Research Article

Comparative Formaldehyde Content in Some Fresh and Ice-packed Fishes Available in Imphal Market, Manipur

Konjengbam Bishwarup*, Chungkham Sarojnalini

Department of Zoology, Manipur University, Canchipur, Imphal, Manipur, INDIA.

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ABSTRACT

Quantitative determination of formaldehyde in three species of fresh and ice-packed fishes namely *Labeo rohita* (Hamilton, 1822), *Pangasius pangasius* (Hamilton, 1822) and *Piaractus brachypomus* (Cuvier, 1818) were conducted. The study observed different concentrations of formaldehyde in different body regions of all the studied ice-packed fishes. Among fresh fishes, formaldehyde was detected only in *P. brachypomus*. In the ice-packed fishes, formaldehyde concentrations were found in the range of 0.80-1.19 µg/g in *L. rohita*, 1.23-1.39 µg/g in *P. pangasius* and 0.86-1.15 µg/g in *P. brachypomus*. The results indicated that there was additional formaldehyde in those ice-packed fishes. Since, formaldehyde is carcinogenic, it will likely cause human health hazards. So, it must be avoided for use in preservation and storage of foods including fishes. The study showed that fresh fishes were better and safer than ice-packed fishes for human health.

Keywords: Carcinogenic, Formaldehyde, Fresh, Ice-packed.

Correspondence:
Konjengbam Bishwarup
Department of Zoology, Manipur University, Canchipur, Imphal-795003, Manipur, INDIA.

Email: bishwarupkonmeitei@gmail.com

INTRODUCTION

According to the State Fisheries Department report (2021-22), the targeted fish production rate of Manipur is around 57,000 metric tonnes however Manipur produced only around 33,000 metric tonnes in a year. In order to meet the demands of the people, the remaining 24,000 metric tonnes of fish need to be brought in and imported from other states and neighbouring countries like Myanmar and Bangladesh. Fishes being highly perishable, their self-life is only around 8-12 hr depending on the species. Transportation to Manipur from the states like Andhra Pradesh, West-Bengal and Odisha took around 4-5 days so fish preservation and processing techniques should be used in proper way to reduce spoilage and in due consideration for human health. Preservation of fish in ice is one of the most effective ways used for retarding spoilage.[1] Icing is the main technique for fish preservation in India. Being a perishable commodity, fish can only be kept fresh in ice for 8 to 14 days depending on the species. Formalin treated Rohu fish can extend shelf life by 6 days comparing to fresh one stored in ice so, many traders used formalin as preservatives considering only for the small profit in business.[2]

Formaldehyde (CH_2O) is classified in the Group-I as carcinogenic to humans by the International Agency for Research on Cancer based on the evidence of nasopharyngeal cancer in humans.[3] Formaldehyde occurs in the gaseous form whereas 37% formaldehyde by weight in the liquid form is known as formalin. It has been reported as one of the chemical mediators to cause programmed cell death called apoptosis and it is also highly reactive, flammable and colourless gas with pungent and irritating odour.[4] It can be decomposed at a temperature above 150ºC. It is used as a fixative in the hospital and anatomical laboratories for biological specimens. Most of the food items including fishes naturally produced formaldehyde endogenously. However, there were many reports on the addition of

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formaldehyde in the marketed fishes. Since fishes are highly perishable, traders used this harmful chemical intentionally to increase the shelf-life of fishes. The main reason behind such malpractices include lack of strict regulatory controls, inadequate cold storage facility, refrigeration, transport and increased demand from consumers.[4] Poor preservation facilities and distant transport reduces the quality of fishes when it reached to consumers.[5] The World Health Organisation (WHO) and Food and Agricultural Organisation (FAO) worked together on risk analysis to tackle the food safety issues.[6] Since formaldehyde is carcinogenic and detrimental to human health, it is very necessary to check the ice-packed fishes marketed everywhere in Manipur specially the Imphal Market as it is the main trading centre. There was an issue of banning those ice-packed fishes for 10 days in Manipur in 2018 in relation to formaldehyde contamination. There were no scientific reports regarding formaldehyde contamination of fishes in the State. The present study was aimed to assess the presence of formaldehyde in some selected freshly harvested and ice-packed fishes sold in the Imphal Market following the modified method of CS Ng using UV-Vis Spectrophotometer.[7]

