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Formaldehyde Contamination in Fish Samples Collected from Selected Wholesale Markets of Guwahati, Assam

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ABSTRACT

Use of adulterants at various stages of transportation from farm to consumers is a common practice to prolong storage life of fresh or chilled fish. In Assam, use of formalin (formaldehyde in water) has been suspected in imported fish to prolong its shelf life and prevent spoilage. In order to test this practice, ICAR-CIFRI conducted a case study in three wholesale fish markets of Guwahati for detection of formaldehyde during November 2023 and May 2024. One out of six muscle samples that were collected from local fish was found to have traces of formaldehyde but below the method detection level (BDL). So, this can be considered as safe for human health and considered as naturally produced during post-mortem degradation. On the contrary, fishes imported from Andhra Pradesh or West Bengal did not have any traces of formaldehyde, which might be due to quick harvest and icing practice before transportation. This implies that if fishes are kept under iced condition immediately after harvesting, formaldehyde will not be naturally produced in fish. Monitoring of fish samples coming to Assam from other states needs to be carried out on regular interval to build confidence among the consumers. It is necessary to build infrastructure/ laboratory with state-of-the-art facilities for detection of contaminants including pathogens that poses risk to the human health.

Keywords Formalin adulteration, Human health concern, Fish muscle, High performance liquid chromatography, Northeast India.

INTRODUCTION

Fish is a significant source of animal protein and plays a key role in human nutrition. However, as a food commodity fish is highly perishable in nature. The use (or abuse) of adulterants and chemicals is not uncommon to maintain freshness of various plant and animal products destined for human consumption. With the time, again much attention has been given to hazardous aldehydes such as formaldehyde, among contaminants. Formalin is the common term for liquid formaldehyde. This is a low-cost chemical reported to have been used in agriculture/food sector

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as disinfectant and preservative (Kundu *et al.* 2019). Formaldehyde is permissible and useful chemical for some industries such as construction, wood processing and household product industries as a bacteriostatic agent (Kim *et al.* 2011, Liteplo *et al.* 2002). However, illegal use of formaldehyde in certain foods i.e., fish and seafood, fruits and vegetables and fruit juice to extend their shelf life was seen as a major health concern for the consumers (Sen *et al.* 2021). The International Agency for Research on Cancer (IARC) has categorized formalin as a Group 1 human carcinogen. The United States Department of Health and Human Services and National Toxicology Program have also listed formalin as carcinogens (IARC 2004).

Formaldehyde (CH_2O, FA) is a kind of colorless gas at room temperature and have an unpleasant smell. It is widely present in the environment, as it is produced from both natural and manmade sources (Stoker *et al.* 2004). It is a metabolic intermediate that is present in most living organisms including fish, at low concentration. Long-term exposure to formalin leads to serious and chronic health effects that causes cancer, lung damage, asthma, leukemia and damage in nervous system (Li *et al.* 2007, Rahman *et al.* 2016). Formalin can be found in fish samples naturally and it can also be added from external source. Naturally, formaldehyde is produced in fish in a very small quantity which may be due to deterioration and ageing. Along with decomposition process, increase in formaldehyde concentration may be due to microbiological, biochemical, enzymatic reaction and other physical activities, (Kimura *et al.* 2003, Gram *et al.* 2002, Mohanty *et al.* 2018). Fish contain a chemical called trimethylamine oxide (TMAO) as an osmolyte contributing to osmoregulation in fish. Trimethylamine (TMA), dimethylamine (DMA), and FA are produced by the postmortem breakdown of TMAO, which is facilitated by the TMAOase enzyme. The US Environmental Protection Agency's highest reference dose of FA is 0.15–0.2 mg/kg bodyweight (BW)/day. The European Food Safety Authority or EFSA (2014) suggested a daily maximum oral intake for humans of 100 mg FA, or 1.7 and 1.4 mg/kg BW for 60 kg and 70 kg, respectively. According to Regulations 148 and 159 (2006) of the Malaysian Food Regulations (1985), FA absorption from smoked fish and meat is allowed during processing, however it cannot go over 5 mg/kg. According to FSSAI (2020), maximum limit of naturally occurring FA in freshwater fishes is 4.0 mg/kg and marine fish species is 4.0-100.0 mg/kg.

In recent years, there have been increasing concern about formalin contamination in fishes that are imported from other states and neighboring countries to northeastern states including Assam, Manipur, Tripura, Nagaland and Meghalaya (Sahu *et al.* 2018). The Unraveling the presence of formalin in imported fishes of Assam has been a matter of concern to the local consumers. Therefore, the present study was undertaken to assess the status of formalin in both local and imported fishes of three wholesale market of Guwahati city, Kamrup (M), Assam.

