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QUANTITATIVE ANALYSIS OF FATTY ACIDS COMPOSITION AND RESIDUAL AMOUNT OF DDTS OF *Mystus gulio* - A BRACKISH FISH

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ABSTRACT

This study deals with the quantitative analysis of the fatty acid profile and the level of pesticides of fish lipid obtained from *Mystus gulio*. Lipid was extracted from *Mystus gulio* by ethylacetate, saponified and converted to methyl ester by using BF_3-MeOH in esterification process. The fish fillet was found to contain 18.22 mg/g of lipid. The TLC analysis revealed that the separation and identification of fatty acids whereas GLC analysis revealed the quantification of fatty acids. Among all the fatty acids oleic acid was predominant (30.29 %). The amount of monounsaturated fatty acid (MUFA) was higher (34.08 %) compared to the polyunsaturated fatty acids (PUFA) whose value was found to 14.42 %. The fish fillet was found to contain ω-3 fatty acid (5.46 %) and ω-6 fatty acid (7.35 %). A very small amount of DDTs was found by GC-ECD in the fish fillet. The ratio of 4,4´-DDT/∑DDT, 4,4´-DDT/DDE and (DDD+DDE)/∑DDT, was 0.109, 0.176 and 0.745 respectively, indicates past input of DDT and also long term biotransformation of DDT to DDD and DDE.

KEYWORDS : Fatty acids, Extraction, Esterification, DDTs, *M. gulio*

Mystus gulio (Hamilton, 1822), locally known as "nona tengra", is a brackish water catfish commonly occurring in coastal region of Bangladesh (M. Begum et al., 2008). *M. gulio* is important due to its delicious taste and high nutritional value in terms of lipid, protein content, the presence of micronutrients and vitamins which are not commonly available in other food (Ara et al., 2006). It was reported that the polyunsaturated fatty acids play as an important factor in health issue, i.e, prevention of the coronary heart disease (CHD), brain development and mental health, hypertension, diabetes, obesity, cancer, thrombosis and lung disease (Ascherio et al., 1995). These are found in large quantities in brain and nervous tissues in the form of complex lipids. Generally Lipids are structural materials i.e. emulsifiers, flavours, vitamins and aromatic compounds. Especially linoleic acid, palmitoleic acids oleic acids and lenolenic acid are essential for the proper functioning of many metabolic processes (Terpstra, 2004; Whigham, 2007).

Environmental chemical contaminants and pesticides in fish pose a potential human health hazard. They may also accumulate in aqua cultured fish through contaminated feed ingredients. DDT is probably the most infamous Persistent Organic Pollutants (POP) and long-term harmful effects of DDT exposure to both humans and wildlife prompted the Environmental Protection Agency to cancel the registration of DDT in 1972. Moreover some people use this DDTs frequently as pesticides in agriculture or required task.

Due to high nutritional value *Mystus gulio* was selected for the quantification of the composition of fatty acids and residual amount of DDTs. The contamination by DDTs can be either dilution by rain water from the field where the pesticides are used as insecticides or the food supplied to feed the fish in gher.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals and reagents used for analysis were of analytical and gas chromatography (GC) grade. All glass apparatus were cleaned by organic solvents and baked at 105ºC in an oven.

Preparation of Sample

Standard sizes of fresh (within 24 hours) *M. gulio* were made bone free, chopped, blended, weighed approximately to 10.0 g and wrapped with aluminium foil and stored in a refrigerator at -20°C until analysis.

METHODOLOGY

Fish fillet (10 g) was extracted by ethyl acetate, saponified with alcoholic potassium hydroxide and converted to methyl Ester by the same procedure (G. Lambertsen, 1972; H. R. Bhuiyan et al., 2006). The methyl esters of samples and standards were placed on thin-layer

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plate (20 cm × 20cm × 0.05 mm) which was coated with silica gel GF254 (12 g/plate) forming thickness 0.25 mm and the plates were developed by ascending technique with suitable solvent system (W. W. Christie, 1976). Standard substances were run simultaneously with the mixture of fatty acid methyl esters which was derived from fish lipid in each of five chromatograms. After development, each of the chromatogram was dried at 150°C for 15 minutes. Then the identification of fatty acids was done by the same procedure (Christie, 1976). For the identification and quantification of fatty acid esters with a "PYE UNICAM" 4500 U model gas chromatograph equipped with a flame ionization detector by using same procedure (M. A. Hossain et al., 1988). For the DDTs, edible parts of the fish samples were extracted by solid phase dispersion method, cleaned up with concentrated H_2SO_4 and finally analyzed by GC-ECD.

