ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.352
IJFAS 2015; 2(6): 94-98

www.fisheriesjournal.com
Received: 17-05-2015
Accepted: 15-06-2015

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Determination of formaldehyde content by spectrophotometric method in some fresh water and marine fishes of Bangladesh

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Abstract
The present study was conducted for quantitative analysis of formaldehyde presence in some important freshwater and marine fish species by spectrophotometric method using Nash reagent in conjunction with TCA extraction. The freshwater fish rohu, *Labeo rohita* ; tilapia, *Oreochromis nilotica;* Thai koi, *Anabas testudineus;* kachki, *Corica soborna;* and marine fish loyitta, *Harpodon nehereus*; chhuri, *Lepturacanthus savala* from local markets and from freshly caught samples were evaluated for determination of formaldehyde concentration. Formaldehyde concentration obtained in fishes from three different wet markets of Mymensingh mechhua bazar was ranged between 1.4 and 7.35 µg/g. On the other hand, formaldehyde concentration in freshly caught fishes rohu, tilapia and Thai koi collected from ponds of Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh showed natural formaldehyde in their muscle having values of 1.45; 1.85 and 2.60 µg/g, respectively. The marine fish *viz.* loyitta and chhuri collected from the landing center of BFDC at Cox's Bazar and investigation in frozen, thawed condition showed to contain naturally occurring formaldehyde as 3.9 and 1.55 µg/g, respectively. Spectrophotometrically determination of formaldehyde concentration showed highest value of 7.35 µg/g in market sample of kachki, and naturally occurring formaldehyde concentration showed higher value of 2.6 µg/g in Thai koi from freshwater and 3.9 µg/g in loyitta fish from marine source. The present study suggested that fish from wet market contained a certain amount of added formaldehyde and fishes from both freshwater and marine sources shows to contain natural occurring formaldehyde in their muscle at different concentration.

Keywords: Formaldehyde, Freshwater fish, Marine water fish, Spectrophotometer

1. Introduction
Fish is a favorite diet of the Bangladeshi people and a prime source of animal protein. It is well known that formalin is generally used as preservatives for tissues. Fish being perishable item many fish sellers spray or dip fish with formalin treated water, where this chemical is endangering public health. It makes the fish enhanced shelf life, stiff and keeps them fresh-look for longer period of time. It is not known whether any local fish are also sprayed with formalin to prevent their spoilage (Kibria 2007) [10]. Many traders may dip the whole fish or inject formalin in the fish body cavity or spread formalin mixed water. Inadequate freezing facilities and ice factories and time consuming transport force the fish traders to resort to such malpractice. Studies conducted at different markets in Dhaka city (Hossain *et al.* 2008; Haque and Mohsin 2009) [7, 5] and Mymensingh Sadar (Yeasmin *et al.* 2010) [19] rationalizes the incidence of adding formalin. Formaldehyde is a toxic material that can kill bacteria and viruses as well as damage human cells. Food manufacturers sometimes add Formaldehyde to foods such as fish, meats, milk, etc to extend its shelf-life. Many ordinary foods such as fish naturally contain small amounts of formaldehyde. However, excess Formaldehyde has been reported in many fish as adulterated by different channels during marketing.
Formaldehyde is an organic compound with the chemical formula, HCHO. It is colorless, pungent and often obtained in the form of formalin with 37% formaldehyde. A high content of accumulated formaldehyde in food poses a threat to human health (Li *et al.* 2007) [11] as formaldehyde is toxic, allergenic and carcinogenic and can cause symptoms like headaches, burning sensation in the throat and difficulty in breathing (Herschkovitz *et al.* 2000) [6]. It has been declared a potential carcinogen and mutagen (Cui *et al.* 2007) [3]. Formaldehyde is possibly carcinogenic to humans at LD_{50} 30 gm for human (WHO 1989) [15].