MATERIALS AND METHODS

Ice-packed fishes namely *Pangasius pangasius* weighing 1025-1045 g with standard length 41.6-41.8 cm, *Piaractus brachypomus* weighing 1080-1185 g with standard length 30.8-31.1 cm and *Labeo rohita* weighing 655-780 g with standard length 29.5-31.1 cm were collected from different fish vendors of Imphal Market and packed in insulated ice box and brought to the Fishery laboratory at the Department of Zoology, Manipur University. The freshly harvested fishes of the above three species of similar sizes and weight were collected from local fish farms. *P. pangasius* and *P. brachypomus* were collected from a farm at Wangoi Makha Leikai, Imphal-West district; Manipur and *L. rohita* were collected from a farm at Nongpok Lourembam Awang Leikai, Thoubal district, Manipur, India. The scales, viscera and fins were removed and the head, dorsal and ventral parts were taken from 6 fishes each of freshly harvested and ice-packed fishes for formaldehyde analysis following the modified method of CS Ng.[7] The different parts were minced separately.

The minced fish samples of 5g each was weighed and added to 20 mL of 5% w/w TCA solution and homogenize properly with homogenizer and make it stand in ambient temperature for 30 min. The mixture was filtered through a Whatman No. 1 filter paper. Again, 10 mL of 5% TCA solution was added to the residue and kept it for another 30 min. and filtered. The combined filtrate was collected and adjusted the pH to 6.0-6.5 with 0.1 N KOH or 0.1N HCl and made upto 50 mL with distilled water. 3 mL of the neutralized filtrate was taken and kept in a deep freezer (-20ºC) for 1 hr. Finally, 3 mL of Nash's reagent was added to the neutralized filtrate and heated the solution in a water bath at 60ºC for 15 min. The absorbance at 412 nm was measured against the blank solution by UV-Vis spectrophotometer (AN-UV-6500N).

The chemicals used were Trichloroacetic Acid (TCA), acetic acid, formaldehyde, acetylacetone of standard quality from Merck, HiMedia and SD's Lab-Chem industry respectively. Nash's reagent was prepared by mixing ammonium acetate (15 g), acetyl acetone (0.3 mL) and acetic acid (0.2 mL) in 100 mL distilled water. It is light sensitive and was kept in a dark-glass bottle. Standard formaldehyde stock solution of 1000 ppm was prepared by dissolving 0.3 mL of 37% formaldehyde in 100 mL distilled water and then diluted the stock solution 100 times to get 10 ppm standard working solution.

Calibration curve was made by using a standard solution of 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ppm concentration of formaldehyde prepared from 10 ppm working solution. 3 mL of Nash's reagent was added to each of the concentration and heated in water bath at 60ºC for 15 min. The absorbance of standard concentration of formaldehyde was determined by measuring the absorbance at 412 nm using the UV-Vis spectrophotometer. A calibration curve was then plotted from the results obtained. Statistical analysis was performed by one way ANOVA using SPSS 21.0 for windows. The formaldehyde content (µg/g) was calculated using the following formula:

$$\text{Formaldehyde } (\mu g/g) = \frac{A}{\text{Vol. of filtrate used}} \times \frac{\text{Total make up vol. of filtrate} \times f}{\text{Weight of sample}}$$

Where, A = Absorbance, f = Factor of formaldehyde.

RESULTS

The fresh *P. pangasius* did not detect any amount of formaldehyde in all the body regions. However, the ice-packed *P. pangasius* had certain significant values in different body regions. The dorsal region had significantly higher values (1.39±0.045 µg/g) followed by ventral (1.31±0.040 µg/g) and head (1.23±0.080 µg/g) regions.

In *P. brachypomus*, formaldehyde was detected highest in the ventral region followed by dorsal and head in all the body regions of both fresh and ice-packed fish. The ventral (1.15±0.000 µg/g), dorsal (1.08±0.040 µg/g) and the head (0.86±0.035 µg/g) regions of ice-packed *P. brachypomus* detected significantly ($p<0.05$) higher contents of formaldehyde than the respective ventral (1.0±0.035 µg/g), dorsal (0.9±0.000 µg/g) and head (0.73±0.030 µg/g) regions of fresh *P. brachypomus*.

Different body regions of fresh *L. rohita* did not detect any amount of formaldehyde; however ice-packed *L. rohita* detected formaldehyde in all the body regions. The highest value (1.19±0.045 µg/g) was detected in the head followed by the ventral (1.11±0.090 µg/g) and the dorsal (0.80±0.045 µg/g) regions. Different body regions of Ice-packed fishes detected different concentrations of formaldehyde significantly ($p<0.05$). The mean formaldehyde content was found highest in the *P. pangasius* followed by *L. rohita* and *P. brachypomus*.

Table 1: Formaldehyde content in different body regions of some selected Fresh and ice-packed fishes.

