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Organochlorine pesticide residues and other toxic substances in salted *Tenualosa ilisha* L.: Northeastern part of India

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Abstract:
Introduction. Fish can sometimes be contaminated with several highly toxic substances at once, e.g. heavy metals, pesticides, and preservatives. In this regard, it is essential to determine the presence of these harmful chemicals in fish products. The research objective was to analyze the level of organochlorine pesticide residues and other toxic substances in *Tenualosa ilisha* L.
Study objects and methods. The study featured organochlorine pesticide residues and other toxic substances in raw and cooked samples of fresh and salted *T. ilisha*, which is a popular dish in Northeast India, especially in the state of Tripura. The analysis involved tests for formaldehyde, pesticides, and heavy metals. Formaldehyde content was estimated using high-performance liquid chromatography, pesticides content – by low-pressure gas chromatography/tandem mass spectrometry, and heavy metals – by inductively coupled plasma/mass spectrometry.
Results and discussion. The salted samples had a high content of formaldehyde, though it remained within the normal range. Both fresh and salted samples demonstrated high concentrations of heavy metals such as zinc, copper, and selenium. The salted sample appeared to have a high content of toxic organochlorine pesticide residues. Frying and boiling of fresh and salted fish decreased formaldehyde and organochlorine pesticide residue contents but did not reduce heavy metal content.
Conclusion. T. ilisha was found to be quite safe for human health.

Keywords: *Tenualosa ilisha*, formaldehyde, pesticides, heavy metals, cooking, chromatography, spectrometry

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INTRODUCTION

Fish is a highly nutritive part of human diet. First of all, it is the primary source of polyunsaturated fatty acids (PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). PUFAs are known to decrease the risk of cardiovascular diseases [1]. Additionally, fish provides proteins, fats, amino acids, essential minerals (mainly iron), as well as vitamins A, B group, and D [2].

Northeast India has huge potential for fisheries due to its many rivers, streams, lakes, and ponds [3]. Fish and rice are the basis of the traditional menu in the state of Tripura. Unfortunately, fish can be contaminated with various harmful substances, e.g. salt, dust, organic toxins, heavy metals, microbes, pesticides, preservatives, etc. [4, 5]. As a result, fish may pose a serious threat to human health. For instance, formaldehyde, which is often used as an antimicrobial preservative, is considered to be carcinogenic to humans, which was also confirmed by the International Agency for Research on Cancer (IARC) [6, 7]. Still, this substance is often used to process foods [8]. In small quantities, formaldehyde is involved in human metabolism. However, high doses can cause pain, vomiting, coma, and possible death [9]. According to the United States Environmental Protection Agency, the acceptable daily intake of formaldehyde is about 0.2 mg/kg b.w. [10]. In addition to being used as a preservative, low concentrations of formaldehyde can also serve as an antiseptic solution due to its antibacterial and antifungal properties. If inhaled, it damages respiratory organs and may cause dizziness and suffocation, not to mention eye, nose, and throat irritation [11]. Exposure to formaldehyde increases the incidence of lung and nasopharyngeal cancer. Ingestion of formaldehyde damages the gastrointestinal tract [11]. The normal range of formaldehyde is 2–50 mg/kg, and the maximum can reach 60 mg/kg in fruits and marine fish [8].

Heavy metals, such as lead, chromium, mercury, arsenic, etc., are well-known contaminating chemicals that cause water and soil pollution [12]. These metals have no beneficial effects on human health. On the contrary, they are generally considered as one of the most toxic elements for humans and animals.

Similarly, pesticides in fish products are a sign of contamination and pose a serious threat to human health as they accumulate in human body fats [13]. Acute symptoms of pesticide poisoning include numbness, incoordination, headache, dizziness, tremor, nausea, abdominal cramps, respiratory depression, etc. [14].

Exposure to organochlorine pesticides mainly occurs through contaminated food, especially fish or shellfish obtained from contaminated rivers and streams. A long term exposure to moderate levels of aldrin and dieldrin can cause headache, irritability, vomiting, and uncontrollable muscle movements. Excessive dieldrin has been established as a 2–7-fold higher risk factor for breast cancer. The lethal dose (LD50) of dieldrin per day is approximately 10 mg per 1 kg of body weight [15].