MATERIALS AND METHODS

Sampling sites

Three sampling sites were selected for the survey based on recommendations from the Department of Fisheries, Govt of Assam. These were (i) Uzanbazar fish landing center and wholesale fish market, Guwahati, Assam, (ii) Betkuchi wholesale fish market, Guwahati, Assam and (iii) Notboma wholesale fish market Guwahati, Assam.

Sampling procedure

Two fish samples (whole) of each species were collected from the wholesale fish markets (Table 1). All the three Indian major carps, namely, catla (*Labeo catla*, average length: 39.3 cm. average weight: 2.15 kg), rohu (*Labeo rohita*, average length 38 cm, average weight 1.95 kg) and mrigal (*Cirrhinus mrigala*, average length: 46.5 cm, average weight: 1 kg) were collected from the Uzanbazar fish landing center and wholesale fish market, Guwahati, Assam. However, four species were collected from Betkuchi wholesale fish market, Guwahati, Assam, viz., catla (average length: 38.5 cm, average weight: 2 kg), rohu (average length: 35 cm, average weight: 1.4 kg), pangas (*Pangasianodon hypophthalmus*, average length: 53 cm, average weight: 1.7 kg) and roopchanda (*Piaractus brachypomus*, average

Table 1. Experimental sampling data of formalin analysis.

Sl. No.	Sampling year	Sampling site	Fish species	Average weight (kg)	Average length (cm)
1	November 2023	Uzanbazar fish landing center	Catla (*Labeo catla*)	2.15	39.3
			Rohu (*Labeo rohita*)	1.95	38
			Mrigal (*Cirrhinus mrigala*)	1	46.5
		Betkuchi wholesale fish market (imported)	Catla (*Labeo catla*)	2	38.5
			Rohu (*Labeo rohita*)	1.4	35
			Pangas (*Pangasianodon hypophthalmus*)	1.7	53
			Roopchanda (*Piaractus brachypomus*)	1.25	40.5
2	May 2024	Betkuchi wholesale fish market (imported)	*Labeo catla*	1.2	41.5
			Labeo rohita	0.97	42.5
			Piaractus brachypomus	1.68	42.8

length: 40.5 cm, average weight: 1.25 kg) which were transported to Assam from Andhra Pradesh and West Bengal under iced condition as informed by the wholesale vendor. In general, this transportation takes 7 days to reach Guwahati. These fishes were brought to laboratory in iced condition, washed thoroughly and wiped then dissected out muscle samples (30-50 g) from each fish. Three such muscle samples were collected from each fish specimen.

Sample packaging and transportation

Muscle samples dissected out from each fish were weighed (30-50 g) and packed in zip pouches and then stored at – 20°C. The frozen samples were sealed packet and transported in iced condition to the laboratory of ICAR-CIFRI, Barrackpore for further analysis.

Sample preparation

Frozen samples were taken out from the deep fridge, thawed and then homogenized with organic solvent. Subsequently homogenate was vortexed, sonicated and centrifuged for extraction. The clear extract was derivatized with 2,4-DNPH for analysis in HPLC.

Analysis

The qualitative and quantitative analysis was carried out in a high performance liquid chromatograph (HPLC) system (Make-Waters) using Photo Diode Array detector. A Surface™ C18 column with 4.6 × 150 mm dimensions and 5 µm film thickness was utilized. The middle phase was methanol and deionized water (50:50 v/v) used in isocratic pump mode at a flow rate of 1 ml/min and detector wave length was set at 355 nm at 1.2 nm resolution. Data analysis and processing was done in the Empower 3 software. The calibration of HPLC was performed prior to sample analysis using the standards of formalin (37% solution) obtained from reliable sources. The analysis was done by comparing the retention duration and peak area of the samples, with those of the calibrated reference standard of the targeted compound. The method detection level was 0.5 mg/kg.

Statistical analysis

Results of HPLC were tabulated. Since majority of the samples were either ND or BDL, no statistical analysis could be applied in the present experiment.

RESULTS AND DISCUSSION

The results of study are presented in Tables 2–3. Formaldehyde concentration was found to have traces (Tr) but below the detectable level (BDL) in one sample out of six fish samples of *L. catla*, *L. rohita* and *C. mrigala* collected from local fish farms of Assam during November 2023. Imported *L. catla* from Betkuchi wholesale fish market and *L. rohita* from Notboma fish market were found to have traces of formaldehyde which was below 1 µg/g. However,

Table 2. Formaldehyde concentration (µg/g) in fish samples of local origin and that of outside origin (imported) during November 2023. BDL: Below detection limit, ND: Not detected.

