Screening of fish from retail markets for the presence of formalin and its effect on the quality of fresh catla

N. K. Mehta^{1*}, R. K. Majumdar¹, D. Pal¹, M. B. Priyadarshini¹ and B. Dhar¹

¹Department of Fish Processing Technology and Engineering, College of Fisheries, Central Agricultural University, Lembucherra - 799 210, Tripura, India

Abstract

In the present study, the illegal usage of formalin for shelf-life enhancement of fish by fish traders in the different markets of Agartala, India was assessed. Total 99 fresh fish and shellfish samples were collected from four different and major markets viz. Lake Chowmuni market, Gol bazar, Lembucherra and Battala market of Agartala, India. Out of these, around 54% of fresh fish and shellfish were found contaminated with formalin. Further, the effect of formalin treatment of 10% formalin on catla fish quality was also analysed. The result of the study showed that the hardness of fish muscle increased by 65%; while solubility and water holding capacity were found to be decreased considerably of fish treated with 10% formalin. The bacteria on fresh fish were log 5.27 that decreased to log 4.6 in 10% formalin treated fish. Formalin is harmful to human health; there is a need to implement strict regulations that prevent adulteration of fish with formalin. Overall, the study deduced that formalin adulteration is not only harmful for human health but also affected the textural properties of fish considerably.

Key words: Contaminants, Fish, Fish market, Formalin, Quality, Texture

Highlights

- A total of 99 fish and shellfish were tested for formalin adulteration.
- Formalin contamination was found in 54% of the tested samples.
- Formalin had an adverse influence on the textural quality of fish muscles.
- Formalin treatment reduced protein solubility and WHC.

INTRODUCTION

India is one of the major fish producer and fresh fish-consuming countries. Around 70-80% of the freshwater fish is consumed in fresh condition. Fish is one of the highly perishable food items and it is important to maintain the prime quality until it reaches the consumers. In most scenarios, the production and consumption sites of fish remain separated, making it extremely difficult to sustain the freshness of the fish in the supply chain. Because of huge production and the dearth of post-harvest management infrastructure, chemicals such as formalin were added to fresh fish to increase shelf-life substantially compared to current short-term preservation methods (Sahu *et al.*, 2018). Formalin is

hazardous and is a concern of safety and public health (Bianchi *et al.*, 2007; Claeys *et al.*, 2009). The International Agency for Research on Cancer categorized formaldehyde in group 1 “as carcinogenic to humans” (IARC, 2004). Furthermore, its carcinogenic impact may result in mutagenicity (formaldehyde induced DNA-protein and protein-protein cross-links) (Claeys *et al.*, 2009; Monakhova *et al.*, 2012). Formalin-treated fish or other foodstuffs, while also direct inhalation, skin or eye contact, would all be possibilities for humans to be exposed to this carcinogen. Kundu *et al.* (2020) conducted an *in vivo* toxicity study on rats and observed the destruction and granulation of the protective mucus layer resulting detachment from the secretory layer. Thus, confirmed that

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*Corresponding Author, E Mail: nareshfishco@gmail.com

continuous consumption of formaldehyde-contaminated carps increases the risk of serious health problems like cancers of the lung, nasopharynx, oropharynx nasal passage and stomach.

Formalin treatment to fish either by dipping or spraying to marketed fish have been reported in many countries especially in South Asia (Hoque *et al.*, 2016; Sahu *et al.*, 2018). Hence, consumers became more concerned about safety due to frequent complaints of formalin in marketed fish. Consequently, the Central Institute of Fisheries Technology, Cochin, India under the aegis of the Indian Council of Agricultural Research (ICAR) came up with a formalin detection kit that detects formalin qualitatively. However, determining the exact quantity of formaldehyde added is challenging since certain fish possess naturally high levels of formaldehyde. Hossain *et al.* (2020) noticed that fish muscle that produced a higher amount of natural formaldehyde had a longer shelf life while having a lower microbial load and pH. The amount of formaldehyde in fish is highly variable and is mostly based on formation stages and environmental factors (more than 200 mg kg⁻¹ in some fish species) (EFSA, 2014). Jaman (2013) reported that natural formation of formalin during post mortem is much lower in freshwater fish compared to marine fish which might be due to high levels of trimethylamine oxide (TMAO) that acts as the natural source of formaldehyde (Bianchi *et al.*, 2007). After the death of the fish, TMAO get reduced to formaldehyde and dimethylamine by trimethylamine-N-oxide demethylase (TMAOase) (Badii and Howell, 2002; Leelapongwattana *et al.*, 2005) and the formation of TMAO increases concomitantly with the storage.

