VII.1. Materials and Methods

VII.1.1 Sample preparation

The sample of nine fish species was prepared as per the procedure mentioned in the **Section IV.1.1** (**Page no. 58**).

VII.1.2. Evaluation of Total Cholesterol, LDL, HDL, VLDL and Triglycerides

The muscle (1 g) of the fish species was mixed and homogenized using methanol and chloroform solution (1:1, 5 mL) for 15 min with a vortex. To separate the solids, the mixture was then centrifuged at 15000 rpm. Thereafter, 1 mL of the extract has been added to the isopropanol and water solution (3:2, 4 mL) followed by the addition of 5M KOH solution (1 mL). The whole solution was taken in vials and stored in a deep freeze for analysis. Total cholesterol, High-density lipoproteins (HDL) and triglycerides were estimated by using the UV-VIS Spectrophotometer (Systronics-117, 200–1100 nm). For the determination of these, the reagent kits were purchased from Span Diagnostics Ltd., India. HDL and total cholesterol contents of the fish species were evaluated by following the assay of CHOD-PAP enzymatic end point (Kaplan and Lavemel, 1983; Herbert, 1984). GPO-PAP end point assay was followed for the determination of triglyceride content (Kaplan and Lavemel, 1983; Herbert, 1984). Using the results of HDL, total cholesterol and triglyceride, LDL (Low-density lipoproteins) and VLDL (Very low-density lipoproteins) were determined using Friedewald's equation given below (Friedewald et al., 1972).

LDL cholesterol = Total cholesterol – (Triglycerides/5) – HDL cholesterol (a)

VLDL = Triglyceride/5 (b)

VII.1.3. Evaluation of Vitamins A and D

Fish fat of selected fish species was extracted as per the procedure mentioned in the **Section IV.1.6** (Page no.). The fat (0.15 g) was mixed with 25 mL of methanol and KOH (15%) and then refluxed for 30 min. Using 50 mL of petroleum ether, the fat-soluble vitamins

were extracted three times. The solvent was concentrated and removed, and then the solid obtained was dissolved in acetonitrile (5 mL). The analyses of vitamins were carried out by injecting the sample solution (100 µL) in the Reversed Phase High Performance Liquid Chromatography (RP-HPLC, 1260) which is equipped with the column C18 (250 × 4.6 mm, 5 µm), and the UV detector. The HPLC mobile phase consisted of solvent A (acetonitrile) and solvent B (methanol). A linear gradient system (Solvent A/Solvent B) was used starting from 50/50 to 70/30 in 20 min. The mobile phase was used at the flow rate of 1 mL/min. The vitamins A and D were identified and quantified by comparing the retention times and the peak areas with those of the standard vitamins (Sigma-Aldrich).

V.1.4. Statistical analysis

Statistical analysis was carried out as mentioned in **Section IV.1.10** (**Page no. 60**).

Table VII.1. Total cholesterol, LDL, HDL, VLDL and triglyceride contents in fish species

Fish species	Total cholesterol (mg/g)	HDL (mg/g)	LDL (mg/g)	VLDL (mg/g)	Triglyceride (mg/g)
B. bendelisis	10.61±0.12^{a}	1.82±0.05^{a}	5.99±0.88^{a}	3.71±0.98^{a}	25.22±0.23^{a}
C. chagunio	8.05±0.22^{b}	1.39±0.08^{a}	nd	7.32±0.02^{b}	36.62±0.13^{b}
G. gotyla	14.12±0.12^{c}	1.48±0.08^{a}	8.27±0.07^{b}	4.36±0.02^{c}	21.84±0.13^{c}
L. pangusia	6.05±0.22^{d}	1.85±0.05^{a}	nd	7.62±0.04^{b}	48.12±0.24^{d}
N. hexagonolepis	3.73±0.06^{e}	0.66±0.02^{b}	1.93±0.02^{c}	1.59±0.02^{d}	5.65±0.12^{e}
R. bola	8.29±0.12^{b}	1.28±0.04^{a}	0.48±0.04^{d}	6.52±0.53^{e}	32.61±0.12^{f}
T. putitora	2.28± 0.06^{f}	0.74±0.04^{b}	nd	2.93±0.02^{f}	14.64±0.12^{g}
C. semiplotum	5.61±0.06^{d}	0.63±0.02^{b}	3.08±0.03^{e}	1.91±0.01^{d}	9.67±0.83^{h}
B. barna	5.53±0.12^{d}	1.35±0.02^{a}	nd	5.99±0.01^{g}	29.95±0.07^{i}

LDL, Low-density lipoproteins; HDL, High-density lipoproteins; VLDL, Very low-density lipoproteins; Results are presented as mean of three replicates ± standard deviation; The different letters in a column indicated that the results are significantly different from each other at $p<0.05$ (one-way ANOVA *t*-test); nd, not detected.

