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Quality Changes in Formalin Treated Rohu Fish (*Labeo rohita*, Hamilton) During Ice Storage Condition

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Abstract: The study was conducted to evaluate the influence of formalin on the quality changes in rohu fish (*Labeo rohita*, Hamilton) during ice storage condition. There are complaints from the consumers that the fish traders in Bangladesh use formalin in fish imported from neighboring countries to increase the shelf life. On the basis of organoleptic characteristics, the formalin treated fishes were found in acceptable condition for 28 to 32 days in ice as compared to the control fish, which showed shelf life of 20 to 23 days. Bacterial load in formalin treated fish was below detection level even after 16 days of ice storage whereas bacterial load was significantly higher in fresh rohu stored in ice and at the end of 24 days of ice storage. NPN content was increased gradually in fresh rohu with the increased storage period in ice. On the other hand, NPN content of formalin treated rohu decreased gradually during the same period of storage. Protein solubility in formalin treated fish decreased significantly to 25% from initial of 58% during 24 days of ice storage as compared to 40% from initial of 86.70% for control fish during the same ice storage period. Gel forming ability of formalin treated fish was reduced significantly due to denaturation of muscle protein indicating poor eating quality and poor digestibility of fish contaminated with formalin.

Key words: Bacterial load, formalin, gel forming ability, ice storage, *Labeo rohita*, NPN, protein solubility, shelf life

INTRODUCTION

Rohu, *Labeo rohita* (Hamilton) is a member of the family Cyprinidae within the order Cypriniformes. It is native to the river systems of Bangladesh, India, Pakistan, Myanmar (Talwar and Jhingran, 1991). Among the world's principal aquaculture species, rohu production ranked seventh (754 677 tonnes) in 1998 (FAO, 2000). In Bangladesh, this species is mostly found in the three river systems including Padma-Brahmaputra and Halda. With the expanding aquaculture practice in the country, rohu culture is practiced in semi-intensive and extensive systems in ponds, low-lying areas like haor and baor. The features that have made this species a potential candidate for aquaculture include faster growth rate, higher market price, greater feed utilization and ability to feed in all three columns in a water body (BBS, 2000). Even though the total fish production in Bangladesh has increased significantly over the last few decades (DoF 2009), it is still not sufficient to meet the growing demand of its population. This paves the way of enter imported fishes from neighboring countries into the domestic market (Kibria, 2007). It was reported that more than 80 MT of fish and fishery products enter into Bangladesh every day through the Teknaf border from Myanmar.

In our preliminary study on the detection of formalin in fishes available in the domestic market, we found that fish imported from neighboring countries were contaminated with formalin ranging from 0.5-1%. Other investigations conducted by the Fish Inspection and Quality Control (FIQC) office of the Department of Fisheries (DoF) showed that formalin was added in imported fish somewhere in the marketing chain after entering the country. Several test conducted by FIQC showed that after landing in Teknaf port, no formalin was detected in fishes whereas formalin contamination was detected when samples were collected from the marketing channel. It is likely to assume that the fishes are dipped into formalin solution for a while by the some fish traders before transporting to the different retail markets (DoF, 2007).

It is well known that formalin is injurious for human health. Formalin is a solution of 37% (w/w) of formaldehyde (H-CHO) in water which is used as a preservative in medical laboratories, as an embalm fluid and as a sterilizer. Formalin is also used in treatment against fish diseases caused by protozoa and fungi. Food and Drug Administration of the United States approved formalin for use in aquaculture as parasiticides and fungicides. It is, however, not approved for usage in

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aquaculture in Europe and Japan because of its association with cancer and tumor development (Wooster, 2005). Use of formalin in food for human consumption is also banned in Bangladesh. However, formalin contamination is reported to occur in table fish marketed in the country.

Although there has been considerable study on the shelf life fish under various storage condition including icing, freezing, drying and other fish preservation methods, little is known about the quality aspects of fish stored using formalin. It is assumed that post-mortem changes are greatly influenced by formalin. The changes include physical, biochemical and bacterial aspects of fish muscle and other nutritional constituents. The present study reports comparative study on the quality aspects of fresh rohu fish with that of formalin treated fish and identifies the quality changes resulting from formalin treatment.