Carbofuran and its major metabolites (3-hydroxy-carbofuran and 3-ketocarbofuran) can cross the placental barrier and affect the maternal-placental-fetal unit [16]. Alachlor is another toxic chemical that dissipates from soil mainly through volatilization, photodegradation, and biodegradation. It is readily absorbed through gastrointestinal tract and distributed to blood, spleen, liver, kidney, brain, stomach, and ovaries. The LD50 of alachlor is between 1.910 and 2.310 mg/kg in mice [17]. Therefore, timely detection of these toxic elements in fish is essential for human health.

Tenualosa ilisha, *Clupeidae* family, is the most popular fish in the Bay of Bengal. *Hilsa*, as they call it in India, occupies the top position among edible fishes due to its unique taste and delicious properties. *T. ilisha* is rich in amino acids, minerals, and fats. In addition, the fish has a high content of high density lipoprotein and a low level of low density lipoprotein, which makes it beneficial for human health [18]. Because of its high lipid content, it cannot be sun-dried. As a result, it is preserved by salting, which is simple and cheap [19]. Both fresh and salted *Hilsa* are very popular among the common people of Tripura. Cooking methods for fresh and salted *Hilsa* include boiling, frying, roasting, etc. Suitable cooking methods minimize the nutrient loss and also improve the digestibility of food [20, 21].

Although some aspects of nutritional composition of *T. ilisha* have already been reported, the estimation of toxic elements in both raw and cooked samples of fresh and salted *T. ilisha* has not yet been explored scientifically [22]. The objective of the present experiment was to analyze the level of organochlorine pesticide residues and other toxic materials in raw and cooked samples of fresh and salted *T. ilisha*.

STUDY OBJECTS AND METHODS

Sample preparation and cooking. Fresh and salted samples of *Tenualosa ilisha* L. were obtained from the local market of Battala, Tripura. The samples were cleaned to remove dust particles. The fresh samples were washed and gutted. In case of fresh samples, the main purpose was to obtain the maximal amount of flesh portion, so the samples were cut approximately parallel to the backbone. The flesh portion was cut into small pieces and prepared for boiling and frying. The salted samples were washed with water and cut into small pieces for further cooking. Fresh and salted samples were boiled for 20 min or deep-fried in vegetable oil for 15 min at 240°C.

Sample preparation to determine formaldehyde. The formaldehyde content was determined according to Claeysa *et al.* [23]. Blank and spiked formalin was added into five-gram samples. After adding 5 mL of acetonitrile, the samples were sonicated for 30 min at 25–30°C and shaken for 30 min in a shaking water bath at 150 rpm at room temperature. Then, they were centrifuged at 6000 rpm at 22°C for 5 min and filtered through a Whatman filter paper (90 mm). After 5 mL of the upper layer of the extract was carefully removed, 2.5 mL DNPH solution (dinitrophenylhydrazine) and vortex were added. Recrystallization of DNPH was carried out by dissolving 10 mL of anhydrous acetonitrile acetate to obtain a saturated solution. The samples were derivatized by shaking at 150 rpm at 40°C for 1 h in a shaking water bath. After incubation, the supernatant was filtered with a syringe micro filter (0.45 μm).

Analytical condition of HPLC. A 10-μL sample solution was analyzed by using a C-18 column (250× 4.6 mm, 5 μm) with a 60% methanol solution as mobile phase and analyzed at 355 nm. The flow rate was 1 mL/min and the operating time was 13 min.

Standard curve preparation. A stock formaldehyde solution (6.2%) was used to prepare standard solutions with concentrations of 0.838, 1.68, 2.51, 3.35, and 5.03 ppm by diluting with distilled water. The absorbance was then measured using a spectrophotometer at 415 nm. The molar concentration of formaldehyde was determined as follows:

$$A = € \cdot C \cdot L \tag{1}$$

where A is absorbance, € is molar absorption coefficient, C is molar concentration, and L is length of the cell.