In addition to the direct health risk, formalin is also a potent dehydrating and protein denaturing agent, thus its impact on fish muscles should be addressed. In the North-Eastern region of India, notably in Tripura state, approximately 95% population consume fish. The per capita fish consumption of the state is

very high (20 kg) (Debnath *et al.*, 2018) compared to the national average (9.2 kg). As per capita consumption is quite high, therefore the supply-demand gap is filled by importing fresh fish from other areas of the nation as well as legal/illegal imports from Bangladesh. The majority of the fresh fish is imported to Tripura in iced condition from distant locations. Therefore, maintaining the prime quality is a challenging task for stakeholders. As a result, the incidence of using formalin in fresh fish in Tripura is highly probable to maintain freshness. Therefore, the objective of the present investigation is to detect the formalin adulteration in the fish samples collected from the four major markets of Tripura, India and its effect on the fish muscle properties.

MATERIALS AND METHODS

Fish collection and formalin treatment: The live catla fish (*Catla catla*) with an average length and weight of 30.56 cm and 1.2 kg respectively were purchased from the market and were transported to the laboratory of the Department of Fish Processing Technology and Engineering. After sacrificing the fishes, adhering dirt, filth and slime were thoroughly cleaned with cold potable water and were subjected to formalin treatment. The formalin treatment was given by dipping fishes in 10% formalin solution for 5 min. Subsequently, the treated fish were stored at refrigerated temperature for 24 h by wrapping in polythene packaging material and further analysis was performed.

Analysis of formalin in fresh fish samples collected from market: Total 99 samples of the different fish and shellfish were collected from four major markets *viz.* Lake Chowmuni market, Gol bazar, Lembucherra and Battala market of Agartala, Tripura, India. The fish samples were wrapped individually in a polythene bag and put in the ice-box during transportation until they reached to the research facility of the Department. Upon the arrival, qualitative detection of formalin was performed

using ICAR-CIFT formalin detection kit. The fish samples found positive for formalin content were subjected to quantitative analysis using the spectrophotometric method.

Analysis of formalin content: The spectrophotometric method with some modification (Benjakul *et al.*, 2003) using Nash's reagent was applied to determine the formalin content (mg/kg) in fish. Nash's reagent (Nash, 1953) was prepared by mixing ammonium acetate (15 g), acetyl acetone (0.3 mL), acetic acid (0.2 mL) and the volume were brought to 100 mL by adding water. Nash's reagent is a light sensitive and was kept in a dark-glass bottle covered with aluminum foil. A 0.1 N NaOH and 0.1 N HCl were used to adjust the pH of the distillate to 7.0.

The fish samples were cut into small pieces. A finely chopped fish (30 g) was added to 60 mL of 6% TCA solution and homogenized uniformly using a mortar and pestle. The mixture was filtered through a Whatman No. 1 filter paper, the filtrate (5 mL) was collected and pH of the filtrate was adjusted to around 7.0 with NaOH or HCl. The extract was then stored in a deep freezer for 30 min. The Nash's reagent

(2 mL) was added to the extract and heated in a water bath at 60°C for 30 min. The absorbance of the extract was measured at 415 nm immediately using a UV-Vis spectrophotometer. The standard curve was prepared using a 10-ppm standard formalin stock solution.