Fish	Body region	Absorbance (ppm)	Formaldehyde content (µg/g)
L. rohita (Ice-packed)	Head	0.027±0.001	1.19±0.045^{b}
	Dorsal	0.018±0.001	0.80±0.045^{a}
	Ventral	0.025±0.002	1.11±0.090^{b}
L. rohita (Fresh)	Head	ND	ND
	Dorsal	ND	ND
	Ventral	ND	ND
P. pangasius (Ice-packed)	Head	0.030±0.002	1.23±0.080^{a}
	Dorsal	0.034±0.001	1.39±0.045^{b}
	Ventral	0.032±0.001	1.31±0.040^{ab}
P. pangasius (Fresh)	Head	ND	ND
	Dorsal	ND	ND
	Ventral	ND	ND
P. brachypomus (Ice-packed)	Head	0.024±0.001	0.86±0.035^{b}
	Dorsal	0.030±0.001	1.08±0.040^{d}
	Ventral	0.032±0.000	1.15±0.000^{d}
P. brachypomus (Fresh)	Head	0.022±0.001	0.73±0.030^{a}
	Dorsal	0.027±0.000	0.9±0.000^{b}
	Ventral	0.030±0.001	1.0±0.035^{c}

The values in the same column having similar superscripts did not differ significantly ($p<0.05$). Significant difference compared for the same species of fresh and ice-packed fish. ND = Not detected.

DISCUSSION

Different body regions of fresh *P. pangasius* and *L. rohita* were not detected any amount of formaldehyde however fresh *P. brachypomus* detected certain amount of formaldehyde ranging from 0.73 µg/g in the head region and 1.0 µg/g in the ventral region. Detection of formaldehyde in fresh *P. brachypomus* revealed that whether the formaldehyde obtained may be from the endogenous production from the fish itself or from the artificial feeds or may be from water treated with formaldehyde meant for treatment of fungal and parasitic actions. Study conducted by Jaman *et al.*[8] reported that formaldehyde was detected in the fresh Rohu (1.45 µg/g), Tilapia (1.85 µg/g) and Thai koi (2.60 µg/g) and concluded that fresh water fishes contained naturally occurring formaldehyde in their muscles. Hossain *et al.*[9] reported that fresh Rohu fish from the pond detected natural formaldehyde to some extent and also found that the imported Rohu had significantly higher concentration of formaldehyde (~3.4 folds) than that of fresh one. Whereas all the three species of ice-packed fishes in the present study detected certain concentrations of formaldehyde in different body regions. The values were found ranging from 0.80 µg/g in the dorsal region of *L. rohita* and 1.39 µg/g in the dorsal region of *P. pangasius*. The results of the present study showed that the ice-packed fishes contain some amounts of added formaldehyde. Different concentrations of formaldehyde in different body regions of ice-packed fishes might be due to differences in the application techniques either by dipping or spraying by the formalin. The mean formaldehyde content in ice-packed fishes were found highest in *P. pangasius* compared to *L. rohita* and *P. brachypomus*. Though the mean values of formaldehyde content in all the three species of ice-packed and fresh *P. brachypomus* were relatively low, the values were higher than 0.2 µg/g which is the maximum daily dose reference for formaldehyde given by the United States Environmental Protection Agency (US EPA).[10] Malaysian Food Regulation, 1985 established maximum limit of 5 µg/g of formaldehyde in fish and fish products. Oral exposure to formaldehyde recommended by European Food Safety Authority or EFSA were 1.7 and 1.4 µg/g body weight per day for 60 kg and 70 kg respectively in humans.[11] Formaldehyde value ranging from 10-20 mg/kg in fishes were not considered acceptable for humans.[12] Joshi *et al.*[13] have observed formaldehyde contents of 0.39-2.32 µg/g in some selected fishes from the wet markets of Kathmandu valley, Nepal. Jaman *et al.*[8] have obtained formaldehyde concentration between 1.4-7.35 µg/g in fishes from three different wet markets of MymenSingh mechhua bazaar. Finfish and shellfish samples of different fish markets have formaldehyde values in the range of 0.33 to 16 mg/kg.[8,9,13-17]