In case of matrix-free calibration, the limit of detection (LOD) was 0.117 ppm and the limit of quantification (LOQ) was 0.384 ppm.

Recovery test. The known concentration of formaldehyde (5, 10, and 25 mg/L) was spiked in fish matrix. Recovery was calculated as follows:

$$\text{\% of recovery} = \text{concentration of formaldehyde quantified in the sample} \times 100/\text{spiked concentration} \tag{2}$$

Pesticide determination. The fish samples were homogenized with an Ultra-Turrax T25 homogenizer and stored in a freezer at –20°C. A standard pesticide

Table 1 Formaldehyde content in raw and cooked samples of fresh and salted *Tenualosa ilisha*

Formaldehyde	Fresh *T. ilisha*			Salted *T. ilisha*		
	Raw ± SEM	Boiled ± SEM	Fried ± SEM	Raw ± SEM	Boiled ± SEM	Fried ± SEM
Content, mg/kg	10.32 ± 2.11	9.24 ± 2.40[a]	9.02 ± 1.80[a]	12.14 ± 1.51	11.23 ± 1.10[b]	10.58 ± 3.70[b]

Five samples were taken to calculate the standard error mean

[a] $P < 0.05$ when compared with raw fresh *T. ilisha*

[b] $P < 0.05$ when compared with raw (uncooked) salted *T. ilisha*

mix, 20 μg/mL in 0.05% formic acid in methyl cyanide, was used as a spiking solution for 100 and 50 ng/g spiking levels. A standard pesticide mix, 500 ng/mL in 0.05% formic acid in methyl cyanide, was used for 5 and 1 ng/g spiking levels in the recovery experiments. A mix of atrazine-d5 and fenthion-d6 at 10 μg/mL was prepared in 0.05% formic acid in methyl cyanide.

Sample preparation. Ten grams of homogenized fish sample was put into a 50-mL polypropylene centrifuge tube. Adding 1000 ng of internal standards (ISTDs) yielded 100 ng/mL. After adding 10 mL of methyl cyanide, the tube was vigorously shaken by hand for 30 s. The entire extract, including tissue, was poured into another 50-mL centrifuge tube with 4 g of $MgSO_4$ (anhydrous) and 1 g of NaCl. After that, the tube underwent a vigorous shaking for 1 min and centrifuging at relative centrifugal field of 3250 for 2 min. Subsequently, 1 mL of the extract and 150 mg of $MgSO_4$ were shaken vigorously in a tube for 30 s and centrifuged at 3250 for 2 min. Then, 0.5 mL of the fish extract was transferred into an auto sampler vial for low-pressure gas chromatography/tandem mass spectrometry (LP-GC/MS-MS). 5 mL of distilled water served as a reagent blank sample. The equivalent tissue concentration per sample extract was 1 g/mL [24].

Five replicates of fish samples were prepared for spiking level. Each batch of samples included a reagent blank and a sample blank. Matrix-matched calibration standards at concentration of 0.5, 1, 5, 10, 25, 50, 100, 250, and 400 ng/mL were used to calculate the recoveries of the pesticides. Fenthion-d6 was used as an ISTD for pesticide quantification, and atrazine-d5 was used as a back-up ISTD if needed. The method detection limits were determined as the pesticide's lowest calibrated levels (LCL).

Determination of heavy metals. Fish tissue was weighed (0.3–0.5 g) and placed in a Teflon digestion vessel with 7 mL of concentrated (65%) nitric acid and 1 mL of 30% hydrogen peroxide. The sample in the vessel containing concentrated nitric acid was then subjected to a microwave program:

Step I: 25–200°C for 10 min at 1000 W;

Step II: 200°C for 10 min at 1000 W.

Digests were made up to 25 mL with deionized water. The heavy metal content was analyzed by inductively coupled plasma/mass spectrometry (ICP-MS) (Model X series, Winsford-cheshire UK) [25].