MATERALS AND METHODS

Raw materials: Live rohu fish with an average body weight of 650 g and size 38 cm were purchased from Bangladesh Agricultural University (BAU) fish market, Mymensingh during the period of June, 2008 and transported to the Department of Fisheries Technology, BAU, Mymensingh in an insulated box in ice (fish, ice ratio 1:1). The fishes were divided into two groups: one group of 20 kg fishes were treated with 5% formalin for 5 min and stored in ice. Another group of 20 kg were stored in ice without formalin treatment. A comparative study on shelf life of the two groups of fish samples was conducted at the laboratory of Department of Fisheries Technology by determining organoleptic, biochemical and bacteriological aspects.

Organoleptic quality assessment: Sensory methods were used to assess the degree of freshness based on organoleptic characteristics such as odour, colour, general appearance, eyes, slime and consistency of flesh. Starting from 0 day, three to four fishes were randomly sampled and their raw sensory attributes were evaluated. The organoleptic characteristics were judged by a trained panel of expert members during the storage period. The grading of fish using score on the characteristics has been followed by Multilingual Guide to EC Freshness Grades for Fishery Products (Howgate *et al*., 1992) to judge the quality of the fish.

Physical and chemical analysis: pH was measured using a pH meter (Corning Model 250) after homogenizing 2 g of fish muscle with 10 mL distilled water in a blender. Amount of non-protein nitrogen (NPN) was determined as described by Perez-Villarreal and Howgate (1987).

Aerobic plate count (APC): About 10-15 g of whole fish sample was blended with appropriate volume of 0.2% peptone water in a sterilized blender for a few minutes until homogenous slurry was obtained. Total APC expressed as colony-forming units per gram of muscle (CFU/g) of the representative samples was determined by standard plate count methods using plate count agar (Hi-media, Mumbai, India) according to Collins and Lyne (1976).

Myofibril preparation and protein solubility determination: Myofibrills were prepared from ordinary and dark muscles immediately after excision according to Perry and Grey (1956) with slight modification. The muscle was chopped by a meat grinder and chilled minced muscle (50 g) was homogenized for 1 min in 5 volumes of 39 mM borate buffer (pH 7.1) containing 25 mM KCl and 0.1mM DTT. The homogenate was centrifuged for 15 min at 600×g. The residue obtained was again homogenized and centrifuged for 15 min. The light-coloured upper layer of the residue consisting mainly of myofibrill was recovered with small volume of 39 mM borate buffer (pH 7.1) containing 0.1M KCl and 0.1 mM DTT. The suspension was centrifuged for 15 min to remove the supernatant. Myofibrils were diluted with 4 volumes of 39 mm borate buffer (pH 7.1) containing 0.1M KCl and 0.1 mM DTT, and coarse materials removed by centrifuging at 400×g. The suspension was centrifuged again for 15 min at 600×g to sediment myofibril. After the pellet was washed three times in the same way, myofibrils were suspended with a desired volume of 39 mm borate buffer (pH 7.1) containing 0.1M KCl to make a concentration of 10-15 mg/ml.

Two mL of myofibrillar suspensions (5 mg/mL) were homogenized with 2 mL of 1M KCl plus 100 mM phosphate buffer (pH 7.0) using a homogenizer. The homogenate was allowed to stand at refrigerated temperature (4ºC) overnight. The suspension was centrifuged for 30 min at 400×g in cool condition. The protein in supernatant was determined by the Biuret method (Gornall *et al*., 1949).

Determination of gel forming ability:

Preparation of meat paste: Dorsal and lateral muscles were excised as fillet form to prepare meat paste. Attention was paid while removing kidney tissues as they form globular masses, which affect both texture and appearance of the product. Skin and belly fats were carefully removed. Manually operated meat mincer was used to mince the excised fillets, which were cooled at 4ºC before every operation. During excision of fillet and preparation of mince, the products were always kept in ice cooled container. Immediately after mincing, it was ground with 3% NaCl by a previously cooled (4ºC)

mortar for 25 min. Due to this grinding with salt the mince transformed in to viscous past. The salt ground paste was then carefully stuffed into heat stable polyvinyledene chloride cylinder manually and the both ends of the cylinder were wrapped with parafilm and polyethylene paper.