Fish species (origin)	Sample	Replicate 1	Replicate 2	Replicate 3
Labeo catla (local)	i	BDL	ND	BDL
	ii	BDL	BDL	BDL
Labeo rohita (local)	i	ND	ND	BDL
	ii	BDL	BDL	BDL
Cirrhinus mrigala (local)	i	BDL	BDL	BDL
	ii	BDL	BDL	BDL
Labeo catla (imported)	i	BDL	ND	BDL
	ii	BDL	BDL	BDL
Labeo rohita (imported)	i	ND	ND	ND
	ii	ND	ND	ND
Pangasianodon hypophthalmus (imported)	i	BDL	BDL	BDL
	ii	BDL	BDL	BDL
Piaractus brachypomus (imported)	i	BDL	ND	BDL
	ii	BDL	ND	BDL

Table 3. Formaldehyde concentration (µg/g) in fish samples of outside origin (imported) during May 2024. BDL: Below detection limit, ND: Not detected.

Fish species (origin)	Sample	Replicate 1	Replicate 2	Replicate 3
Labeo catla (imported)	i	BDL	BDL	ND
Labeo rohita (imported)	i	BDL	ND	ND
	ii	ND	ND	ND
Piaractus brachypomus (imported)	i	ND	ND	ND
	ii	ND	ND	ND

other fish samples have not detected (ND) any formaldehyde content during both sampling period.

The present investigation showed that fishes (one out of six muscle samples) that were farmed and marketed locally in Uzanbazar wholesale market during November 2023, imported *L. catla* fish from Betkuchi wholesale fish market and *L. rohita* from Notboma fish market during May 2024 were found to have traces of formaldehyde. The formalin values were BDL and so considered as safe for human health as per FSSAI guidelines of maximum 4 mg/kg in freshwater fishes FSSAI (2019). Traces of formaldehyde detected in 16.7% of the muscle samples collected from local fishes that are freshly dead, may be due to intrinsic / natural factors. These fishes would have undergone some post-mortem changes such as breakdown of trimethyl amine oxide (TMAO) into tri-methylamine, di-methylamine and finally into formaldehyde. It has been observed that bacteria or enzyme reactions in fish muscle naturally create formaldehyde (Sikorski 1982). For example, endogenous formaldehyde residues ranging from 0.1—31.8 µg/g were found in various fish species, including striped bass (*Morone saxatilis*), Nile tilapia (*Tilapia nilotica*), eel (*Anguilla japonica*) and banana shrimp (*Penaeus merguiensis*) (Xu and Rogers 1995, Yamagata and Low 1995). Kundu *et al.* (2020) determined formaldehyde content using HPLC in freshwater carp and non-carp fish species from eastern region of India, where they observed negligible amount of formaldehyde in live freshwater carp (*L. catla* and *L. rohita*). Mehta *et al.* (2021) also reported low level of formalin (1.66 mg/kg) in freshwater fish collected from various fish markets of Agartala, India, which may be due to natural formation of formalin during post mortem changes. Das *et al.* (2018) reported formaldehyde concentration from Mumbai fish markets in *L. rohita* and *L. catla* ranging from 2.96-3.11 µg/g and 2.76-2.88 µg/g, respectively. On the contrary, imported fishes from Andhra Pradesh or West Bengal did not have any traces of formaldehyde in the muscle samples. This might be due to quick harvest and icing. Generally, fishes are netted out from ponds after 1-2 days of no-feeding-till-harvest and immediately put in thermocoal boxes and iced. This process actually reduces the chances of chemical, bacterial or enzymatic degradation and prolongs rigor mortis stage (stiffening of muscles) in fishes.

CONCLUSION

Though trace levels of formaldehyde were detected in one out of six muscle samples collected from local fishes during November, 2023, *L. catla* from Betkuchi wholesale fish market and *L. rohita* from Notboma fish market during May 2024 but values were BDL and thus consider safe for human consumption. This is mostly because the formaldehyde produced was due to degradation during post-mortem period. This also implies that if fishes are kept under iced condition, formaldehyde will not be produced as found in imported fishes in the present case. However, monitoring

of fish samples coming into Assam from other states needs to be carried out on regular interval to build confidence among the consumers. It is suggested that state-of-the-art infrastructure/ laboratory facilities has to be built for detection of formaldehyde and other contaminants posing consumer health issues. The present study was conducted on a limited scale (in three wholesale fish markets and two times in six-month interval). However, urgency of the matter warrants continuous and wide-scale survey and monitoring, that might restrain the wrong-doers, if any and improve consumer confidence on the fishes coming to the Assam from other states.

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