Formaldehyde occurs naturally in fish and seafood. As soon as fish undergo post mortem, trimethylamine oxide (TMAO) is broken down to dimethylamine and formaldehyde as its main product. TMAO is mainly found in marine fish (Jiang *et al.* 2006) [8]. Formaldehyde may be formed during the ageing and deterioration of fish flesh. Besides natural formation of formaldehyde in fish and seafood by enzymatic reaction, other biochemical reactions can also occur such as oxidation of lipids as a result of microorganism activities. This will eventually result in physical damage of fish or production of chemical metabolites such as biogenic amines or other unpleasant compounds (Gram *et al.* 2002; Arashisar *et al.* 2004) [4, 1].

Freshness is a property of fish that has a considerable influence on its quality (Connell 1995) [2]. Now a days consumer is becoming more conscious of the application of formaldehyde in fish and also its side effects. There has been claim that fishes in Bangladesh are formalin contaminated in the supply chain. Therefore, the objective of the present study is to determine or quantify the formaldehyde content of some important freshwater and marine fishes of Bangladesh by a method of precision spectrophotometrically using Nash's reagent.

2. Materials and methods

The experiment was conducted in the laboratory of the Department of Agri-Chemistry and Department of Fisheries Technology, Bangladesh Agricultural University, Mymensingh.

2.1 Collection of fish sample

Fish samples were collected from different wet markets such as K.R. (Bangladesh Agricultural University) market, peri-urban market- Kewatkhali bazar, and Machua bazaar, Mymensingh town. Three types of fresh fish species namely Indian major carp, rohu (*Labeo rohita*), tilapia (*Oreochromis nilotica*) and Small Indigenous Species (SIS) kachki (*Corica soborna*) were collected from these markets. Collected fish was kept in iced condition in insulated ice box and carried to the laboratory for determination of formaldehyde.

For other experiment, freshly caught rohu (*Labeo rohita*), tilapia (*Oreochromis nilotica*) and climbing perch, Thai koi (*Anabus testudineus*) were collected from ponds of Freshwater Station, Bangladesh Fisheries Research Institute (BFRI), Mymensingh. Marine fishes *viz.* bombay duck, loyitta (*Harpodon nehereus*) and ribbon fish, chhuri (*Lepturacanthus savala*) were collected from Bangladesh Fisheries Development Corporation (BFDC) landing center of Cox's Bazar. Immediate after collection the fishes were frozen and brought to the laboratory in insulated box which took about 12 hours journey. Then the fishes were kept frozen for about 2-3 days until the detection of formaldehyde was done.

2.2 Chemicals and reagent used

Trichloroacetic 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. Fifteen 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 at all time. 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 p^H meter.

2.3 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. × C

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

2.4 Sample preparation for determination of formaldehyde

The fish samples under verification were cut into small pieces. Then fish flesh was taken into blender for homogenization and blended for 10 minutes. Then a 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. Then pH of the solution was determined by a pH meter. Though the addition of tri-chloro-acetic acid reduced the pH value of the sample it was adjusted the pH between 6.00-7.00 of the sample by using Potassium hydroxide (KOH) and Hydrochloric acid (HCl). Then 5 ml of sample solution was taken in a 50 ml of volumetric flask. Then the sample was kept in a freeze (- 20^0C) for 1 h. During analysis, the sample was taken out of the freeze 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.

3. Result and discussion

For the calculation of the absorbance from fish sample, at first a 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.

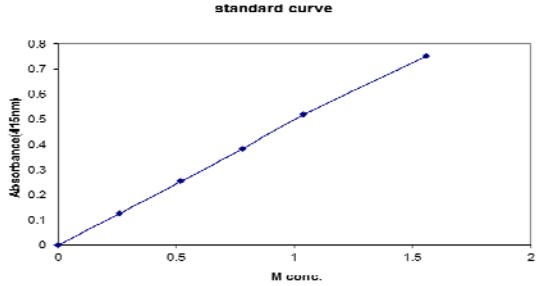

Fig 1: Standard curve of formaldehyde concentration calculated as on the basis of absorbance vs molar conc.