Preparation of gel: The paste in cylinders was heated to produce gel. Some samples were heated once only in well stirred water bath, whilst the rest were heated twice. For convenience, the former method of heating is called one-step heating and the latter two-step heating. All heating treatments were triplicates. In the one-step heating, samples were heating for 120 min. in water bath at temperature of 30º, 40º, 50º, 60º, 70º, and 80ºC. In the two-step heating, the first heating was for 120 min. in water bath at temperature 30º, 40º, 50º, 60º, 70º and 80ºC. This first heating will be conveniently called pre-heating from now on. After this pre-heating treatment, they were immediately heated for another 30 min. in water bath at 85ºC. After heat treatments, the samples were taken out of the water bath, kept in iced water for 1 hour and subjected to measurement of pH and gel strength (Poon *et al*., 1981).

Statistical analysis: Data from different biochemical measurements were subjected to *t*-test ($p<0.05$). The statistical analysis package statview 5.0 for Macintosh (SAS Institute Inc., NC, USA) was used to explore the statistical significance of the results obtained.

RESULTS AND DISCUSSION

Changes in organoleptic qualities of formalin treated fish: Studies were conducted to evaluate the changes in organoleptic quality of rohu fish treated with formalin during ice storage in an insulated box. Immediately after dipping the fish in 5% formalin for 5 min and putting into ice, there was strong formalin odour emitting from fish. The gill became slightly blackish and there was slight loss of brightness. The eye became sunken and the muscle texture became slightly hard. At that time the fishes were found acceptable condition and were found in acceptable conditions for 28 to 31 days of ice storage. The fishes were rejected after 32 days of storage. On the other hand, shelf life of fresh control rohu fish during ice storage was found to be 20 to 23 days. Shelf life *Catla catla* and *Labeo filbriatus* was reported to be 18 days in ice storage (Bandyopadhyan *et al*., 1985). Faruk *et al.* (1994) have reported on Indian major carps and other commercially fish species and found that the fish can be kept in ice in edible condition up to 2-3 weeks. The present study demonstrated that the use of formalin in fish increased the shelf life of rohu by 6 days compared to those of the control fish stored in ice. It is likely to assume that formalin reduces the water activity inside fish muscle, which reduces action of some spoilage bacteria.

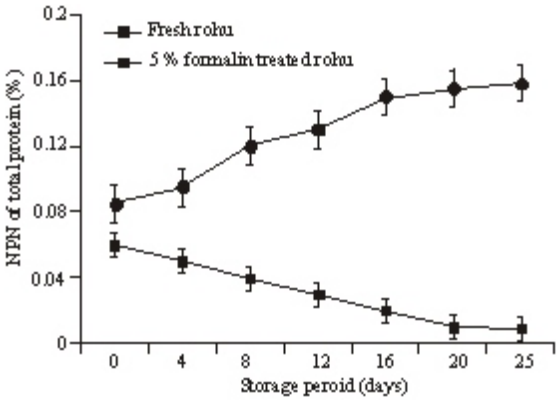

Fig. 1: Non-protein nitrogen (NPN) of fresh and formalin treated rohu fish (*Labeo rohita*) during ice storage. Vertical bars denote SD

