Short Communication

QUALITY DETERMINATION OF ROHU (*Labeo rohita*) DURING ICE STORAGE

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ABSTRACT

An investigation on quality assessment of rohu (Labeo rohita) stored in ice was carried out through sensory, microbial and chemical analyses. Significant changes in the proximate composition of ice stored fish in different days were observed (P < 0.01) except for minerals (P > 0.01). The organoleptic quality of the ice stored fish was significantly (P < 0.01) influenced by the Total Volatile Base Nitrogen (TVBN), Trimethylamine Nitrogen (TMAN) Thio Barbutric acid (TBA), Free Fatty Acids (FFA) and Water Holding Capacity (WHC) and microbial counts. Total Plate Count (TPC) of mesophilic bacteria and Staphylococcus aureus decreased continuously while Psychrophiles, Pseudomonas and Aeromonas spp. increased in count. Escherichia coli, Faecal streptococci , Vibrio spp., Salmonella spp. and Listeria monocytogenes were completely absent throughout the study. Organoleptic quality tests showed that the maximum shelf life of rohu in ice was 17 days.

Key wods: Quality assessment, Ice storage, Rohu, Biochemical changes, Microbial changes

INTRODUCTION

Preservation of fish in ice is one of the most efficient ways of retarding the spoilage. Proper icing preserves the fish in an acceptable condition for reasonable periods. A regular examination of the fish is required to establish its hygienic status which is directly related to the health and hygiene of the consumers. The spoilage of fish during ice storage is due to the contamination by the psychrotropic bacteria (Shewan and Ehrenberg, 1977; Surendran and Gopakumar, 1981), which easily grow and multiply at low temperatures. The shelf life of fish preserved in ice varies with the species, method of capture, location of fishing grounds

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and size of fish (Lima dos Santos et al., 1981). The storage life of iced fish is usually affected by initial microbial load of the fish and storage temperature (Church, 1998*)*. Advantages of the ice preservation are maximum possibility of preserving the natural nutritional and functional properties of the fish.

Major carps are the most important species that support freshwater fisheries of India. Among the Indian major carps, *Labeo rohita* (Rohu) is highly preferred species for culture and contributes more than 80% of the major carp culture of India. It is mainly due to its high commercial value, the taste and good looking white colour of the meat. Keeping in view of all these, the present study was undertaken to determine the shelf life of rohu, preserved in ice, through sensory, microbial and chemical assessment.

MATERIALS AND METHODS

Labeo rohita (rohu) samples were collected in the early morning from a fish farm located at Muthukur and brought to the Institute laboratory in insulated containers without ice. The fishes were washed immediately with ice cold potable water to remove dirt, sand and unwanted matter. After washing, the length and weight of fish were measured and the freshness of fish was assessed by torry meter. The average length and weight of fish were 36.75 ± 2.98 cm and 884.40 ± 114.77 grams, respectively. The fishes were later divided into four lots and stored with flake ice in alternate layers 1:1 (w/w) (Lima dos Santos et al., 1981) in insulated boxes. Four fishes were randomly selected for quality assessment. The results obtained were treated as initial readings ('0' day) of quality of ice stored fish. Sampling was carried out every alternate day by randomly selecting one fish from each lot. Totally four fishes were selected and the sampling was continued up to18 days. Out of four fishes, three fishes were used for the estimation of various quality parameters and one fish for sensory assessment.

Proximate composition of the fish was analyzed by the method described in AOAC (2000). TVBN and TMA were determined by the Conway method (1962) and expressed as mg/100 g of meat. Peroxide value was determined using method adopted by Jacobs (1958). TBA value was determined as described by Tarladgis et al. (1960) and expressed as mg malonaldehyde per kg of fish sample. Free fatty acid was estimated by Olley and Lovern (1960) method. Water holding capacity (WHC) of fish muscle was measured by modified centrifugation method described by Del valle and Gonzales- Inigo (1968) and expressed in percentage (%). The microbiological analyses were carried out following the methods described by APHA (1992). This study included Total Plate Count (TPC) and growth of *Staphylococcus aureus, Escherichia coli, Faecal streptococci*, total *Psychrophiles*, total H_2S producers, *Aeromonas* spp., *Pseudomonas* spp*.*, *Vibrio* spp*.*, *Salmonella* spp. and *Listeria monocytogenes*.

