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Detection of Formaldehyde Content in Selected Fishes from Three Different Retail Markets at Mumbai

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A B S T R A C T

The use of formaldehyde illegally in fish preservation by fishermen and fish vendors became emerging issue recently. Thus, the present study was conducted to verify the presence or absence of formaldehyde from three different retail fish markets in Mumbai. Rohu (*Labeo rohita*), Catla (*Catla catla*), Boyal (*Wallago attu*), Indian Mackerel (*Rastrelliger kanagurta*) and Bombay duck (*Harpodon nehereus*) were collected and tested for the presence of formaldehyde. Catla fish collected from the four Bunglows fish market contained formaldehyde conc. of 2.76 µg/g and from Andheri fish market, fish contained formaldehyde conc. of 2.88 µg/g. Rohu fish collected from Four Bunglows and Andheri fish market showed the presence of formaldehyde with 3.11 and 2.96 µg/g respectively. Boyal fish collected from Four Bunglows and Andheri fish market has formaldehyde content of 2.38 and 2.22 µg/g correspondingly. Bombay duck fish collected from the same markets contain 1.48, 1.71, 2.08 µg/g whereas Indian Mackerel fish collected were found to have 1.81, 2.27, 2.35 µg/g of formaldehyde content. Both marine and freshwater fishes were noticed to have formaldehyde content in their flesh. Even though, marine fish produces formaldehyde due to their natural process, it was lesser than the freshwater fishes probably due to adulteration in marketing chain which can be concluded through comparison with several other authors.

Introduction

Fish and seafood are important source of animal protein and considered as one of the delicious diet in India. As fish is highly perishable, different types of preservation methods such as freezing, drying, pickling, marinating etc. has been widely practiced to achieve food safety and quality. In order to accomplish this cheaply, fish sellers spray or dip fish with formalin treated water which is injurious to human health. Many traders make up common practices of dipping the whole fish or inject formalin in the fish body cavity or spreading formalin mixed water. Inadequate freezing facilities and time consuming transportation forces the fish traders to resort to such malpractice. Several studies conducted

at different markets in several countries (Hossain *et al.,* 2008; Haque and Mohsin 2009; Yeasmin *et al.*, 2010) which rationalizes the incidence of adding formalin.

Although Formaldehyde acts as a bactericide, it is a toxic substance that causes corrosive damage to gastrointestinal mucosa, leading to nausea. Food manufacturers sometimes add Formaldehyde to foods in safe level such meats, milk etc to improve its shelf-life. Despite, marine fishes naturally contain small amounts of formaldehyde due to degradation of bodily replace with post mortem, compounds. With the onset of post mortem trimethylamine oxide (TMAO) in fish body is broken down to dimethylamine and formaldehyde as its main product. TMAO is mainly found in marine fish (Jiang *et al.,* 2006). During the ageing and deterioration of fish flesh, Formaldehyde may be formed. Along with the natural formation of formaldehyde, enzymatic reaction and other biochemical reactions such as oxidation of lipids and microbial activity can also cause formaldehyde formation in fish and seafood. This may eventually cause physical damage in fish flesh or production of chemical metabolites such as biogenic amines or other unpleasant compounds (Gram *et al.,* 2002; Arashisar *et al.,* 2004).

Detection of formaldehyde content more than safe limit has been reported in many fishes due to adulteration by different marketing channels. Formaldehyde is an organic compound with the chemical formula, HCHO. It is colorless, pungent and generally obtained in the form of formalin with 37% formaldehyde. Excess amount of formaldehyde in food material poses a serious threat to human health (Li *et al.,* 2007). Formaldehyde has been classified as Group 1 Carcinogenic according to International Agency for Research on Cancer (IARC). WHO, 1989 declared a potential carcinogen and mutagen to humans at LD_{50} 30 g declared by (Cui *et al.,* 2007). As it is toxic and allergenic, it can cause symptoms like headaches, burning sensation in the throat and difficulty in breathing (Herschkovitz *et al.,* 2000).

Freshness property of a fish has a considerable influence on its quality and market value. Now a day's consumers are becoming more conscious over the issue and also its probable side effects. There has been claim that fishes in Indian market are formalin contaminated in the supply chain. Therefore, the objective of the present study was framed to determine the presence of formaldehyde content of some important freshwater and marine fishes of Mumbai fish market by a method of precision spectrophotometrically using Nash's reagent.

Materials and Methods

Collection of fish sample

The experiment was conducted in the laboratory of Post-Harvest Technology discipline under FRHPHM division of ICAR-Central Institute of Fisheries Education, Mumbai from August to September, 2015. Fish samples were collected from three different fish markets such as Versova fish market, Four Bungalows Fish Market, and Andheri Fish Market. Three types of fresh fish species namely Rohu (*Labeo rohita*), Catla (*Catla catla*) and Boyal (*Wallago attu*) and two marine species namely Indian Mackerel (*Rastrelliger kanagurta*) and Bombay duck (*Harpodon nehereus*) were collected from these markets. Collected fish was carried to the laboratory in iced condition in insulated ice box for determination of formaldehyde.

