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RESEARCH ARTICLE

Formalin in fish trading: an inefficient practice for sustaining fish quality

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Abstract. The illegal use of formalin for quality control purposes is a widespread practice in today's fish industry. Therefore, the present study was undertaken to assess the quality characteristics of formalin treated (5% formalin for 30 min) mrigel carp, *Cirrhinus mrigala*, Hamilton, stored on ice. The formalin detected was 12.19 ± 0.814 μg g^{-1} for freshly treated samples and 8.10 ± 0.068 μg g^{-1} for treated samples stored on ice. In the control fish, the formalin concentration was almost negligible. The assessment of the organoleptic characteristics of the treated fish revealed that the gills had blackened, the eyes had become whitish and opaque, the muscles had lost tenderness, and the fish had stiffened. The quality assessment score of the control batch was significantly higher than that of the treated batch. The microbes in the fish were still lower than the amount specified for fish spoilage. Lipid hydrolysis in the treated fish was much higher than that of control, especially when storage time increased. High levels of trichloroacetic acid (TCA) soluble protein in the treated fish, as compared to that of the control fish, were observed throughout the storage period. Considering the low organoleptic status and poor nutritional quality, formalin treatment in the fish industry is not recommended even though the formalin content and the microbiological counts were still below permissible limits.

Keywords: formalin, ice, poor nutritional quality, quality control, TCA soluble protein

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Introduction

Fish is a high-value crop with increasing consumer demand. Fish are prone to rapid post-harvest spoilage because of their composition (Ismail 2005). The freshness of this extremely perishable food item influences considerably its acceptability to the consumer (Sikorski et al. 1976, Shenouda 1980, Connel 1995). Inadequate preservation facilities and lengthy transport from distant places lead to considerable decreases in fish quality by the time the products reaches consumers (Haque and Mohsin 2009). In this scenario, many fish traders use formalin to prevent spoilage and increase shelf life (Yeasmin et al. 2010).

Recently, several media outlets have reported on formalin preserved fish across Asia. In Hong Kong, noodle fish are regularly found to be contaminated with formalin (Aminah et al. 2013). The Malaysian

government have information that imported fish like cod, salmon, and tuna have formalin in them. Fish are dipped in formalin before being transported from fishing ports to the inland markets in Sri Lanka. In Tanzania, Indonesia, China, and Ghana, the use of formalin to store fish is widespread (Goon et al. 2014). Many studies done by the Bangladesh government show evidence of high formalin levels in imported freshwater fish, while the amounts in local varieties of the same species are almost negligible (Hossain et al. 2008). The high formalin during content in imported freshwater fish definitely confirms this illegal practice of formalin preservation (Bianchi et al. 2007). The natural formation of formalin post mortem is reported to be much lower in freshwater fish than in marine fish (Jaman 2013). Formalin contamination is also reported in India in fish of the genus *Pangasius*, a type of Vietnamese catfish farmed in Andhra Pradesh.

Among different fixatives, attention has been paid to formalin as it is listed as a group 1 carcinogen by the International Agency for Research on Cancer (IARC 2004). Thus, even smoked fish are not permitted to absorb formalin in excess of 5 μg g^{-1} during processing (Malaysian Food Regulations 1985 and 2006). The established fact is that fish contaminated with formalin have a longer shelf life than fish stored on ice (Yeasmin et al. 2010), but little is known about the quality aspects of fish contaminated with formalin. Prolonged dietary formalin exposure could be potentially lethal even in low amounts. The present paper, therefore, focuses on estimating formalin levels and nutritional status assessments of fish contaminated with formalin.

Materials and Methods

Mrigel carp, *Cirrhinus mrigala*, Hamilton, weighing between 50 to 55 g (1.4 kg in total) were purchased from Maniktala market in Kolkata, West Bengal, India from July to October 2014. Samples were transported to the Bethune College laboratory within a half an hour in an insulated ice box (at a fish to ice ratio of 1:1). The fish were then separated in two different lots for analysis, i.e., ice stored fish with (treated) or without (control) formalin treatment. Fish from the treated group were bathed in a 5% formalin solution for 30 min before ice storage. The fish specimens from the two lots were kept in separate ice boxes surrounded by crushed ice at a 1:1 fish to ice ratio. The boxes were stored in a refrigerator at 4°C. When required, the ice was renewed. Control and treated fish samples were taken for analysis on days 0, 8, 12, and 14 of ice storage.