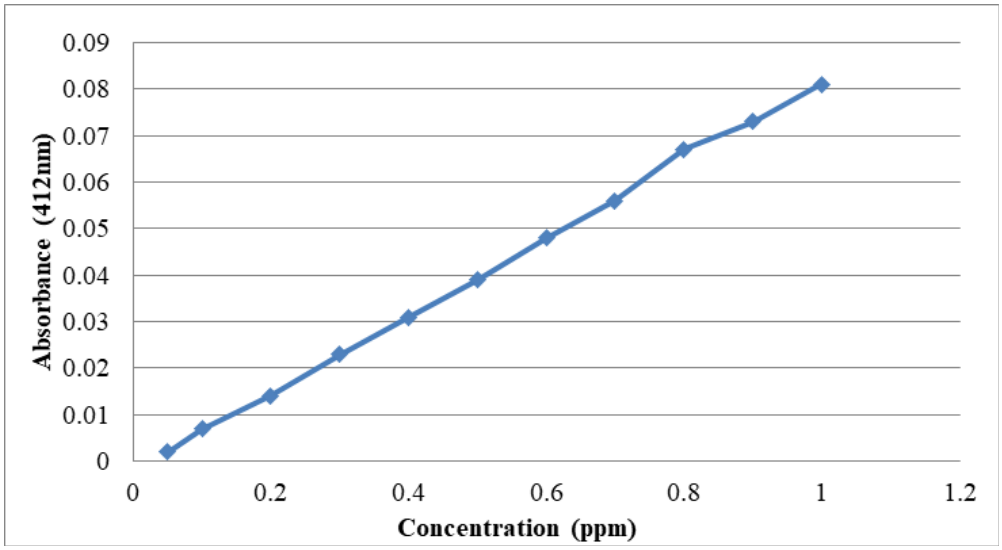

Figure 1: Calibration curve of standard formaldehyde solution.

Presence of naturally occurring formaldehyde in marine fishes is an established fact due to the presence of Trimethylamine Oxide (TMAO). During enzymatic reaction, TMAOase catalyses the breakdown of TMAO to equimolar amounts of formaldehyde and Dimethyl Amine oxide (DMA).[18-20] Formalin was used in cultured fish to control external parasites. It was approved by the United States Food and Drug Administration (USFDA) for the control of parasites and fungi in marine fish farming. Used of formalin in aquaculture was not permitted in Australia, Europe and Japan due to its carcinogenic effect.[5] Ingestion of 30 mL of formalin can cause death of an adult human being.[21] According to Zhang *et al.*[22] chronic inhalation of formaldehyde can cause respiratory symptoms and irritation of eyes, nose and throat. People who are engaged in spraying or injecting formalin for long period of time may have blindness, asthma and lung cancer.[23] Moreover, formaldehyde reacts with protein and subsequently causes protein denaturation and muscle toughness during improper storage of fish.[24] Not only protein denaturation and muscle toughness, it also reduces water holding capacity[25] leading to lower acceptability as well as functionality.[26] Thus, affecting the nutritional quality of fishes as well. Sanyal *et al.*[27] reported that the added formaldehyde content can reduce to some extent during ice storage of fish due to loss along with ice melt water. It was also reported that roasting and boiling helped in decreasing the concentration of formaldehyde by evaporation.[28]

CONCLUSION

The present study observed different concentrations of formaldehyde in different body regions of all the studied ice-packed fishes. The results indicated that there was additional formaldehyde in those ice-packed fishes. The study showed that fresh fishes were better and safer than ice-packed fishes for human health.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Since formaldehyde is carcinogenic, consumption of fishes treated with formaldehyde in lower doses or concentration will also give several health issues accordingly. Therefore, use of formaldehyde in every food item as food preservatives may likely cause human health hazards. Proper handling, storage, packaging of food items must be maintained. Therefore a proper surveillance and implementation of food safety measures should be followed for better human health.

REFERENCES

1. Ozogul Y, Ozogul F, Kuley E, Ozkutuk AS, Gokbulut C, Kose S. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage. Food Chem. 2006;99:752-8.
2. Goon S, Bipasha MS, Islam MS. Fish Marketing Status with Formalin Treatment in Bangladesh. Int J Med Sci Public Health. 2014;3(2):95-100.
3. Bosetti C, Mclaughlin JK, Tarone RE, Pira E, La Vecchia C. Formaldehyde and cancer risk: A quantitative review of cohort studies through 2006. Ann Oncol. 2008;19:29-43.