Chemicals and reagent used

The experiment was conducted as described by Ng., 1987. Trichloro acetic acid (TCA) (60 ml of 6%) was used for fish sample extraction purposes. Nash's Reagent (Nash, 1953) was

used as an indicator to detect the absorbance of formaldehyde. 15 g ammonium acetate was diluted in a 100 ml Erlenmeyer flask with an addition of 0.3 ml of acetyl acetone and 0.2 ml of acetic acid. Nash's Reagent is light sensitive and was kept in dark-glass reagent bottle. A 0.1N potassium hydroxide (KOH) and 0.1N hydrochloric acid (HCl) was used to adjust the pH of the distillate to be in range of 6.0 to 6.5 by a pH meter.

Standard curve establishment

The standard curve was obtained by plotting absorbance of known formaldehyde concentration (*viz.* 0.838, 1.68, 2.51, 3.35 and 5.03 ppm) from a stock solution of formaldehyde having 6.2% concentration (Fig. 1). The different concentration of formaldehyde solution was added with Nash reagent to get the respective absorbance on spectrophotometer (using 415 nm). The molar concentration of the formaldehyde sample ranged "between" 0.26×10^{-4} to 1.56×10^{-4} using following formula, $A = €Cl$

Where, A = Absorbance; € = molar absorption co-efficient; C = Molar concentration; l = length of the cell

$A = €l \times C$
$A = Const. \times C$

The model used for the equation was, Y = mx equation, the straight line passing through the origin

Sample preparation for determination of formaldehyde

The fish samples were chopped to small pieces and it was homogenized for 10 minutes. Then 60 ml of 6% tri-chloro-acetic acid was added for extraction of formaldehyde from the fish flesh. The extracted solution was then filtered by a Whatman No.1 of filter paper. pH was adjusted between 6.00-7.00 using Potassium hydroxide (KOH) and Hydrochloric acid (HCl) by pH meter. Then 5 ml of sample solution was transferred in a 50 ml of volumetric flask. Then the sample was kept in a freezer (- 20^0C) for 1 h. During analysis, the sample was taken out of the freezer and 2 ml of previously prepared Nash's reagent was added as indicator. Fish sample was then heated in the water bath at 60^0 C for 30 minutes. The absorbance of the sample in cuvette was measured at 415 nm immediately by UV/v spectrophotometer (Thermo Fisher Scientific, Waltham, MA). Triplicate of the absorbance was made for each sample and recorded for further calculation. The sample reading was placed in the standard curve for the calculation of formaldehyde content of the sample.

Results and Discussion

For the calculation of the absorbance from fish sample, a known concentration of formaldehyde solution was analyzed in different fraction and the reading was recorded by UV-spectrophotometer. The recorded concentration was then used for the preparation of a standard curve. From this standard curve (Fig. 1) formaldehyde concentration in different fish samples were compared and result was tabulated.

Determination of formaldehyde content in freshwater fishes from different markets

Mean formaldehyde content in different fishes collected from various retail markets were estimated from the absorbance and molar concentrations of standard curve. The formaldehyde content of five different fishes from three different markets is presented in Table 1. From the present experiment it was evident that the three freshwater species of two different market *viz.* Catla, Rohu and Boyal showed a range of 2.22 to 3.11µg/g

formaldehyde. From the standard curve, the result obtained that the Catla fish collected from the Four Bangalows fish market contained formaldehyde conc. of 2.76 µg/g and fish collected from Andheri Fish market contained formaldehyde conc. of 2.88 µg/g (Table 1).

There was no significant difference of formaldehyde conc. in Catla fishes from two different markets. In case of Rohu fish collected from Four Bunglows and Andheri Fish market contained formaldehyde with values of 3.11 and 2.96 µg/g respectively (Table 1). There was no significant difference of formaldehyde concentration among two market fishes. In case of Boyal fish collected from Four Bunglows and Andheri Fish market contained formaldehyde with values of 2.38 and 2.22 µg/g respectively (Table 1). There was no significant difference of formaldehyde concentration among two market fishes. Marine fishes generally contain higher formaldehyde content as compared to freshwater fishes due to degradation of bodily compound.