Observations on the changes in appearance, colour, odour, taste and texture of fish sampled studied were registered. The sensory evaluation for overall acceptability was carried out after cooking the selected fish and it was done by 8 trained panelists using 10 point hedonic scales *viz*., excellent (10), good (9), fair (8), acceptable (7), borderline of acceptance (6), and not acceptable (5). Torry meter readings were taken to study the correlation between sensory score and instrumental score.

The SPSS 16 (IBM, 2010) Statistical Package for Social Sciences was used for analysis of the experimental results. The results were expressed as mean ± standard deviation (SD). Correlations were established between the various characteristics by using "Post Doc" coefficient of SPSS. Sensory scores for overall acceptance of the product were correlated with the storage time, and the shelf life of ice stored rohu using linear regression plot.

RESULTS AND DISCUSSION

Proximate composition of the fish meat obtained from ice-stored whole fish showed significant variations in moisture, total lipids, total protein and ash levels. Moisture content of the ice stored fish increased from the initial value of 78.55% to 80.16% till 10th day. It could be due to absorption of melted ice water by the fish muscle. Later gradual decrease was observed up to 77.69% on 18th day. It might be due to the evaporation of moisture from the surface which relies on various factors like geometric shape, chemical composition of product, storage temperature and relative humidity. Similarly, the percentage of total lipid content of the stored fish progressed from '0' day (3.29 %) to 8th day of storage (3.52%) and again it started decreasing till the end of storage period (2.51%). Lipid breakdown might be causative for such variations in the lipid content of the ice stored fish as observed by Devadasan and Nair (1977) and Meenakshi et al (2010) in *Cyprinus carpio*. Protein content decreased from 18.78% at '0' day to 16.85% at the end of the storage period. Decrease in true protein content during the storage might be due to leaching out of water-soluble protein components and dilution effect caused by water uptake. It is further implicit that, proteolytic enzymes might have caused such effect by splitting the peptide bonds. Similarly ash content was also reduced from 1.23% to 0.94 % at the end of storage period.

The results of the biochemical analysis are presented in Table 1. The results of present study were similar with the studies conducted by Joseph et al (1988) in rohu. However, TMAO values were less compared to TVBN in the present study. It might be due to the feed containing low TMAO fed to the cultured species. It might have modified the level of TMAO in osmotic regulation in the cultured fish (Kyrana et al., 1997). Further, it could have reduced the level of TVBN content in the preserved fish during the ice storage.

The values of PV, TBA and FFA showed a gradual increasing trend till the end of storage period of rohu fish (Table 1). Although a high degree of correlation was found between TBA values and storage time, the difference between initial and final values was very small. It indicated the occurrence of limited oxidative rancidity. The WHC of fish meat serves as an indicator in evaluating the fish quality and any decrease in WHC of fish meat results in loss of texture.

The mesophilic and psychrophilic plate counts are given in Table 2. *E. coli* and *F. streptococci* were absent throughout the storage period. The counts of *Psychrophiles*, sulphur producing bacteria, *Pseudomonas* and *Aeromonas* spp. were increased during the storage period. However, *Pseudomonas* appeared from 6th day of storage and sulphur producing bacteria from 4th day onwards and both were in increasing trend. The count of sulphur producing bacteria and *Aeromonas* spp. also increased. The growth of all these bacteria increased significantly since the temperature of the storage condition favoured their growth. Pathogenic microorganisms like *Vibrio* spp., *Salmonella* spp., and *Listeria monocytogenes* were absent throughout the storage period.

The organoleptic scores decreased gradually with increasing storage time. The sensory score of iced rohu fish analysed by the panelists decreased from the 0' day score of 10 ± 0.00 'to storage end period score of 5.90 ± 0.08. A negative correlation was observed between storage time and sensory score (r = - 9.996) and the corresponding regression equation is $y = -0.230x + 9.915$. This negative correlation might be due to reduction of sensory scores with increase of storage days.