RESULTS AND DISCUSSION

On chromatographic plates, eight spots of dark yellow-brown were appeared within few minute and identified by comparing the R^f values of methyl esters of standard fatty acids (Table 1). Using the different solvent systems, spray reagent and UV lamp, the spots were identified as Myristic acid ($C_{14:0}$), Palmitic acid ($C_{16:0}$), Stearic acid ($C_{18:0}$), Oleic acid ($C_{18:1}$), Linoleic acid ($C_{18:2}$), Linolenic acid ($C_{18:3}$), Arachidic acid ($C_{20:0}$) and Behenic acid ($C_{22:0}$).

The peaks of analyzed fatty acids mixtures (figure-2) were tentatively identified by comparing their relative retention time with relative retention time of the standard fatty acids mixtures (figure-1). Table 2 reveal that the fish lipid contains 6.33% Myristic acid, 24.23% Palmitic acid, 8.42% Stearic acid, 30.29% Oleic acid, 7.35% Linoleic acid (ω-6), 5.46% Linolenic acid (ω-3), 2.47% Arachidic acid, 4.25% Behenic acid and 11.13% unknown acid. The percentage of saturated fatty acid, monounsaturated fatty acid and polyunsaturated fatty acid was 51.42%, 34.08% and 14.42% respectively. The ω-6 fatty acid lowers low-density lipoprotein (LDL) and triglyceride levels, while increases high-density lipoprotein (HDL) concentration

Table 1 : Identification of Fatty Acid Methyl Esters of Standard and Fish Lipid by TLC

Name of the Sample	Fatty Acid Methyl Esters Standard	R_f Value Obtained From The Spot In Developing Solvent System				
		P:E (80:20)	P:E (60:40)	P:E:A (85:15:1)	P:E:A (80:20:1)	H:E (80:20)
Standard	Methyl laurate ($C_{12:0}$)	0.925	0.672	0.857	0.911	0.875
	Methyl myristate ($C_{14:0}$)	0.892	0.765	0.812	0.623	0.729
	Methyl palmitate ($C_{16:0}$)	0.635	0.530	0.637	0.429	0.552
	Methyl palmitoleate ($C_{16:1}$)	0.523	0.497	0.561	0.552	0.469
	Methyl stearate ($C_{18:0}$)	0.765	0.829	0.911	0.752	0.833
	Methyl oleate ($C_{18:1}$)	0.466	0.323	0.492	0.411	0.385
	Methyl linileate ($C_{18:2}$)	0.565	0.395	0.433	0.295	0.356
	Methyl linolineate ($C_{18:3}$)	0.615	0.556	0.492	0.523	0.442
	Methyl arachidate ($C_{20:0}$)	0.632	0.721	0.554	0.332	0.497
	Methyl arachidonate ($C_{20:4}$)	0.332	0.352	0.491	0.432	0.450
	Methyl behanate ($C_{24:0}$)	0.910	0.812	0.937	0.650	0.720
Fish Lipid	Methyl myristate ($C_{14:0}$)	0.896	0.760	0.816	0.627	0.723
	Methyl palmitate ($C_{16:0}$)	0.634	0.538	0.632	0.423	0.556
	Methyl stearate ($C_{18:0}$)	0.762	0.822	0.911	0.759	0.836
	Methyl oleate ($C_{18:1}$)	0.467	0.329	0.495	0.417	0.382
	Methyl linileate ($C_{18:2}$)	0.569	0.398	0.438	0.298	0.352
	Methyl linolineate ($C_{18:3}$)	0.613	0.553	0.494	0.527	0.448
	Methyl arachidate ($C_{20:0}$)	0.639	0.728	0.559	0.338	0.492
	Methyl behanate ($C_{24:0}$)	0.913	0.819	0.933	0.656	0.725

Table 2 : Fatty Acid Composition and Weight % of Fish Lipid With Respect to Standard Fatty Acids