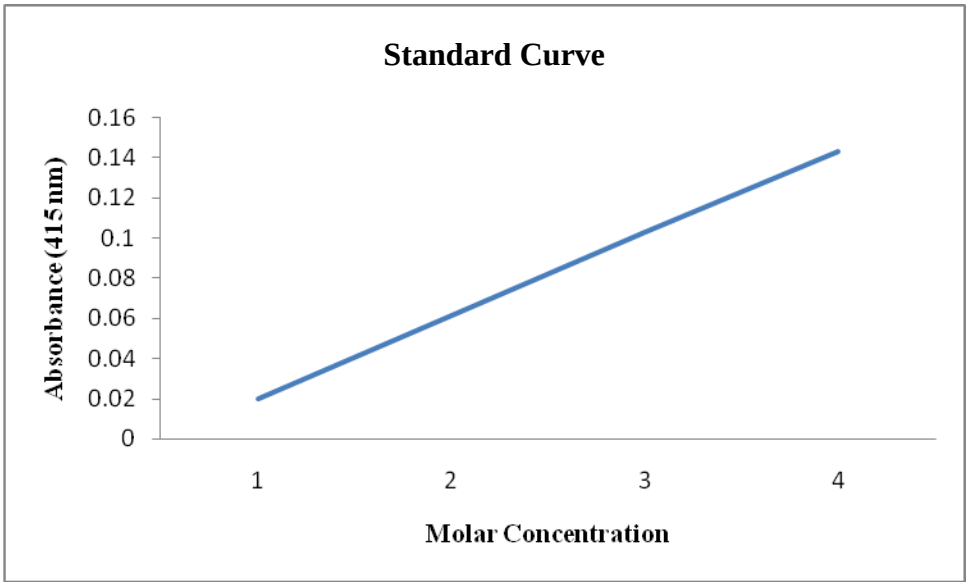
In this study it is found that Indian mackerel collected from three different markets viz. Versova, Four Bunglows and Andheri fish market contain 1.81, 2.27, 2.35 µg/g of formaldehyde respectively (Table 1). There is no significant difference among Indian Mackerel sample of three markets.

Bombay duck fish collected from the same markets contain 1.48, 1.71, 2.08 µg/g formaldehyde respectively (Table 1). There is also no significant difference among Bombay duck fish sample of the three markets.

Table.1 Formaldehyde content of different fishes collected from different market

Sample	Market	Conc. of formaldehyde (µg/g)
Catla	Four Bunglows market	2.76
	Andheri market	2.88
Rohu	Four Bunglows market	3.11
	Andheri market	2.96
Boyal	Four Bunglows market	2.38
	Andheri market	2.22
Indian Mackerel	Versova Fish market	1.81
	Four Bunglows market	2.27
	Andheri market	2.35
Bombay Duck	Versova Fish market	1.48
	Four Bunglows market	1.71
	Andheri market	2.08

Fig.1 Standard curve of formaldehyde concentration calculated as on the basis of Absorbance vs molar conc.



D.P. Sen, 2006 depicted 1 ppm and 1-5 ppm formalin as safe level for formalin concentration in fish. From that view point all freshwater fishes are unacceptable. Although Marine fishes naturally should contain higher portion of formaldehyde content compared to fresh water fishes, but in this present study the formaldehyde content of marine fishes are in the lower side. This may be due to the fresh availability of the marine fish from Arabian sea. But comparatively higher content of formaldehyde in freshwater fish is showing the sign of formalin adulteration. The results obtained in the present study are comparable with the findings of Hossain (2008) in case of rohu. Jaman *et al.,* 2015 found that fresh rohu fish contains 1.45 µg/g formaldehyde which is much lower than value of the rohu in the current study. The imported rohu fish had significantly higher formaldehyde conc. (≈3.4 folds) than that of fresh rohu fish from pond indicating presence of natural formaldehyde to some extent in fresh rohu fish (Hossain 2008). Haque and Mahasin, 2009 found 44% rohu followed by 22% catla were treated with formalin among all the samples collected from several markets of Dhaka, Bangladesh. Shahjalal *et al.,* 2008 found 3.95 nmol/mg and 13.40 nmol/mg formaldehyde in fresh and imported rohu respectively. Hasan *et al.,* 2006 reported 0.65 – 4.87 µg/g of formalin in rohu fish. Tunun *et al.,* 1996 reported 0.2 ppm formalin from fresh king mackerel. It is also true that TMAO is much more available in marine fish than in freshwater fish (Jung *et al.,* 2001). The formaldehyde thus produced naturally in the fish muscle by either bacteria or enzyme reaction became covalently bonded for a cross-linkage among peptide chains (Siskorski *et al.,* 1982).

Also endogenous formaldehyde residues ranging from 0.1-31.8 µg/g were detected in several species including eel (*Anguilla japonica*), striped bass (*Morone saxatilis*), Nile tilapia (*Tilapia nilotica*) (Xu and Rogers 1995) and banana shrimp (*Penaeus merguiensis*) (Yamagata and Low 1995). From the study of Noordiana *et al.,* (2011) the amount of formaldehyde in Bombay duck in Malaysian wet market was 15.75 µg/g which is much higher than the present study (3.9 µg/g). Jaman *et al.,* 2015 found little bit higher formaldehyde conc. of 3.9 µg/g Marine

frozen fish i.e. Bombay duck. Certain marine fish during frozen storage showed a level of formaldehyde as reported to be up to 400 mg/kg in Bombay duck after cold storage and less then equal to 140µg/g in fresh Bombay duck (Jaman *et al.*, 2015). However, there were some limitations in this study such as the temperature change, time of storage and handling could possibly influenced the concentrations of formaldehyde since it is a volatile compound. Additionally, only edible parts of fish were analyzed and no results were shown in the bones and fins.

In Conclusion, the present study found that both types of fishes contain formaldehyde in their flesh. In case of marine fish this may be due to the natural process. But in freshwater fishes it probably due to adulteration in marketing chain which can be concluded through comparison with several other authors. Prolonged consumption of fish having formalin may increase the risk of serious health hazards, like cancer, in the population. This causes increased morbidity and mortality, and also increases the health care costs of the country.

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