Changes in chemical characteristics of formalin treated fish: Changes in fish muscle pH of ice fish during 24 days of ice storage was determined where the initial pH of fresh rohu was in the range of 6.09-6.39, reflecting good nutritional state of fish, which increased gradually to a value up to 7.92-8.02. As for the formalin treated fishes, pH was found to be significantly lower as compared to control with no significant change with lapse of ice storage period. NPN content of rohu treated with formalin during ice storage is represented in Fig. 1. In fresh rohu, the initial NPN content was 0.085% which increasing of almost linearly with the increased of the storage period where NPN content reached to 0.158% after 24 days of storage. On the other hand, the initial NPN content of the formalin treated fish was higher with a value of 0.06%. With the lapse of storage period NPN content decreased significantly and at the end of 24 days of ice storage, it reached to 0.009%. The deteriorative changes in fish muscle are associated with the hydrolysis of cellular compounds by intracellular enzyme and bacterial enzyme during post-mortem period. Hydrolysis of protein and other nitrogenous compounds by autolytic enzyme together with bacterial action leads to an increased in the NPN content. There was a clear trend to increasing NPN content with the laps of storage period but the values were within the limit set for fresh fish as reported by Tarr (1965) for teleosts. Faruk (1994) reported increasing NPN content of rohu with increasing ice storage period, where bacterial load also increased. Non-protein nitrogenous compounds as a whole form a relatively small proportion of total nitrogen. Normally the NPN values may vary considerably from species to species and even the individual of the same species due to variety of causes such as sex, age, season, feeding habit

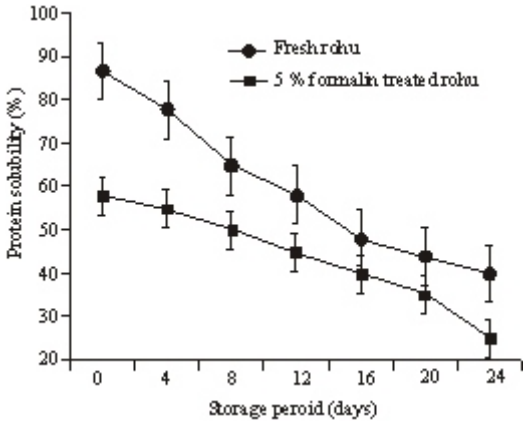

Fig. 2: Changes in myofibrillar protein solubility of fresh and formalin treated rohu fish (*Labeo rohita*) during ice storage. Vertical bars denote SD

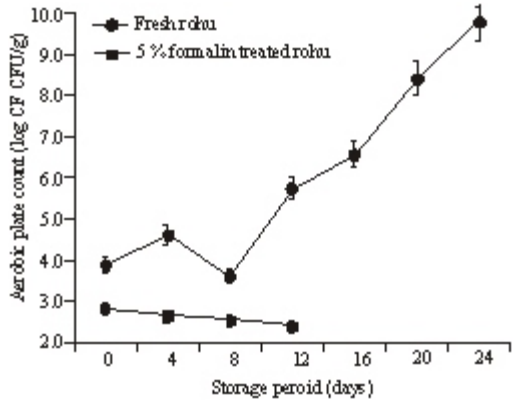

Fig. 3: Bacterial loads of fresh and formalin treated rohu fish (*Labeo rohita*) during ice storage. Vertical bars denote SD

and spawning cycle. The result of the present study indicated that the low bacterial load in formalin treated fish during ice storage is the reason for low NPN content. There is a direct relationship between the bacterial load and NPN content where NPN content was higher with the higher bacterial load.

Changes in myofibrillar protein solubility in formalin treated fish: Studies were also conducted on the changes in protein solubility in rohu fish treated with formalin during ice storage (Fig. 2). In fresh rohu, initial myofibrillar protein solubility was 86.70%, which decreased gradually with the increasing of storage period. At the end of 24 days of storage, solubility was reduced to 40%. On the other hand, initial myofibrillar protein solubility of formalin treated fish was significantly lower than that of fresh fish with a value of 58%, which also decreased gradually during the ice storage. At the end of 24 days of storage myofibrillar protein solubility of formalin treated fish reduced to 25%. Seki *et al.* (1979) reported that solubility of carp myofibril decreased from 95 to 20% during ice storage within 2-3 weeks. The large fall in solubility during ice storage in fish muscle was caused by the decrease in pH (Kramer, 1981) and aggregation of myofibrillar proteins (Reddy and Srikar, 1991). The rapid decline in solubility of myofibrillar protein during the storage period is also a clear indication of myofibrillar protein denaturation. Faruk *et al.* (1994) reported that myofibrillar protein solubility immediately after death was 87% of rohu, which decreased gradually to 32% at the end of 24 days. The present study suggests that the formalin contamination results considerable denaturation of the muscle protein.