Name of Sample	Peak No.	Chain length	Relative Retention time	Name of fatty acids	Weight%
Standard	1	$C_{12:0}$	4.42	Lauric	-
	2	$C_{14:0}$	6.16	Myristic	-
	3	$C_{16:0}$	8.06	Palmitic	-
	4	$C_{18:0}$	10.52	Stearic	-
	5	$C_{18:1}$	11.18	Oleic	-
	6	$C_{18:2}$	12.06	Linoleic	-
	7	$C_{18:3}$	13.15	Linolenic	-
	8	$C_{20:0}$	14.11	Arachidic	-
	9	$C_{22:0}$	18.51	Behenic	-
	10	$C_{24:0}$	25.68	Lignoceric	-
Fish lipid	1	$C_{14:0}$	6.15	Myristic	6.33
	2	$C_{16:0}$	8.06	Palmitic	24.23
	3	$C_{18:0}$	10.52	Stearic	8.42
	4	$C_{18:1}$	11.18	Oleic	30.29
	5	$C_{18:2}$	12.05	Linoleic	7.35
	6	$C_{18:3}$	13.16	Linolenic	5.46
	7	$C_{20:0}$	14.12	Arachidic	2.47
	8	$C_{22:0}$	18.50	Behenic	4.25
	9	Unknown	21.95	Unidentified	5.47
	10	Unknown	25.68	Unidentified	2.33
	11	Unknown	26.62	Unidentified	3.33

Table 3 : Residual *A*mounts of DDTs in *Mystus gulio* Fish Fillet

4,4′-DDT ppb	2,4′-DDT ppb	DDD ppb	DDE ppb	(DDD+DDE) /∑DDT	4,4′-DDT /∑DDT	2,4′-DDT /∑DDT	4,4′-DDT /DDE
0.006	0.008	0.007	0.034	0.745	0.109	0.145	0.176

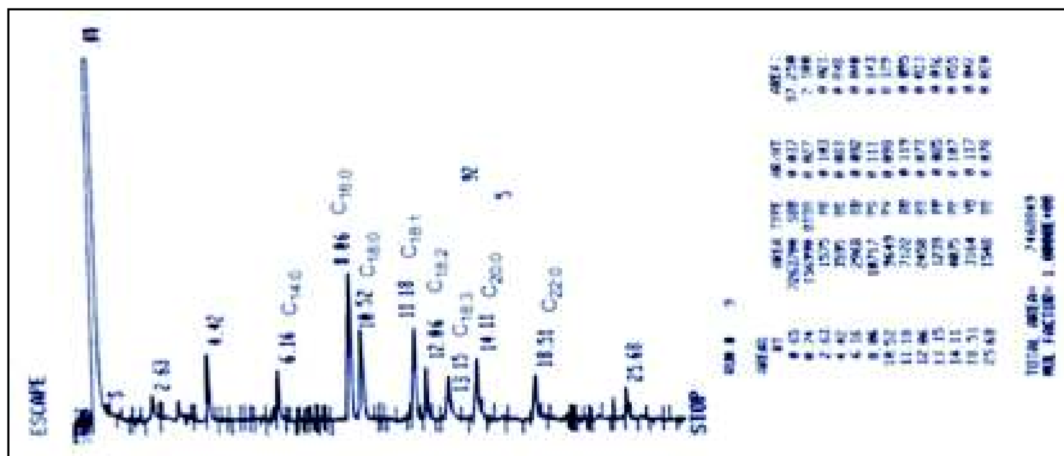

Figure 1 : GLC Separation of Standard Fatty Acid Methyl Esters on a Polar Stationary Phase

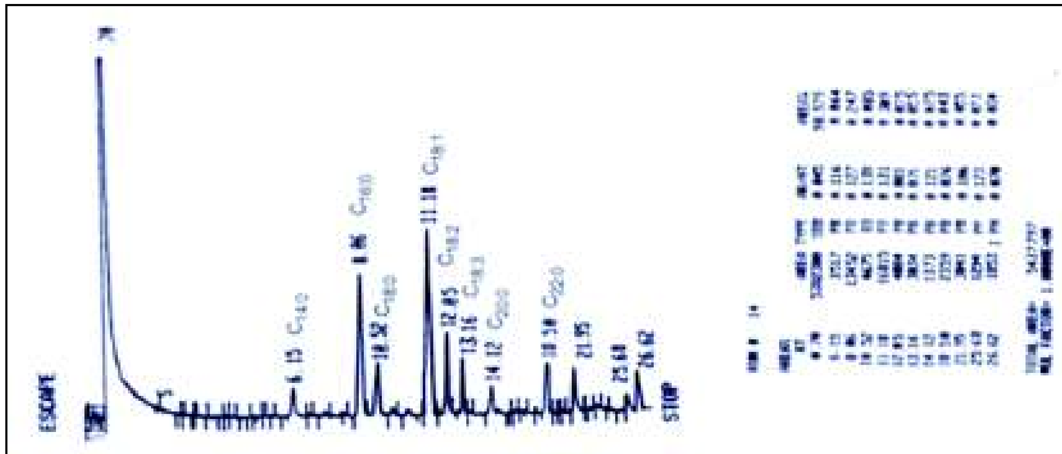

Figure 2 : GLC Separation of The Fatty Acid Methyl Ester Mixture Derived From Fish Lipid on Polar Stationary Phase