VII.2. Results and Discussions

VII.2.1. Total Cholesterol, HDL, LDL, VLDL and Triglycerides

The total cholesterol, HDL, VLDL, LDL and triglyceride contents of nine fish species are presented in **Table VII.1**. *G. gotyla* displayed the highest quantity of cholesterol (14.12 ± 0.12 mg/g) followed by *B. bendelisis* (10.61 ± 0.12 mg/g) and *R. bola* (8.29 ± 0.12 mg/g). In the other fish species, total cholesterol ranged from 2.28±0.06 mg/g (*T. putitora*) to 8.05 ± 0.22 mg/g (*C. chagunio*). However, Azrina et al. (2015) reported higher levels of cholesterol in fish species and seafood samples which are found in the range of 27.13–353.97 mg/100 g. Sarma (2015) reported cholesterol content in the muscle of some minor carps which varied from 76.688 ± 0.14 to 127.766 ± 0.15 μg/100 mg. Bibi et al. (2016) studied the size-dependent variation in cholesterol of *W. attu* and reported that cholesterol contents in different tissues of small and large-sized groups of *W. attu* showed significant variations which varied from 40.1 to 183.9 mg/100 g. Nanda and Sahu (2005) reported free cholesterol in some freshwater fish species which varied from 73.29 to 87.74%. Donmez (2009) reported cholesterol levels of some freshwater fishes that ranged from 94.68 ± 3.13 to 179.84 ± 6.75 mg/100 g. Imre and Saglik (1998) investigated the cholesterol levels of some Turkish fishes which varied from 40.3 to 75.3 mg/100 g. Jeyasanta et al. (2013) also investigated the cholesterol contents of six fish species from Tuticorin, India and found them to be in the range of 38 ± 2.49–107 ± 2.64 mg/100 g. In the present study, the HDL concentration was observed to be the highest in *L. pangusia* (1.85±0.05 mg/g) followed by *B. bendelisis* (1.82 ± 0.05 mg/g). However, almost similar results of HDL (0.63 ± 0.02–1.39 ± 0.08 mg/g) were noticed in all the other fish species of this study. High-density lipoproteins (HDL) are considered the good cholesterol that can transport the cholesterol and its esters from the peripheral tissues to the liver for its scavenging action or catabolism (Sujatha et al., 2013; Karatas et al., 2014). The highest concentration of LDL (Low-density lipoproteins) was found in *G. gotyla* (8.27 ± 0.08 mg/g) followed by *B. bendelisis* (5.99 ± 0.88 mg/g). The lowest amount of LDL was observed in *R. bola* (0.48 ± 0.04 mg/g). LDL was not found in the other four fishes *viz. L. pangusia*, *C. chagunio*, *B. barna* and *T. putitora*. LDL, known as the bad cholesterol, can control the synthesis of cholesterol in extra-hepatic tissue (Sujatha et al., 2013). The highest amount of VLDL cholesterol was noticed in *L. pangusia* (7.62 ± 0.05 mg/g), which is followed by *C. chagunio* (7.32 ± 0.03 mg/g) and *R. bola* (6.52 ± 0.53 mg/g). The lowest level of VLDL cholesterol was exhibited by *N. hexagonolepis* which contained 1.59 ± 0.02 mg/g. VLDL can transport cholesterol and its esters, endogenous triglycerides, and phospholipids. VLDL can work for the mechanism of internal transport of the body for