4. Laly SJ, Priya ER, Panda SK, Zynudheen AA. Formaldehyde in seafood: A review. Fish Technol. 2018;55:87-93.
5. Haque E, Mohsin ABM. Intensity of Formalin use for consumable fish preservation in Dhaka city Bangladesh. J Fish Int. 2009;4:52-54.
6. World Health Organization. Global strategy for food safety: safer food for better health, World Health Organization, Geneva, Switzerland. 2002.
7. Ng CS. Determination of formaldehyde in fish meat using Nash's reagent. Hasegawa H. (Ed.). Laboratory Manual on Analytical Methods and Procedures for Fish and Fish Products. Marine Fisheries Research Department, Southeast Asian Fisheries Development Centre, Singapore. 1987; 5.1-5.4 B
8. Jaman N, Hoque MS, Chakraborty SC, Hoq ME, Seal HP. Determination of formaldehyde content by spectrophotometric method in some freshwater and marine fishes of Bangladesh. Int J Fish Aquat. 2015;2:94-8.
9. Hossain MS, Rahman MA, Sharkar TK, Shahjalal HM. Formaldehyde content in the Rui fish *(Labeo rohita*) in Bangladesh and effect of formaldehyde on lipid peroxidation in Rat liver and Intestinal tissues. J Med Sci. 2008; 8(4): 405-409.
10. Wang S, Cui X, Fang G. Rapid determination of formaldehyde and sulphur dioxide in food products and Chinese herbals. Food Chem. 2007;103:1487-93.
11. European Food Safety Authority. Endogenous formaldehyde turnover in humans compared with exogenous contribution from food sources. EFSA Journal. 2014;12:3550.
12. Yasuhara A, Shibamoto T. Quantitative analysis of volatile aldehydes formed from various kinds of fish flesh during heat treatment. J Agric Food Chem. 1995;43:94-7.
13. Joshi R, Bhatta R, Paudel PN, Kafle BK. Formaldehyde content of selected fish from the wet markets of Kathmandu valley. Int Food Res J. 2015; 22: 1434-1437.
14. Wahed A, Razzaq A, Dharmapuri S, Corrales M. Determination of formaldehyde in food and feed by an in-house validated HPLC method. Food Chem. 2016; 202: 476-483.
15. Aminah AS, Zailina H, Fatimah AB. Health risk assessment of adults consuming commercial fish contaminated with formaldehyde. Food Pub Health. 2013;3:52-8.
16. Noordiana N, Fatimah AB, Farhana YCB. Formaldehyde content and quality characteristics of selected fish and seafood from wet markets. Int Food Res J. 2011;18:125-36.
17. Bhowmik S, Begum M, Hossain A, Rahman M, Nowsad Alam AKM. Determination of formaldehyde in wet marketed fish by HPLC analysis: A negligible concern for fish and food safety in Bangladesh. Egypt J Aquat Res. 2017;43:245-8.
18. Amano K, Yamada K. A biological formation of formaldehyde in the muscle tissue of gadoid fish. Bulletin of the Japanese Society for the Science of Fish. 1964; 30: 430-435.
19. Lundstrom RC, Correia FF, Wilhelm KA. Enzymatic dimethylamine and formaldehyde production in minced American plaice and backbone flounder mixed with a red hake TMAOase active fraction. J Food Sci. 1982;47:1305-10.
20. Parkin KL, Hultin HO. Some facts influencing the production of dimethylamine and formaldehyde in minced and intact red hake muscle. J Food Process and Preser. 1982;6:73-97.
21. Immaculate J, Jamila P. Quality characteristics including formaldehyde content in selected sea foods of Tuticorin, southeast coast of India. Int Food Res J. 2018;25(1):293-302.
22. Zhang L, Steinmaus C, Eastmond DA, Xin XK, Smith MT. Formaldehyde exposure and leukemia: A new meta-analysis and potential mechanisms. Mutat Res. 2008;681:150-68.
23. Hossain MM. Consumption in rural Bangladesh: Households, lifestyles and identities. Academic dissertation presented to consumer economics unit, Department of economics and management, University of Helsinki, Finland. 2011.
24. Sotelo CG, Piñeiro C, Pérez-Martín RI. Denaturation of fish proteins during frozen storage: Role of formaldehyde. Eur Food Res Technol. 1995;200:14-23.
25. Haard NF, Simpson BK. Seafood Enzymes: Utilization and Influence on Postharvest Seafood Quality, CRC Press, Taylor & Francis Group, Boca Raton, London, New York. 2000; 696 p.
26. Li J, Zhu J, Ye L. Determination of formaldehyde in squid by high performance liquid chromatography. Asia Pac J Clin Nutr. 2007;16:127-30.
27. Sanyal S, Sinha K, Saha S, Banerjee S. Formalin in fish trading: an inefficient practice for sustaining fish quality. Arch Pol Fisheries. 2017;25:43-50.
28. Bianchi F, Careri M, Corradini C, Musci M, Mangia A. Innovative method for ultratrace determination of formaldehyde in frozen fish: SPME extraction and GC-ITMS/MS analysis. Curr Anal Chem. 2007;1:129-34.

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