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Physico-chemical and microbiological investigation of the lipid from Bangladeshi fresh water fish *Mystus vittatus*

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Abstract : This study dealt with physical, chemical and microbiological characteristics of the lipid obtained from *Mystus vittatus* and some nutritional properties of the fish. The fish was found to contain moisture 76.98%, calcium 521.072 mg / 100gm, vitamin C 11.276 mg / 100 gm., lipid 1.89%, and protein 10.94% respectively. The specific gravity, refractive index and co-efficient of viscosity of this lipid were recorded as 0.935 at 30°c, 1.4662 at 30°c and 409.26 millipoise at 30°C respectively. The saponification value, iodine value, peroxide value, acid value, percentage of free fatty acid, percentage of unsaponifiable matter , Reicher-Meissel value, Polenske value and Acetyl value were found to be 218.12, 96.17, 1.79, 1.92, 0.968, 0.632, 0.932, 0.652 and 11.97 respectively. The GLC analysis revealed that the lipid is composed of palmitic acid (30.79%), oleic acid (29.50%), palmotelic acid (15.97%), stearic acid (11.89%), linoliec acid (5.43%), myristic acid (5.15%), lauric acid (1.07%), arachidic acid (.57%). The chloroform extract of this lipid showed some microbiological actively. The minimum inhibitory concentration of the extract was 0.5 µl/ ml against Staphylococcus aureus (107 cells/ ml) and 0.125µl/ ml against Shigella dysenteriae (107 cells/ ml).

Key words: Physico-chemical, fish nutrition, M. vittatus

Introduction

The small indigenous fresh water fish *Mystus vittatus* is important due to its high nutritional value in terms of lipid, protein content, the presence of micronutrients and vitamins which are not commonly available in other food. *M vittatus* was studied to obtain detail information on their chemical composition. The study reports about the moisture, crude-fiber, drymatter, calcium, vitamin-C and protein content of the fish fillet and fatty acid composition of the lipid with its physical, chemical and microbiological characteristics.

The effectiveness of fish oil to reduce cardiovascular problems has attracted the investigators extensively to analyze the fish oils of both marine fishes and fresh water vertebrates (Kromhout *et al.*, 1995; Herold, *et al.*, 1996; Metcalf *et al.*, 2007). Polyunsaturated fatty acids were found to present in high ratios in marine fish of which fatty acids of ω-3 and ω-6 configurations are predominant (Raclof & Grocolas, 1994; Connor, 2001). Polyunsaturated fatty acid were reported to be an is important factor to reduce the risk of heart attack (Chin *et al.*, 1993; Siscovick, *et al.*, 1995; Ascherio *et al.*, 1995; Daviglus *et al.*, 1997)

Lipids are structural materials, reserve supply of fuels, vitamins, emulsifiers, flavours, aromatic compounds and barriers to the environment (Harris, 1989; Nestel, 1990). In the form of complex lipids, they are found in large quantities in brain and nervous tissues, indicating the important roles these substances play in the living organism (Lunberg, 1958; Goodpaster *et al.*, 2004). The polyunsaturated fatty acids, especially linoleic acid and lenolenic acid are necessary for the proper functioning of many metabolic processes (Ascherio, 1995; Terpstra, 2004; Whigham, 2007). Beside the use in medicine and in diet, fats and lipids also have some industrial application in the manufacture of soap, paints, vernishes, and cosmetics.

Materials and Methods

Standard sizes of living *M. vittatus* were collected from Shaheb Bazer, Rajshahi. Only the middle portion of the body was used for nutritional studies and extraction of lipid and the content of moisture, ash, dry matter, crude fibre, calcium, vitamin-C of the fish fillet were determined by standard methods.

Lipid of this fish was extracted by Bligh and Dyer method (Bligh *et al.*, 1959). A mixture of chloroform and methanol (2:I V/V) was used in this process. Standard methods were used for determining various physical and chemical characteristics of this lipid sample.

The fatty acid compositions of this lipid were determined by TLC and GLC analysis of the methyl ester mixture obtained from the fish lipid. At first, the lipid was saponified with alcoholic potassium hydroxide, then cooled and diluted with water. After evaporation of alcohol, the acidified aqueous mixture was extracted with ether. Ether was then removed from the extract to give fatty acid mixture. The fatty acid mixture was esterified with methanolic solution of sulphuric acid (25M, 5 ml per gm. of acid). After esterificaton, the reaction mixture was dissolved in

diethyl ether in a separating funnel and was washed with dilute sodium carbonate solution until the effervescence ceased. It was then washed with water, dried over anhydrous sodium sulphate and finally ether was removed to give methyl ester mixture. The methyl esters were charged on thin-layer plate (20 cm × 20cm × 0.05 mm) coated with silica gel G and the plates were developed by ascending technique with suitable solvent system. GLC experiment (Molla *et al.*, 1994) was carried out with a "PYE UNICAM 4500 U model gas chromatograph" equipped with a flame ionization detector.