The torry meter readings of ice stored fish decreased from 15.10 ± 0.08(0 day) to 7.30 ± 0.21 (18 day). A significant negative correlation was observed between storage periods and torry meter readings (r = - 0.991) and its corresponding regression equations is $y = -0.410x + 14.71$. As the freshness decreases with increasing storage time, the electrical conductivity decreased which resulted in decrease of torry meter readings.

A significant ($P<0.01$) positive high correlation was obtained between storage days and PV (0.991), FFA (0.981), TBA (0.862), TVBN (0.977) and TMAN (0.995) in rohu fish sample during ice storage.

Acceptability limits obtained during study for the different freshness parameters of ice stored fish are 7.74, 37.72 meqO_2/kg fat, 1.30 mg MA/kg of sample, 0.03 % of oleic acid, 4.88 mg/100grams of meat and 2.51 mg/100grams of meat for Torry meter readings, Peroxide value, Thiobarbutric acid, Free fatty acid, Total volatile base nitrogen and Trimethylamine nitrogen content respectively. On correlating the overall sensory scores with storage period, the rohu fish sample was acceptable for 17 days in ice stored condition.

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Table 1. Changes in biochemical parameters of rohu fish stored in ice.

Days of Ice Storage	**P^{H}**	**TVBN**(mg/100g of meat) meat)	**TMAN** (mg/100g of of fat)	**PV** (meq O_2/kg sample)	**TBA** (mg of MA/kg of	**FFA** (% oleic acid)	**WHC**
0 day	6.51 ±0.00^{ab}	0.98 ± 0.01^{a}	Nil	5.8 ± 0.28^{a}	0.40±0.00^{a}	0.0008±0.0^{a}	78.85±0.14b^{c}
2 day	6.59 ± 0.02^{c}	2.03 ± 0.04^{b}	0.18 ± 0.01^{ab}	7.0 ± 1.41^{a}	0.45±0.00^{b}	0.01±0.00^{a}	80.35± 0.10^{d}
4 day	6.63 ±0.02^{cd}	2.52 ± 0.02^{c}	0.35 ± 0.06^{ab}	11.8 ±0.28^{b}	0.49±0.01^{c}	0.01±0.00^{b}	81.19 ±0.25^{e}
6 day	6.70 ± 0.02^{f}	2.91 ± 0.15^{d}	0.66 ± 0.04^{ab}	13.4±0.56^{b}	0.56±0.09^{d}	0.02±0.00^{c}	80.28±0.19^{d}
8 day	6.77 ± 0.01^{f}	3.36 ± 0.00^{e}	1.03 ± 0.06^{ab}	17.0 ±0.28^{c}	0.60±0.00^{e}	0.03±0.00^{c}	79.40 ± 0.26^{c}
10 day	6.70 ± 0.01^{e}	3.72 ± 0.11^{f}	1.40 ±0.02^{abc}	21.2±0.74^{d}	0.65±0.01^{f}	0.03±0.00^{d}	78.43±0.20^{ab}
12 day	6.68 ±0.01^{de}	4.06 ± 0.05^{g}	1.71 ± 0.01^{bc}	28.4±0.28^{e}	0.63±0.00^{f}	0.04±0.00^{d}	78.11 ± 0.22^{a}
14 day	6.64± 0.01^{cde}	4.21 ± 0.01^{g}	1.81 ± 0.05^{bc}	32.0 ±0.74^{f}	0.72±0.00^{g}	0.04±0.00^{e}	79.32 ± 0.21^{c}
16 day	6.53 ± 0.02^{b}	4.57 ± 0.03^{h}	2.15 ± 0.09^{bc}	35.0 ±0.56^{g}	1.33±0.00^{h}	0.04±0.00^{e}	78.54±0.13^{ab}
18 day	6.47 ±0.03^{a}	4.84 ± 0.06^{i}	2.58 ± 0.08^{c}	41.2±0.28^{h}	1.45±0.00^{i}	0.05±0.00^{e}	78.22 ± 0.09^{a}

^{abcdefghi} Means followed by the same superscript with in a column are not significantly different ($p > 0.01$).