Preparation of formalin standard curve: The commercial formaldehyde solution was used to make 10 ppm standard stock solution. Thereafter, the standard curve was prepared by diluting 10 ppm stock solution of formalin into 0.0, 0.5, 1.0, 2.5, 5.0 and 10.0 ppm concentration. From each standard concentration, 5 mL was taken and 2 mL Nash's reagent was added and heated on water bath at 60°C for 30 min. The absorbance of each standard concentration of formalin was determined by measuring the absorbance at 415 nm using the UV-Vis spectrophotometer. A calibration curve was then plotted from obtained data (Fig. 1).

pH: A 5 g of fish meat sample was macerated with 45 mL of distilled water using pestle and mortar and the pH of slurry was measured using pH meter (Labman LMMP-30). Before

Fig. 1. Standard curve for formalin determination in unknown samples

measuring the pH, pH meter was calibrated with standard buffer solution of pH 4.2 and 9.2.

Protein solubility: The solubility of protein extracted from fish was determined using phosphate buffer (15.6 mM Na_2HPO_4 , 3.5 mM KH_2PO_4 , containing 1.1 M Potassium chloride, pH 7.5). The fish muscles were homogenized in phosphate buffer at 9000 rpm for 2 min with the help of a laboratory homogenizer and then centrifuged ($9000 \times g$) at 4°C for 15 min. The pellet homogenate before centrifugation and the supernatant obtained after the centrifugation were analysed for the amount of soluble protein (Robinson and Hogden, 1940). The protein solubility was calculated as: Protein solubility = (amount of soluble protein before centrifugation/ amount of soluble protein after centrifugation) $\times$ 100

Water holding capacity (WHC): The WHC for fish samples were measured according to the method of Jauregui *et al.* (1981) with slight modification. Meat sample of 5 g was weighed and put between two layers of filter paper (Whatman No. 1). Sample was placed at the bottom of 50 mL centrifuge tubes and centrifuged at $5000 \times g$ for 10 min at 4°C. Immediately after centrifugation, the sample was removed and reweighed. WHC was calculated as follows:

$$\text{WHC (\%)} = (W_1 - W_2) / W_1 \times 100$$

Where, W_1 is the initial weight (g) and W_2 is the final weight. Average of three replicates was reported as value of WHC.

Texture profile analysis (TPA): The TPA was performed for fresh and formalin-treated fish fillets using Texture Analyser (TA. XT2i Stable Micro System, Surrey, England) using a 75 mm compression plate with 50 kg load cell. The size of fish fillets used for TPA test was $2 \times 2 \times 2$ cm. The hardness, cohesiveness, springiness, gumminess and chewiness were measured.

Total plate count: Total plate count was determined for fresh and formalin treated samples by pour plate technique. About 10 g

of fish meat was taken and macerated in 90 mL 0.9% saline and the serial dilutions were prepared. From each dilution 1 mL of sample was mixed carefully to molten media (TPC agar) and poured onto sterilized Petri dishes. After solidification, the plates were incubated at $37 \pm 1^\circ\text{C}$ for 24 h. Colonies obtained were enumerated manually.

Statistical analysis: The data obtained were analysed by one way analysis of variance (ANOVA) using SPSS 16.0 software.

RESULTS

Screening of formalin contamination in the different marketed fish samples: The fish samples were collected from four important market viz. Lake Chowmuni market, Gol bazar, Lembucherra and Battala market of Agartala, India. In Lake Chowmuni market, total 38 fish and shellfish samples were collected. Out of these, 24 samples were found positive for formalin contamination (Table 1). In case of Gol bazar, 15 samples were detected with formalin presence out of 17 samples collected. Similarly, Battala market had contamination in 15 samples out of total 19 samples (Table 2). However, no sample found to be positive for formalin in Lembucherra market (Table 1).