Statistical analysis. The statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) v. 16.0 for windows (SPSS, SAS Institute Inc. Cary, USA). The data were analyzed to determine the descriptive statistics, such as standard error of mean (SEM), standard deviation (SD), statistic mean, minimum and maximum value, and ranges of variables. A one-way analysis of variance (ANOVA) was performed to determine a 5% level of significance.

RESULTS AND DISCUSSION

Table 1 shows the formaldehyde content in the raw and cooked samples of fresh and salted *Tenualosa*

Table 2 Pesticide residues in raw and cooked samples of fresh and salted *Tenualosa ilisha*

Pesticides	*T. ilisha* (fresh)			*T. ilisha* (salted)		
	Raw ± SEM	Boiled ± SEM	Fried ± SEM	Raw ± SEM	Boiled ± SEM	Fried ± SEM
Aldrin	11 ± 2	10 ± 2*	10 ± 1*	78 ± 9	76 ± 7**	68 ± 8**
Alachlor	n.d.	n.d.	n.d.	88 ± 5	82 ± 6**	78 ± 4**
Carbofuran	17 ± 3	12 ± 2*	10 ± 4*	64 ± 3	62 ± 2**	58 ± 3**
Dieldrin	26 ± 5	22 ± 2*	18 ± 3*	66 ± 4	64 ± 2**	62 ± 4**
Endosulfan sulfate	17 ± 4	15 ± 2*	14 ± 3*	34 ± 9	32 ± 7**	31 ± 8**
o,p′-DDT+ p,p′-DDD	47 ± 8	46 ± 6*	45 ± 8*	118 ± 13	113 ± 17**	112 ± 12**
p,p′-DDT	19 ± 6	17 ± 2*	16 ± 1*	38 ± 4	36 ± 7**	32 ± 8**
Hexachlorobenzene	n.d.	n.d.	n.d.	67 ± 5	65 ± 2**	64 ± 6**

The pesticide values are expressed as spiking level (5 ng/g)

n.d. – not detected

* $P < 0.05$ when compared with raw fresh *T. ilisha*

** $P < 0.05$ when compared with raw (uncooked) salted *T. ilisha*

Table 3 Heavy metal content in raw and cooked samples of fresh and salted *Tenualosa ilisha*

Heavy metals	*T. ilisha* (fresh)			*T. ilisha* (salted)		
	Raw ± SEM	Boiled ± SEM	Fried ± SEM	Raw ± SEM	Boiled ± SEM	Fried ± SEM
Total arsenic, μg/g	11.90 ± 0.005	11.02 ± 0.002	10.98 ± 0.002	11.74 ± 0.005	11.06 ± 0.002	10.85 ± 0.005
Inorganic arsenic, μg/g	0.049 ± 0.0002	0.042 ± 0.0005	0.041 ± 0.0005	0.061 ± 0.0002	0.060 ± 0.0002	0.058 ± 0.0005
Mercury, mg/kg	0.101 ± 0.0005	0.098 ± 0.0002	0.090 ± 0.0002	0.102 ± 0.0005	0.097 ± 0.0005	0.095 ± 0.0002
Lead, mg/kg	0.81 ± 0.002	0.80 ± 0.005	0.80 ± 0.005	0.80 ± 0.005	0.78 ± 0.002	0.78 ± 0.002
Cadmiun, mg/kg	0.51 ± 0.002	0.50 ± 0.002	0.50 ± 0.002	0.52 ± 0.005	0.51 ± 0.002	0.51 ± 0.002
Chromium, mg/kg	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Copper, mg/kg	54.02 ± 0.005	54.00 ± 0.002	54.00 ± 0.002	53.88 ± 0.005	54.00 ± 0.002	53.87 ± 0.005
Nickel, mg/kg	1.82 ± 0.005	1.80 ± 0.002	1.81 ± 0.002	1.79 ± 0.005	1.78 ± 0.002	1.78 ± 0.002
Selenium, mg/kg	11.20 ± 0.006	10.88 ± 0.005	10.85 ± 0.005	11.00 ± 0.002	10.98 ± 0.006	10.97 ± 0.005
Zinc, mg/kg	122.0 ± 0.035	121.5 ± 0.032	121.3 ± 0.032	121.6 ± 0.057	121.4 ± 0.057	121.3 ± 0.032