Changes in APC in formalin treated fish: Figure 3 shows the changes in bacterial load in formalin treated rohu with that of control fish during ice storage. The result showed that there was significant decrease of bacterial load in 5% formalin treated rohu which may be related to the bactericidal affect of formalin itself. In fresh rohu, immediately after death the initial bacterial load was 7.5×10^3 CFU/g. But immediately after dipping in 5% formalin for 5 min, bacterial load decreased to 6.2×10^2 CFU/g. During the ice storage, bacterial load further decreased and at the end of 16 days of ice storage, it significantly reduced below detection level. On the other hand, in fresh rohu immediately after killed bacterial load in the muscle was 7.5×10^3 CFU/g, which increased gradually with the increasing of storage periods. At the end of 16 days of storage, the bacterial load increased to 3.5×10^6 CFU/g, which is this upper limit for the chilled and frozen fishery products. However, at the end of 24 days of storage the bacterial load increased of 6.2×10^9 CFU/g and at that time the fish became spoiled and the samples became organoleptically unacceptable. The initial decrease in bacterial population in fish muscle after the first days of ice storage might be due to cold shock or leaching of surface flora by washing with melted ice. This is an agreement with those reported for some ice stored fresh water fish (Bandyopadhyan *et al.*, 1985). It has been also been reported that the number of bacteria in gill, intestinal content and the skin of newly caught fish vary from species to species and also depend on the microbial load of the waters in which they live (Frazier and Westhoff, 1990). The changes in bacterial load were found to be positively correlated, the changes in NPN level and muscle pH.

Gel forming ability at time interval: Figure 4 shows the gel forming ability in term of breaking force during 8 h ice storage in both formalin treated and untreated fish. The result showed that the highest breaking force of 1240 (±1 g) was obtained in fresh rohu immediately after killed,

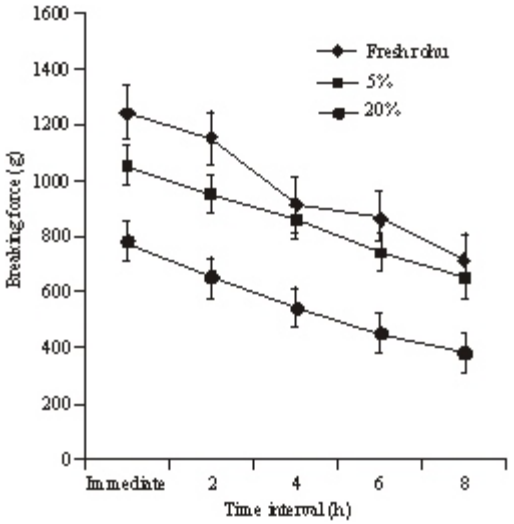

Fig. 4: Breaking force of fresh and formalin treated rohu fish (*Labeo rohita*) at time interval (hour). Vertical bars denote SD

which declined with the increasing period of ice storage. Similar result were obtained in 5 and 20% formalin treated fish during ice storage but breaking force reduced significantly in formalin treated rohu compared to that of control samples at same time interval. Shimizu and Kaguri (1986) reported that low gelling potential of tilapia might be due to the denatured of myofibrillar proteins that occurred more rapidly in acidic condition of pH 6.0. Khan (1996) reported that fine quality products were prepared from surimi of fresh rohu (*L. rohita*). On the other hand, Kongpun *et al.* (2001) reported that the gel forming ability of dorab meat was affected with addition of various concentration of formalin solution, which not only affected the gel forming ability of myofibrillar protein but also the conformation of those proteins.

CONCLUSION

The study conducted to determine the influence of formalin on the quality of rohu fish indicated that on the basis of organoleptic characteristics the formalin treated fishes were found acceptable conditions for longer than those fresh fish during ice storage with slight acrid odor of formalin itself. The low bacterial load in formalin treated fish during ice storage is the main reason for low NPN content in formalin treated fish. Protein solubility and gel forming ability was greatly reduced due to denaturation of muscle protein indicating poor eating quality of formalin contaminated fish. The government is taking initiative to ban marketing of such fish and fishery products to protect public health throughout the country.

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