The protein content of this fish was estimated by Micro-kjeldahl's method. At first the fish fillet was defatted, then the interfering materials were removed by 5% TCA solution. In this process, protein was estimated by titrimetric estimation of ammonia.

The micro-biological characteristics of the lipid of *M. vittatus* was determined by "Disc diffusion method" (Bauer *et al.*, 1966). The minimum inhibitory concentration (MIC) of the lipid was carried out by serial dilution technique using a broth medium.

Results and Discussion

In the present investigation the lipid, protein, micronutrients, vitamins and other related substances in the fish fillet of *M. vittatus* were recorded. The results have been shown in the following tables.

Table-I : The nutrients in *M. vittatus*

Moisture content	76.49%
Dry matter content	23.087%
Ash content	1.013%
Crude fiber content	6.013%
Lipid content	1.89%
Protein content	10.94%
Calcium content	521.072 mg/100gm
Vitamin-C content	11.276 mg/100gm

The lipid content of the fish *M. vittatus* was 1.89 percent. The physical and chemical characteristics of the oils and fats varied within a small so that they seem to be constant. Although the chemical constants are more important to characterize an oil, but physical constants are also often capable of giving valuable information. The physical and chemical constants of the fish oil are given in tables II and III. The saponification value of the lipid of *M. vittatus* was found to be 218.12, when the saponification equivalent of the fish lipid was observed to be 257.19. This wascalculated from saponification value as described in the materials and methods. It is directly proportional to the average chain length of fatty acid present. Fats or oils consisting largely of C_{18} fatty acids, along with some myristic acid, palmitic acid, a little unsaponifiable matter and a low free acid, generally have a saponification equivalent of around 290.80; higher value indicates the presence of appreciable quantity of higher acid. The results indicate that the lipid of *M. vittatus* fish contain mainly fatty acids of C_{18} molecular weight along with some palmitic acid.

Table-II: The physical constants of the fish oil.

Physical constant	Value
Specific gravity	0.935 at 30°C
Refractive index	1.4662 at 30°C
Viscosity	409.26 millipoise at 30°C
Energy of activation	24.95 kjmole^{-1}

Table – III: The chemical constants of the fish oil.

Chemical constant	Value
Saponification value	218.1
Saponification equivalent	257.2
Iodine value	96.2
Peroxide value	1.79
Acid value	1.93
Free fatty acid as oleic (%)	0.968
Unsaponifiable matter (%)	0.632
Reichert-Meissel value	0.932
Polenske value	0.652
Acetyl value	11.97

Iodine value gives an estimation of the degree of unsaturation and the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat. It may be suggested that the oil under investigation contains higher amounts of unsaturated fatty acids as its iodine value was calculated to be 96.17. A higher percentage of free fatty acid (above 1.5%) is a determination or indication of unsuitability of the oil for edible purpose. So the fish oil of *M. vittatus* might be suitable for edible purposes as it contains free fatty acid less that 1.5%.

The fatty acid methyl ester mixture obtained from the fish oil was subjected to TLC examination

and their fatty acids composition were identified by comparing the R_f values of methyl esters of standard fatty acids (Table-IV).

Table-IV: **Thin layer chromatographic examination of the methyl esters mixture obtained from the lipid of *Mystus vittatus*.**

Name of the sample	Developing solvent system	Rf value obtained from the spot									
		Methyl Palmitate	Methyl Stearate	Methyl Oleate	Methyl Linoleate	Methyl Myristate	Methyl Arachidate	Methyl ester mixture			
Lipid of *M. vittatus*	P:E (80:20)	0.962	0.931	0.901	0.933	0.282	0.885	0.900	0.936	0.251	
	P:E (60:40)	0.978	0.951	0.952	0.953	0.323	0.892	0.731	0.852	0.950	
	P:E:A (85:15:1)	0.844	0.852	0.805	0.812	0.319	0.971	0.679	0.812	0.850	
	P:E:A (80:20:1)	0.810	0.862	0.880	0.821	0.419	0.932	0.792	0.861	0.722	0.810
	H:E (80:20)	0.840	0.852	0.866	0.940	0.191	0.891	0.867	0.774	0.800	

