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Residual DDTs and Fatty Acid Compositions in Popular Two Sea Fish Samples

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Abstract

Fatty acid compositions, DDT and its metabolites of popular two sea fishes i.e., *Pampus argenteus* (Pomfret) and *Lates calcarifer* (Vernacular) were determined. Lipid was extracted by ethylacetate, saponified and converted to methyl ester using BF_3-MeOH to determine fatty acid compositions by GC-FID.For 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. The LOD and LOQ for DDE, DDD, 2, 4´-DDT and 4, 4´-DDT were found to be 0.39, 0.39, 1.56, 1.56 and 1.36, 1.36, 4.89 and 4.89 µg/kg respectively. Internal Standard CB53 (0.25mg/g) was used to determine the recovery of the experiment and was within range of 77.48-92.35 %. The residual amount of DDT (2, 4´-DDT+4, 4´-DDT), DDE and DDD in pomfret and vernacular were found to be in the range of 13.56-81.05, 3.86-21.08, 4.32-35.4, and 23.44-85.59, 2.90-5.81 and 6.33-12.44 µg/kg respectively. The ratios of DDT/∑DDTs in pomfret and vernacular were 0.675 and 0.773 respectively. Among the saturated fatty acids, palmitic acid was predominant (62.84% for pomfret and 41.81% for vernacular) whereas among the unsaturated fatty acids oleic acid was predominate and higher in pomfret (15.11 %).

KEYWORDS: DDTs, Fatty acid, Pomfret, Vernacular and GC.

I. Introduction

Bangladesh is an agro-based developing country. Rice, pulses, maize, wheat, jute, potato, sugarcane, vegetable and tea are the major agricultural crops. In-spite of being eighth populous country of the world, Bangladesh is ensuring food security. To ensure food security, it is necessary to increase bumper production of crops which is possible by the use of different kinds of pesticides and fertilizers. But indiscriminate use of various kinds of pesticides is making the environment hazardous.

Organohalogenated compounds such as dichlorodiphenyltrichloro ethane (DDT), polychlorinated biphenyls (PCBs) and dioxins that are also known as persistent organic pollutants (POPs), are still being found in our natural ecosystems, despite of substantial reduction in uses and emissions[1-2]. Pomfret and vernacular are popular two sea fish species and widely available in Bangladesh. Local people eat them all most every day. DDT, being environmental contaminate may accumulate into marine fish samples as it has the ability of wide range transport even in sea through current.

Therefore, analysis of bioaccumulation of DDT in marine fish samples has its great importance. In marine fish, polyunsaturated fatty acids were found to be present in high ratios of ω-3 and ω-6 configurations fatty acids[3]. It was reported that the polyunsaturated fatty acids play as an important factor in health issue and can reduce the risk of heart attack[4].

Although Government of Bangladesh banned the use of DDT and HCH in the country, our earlier findings reported that DDT and its metabolites were found in fish, dry fish, chicken meat and human blood samples, and indicated that it is still being illegally used as pesticides in agricultural fields in different parts of the country [5-8]. In order to evaluate the presence of DDTs and fatty acid compositions in fish oil of other popular fish species, pomfret and vernacular, have been selected for this study which has not been analyzed earlier.

II. Materials and Methods

Chemicals and Reagents

Analytical grade chemicals ethyl acetate, n-hexane, acetone and sulphuric acid (95-98%) were used for all the analyses and purchased from E. Merck, Germany and BDH, England. Anhydrous sodium sulphate was heated at 200 ºC for overnight to make free from adhering moisture. All glass apparatus were cleaned with washing detergent followed by organic solvents and baked at 105 ºC in an oven.

Collection and preparation of samples

Six fishes of each kind were collected from different markets of Dhaka city. All the collected fish samples 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.

Preparation of standard and internal standard solution

Approximately 10.0 mg standard of DDT (purity 99.5 %), DDD (purity 99.0 %) and DDE (purity 98.5 %) were taken individually in a 100 mL volumetric flask and was up to the marked with n-hexane to make the concentration 100.0 µg/kg. Similarly internal standard CB-53 was prepared in a 100.0 mL volumetric flask with n-hexane to make 100.0 µg/kgsolution. Then these solutions were diluted to different concentrations. These solutions were labeled indicating name of the standard, solvent, concentration, date of preparation and signature. The meniscus of the solutions was marked with permanent ink. These solutions were stored in the freezer of a refrigerator.

Extraction and clean-up of fish sample for DDTs

About 10.0 g of each fish fillet was taken and extraction was carried out by solid phase dispersion method, cleaned up by

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conc. H_2SO_4 and the cleaned extracts were analyzed by GC-ECD [9].

Saponification and esterification of fish oils

Approximately 100 mg of fish oil extracted from fish sample was taken in a pear shaped flask and saponified by methanolic NaOH. After that it was esterified to its methyl ester by BF_3-MeOH by the same procedure[10] and analyzed by GC-FID to find out the fatty acid composition of fish oil.