Effect of formalin treatment on quality of catla fish: The initial formalin content in the untreated fish was 1.66 mg that increased to 23.24 mg/kg on dip treatment for five minutes in 10% formalin solution. The pH of the fresh fish was 6.48 which decreased to 6.17. The solubility and WHC of the fresh fish were 69.67% and 87.34% respectively which found to be decreased after formalin treatment. The initial TPC of fresh fish was log 5.27 that decreased to log 4.6 due to formalin.

The hardness, springiness, cohesiveness, gumminess and chewiness value of the fish fillets of untreated fish was 19.75 kg, -0.25, 0.45, 0.23, 45.15 and 20.65 which enhanced to 32.60, -0.77, 0.51, 0.28, 89.65 and 45.7 respectively after treatment with formalin.

Table 1. Qualitative and quantitative formalin detection in fish and shellfish of retail markets of Lake Chowmuni and Lembucherra

Lake Chomuni market (total samples-38)				Lembucherra market (total samples-25)		
Sl. no.	Fish species	No of samples	Qualitative analysis of formalin	Quantitative analysis of formalin (mg/kg)	Fish species	No of samples
1.	Rohu	1	+ve	1.355	Rohu	5
2.	Catla	2	-ve	ND	Catla	5
3.	Pomfret	2	-ve	ND	Pabda	5
4.	Tengra	5	-ve	ND	Kechki	5
5.	<i>Nandus nandus</i>	3	-ve	ND	Puntius	5
6.	Kechki	6	+ve	2.896		
7.	<i>Chanda nama</i>	1	+ve	2.939		
8.	<i>L. vannahmei</i>	2	+ve	0.942		
9.	Hilsha	2	-ve	ND		
10.	Mola	4	+ve	0.827		
11.	Esomus	2	+ve	0.927		
12.	<i>M. rogenbergii</i>	3	+ve	4.494		
13.	Prawn (small)	3	+ve	9.715		
14.	<i>Puntius</i> spp.	2	+ve	10.357		

+ve = Formalin presence, -ve = Formalin Absence, ND- Not Done, Tengra (*Mystus gulio*), Kechki (*Corica soborna*), Hilsa (*Tenulosa ilisha*), Mola (*Amblypharyngodon mola*), Esomus (*Esomus danricus*), Pabda (*Ompok bimaculatus*), *Puntius sophore*

Table 2. Qualitative and quantitative formalin detection in fish and shell fish from Gol bazar and Battala market

Gol bazar (total samples-17)				Battala market (total samples-19)		
Sl. no.	Fish local name/ scientific name	No of samples	Qualitative analysis of formalin	Quantitative analysis of formalin (mg/kg)	Fish species	No of samples
1.	Awanya	2	+ve	1.312	Kechki	4
2.	Pabda	2	+ve	0.742	<i>L. vannahmei</i>	3
3.	Kechki	4	+ve	0.813	Catla (<i>C. catla</i>)	2
4.	<i>Ailia coilia</i>	2	+ve	1.084	Rohu (<i>L. rohita</i>)	2
5.	Tengra	3	+ve	0.428	Bombay duck	1
6.	Catla (<i>C. catla</i>)	2	-ve	ND	(<i>Herpedon neharicus</i>)	
7.	Rohu (<i>L. rohita</i>)	2	+ve	0.670	<i>Glossogobius giuris</i>	3
					Tengra	4

+ve= Formalin presence, -ve= Formalin Absence, ND-Not Done

DISCUSSION

Formalin in the different market fish samples:

Considering the concerns of formalin contamination in marketed fish, a fish survey was undertaken to detect the formalin contamination in four main market places in Agartala, India. During the survey, total 99 fresh fish and shellfish samples were collected from four major markets. More than 54% of fresh fish and shellfish were found to be contaminated with formalin (Table 1 & 2). Amongst, 99 fish and shellfish samples collected, Bombay duck was found to have the highest formalin content of 42.75 mg/kg fish. Similarly, a study conducted in Kewatkhali market of Bangladesh also reported having significantly higher concentration of formaldehyde in Bombay duck (Noordiana *et al.*, 2011). The scientific panel of Food Safety and Standard Authority of India (FSSAI) fixed an adhoc maximum limit for formalin content with 4 mg/kg for freshwater fish and 100 mg/kg for brackish and marine water fish (FSSAI, 2019). As per the limit of FSSAI, only 12 % of fish and shellfish were found beyond the maximum limit. Among the species collected *Puntius* spp, freshwater prawn (*M. rogenbergii*) and small prawn from Lake Chowmuni market were detected to have higher value of beyond the level of acceptability. Among the four different markets, fish from Lembucherra was found to be free from formalin contamination whereas; Lake Chowmuni fresh fish samples were detected to have maximum (63%) formalin contamination (Table 1). It is crucial to emphasize that the Lembucherra market exists outside of Agartala city and receives the majority of its fish supply from local sources, whilst the other three markets import fish from other Indian states such as Andhra Pradesh, West Bengal and legally or illegally from Bangladesh. Therefore, the chances of formalin contamination were more likely in the other three markets fish samples. Devaraj *et al.* (2021) also investigated the occurrence of formaldehyde in fish and other seafood obtained from five important fish markets in

Chennai, India and revealed that formalin contamination varied from 58 to 100% of the fish samples. Kundu *et al.* (2020) reported the presence of formaldehyde in market samples of rohu and catla from local markets of the eastern region of India. A similar survey was conducted in three retail markets of Bombay and reported formalin content in rohu (*Labeo rohita*), catla (*Catla catla*), boyal (*Wallago attu*), Indian mackerel (*Rastrelliger kanagurta*) and Bombay duck (*Harpodon nehereus*) (Das *et al.*, 2018).

Effect of formalin treatment on residual formalin content in catla fish:

The formalin content in fresh fish was 1.66 mg/kg fish (Table 3). The result reveals that the fish developed formalin naturally following death due to degradation of TMAO. The occurrence of naturally high levels of formaldehyde may range from 0.1-0.3 mg/kg in milk to over 200 mg/kg in some fish species (EFSA, 2014). Jaman (2013) reported that the natural formation of formalin during post mortem is much lower in freshwater fish compared to marine fish. After treatment of the experimental fish with 10% formalin solution for 5 min, the content enhanced to 23.24 mg/kg fish (Table 2). Hoque *et al.* (2018) established that fish treated with formalin at increasing concentrations and exposure time showed increased values of formaldehyde absorption in fish muscles irrespective of fish species and analytical methods used.

Effect of formalin treatment on pH of fish muscle:

The pH of the fresh fish was 6.48 which indicates the good quality of the fish. On treating with formalin, the pH decreased (Table 3). The decreased pH values can be correlated to the lower pH of 10% formalin solution and also due to the formation of post mortem lactic acid.

Effect of formalin treatment on protein solubility of fish muscle:

The protein solubility of untreated fish in the present study was found

Table 3. Effect of formalin treatment on fish quality parameters

Parameters	Fresh Fish	Fish treated with 10% formalin
Formalin content(mg/kg)	1.66±0.04 ^a	23.24±0.08 ^b
pH	6.48±0.03	6.17±0.04
WHC (%)	69.67±1.5 ^a	59.04±1.28 ^b
Protein solubility (%)	87.34±1.98 ^a	69.33±4.34 ^b
TPC(log ₁₀ cfu/g)	5.27±0.14 ^a	4.6±0.05 ^b
Hardness (kg)	19.75±2.49 ^a	32.60±1.28 ^b
Adhesiveness(g-sec)	-0.25±0.09 ^a	-0.77±0.3 ^b
Springiness	0.45±0.16 ^a	0.51±0.04 ^b
Cohesiveness	0.23±0.01 ^a	0.28±0.02 ^b
Gumminess	45.15±4.63 ^a	89.65±3.79 ^b
Chewiness	20.65±9.58 ^a	45.7±2.47 ^b

Values are means ± SD, n=3, p< 0.05, value in the same row bearing unlike letters differs significantly

to be 87.34% and the value further reduced significantly in the fish samples treated with 10% formalin solution (Table 3). The reduction in the protein solubility can be due to the protein denaturation causing the aggregations effected by the formalin treatment (Morisasa *et al.*, 2020). The marked reduction in the pH value also found to be concurrent with the protein solubility and this might be due to shifting of isoelectric pH of the muscle protein. A similar reduction in protein solubility was reported for the protein extracted from rohu fish treated with 5% formalin solution (Yeasmin *et al.*, 2010).