3.1 Determination of formaldehyde content in freshwater fishes from different markets

Mean formaldehyde content in different fishes collected from various retail markets was estimated from the absorbance and molar concentrations of standard curve. The formaldehyde content of three different freshwater fishes from different markets is presented in Table 1. From the present experiment it was evident that the three species of market fishes *viz.* rohu, tilapia and kachki showed a range of 1.4 and 7.35 µg/g formaldehyde. From the standard curve, the result obtained that the rohu fish collected from the K.R market contained formaldehyde conc. of 1.4 µg/g and fish collected from Kewatkhali market contained formaldehyde conc. of 1.5 µg/g (Table 1). There was no significant difference of formaldehyde conc. in rohu fishes from two different markets. In case of Tilapia, fish collected from K.R and Kewatkhali market contained formaldehyde with values of 2.53 and 2.5 µg/ respectively (Table 1). There was no significant difference of formaldehyde concentration among two market fishes. On the other hand, kachki fish samples were collected from three different markets of Mymensingh as mentioned in Table 1. Among the three different market source of kachki fish, it was observed that kachki fish collected from Mechua bazar showed the highest formaldehyde content (7.35 µg/g) (Table 1) in contrast to formaldehyde content of 7.2 and 7.0 µg/g in fish from Kewatkhali market and K.R market, respectively. However, the estimates did not show any significant difference of formaldehyde conc. among kachki fishes from three different markets. Moreover, formaldehyde conc. among fishes from 3 different market samples did not vary significantly. The 3 fish species collected from K.R market (BAU); Kewatkhali market and Mechua bazaar, Mymensingh were found to contain formaldehyde concentration of 1.4 to 1.5µg/g in rohu, 2.5 to 2.53µg/g in tilapia and 7.0 to 7.35µg/g in kachki fish. The results obtained in the present study are comparable with the findings of Hossain (2008) [7]; Xu and Rogers (1995) [16] in case of rohu and tilapia, but much lower than the findings of (Noordiana *et al.* 2011) [13] in case of Bombay duck (loittya). 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) [7].

Table 1. Formaldehyde concentration in different fresh water fish from different market in Mymensingh calculate as on the basis of absorbance (415nm) vs molar conc.

Fish Species	Market/Source	Absorbance	Molar conc.	Formaldehyde content (µg/g)
Rohu	K.R. market	0.146 ± .001	0.28 $\times 10^{-4}$	1.40
	Kewatkhali	0.140 ± .0015	0.30 $\times 10^{-4}$	1.50
Tilapia	K.R. market	0.256 ± .008	0.509 $\times 10^{-4}$	2.53
	Kewatkhali	0.245 ± .001	0.501 $\times 10^{-4}$	2.50
Kachki	K.R. market	1.636 ± 0.53	1.40 $\times 10^{-4}$	7.00
	Kewatkhali	1.704 ± 0.024	1.44 $\times 10^{-4}$	7.20
	Mechua bazaar	1.770 ± 0.017	1.47 $\times 10^{-4}$	7.35

3.2 Determination of formaldehyde content in fresh fishes from the BFRI ponds

Table 2 showed the formaldehyde content in three different fresh fishes from the BFRI ponds. The present experiment evident that freshly caught rohu, tilapia and Thai koi fish had a small quantity of natural formaldehyde in their muscle having values ranging from 1.45 to 2.60 µg/g. The formaldehyde content in these fresh fishes was obtained as 1.45, 1.85 and 2.60µg/g for rohu, tilapia and Thai koi respectively (Table 2). The present observation revealed that freshwater fishes contain naturally occurring formaldehyde in their muscle. On the other hand, fresh fishes *viz* rohu, tilapia and Thai koi collected from ponds of Freshwater Station, BFRI, Mymensingh contained naturally occurred formaldehyde of 1.45, 1.85 and 2.6 µg/g respectively. Despite use of Formalin detection kit developed by Bangladesh Council of Scientific & Industrial Research (BCSIR) and Z-300 Formaldehyde Meter used by Department of Fisheries (DoF), the present study was an initiative to determine formaldehyde concentration naturally occurred in freshwater and marine fishes in addition to suspected market samples with formalin. The formaldehyde concentration in selected fishes from different wet markets varied from market to market and also among species sampled. In market samples, the highest amount of formaldehyde was found in kachki at 7.35 µg/g while rohu contained the lowest amount of formaldehyde at 1.4 µg/g level. These results indicated that market fishes are somehow treated with formalin by the traders. Yeasmin (2010) [19] also reported the presence of formalin in imported rohu ranged 0.5% to 1%.