Determination of formalin

The formalin content of the fish samples was determined with the spectrophotometric method of Nash (Castell and Smith 1973) in a Trichloroacetic acid (TCA) extract of the fish muscle (Benjakul et al. 2003). Absorbance was measured at 415 nm, and the formalin content was calculated from a standard curve (Noordiana et al. 2011).

Organoleptic qualities

Organoleptic changes were evaluated in terms of five primary quality parameters of the fish, namely: general appearance (skin, stiffness, and flesh texture); eyes (pupil); gills (color); and abdomen (peritoneum and intestine) according to EEC guidelines (EEC 1976) of fish quality assessment. Samples were analyzed for organoleptic qualities on days 0, 6, 8, 12, and 14 of ice storage.

Muscle pH estimation

Measurements of pH were taken with a pH meter (pH TUTOR, Eutech) after homogenizing 4 g of muscle in 20 ml distilled water (Yeasmin et al. 2010).

Aerobic plate count (APC)

Standard plate count method at 37°C on Tryptone glucose yeast extract agar was used to determine aerobic plate count of bacteria (APHA 1998).

Acid value (AV) (free fatty acid (FFA)) measurement for oxidative lipid damage

Samples of 0.8 g of muscle were homogenized in 10 ml of neutral solvent and then titrated by 2.02 N KOH (MERCK) (Sadasivam and Manikam 1996).

TCA soluble protein assay (TSP) for proteolytic degradation assessment

For total protein, 0.5 g of muscle was homogenized in 5 ml of PBS buffer (pH 7.4) with a manual homogenizer (Borosil) on ice. The homogenate was then centrifuged (Cecilce 4002 : 4000 Series) at 10,000 rpm for 30 min at 4°C. The supernatant was collected and subjected to the Lowry protocol (Lowry et al. 1951) for protein estimation using a UV-VIS Spectrophotometer (Shimadzu; Model UV-1700 Pharma Spec). For the TCA soluble protein assay (TSP) assay, 1.11 g of muscle was homogenized in 10 ml of 5% TCA (Merck) solution with a manual homogenizer (Borosil) on ice. The homogenate was then incubated on ice for 1 h and centrifuged (Cecilce 4002 : 4000 Series) at 8500 rpm for 15 min at 4°C. The supernatant was collected for TSP estimation with the Lowry protocol (Lowry et al. 1951) for protein estimation using a UV-VIS Spectrophotometer (Shimadzu; Model UV-1700 Pharma Spec) (Benjakul et al. 2003). The percentage of TSP estimated with the following formula: (TCA soluble protein × 100/Total Protein).

Statistical analysis

The One-Way ANOVA test was performed to examine whether there were significant differences in the formalin contents of treated fish analyzed that had been stored on ice for different periods. Data from the various biochemical measurements were subjected to the paired *t*-test for means ($P < 0.05$). The statistical analysis was done in Excel 2003 (Microsoft Seattle, WA, USA) with the add-in software Statcel 2 (Yanai 2004).

Results and Discussion

The highest amounts of formalin ranging from 11.52 to 13.10 μg g^{-1} were detected in the fish muscle immediately after treatment. The formalin concentrations estimated in treated fish after 14 days of ice storage varied from 8.49 to 7.93 μg g^{-1} at a mean of 8.10 μg g^{-1}. The formalin content observed in the treated fish on day 8 that was lower than that of the fresh treated fish (Table 1) could have stemmed from the formalin washing off along with the melting ice or the conversion of formaldehyde to other chemical compounds (Tsuda et al. 1988). Further, no significant difference (ANOVA Single factor, $F_{2,5} = 0.44$; $P > 0.05$) was found in the recovery rate of formalin from treated samples on days 8, 12, and 14 of storage. The results indicated that a constant level of formalin was retained by the fish muscle after initial washing (Table 1). The formalin concentrations in the control fish varied between 0.001 to 0.067 μg g^{-1} under iced conditions (Table 1). Negligible amounts of formalin in control fish throughout the storage time indicates the formation of a low amount of naturally occurring formalin in fresh water varieties. This fact was further confirmed by obtaining no significant change ($P > 0.05$) in formalin recovery from the ice stored treated fish on days 8, 12, and 14. Thus, the high formalin content in the treated fish definitely stemmed from the added formalin that does not occur naturally. The formalin level was reduced, but not fully removed, throughout the storage period.