Effect of formalin treatment on water holding capacity (WHC): In the present investigation, the WHC of the untreated fish muscle was 69.67% that was significantly reduced to 59.04% when treated with 10% formalin (Table 3). Due to the strong reactivity of the formaldehyde, cross links between the myofibrillar protein and formaldehyde are formed causing the toughening of the flesh and a reduction in water holding capacity (Haard and Simpson, 2000) leading to lower acceptability as well as functionality (Li *et al.*, 2007).

Effect of formalin treatment on muscle texture: In rigor or pre-rigor stage, fish remains stiffened and flesh hardness is one of the criteria

to assess the freshness of the fish. Further, soon after the resolution of the rigor mortis, the fish flesh becomes softer. Therefore, to understand the effect of formalin treatment on fish flesh hardness, a compression test was performed using a texture analyser. In the present study, the hardness of the fresh fish fillets without formalin treatment was 19.75 kg and the value increased to 32.60 kg after treatment with 10% formalin (Table 3). Yeasmin *et al.* (2010) found that the muscle texture of rohu fish (*Labeo rohita*) became slightly hard after dipping in 5% of formalin for 5 minutes. Similarly, Morisasa *et al.* (2020) measured the hardness of the sample that was soaked with 1000 ppm formaldehyde (FA) and found that the samples exhibited significantly harder values in comparison to samples soaked with 0 ppm FA. The reason for the increase in the hardness could be due to reduced water holding capacity and dehydration of muscles. In addition, the protein aggregation stimulated by formalin treatment is also one of the important factors for muscle stiffening (Morisasa *et al.*, 2020).

Effect of formalin on the total plate count (TPC): In the present investigation, the initial microbial load in the fish samples was about 5.27 log₁₀ CFU/g (Table 3) indicated good quality of the fish. The microbial load for the

good quality fish had been reported to range from 2 to 6 log₁₀ CFU/g (Huss, 1995). This load varies from species to species depending on the place of harvest, condition, and temperature of post-harvest operations (Perigreen *et al.*, 1987). Further, in the formalin treated fish, the TPC value found to be decreased to 4.6 log₁₀ CFU/g. Uddin *et al.* (2014) established that formaldehyde acts as an antibacterial and antifungal agent; therefore, it may inhibit the microbial infestation on the fish skin or in fish fillet, and delay the spoilage of formalin treated fish samples.

The study showed that around 50% of the fish samples collected and tested for formalin were found positive for the test. The endogenous production of formaldehyde, on the other side, may pose a difficulty in actual measurement. Apart from the known carcinogenic impact of formaldehyde, the study found that it degraded the textural and nutritional properties of the fish. As a result, stringent measures should be taken to avoid such malpractices and assure consumer food safety. Government agencies should take proactive measurements such as public

awareness and random check for formalin adulteration in the markets in different season.

Conflict of interest: Authors have no conflict of interest in this study.

Author's contribution: NKM: Involved in work execution, manuscript drafting and planning the experiments; RKM: Involved for planning and monitoring of the experiments; DP: Concerned with the execution and data analysis of the experiments; MBP: Engaged in interpretation of the results and manuscript reviewing; BD: Involved in data analysis and manuscript editing.

ACKNOWLEDGEMENTS

The authors are thankful to the Dean, College of Fisheries, Central Agricultural University (Imphal), Lembucherra for providing us the facilities to carry out the work. Authors are also thankful to the Department of Biotechnology under the project- Centre of Excellence- DBT-NER/LIVS/05/ 2011 Phase II for providing fund of the research work.

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