the lipids (Karatas et al., 2014). In this study, the triglyceride content of the fish species was found to be ranging from 5.65 ± 0.12 mg/g (*N. hexagonolepis*, the lowest) to 48.12 ± 0.24 mg/g (*L. pangusia*, the highest). Higher triglyceride contents were also noticed in *G. gotyla* (21.84 ± 0.14 mg/g), *B. bendelisis* (25.22 ± 0.24 mg/g), *B. barna* (29.95 ± 0.07 mg/g), *R. bola* (32.61 ± 0.12 mg/g) and *C. chagunio* (36.62 ± 0.14 mg/g). Similarly, Sujatha et al. (2013) determined triglyceride content of some fish species from Kasimodu fish landing centre, Tamil Nadu, which was found in the range of 58.22 ± 1.55–98.55 ± 1.59 mg/100 g (muscle), and 50.58 ± 0.68–79.50 ± 1.57 mg/100 g (brain). Triglyceride that consists of around 98% of the total dietary lipids is one of the most abundant components of all the lipids and the remaining about 2% are the components of the cholesterol, cholesterol esters and phospholipids (Sujatha et al., 2013). Sarma (2015) investigated the triglyceride content in the muscle of some minor carps that varied from 12.632 ± 0.095 to 21.514 ± 0.054 μg/100 mg, which is lower compared to our investigation. Dey et al. (2016) mentioned that consumption of fish meat has a beneficial role on human health as it minimizes the appearance of cardiovascular diseases by decreasing the levels of triglyceride and cholesterol, controls the inflammatory response and improves carbohydrate metabolism. Therefore, the present study is a documentary report for lipid profiling from fish oils. The lipid components determined from the nine fish species collected from *Hel* river will be a well-documented record for future reference work.

Table VII.2. Vitamins A and D contents of fish species

Fish species	Vitamin A (μg/100 g)	Vitamin D (μg/100 g)
B. bendelisis	100.37	129.70
C. chagunio	21.46	199.79
G. gotyla	15.85	124.04
R. bola	43.01	105.95
C. semiplotum	50.04	593.83
B. barna	25.09	176.54
N. hexagonolepis	1287.0	318.0
L. pangusia	625.0	45.0
T. putitora	172.0	95.0

VII.2.2. Evaluation of Vitamin A and D contents

The results of vitamins A and D analyses of nine fishes using RP-HPLC (**Appendix Section; Fig. VII.A.1 – Fig. VII.A.9**) are represented in **Table VII.2**. The highest vitamin A content of 1287.0 µg/100 g was exhibited in *N. hexagonolepis.* A high amount of vitamin A was also noticed in *L. pangusia* (625.0 µg/100 g), and the lowest level of vitamin A was observed in *G. gotyla* (15.85 µg/100 g). Similarly, Dhaneesh et al. (2012) reported the vitamin A content of some fishes of Lakshadweep, India, which was found in the range of 190 – 720 µg/100 g. Stancheva et al. (2012) also reported the vitamin A contents of Garfish (21.7 µg/100 g) and *Psetta maxima* (7.2 µg/100 g), which are lower in comparison to our result (**Table VII.2**). The highest vitamin D content was observed in *C. semiplotum* (593.83 µg/100 g) and the lowest amount of vitamin D was detected in *L. pangusia* (45.0 µg/100 g). Dhaneesh et al. (2012) also investigated the vitamin D contents of fish species and found to be in the range of 370 – 770 µg/100 g, and the two fishes of the present study *viz. C. semiplotum* and *N. hexagonolepis* (**Table VII.2**) indicated similar results of vitamin D content. However, lower levels of vitamin D were detected in other fish species of this study. The fish species of our study displayed higher amounts of vitamin D contents compared to vitamin D contents of Turbot (4.6 µg/100 g) and Garfish (5.8 µg/100 g) reported by Stancheva et al. (2012). Vitamins like fat-soluble vitamins can control several important biological processes of the human body. Vitamin A that possesses antioxidant property has the capacity to protect the skin and body against various infections. Vitamin A is required for normal vision, growth of teeth and bones, regulating gene expression and cell division, photoreception, reproduction and embryonic development. Vitamin D is needed for the metabolism and absorption of calcium and phosphorus. Vitamin D owns corrective properties for several diseases such as cancer, diabetes, hypertension, osteoporosis and cardiovascular diseases (Stancheva et al., 2012; Mahanty et al., 2014; Salma and Nizar, 2014).

Conclusion

In this study, the nine fish species exhibited varying concentrations of lipid components. The vitamin A content found in the fish species varied from 15.85 to 1287.0 µg/100 g and the vitamin D content was found in the range of 45.0–593.83 µg/100 g. The fish species contain good sources of vitamins and lipid components, and consumption of these fishes can provide good nutrition for human health. The lipid components determined from the nine fish species collected from the *Hel* river will be a well-documented record for future reference work.