Table 1
Amount of formalin (mean± SE) in fish muscle during the storage period

Storage period	Treated fish	Control fish
0 d	12.197 ± 0.814^a	0^b
8 d	8.095 ± 0.077^a	0.067^b
12 d	8.153 ± 0.296^a	0.070^b
14 d	8.003 ± 0.063^a	0.066^b

*Different superscripts represent significant statistical differences, $t_{(4)} = 8.53$; $P < 0.01$

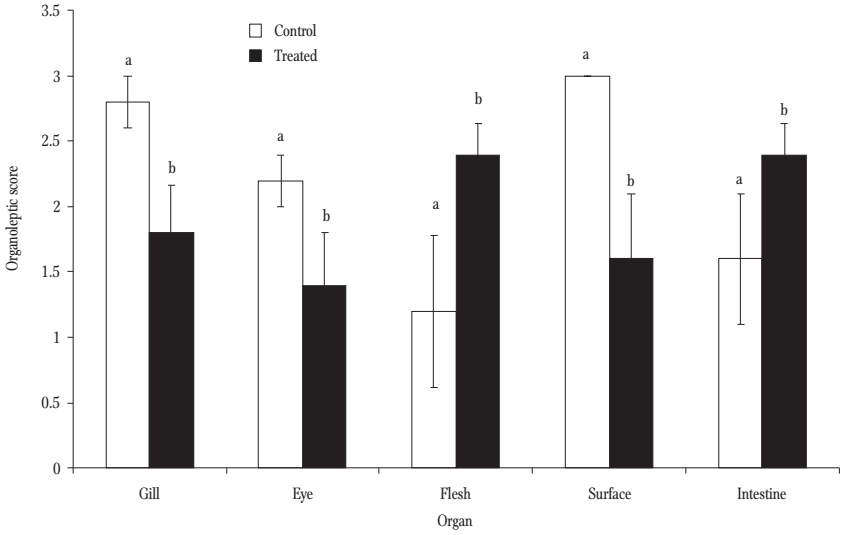

Figure 1. Sensory evaluation of control and treated samples stored on ice. Data with different letter index in the organs differ significantly statistically ($P < 0.05$).

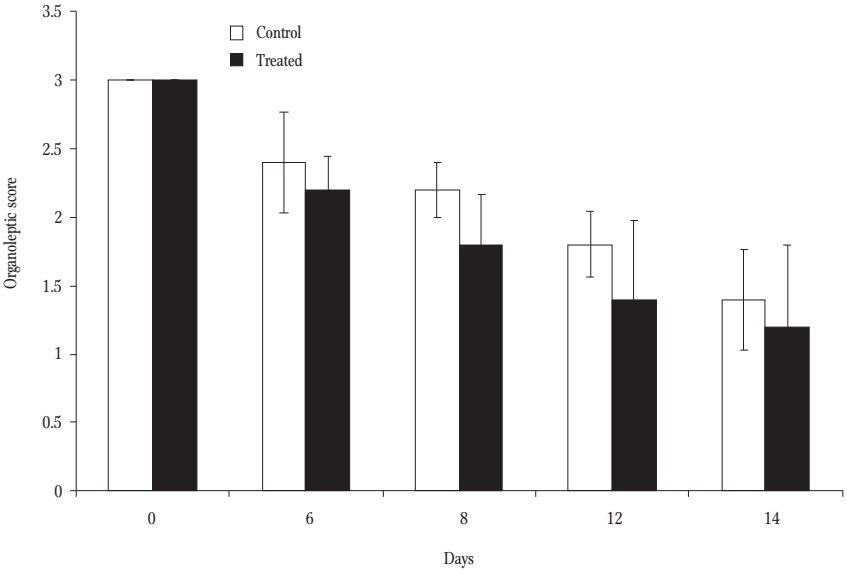

Figure 2. Relationship between organoleptic score of control and treated samples on the days of storage.