Table 2: Formaldehyde contents in fresh fishes from BFRI ponds calculated as on the basis of absorbance (415nm) vs molar conc.

Fish species	Source	Absorbance	Molar conc.	Formaldehyde content (µg/g)
Rohu	BFRI pond	0.143 ± .002	0.29	1.45
Tilapia	,,	0.184 ± .002	0.37	1.85
Thai koi	,,	0.250 ± .001	0.52	2.6

3.3 Determination of formaldehyde content in marine fishes

Formaldehyde content in two different marine fish species are presented in Table 3. The frozen marine loyitta and chhuri fishes showed formaldehyde content of 3.9 µg/g and 1.55 µg/g, respectively (Table 3). The study revealed higher concentration of formaldehyde was found in marketed kachki fish, freshly caught Thai koi and frozen marine loyitta fish. Based on the result, there were significant differences in the concentration of formaldehyde between fish samples from freshwater and marine origin. Two marine fishes collected from landing centers of Cox's Bazar and were analyzed in frozen condition found to contain formaldehyde of 1.55µg/g in chhuri and 3.9 µg/g in loittya fish. On the other hand, fresh fishes from ponds was found to contain naturally occurred formaldehyde lowest in rohu fish (1.45 µg/g) and highest in Thai koi (2.6 µg/g). It is also true that TMAO is much more available in marine fish than in freshwater fish (Jung *et al.*, 2001) [9]. 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) [14]. Also endogenous formaldehyde residues ranging from 01-31.8 µg/g were detected in several species including eel (*Anguilla japonica*), striped bass (*Moronesaxatilis*), Nile tilapia (*Tilapia nilotica*) (Xu and

Rogers 1995) [16] and banana shrimp (*Penaeus merguiensis*) (Yamagata and Low 1995) [17]. From the study of Noordiana *et al.* (2011) [13] 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). Marine frozen fish i.e. loittya contained little bit higher formaldehyde conc. of 3.9 µg/g. 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 YAU (2007) [18]. 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.

Table 3: Formaldehyde content in frozen marine fishes from BFDC landing center in Cox's Bazar calculated as on the basis of absorbance vs molar concentration using 415 nm

Fish species	Source	Absorbance	Molar conc.	Formaldehyde content (µg/g)
Loyitta	BFDC landing center	0.919 ± .002	0.78×10^{-4}	3.9
Chhuri	,,	0.149 ± .002	0.31×10^{-4}	1.55

4. Conclusion

The present study revealed the presence of formaldehyde in market samples of rohu, tilapia and Kachki from 3 markets of Mymensingh Sadar with the different ranges of 1.4 µg/g to 7.35 µg/g. Based on the findings by different authors, the present situation of fish adulteration in wet markets is a fact which presently shows improving due to public awareness, government initiatives against formalin use in markets. Naturally occurred formaldehyde in marine fishes is an established fact. This experiments based on Nash test in conjunction with TCA extraction with freshly caught fishes like rohu, tilapia and Thai koi also showed the presence of naturally occurred formaldehyde with the range of 1.45 µg/g—2.6 µg/g. However, the present study was and the estimation of formaldehyde was calculated in line with standard curve obtained from concentration of formaldehyde solution used for this study.

5. Competing interests

The authors declare that they have no competing interests.

6. Authors' contributions

SCC and MEH formulated the hypothesis and designed the studies. MNJ carried out the experimental where HPS provide full technical advice and assistance during conducting experiment. MNJ and MSH analyzed data and prepared the manuscript. SCC made the comments on the results-duscussion and MSH finalizes the manuscript. All authors read and approved the final version of the manuscript.

7. Acknowledgements

The authors would like to express their sincere thank to Department of Fisheries Technology and Department of Agri-Chemistry under Bangladesh Agricultural University and Bangladesh Fisheries Research Institute, Mymensingh, Bangladesh for the institutional support.

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