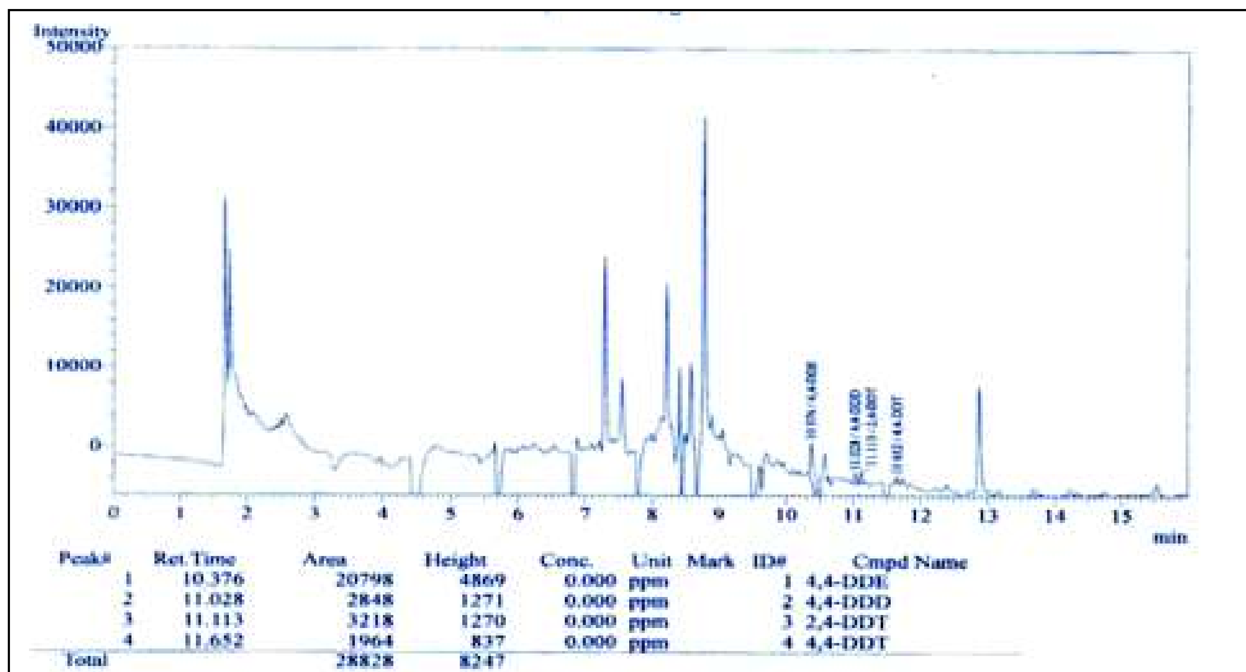

Figure 3 : Chromatogram of DDTs Analysis of *Mystus gulio* Lipid by Gas Chromatography

(Guivernau M. et al., 1994). The ω-3 fatty acids can reduce blood pressure levels in people with high blood pressure and prevent coronary heart diseases also.

In Table 3, the concentrations of DDTs were calculated from the area of the chromatogram and shown in figure 3 where the sample contained DDT, DDD and DDE as 0.0139 ppb, 0.0074 ppb and 0.034 ppb respectively i.e. DDE is higher than DDD and DDT. This suggests that the source of the fish or the fish feeds have been contaminated with DDT because with time period DDT was converted to DDD and DDE.

The ratio of 4,4´-DDT/DDE > 0.5 may indicate recent input of DDT and in contrast of < 0.3 may imply past input DDT (B. Strandberg et al., 1998). In this study the ratio of 4,4´-DDT/ΣDDT and 4,4´-DDT/DDE was 0.109 and 0.176, respectively (Table 3). In addition to, the ratio of

(DDE+DDD)/ΣDDT > 0.5 indicates for a long-term biotransformation of DDT to DDD and DDE, while a ratio of less than 0.5 may be recent input (R. A. Dong et al., 2002). In the present study, the ratio of (DDE+DDD)/ΣDDT was 0.745, which indicates that a long term biotransformation of DDT to DDD and DDE. Since its MRL value is 50 ppb (Battu RS et al., 2004) and our finding of DDTs in *M. gulio* is lower than the MRL value so it can be consumed for its great nutritional value.

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