Using the different solvent systems, spray reagent and UV lamp, the spots were identified as oleic acid ($C_{18.1}$), linoleic acid ($C_{18.2}$), palmitic acid ($C_{16.0}$) and stearic acid ($C_{18.0}$). Fatty acid analysis of the fish lipid was carried out by GLC after trans-esterification of the glycerides to their methyl esters. The identification of fatty acid components from GLC analysis was carried out on the basis of relative retention time and were quantified by measuring the peak area in comparison with standard. The analytical data were summerised in the table V. It is evident from the table that the lipid of *M. vittatus* contain palmitic acid (30.79%), oleic acid (29.50%), palmotelic acid (15.79%), stearic acid (11.89%), linoleic acid (5.43%), myristic acid (5.15%), lauric acid (1.07%) and arachidic acid (0.57%).

Table-V: Fatty acid composition of the fatty acid methyl ester mixture derived from the lipid of *M. vittatus* (by GLC analysis).

Retention time (min)	Area	Name of acid	Relative percentage (%)
7.67	609	Lauric	1.07
12.55	2933	Myristic	5.15
17.16	8765	Palmotelic	15.39
17.75	17520	Palmitic	30.79
21.82	3091	Linoleic	5.43
22.04	16783	Oleic	29.50
22.64	6766	Stearic	11.89
27.21	429	Arachidic	0.57

Protein plays crucial roles in virtually all biological processes. All animals, including human, must have an adequate source of protein in order to grow and maintain themselves. The amount of protein present in *M. vittatus* was 10.94%. It was determined by micro-kjeldahl's method.

In the microbiological investigation, it was found that the chloroform extract of the lipid of *M. vittatus* was active against all the gram-positive bacteria tested. The extract was also sensitive against most of the gram-negative bacteria. The minimum inhibitory concentration (MIC) of the lipid of *M. vittatus* was determined against two test organisms, *Staphylococcus aureus* and *Shigella dysenteriae.* The results are in table VI.

Table –VI: Minimum inhibitory concentration of the lipid of the *M. vitattus* against *S. aureus.*

Test tube number	Nutrient broth medium added (ml)	Diluted solution of lipid sample µl/ml	Inoculum added (µl)	Observa-tion
1	1	8.000000	10	NG
2	1	4.000000	10	NG
3	1	2.000000	10	NG
4	1	1.000000	10	NG
5	1	0.500000	10	NG
6	1	0.250000	10	G
7	1	0.125000	10	G
8	1	0.062500	10	G
9	1	0.031250	10	G
10	1	0.015625	10	G
C_s	1	8.000000	00	NG
C_1	1	0	10	G
C_M	1	0	00	NG

G= Growth
NG = No growth
Number of cells: 10^7/ml

From table VI, it was found that the MIC of the lipid of *M. vitattus* against *S. aureus* is 0.500000 µl/ml.

Table –VII: Minimum inhibitory concentration of the lipid of the *M. vitattus* against *S. dysenteriae.*

Test tube number	Nutrient broth medium added (ml)	Diluted solution of lipid sample µl/ml	Inoculum added (µl)	Observation
1	1	8.000000	10	NG
2	1	4.000000	10	NG
3	1	2.000000	10	NG
4	1	1.000000	10	NG
5	1	0.500000	10	NG
6	1	0.250000	10	NG
7	1	0.125000	10	NG
8	1	0.062500	10	G
9	1	0.031250	10	G
10	1	0.015625	10	G
C_s	1	8.000000	00	NG
C_1	1	0	10	G
C_M	1	0	00	NG

G= Growth
NG = No growth

It was found from the above table that the minimum inhibitory concentration of the lipid of *Mystus vitattus* against *Shigella dysenteriae* is 0.125000 µl/ml.

From the foregoing evidences, it may be concluded that the fish *Mystus vitattus* along with its lipid is suitable for edible purpose. The fish and fish products are the important food sources and sources of protein in Bangladesh, which are essential in consideration to their market value. It is the basic fisheries item of Bangladesh. Lipid of the fish contains physiologically active polyunsaturated fatty acids. This is an important indication of the fish lipid to inhibit the activity of HMGCoA-reductase which is the regulatory enzyme in cholesterol biosynthesis. The cholesterol lowering effect of polyunsaturated fatty acids in fish oils have antiaggregating effect and thus reducing the risk of heart attack (Fehily *et al.*, 1982 & Herold *et al.*, 1986)

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