Thus, the conclusion reached is that once it is added, formalin cannot be removed.

The organoleptic scores for each parameter throughout the storage period are presented in Fig. 1. The decrease in sensory scores (Fig. 2) was highly correlated ($r = - 0.99$) with storage time, which indicated quality deterioration. The total points calculated up to day 14 of ice storage were significantly higher in the control (2.16 ± 0.27) than in the treated samples (1.9 ± 0.32) ($t_{(4)} = 3.20$; $P < 0.05$). The condition of fish preserved with formalin was unacceptable in terms of the blackish gills and opaque, milky eyes. Loss of brightness along with definite dullness was evident in treated samples at 6, 8, 12 and 14 d of storage. The treated fish also lost their natural odor and were found to emit strong formalin odor. The only good characteristic observed in the treated fish even on day 14 of ice storage was a firm body (although a little too hard) and the smooth surface without wrinkles compared to the fresh fish, which had soft, flaccid bodies and wrinkled surfaces.

Deteriorative changes in the gills, eyes, and surfaces (dull) were the primary cause for low organoleptic points in the treated fish (Belton et al. 2011). Further, the addition of formalin to ice-stored fish resulted in a rubbery texture. The aggregation of myofibrillar proteins by formalin leads to this textural change (Haard 1992). The current study on the use of formalin presents controversial results in light of earlier studies (Hossain et al. 2008, Haque and Mohsin 2009), in which formalin was shown to enhance to fish shelf life and freshness. However, the formalin treated samples were in more acceptable condition than were the controls in terms of firm texture, smooth (wrinkle-less) surface, and good (sticky) peritoneum. This was likely caused by formalin reducing the spoilage activity of some bacteria.

The pH of the fresh control fish was 6.72 ± 0.02, which gradually increased to 6.86 ± 0.01 at the end

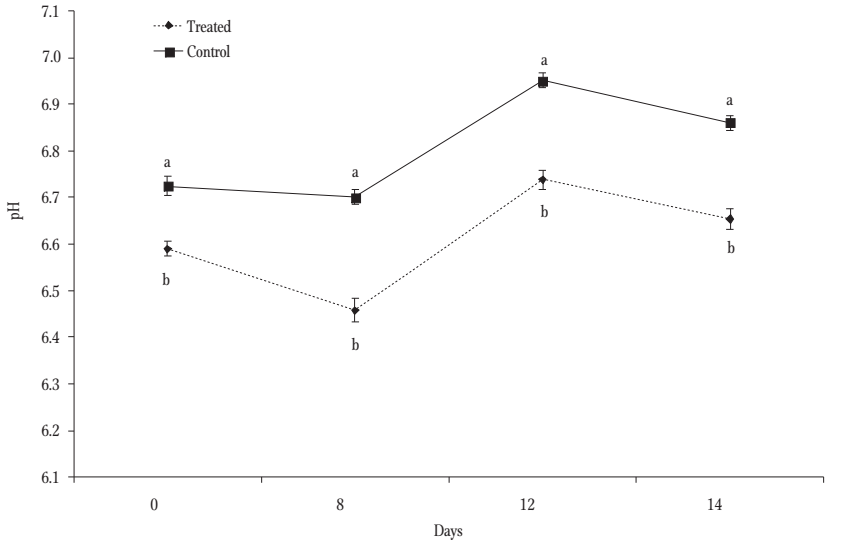

Figure 3. pH in control and treated fish during storage period. Data with different letter index in the same day differ significantly statistically ($P < 0.05$).