The result is significant at $P < 0.05$

ilisha L. The salted sample contained a higher amount of formaldehyde (12.14 mg/kg) than the fresh sample (10.32 mg/kg), which could be because formaldehyde served as a preservative. However, its content reduced after boiling and frying. Again, cooking had some effect on the formaldehyde content. This toxic element is known to degrade after thermal treatment [26]. The concentration of formaldehyde mainly depends on different levels of trimethylamine n-oxide (TMAO) [27]. Tri-methylamine, di-methylamine, and formaldehyde are formed after the breakdown of TMAO [28].

The normal range of formaldehyde is 2–50 mg/kg, so the formaldehyde content in the present experiment was within the normal range for both fresh and salted fish samples.

Table 2 demonstrates the pesticide content (spiking level 5 ng/g) in the raw and cooked samples of fresh and salted *T. ilisha*. Alachlor and hexachlorobenzene were detected neither in the raw nor in the cooked samples. On the other hand, the levels of aldrin, alachlor, carbofuran, dieldrin, endosulfan sulfate, o,p′-DDT+ p,p′-DDD, p,p′-DDT, and hexachlorobenzene were higher in the salted sample. After cooking, the pesticide content went down. The toxic organochlorine pesticides residue was higher in the salted sample, which may be due to the fact that these substances were added to the fish as preservatives. After cooking, the organochlorine pesticides residue decreased because cooking process increases volatilization, hydrolysis, or other chemical degradation and leads to the decomposition by applying heat [29, 30].

Table 3 shows the heavy metal content in the raw and cooked samples of fresh and salted *Hilsa*. The mercury content was found to be 0.101 mg/kg in the fresh sample and 0.102 mg/kg in the salted sample. However, it went down after cooking. The above result is acceptable for fish, considering that the proposed upper limit for mercury is 0.5 mg per 1 kg of fresh weight. The cadmium content was high in both fresh and processed samples. For general fish muscle, cadmium level is 0.05 mg per 1 kg of fresh weight [30].

Copper is essential for maintaining good health, but a long term exposure may cause toxic effects, e.g. Wilson's disease [31]. In the present experiment, the copper concentration was 54.02 and 53.88 mg/kg in fresh and salted *T. ilisha*, respectively. The chromium content did not exceed 1.0 mg/kg for all raw and cooked samples of fresh and salted *T. ilisha*. Zinc is another essential nutrient. In moderate quantities, it improves immune system and metabolism, whereas a high level of zinc can be harmful. According to FAO, the limit for zinc is 30 mg/kg [32].

The limit of zinc acceptability exceeded the norm in both raw and cooked samples of fresh and salted fish. Selenium is an essential trace element required in small amounts for animals and humans. However, a higher content of selenium is toxic. Cooking appeared to have no significant effect on selenium content. The nickel content was within acceptable limits. Cooking had no significant effect on lead contamination either. Arsenic, another toxic element, can be found as a contaminant in fish, shellfish, and other seafood. In the present experiment, the arsenic content was within the normal range. According to FAO/WHO, the maximal intake of arsenic is 15 μg/kg b.w. Both raw and cooked samples of fresh and salted *T. ilisha* contained a high amount of arsenic [33].

CONCLUSION

The organochlorine pesticide residues were higher in the salted samples of *Tenualosa ilisha* L., but boiling and frying reduced their amounts. The salted samples of *T. ilisha* contained a high amount of such heavy metals as zinc, copper, and selenium. Fresh *T. ilisha* proved to be safe for human health as thermal treatment had some positive effects on the potentially harmful substances.

CONTRIBUTION

Dr. Kuntal Manna designed the research; Sanchari Goswami collected the samples, performed the analysis, and wrote the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests regarding the publication of this article.

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