Instrument

A GC-17 Shimadzu Gas Chromatography having ECD detector was used for identification and quantification of DDTs. A HP-5MS fused silica capillary column (30 m × 250 µm i.d. and 0.25 µm film thickness) was used. Nitrogen was used as the carrier (flow rate, 2 $mLmin^{-1}$)and make-up gas (flow rate, 60 $mLmin^{-1}$). Oven temperature was programmed as 80 °C (1 min hold) and increased from 20 °C min^{-1} to 260 °C (6 min hold). The injector and detector temperatures were 250 °C and 280 °C respectively. All injections were made in splitless-split mode. Injection volume was 1 µL.

A GC Shimadzu 2025 Gas Chromatograph having FID Detector was used for identification and quantification of fatty acids. Separations were performed on WCOT quartz capillary (DB-5) column (30 m in length and 0.25 mm in diameter). The temperature program in the oven was as followed: 120˚C – 1 min (hold) – increased by 7˚C/min to 270˚C and again hold for 6 min. N_2 was used as carrier gas with a column flow rate of 1.78 mL/min. Air and hydrogen gases were used as fuel for FID. The injector and detector temperatures were 280 °C and 290 °C respectively. All injections were made in split mode (split ratio 1:90). Injection volume was 1 µL.

Calibration curve

Working standard solutions were serially diluted to 0.125 mg/g, 0.05 mg/g, 0.025 mg/g,0.01mg/g, and 0.005 mg/g. Each of the diluted standard solutions was injected to GC-ECD. The calibration curve of each of standard was made by plotting Peak area vs concentration (Fig. 1).

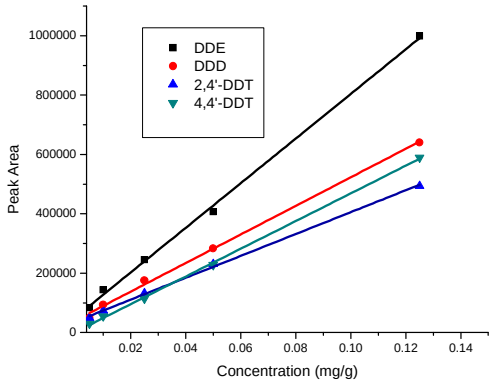

Fig. 1. Calibration curve for standard DDE, DDD, 2, 4′-DDT and 4, 4′-DDT

LOD, LOQ and Recovery experiments

The limit of detection (LOD) was determined by injecting serially diluted mixtures of standard DDTs solution in GC-ECD from the lowest concentration to higher level. For LOD the peak area of each standard was considered 3 times higher than the base line noise *i.e*., signal to noise ratio was 3. For LOQ the peak area of each standard was considered 10 times higher than the base line noise i.e., signal to noise ratio was 10. The LOD and LOQ for DDE, DDD, 2, 4´-DDT and 4, 4´-DDT were found to be 0.39, 0.39, 1.56, 1.56 and 1.36, 1.36, 4.89, and 4.89 µg/kg respectively.

For recovery experiment fresh fish fillet (10 g) was used. Internal standard (CB53**)** was spiked (0.25 mg/g) to the fish tissue and allowed the sample to stand for 30 min to let the pesticides be absorbed into the samples. The samples were extracted and cleaned-up by following the same procedure as described above and made final volume to 1 mL. The percentage of recovery was found in the range from 77.48-92.35 %.

III. Results and Discussion

Two species of popular sea fish samples (pomfret and vernacular)were analyzed for the presence of DDTs. DDT (2,4′-DDT+4,4′-DDT), DDE and DDD and were found to be in the range of 13.56-81.05, 3.86-21.08, 4.32-35.4, and 23.44-85.59, 2.90-5.81 and 6.33-12.44 µg/kg respectively (Table 1).The chromatogram of standard DDTs and a sample is shown in (Fig.2).

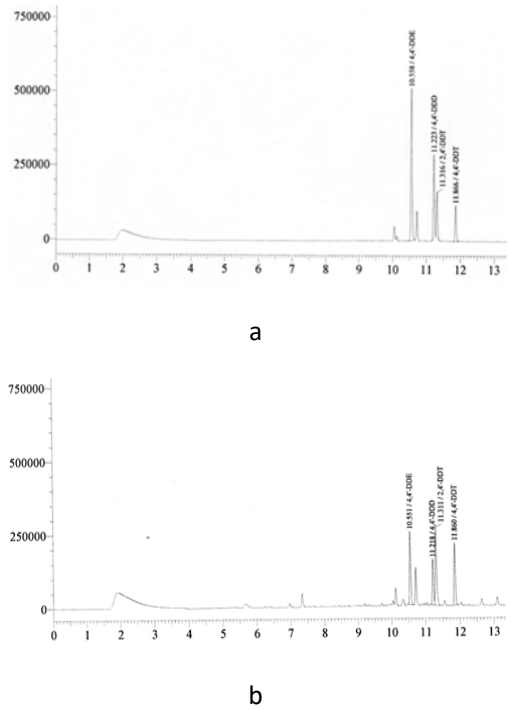

Fig. 2. GC Chromatogram of standard (a) and sample (b)