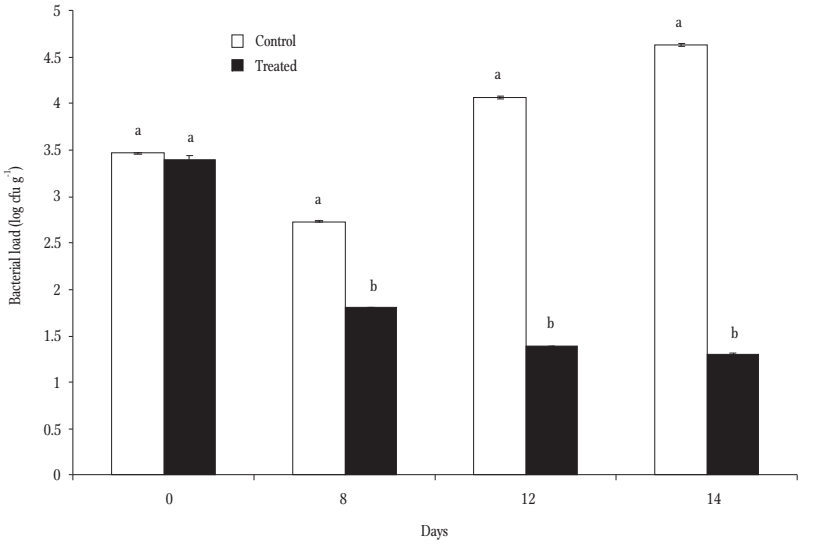

Figure 4. Bacterial load (in log $_{10}$(cfu × g^{-1} of muscle)) in fresh and formalin treated fish. cfu = colony forming unit. Data with different letter index in the same day differ significantly statistically ($P < 0.05$).

of 14 days of ice storage (Fig. 3). On day 0, the pH value of the treated fish was 6.59 ± 0.01, while during chilled storage, the values ranged from 6.45 ± 0.02 to 6.65 ± 0.02 (Fig. 3). However, the pH of the treated batch was significantly lower ($t_{(19)} = 19.53$; $P < 0.001$) than was that of the control.

The bacterial load remained at a significantly high level ($t_{(14)} = 8.54$; $P < 0.001$) in the control compared to the treated mrigel during the storage period (Fig. 4). Moreover, the aerobic plate count of the control fish at the end of 14 days of ice storage was much less than 8-9 log cfu g^{-1} (Huss 1995); therefore, it was still not considered to be spoiled.

The low bacterial counts in the treated fish were related to the bactericidal effects of formalin. The production of alkaline bacterial metabolites coinciding with high APC counts might be responsible for the high pH of the control samples. The low pH of the fish treated with formalin retarded bacterial growth.

The acid value in both the treated (except day 12) and control fish (except days 8 and 12) increased over the course of iced storage. As for the formalin treated fishes, the acid value was found to be high ($t_{(24)} = 3.61$; $P < 0.01$) as compared to the control (Fig. 5). Lipid hydrolysis in the tissue resulted in the accumulation of free fatty acid (Aubourg et al. 2004, Rodriguez et al. 2007), thus raising the acid value. Free fatty acid has a pro-oxidant effect on lipids (Rodriguez et al. 2007).

TCA-soluble peptides in both the control and treated fish increased through to day 22 of iced storage (Fig. 6), which suggests the degradation of the fish protein (Benjakul et al. 2003). The treated fish had more TCA-soluble peptides than did the control fish ($t_{(19)} = 6.42$; $P < 0.001$), even in the initial days of storage. On days 8, 12, and 14, the TCA-soluble peptides in the treated samples were 1.84, 1.82, and 1.37 times higher than the values determined in the control samples. Higher TCA soluble peptide formation in the treated samples than in the control samples indicates the profound effect of formalin in protein degradation (Parkin and Hultin 1982).

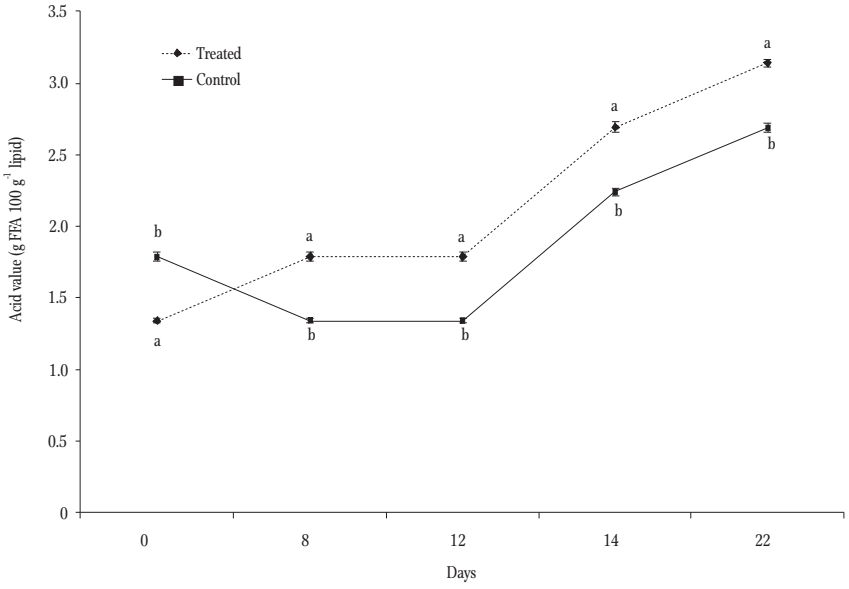

Figure 5. Acid value in control and treated fish during storage period. Data with different letter index in the same day differ significantly statistically ($P < 0.05$).

An increase in TCA-soluble proteins was noted as the storage period progressed; however, at that time the treated fish contained much lower amounts of formaldehyde. This suggests that the initiation of protein degradation by formalin requires some time. Once conformational change is initiated, formalin reacts more efficiently with already denatured proteins and accelerates further denaturation. This fact is further evidenced by the rubbery texture of the late-stage treated fish. Moreover, the acid value in treated mrigel was attributed to formalin content in a way similar to the TSP (%) assay.

The low pH of the treated fish muscle during ice storage contributed to muscle degradation as evident from the rise of TCA soluble proteins (Reddy and Srikar 1991). The net surface charge and water holding capacity of muscle proteins decrease as pH drops. This leads to textural changes such as loss of juiciness and a hard fibrous product (Careche et al. 1999). Changes in the levels of acid values corresponded to the values of protein deterioration. Thus, the results agree with previous reports that free fatty acids interact with proteins resulting in quality degradation. Microbial proteinases (Benjaku et al. 2003) and lipases (Farag 2012) play essential roles in protein and lipid degradation (Aubourg et al. 2004). Microbial activity generates small peptide fragments (Griswold et al. 1999). In spite of low bacterial activity, the higher values of TCA soluble proteins and free fatty acid in the treated samples in comparison to those in the control samples confirmed the effect of formalin in protein degradation and lipid hydrolysis.

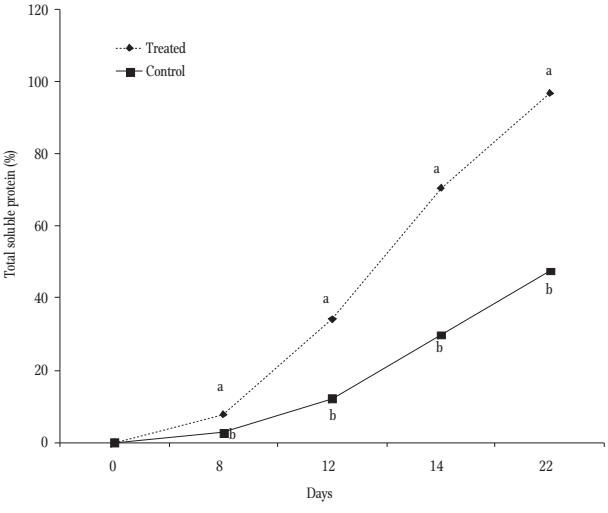

Figure 6. Total soluble protein (TSP,%) value in control and treated fish during storage period. Data with different letter index in the same day differ significantly statistically ($P < 0.05$).

Conclusions

The study clearly shows that once added, formaldehyde content decreased but could not be fully removed from the samples. Nevertheless, the continuous ingestion of formaldehyde, even in low doses, in fish could be hazardous to the human body. Additionally, the presence of high TCA soluble protein solubility stemming from the denaturation of muscle proteins clearly indicates that formalin contaminated fish cannot be considered as a principle source of animal protein. Hence, the procedure

should not be applied on a commercial basis for the post-harvest storage of fish for human consumption.

Author contributions. S.S. and K.S. conceived of and designed the experiments, analyzed the data, S.W.S. and K.S. performed the experiments, S.S. and S.B. interpreted the data and wrote the paper. Both S.S. and S.B. supervised the whole work and edited and revised the manuscript.

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