The ratios of DDT/ΣDDTs in pomfret and vernacular in the present study were 0.675 and 0.773 respectively and indicated the exposure is recent. The ratio of (DDT)/ΣDDTs > 0.5 indicates for a recent biotransformation of DDT to DDD and DDE, while a ratio of less than 0.5 may be recent old [11]. DDTs are accumulated in the fat tissue, so higher the fat content higher would be the DDTs if the samples are from the same habitats. From Table 2 it is clear that the amount of fat (mg/g f.w.) was higher in pomfret (34.98 %) compared to vernacular (27.47 %). Our findings are very consistent as we found higher DDTs in pomfret compared to vernacular. The findings showed that both the fish sample contained DDT, DDD and DDE but below the MRL value (5 mg/kg)[12]. But continuous consumption of pomfret and vernacular fish will accumulate DDTs in our body which may lead to the concentration enough to cause a threat to our health. Both the fish oil contained saturated and unsaturated fatty acids. It is observed that saturated fatty acids contained most of the relative fatty acids composition in pomfret(86.88 %) while in the vernacular fish(53.80 %) (Table2). Among the saturated fatty acids, palmitic acid was predominant for both fishes and its mean relative percentage was 57.50 % in pomfret while 37.71 % was found in vernacular. Least percentage was contained in pomfret by arachidic acid (1.75%) but it was not found in vernacular. Other major fatty acids, stearic acid (17.39%) & myristic acid (10.74 %) were found in higher amount in pomfret compared to vernacular. This has been found to be similar to that reported by Eboh *et al*, where palmitic acid was found to be the most prevalent saturated fatty acids in catfish, tilapia, hilsha, bonga and mudskipper [13].

Table 1. Amount of DDTs in pomfretand vernacular

Fish Name	DDT (2,4′-DDT +4,4′-DDT) µg/kg		DDD µg/kg		DDE µg/kg		DDT /∑DDTs
	mean	range	mean	range	mean	range	
Pomfret (n=6)	54.98	13.56-81.05	17.33	4.32-35.44	9.06	3.86-21.08	0.675
Vernacular (n=6)	45.65	23.44-85.59	9.00	6.33-12.44	4.36	2.90-5.81	0.773

Table 2. Amount of fatty acids composition in pomfretand vernacular

	Pomfert (n=6) (%)		Vernacular(n=6) (%)	
	mean	Range	mean	Range
Amount of fat (mg/g f.w.)	34.98	20.36-40.88	27.47	14.53-41.33
Percentage of fate	3.50	2.04-5.24	2.74	1.45-4.13
Myristic	10.74	8.75-13.58	6.43	4.32-9.23
Palmotelic	3.86	2.93-4.67	13.88	7.26-17.83
Palmitic	57.50	48.30-66.62	37.71	35.99-41.81
Linoleic	4.57	0.97-9.75	1.27	0.50-1.68
Oleic	1.88	0.54-2.72	15.11	7.04-26.15
Stearic	17.39	16.19-19.77	8.31	7.06-10.61
Arachidic	1.75	0.94-2.10	-	-
MUFA	2.93	0.54-4.67	15.89	7.04-26.15
PUFA	4.57	0.97-9.75	1.21	0.50-1.68
SFA	86.88	0.94-66.62	53.80	4.32-41.81
PUFA/SFA	0.05	-	0.02	-

Among unsaturated fatty acids palmotelic acid (13.88 %) and oleic acid (15.11 %) were found in higher amount in vernacular than pomfret (3.86 % and 1.88% respectively) whereas linoleic acid (4.57 %) was higher in pomfret compared to vernacular (1.27 %).In both human and animal studies, Linoleic acid (ω-6 fatty acid) reduces the tendency of platelets to aggregate or clump together within small blood vessels[14-15]. This activity is related to its effect of reducing activity of thromboxane, a signaling molecule involved in blood clotting and may be helpful in reducing the risk of heart attack and strokes[15-16]. The ω-6 fatty acid also shows promise in lowering low-density lipoprotein (LDL) and triglyceride levels, while increasing high-density lipoprotein (HDL) concentration[16]. From Table 1 we see that Linoleic acid (ω-6 fatty acid) is higher in pomfret compared to vernacular indicates that pomfret is more beneficial to reduce the risk of heart attack and strokes.

IV. Conclusion

From the recent study, findings of DDT in higher amount than that of DDD and DDE represented that DDT is still being used although it is banned since long. Although the amount of DDTs were found to be lower than the MRL values, but continuous consumption might cause a threat to human health as a result of biomagnifications. And both the fishes contain ω-6 fatty acid can reduce the risk of heart attack and strokes.

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