Table 2. Mesophilic and Psychrophilic plate count in ice stored rohu fish

Storage period Day	#Time	TPC (cfu/gram of meat)	*S.aureus* (cfu/gram of meat)	Psychrophiles (cfu/gram of meat)	Pseudomonas (cfu/gram of meat)	H_2S producing bacteria (cfu/gram of meat)	Aeromonas spp. (cfu/gram of meat)
0	24h	6.3x10^{2}(2.80)	3.4x10^{2}(2.53)	$	Est< 1	Est< 1	2.5x10^{1}(1.39)
	48h	6.6x10^{2}(2.81)	3.8x10^{2}(2.57)	$	Est<1	Est<1	2.5x10^{1}(1.39)
2	24h	3.6x10^{2}(2.55)	2.7x10^{2}(2.43)	1.7 x 10^{2}(2.25)	Est<1	4.2x10^{1}(1.62)	5.6x10^{1}(1.74)
	48h	3.9x10^{2}(2.59)	3.0x10^{2}(2.48)	1.8 x10^{2}(2.25)	Est< 1	4.4x10^{1}(1.64)	6.2x10^{1}(1.79)
4	24h	2.5x10^{2}(2.41)	8.6x10^{1}(1.93)	2.6 x 10^{2}(2.41)	Est<1	6.4x10^{1}(1.81)	1.3x10^{2}(2.11)
	48h	2.7x10^{2}(2.44)	9.2x10^{1}(1.96)	2.6 x10^{2}(2.42)	Est< 1	6.8x10^{1}(1.83)	1.3x10^{2}(2.12)
6	24h	2.3x10^{2}(2.36)	6.2x10^{1}(1.79)	3.8 x 10^{2}(2.58)	0.4 x 10^{1}(0.60)	8.6x10^{1}(1.93)	1.9x10^{2}(2.29)
	48h	2.5x10^{2}(2.40)	6.8x10^{1}(1.83)	3.9 x10^{2}(2.59)	0.4x10^{1}(0.60)	1.1x10^{2}(2.05)	1.9x10^{2}(2.47)
8	24h	1.8x10^{2}(2.26)	5.6x10^{1}(1.74)	1.0 x 10^{3}(3.03)	0.6 x 10^{1}(0.77)	1.2x10^{2}(2.07)	2.3x10^{2}(2.37)
	48h	2.0x10^{2}(2.30)	5.8x10^{1}(1.76)	1.0x10^{3}(3.03)	0.6 x 10^{1}(0.77)	1.3x10^{2}(2.11)	2.4x10^{2}(2.38)
10	24h	1.3x10^{2}(2.13)	3.2x10^{1}(1.51)	2.4 x 10^{4}(4.38)	1.3x10^{2}(2.12)	2.2x10^{2}(2.34)	2.8x10^{2}(2.45)
	48h	1.4x10^{2}(2.15)	3.4x10^{1}(1.56)	2.4 x10^{4}(4.38)	1.4x10^{2}(2.16)	2.2x10^{2}(2.35)	2.8x10^{2}(2.45)
12	24h	9.1x10^{1}(1.96)	1.6x10^{1}(1.20)	1.1 x 10^{5}(5.05)	2.2x10^{2}(2.35)	2.6x10^{2}(2.42)	3.4x10^{2}(2.53)
	48h	9.4x10^{1}(1.97)	2.0x10^{1}(1.30)	1.1 x10^{5}(5.05)	2.3x10^{2}(2.37)	2.8x10^{2}(2.44)	3.4x10^{2}(2.53)
14	24h	8.2x10^{1}(1.92)	0.9x10^{1}(0.95)	3.4 x 10^{5}(5.53)	3.3x10^{2}(2.52)	3.4x10^{2}(2.54)	4.0x10^{2}(2.60)
	48h	9.0x10^{1}(1.95)	1.4x10^{1}(1.14)	3.4 x10^{5}(5.53)	3.4x10^{2}(2.53)	3.5x10^{2}(2.55)	4.0x10^{2}(2.60)
16	24h	8.2x10^{1}(1.91)	0.6x10^{1}(0.77)	5.0x10^{5}(5.70)	3.6x10^{2}(2.55)	4.8x10^{2}(2.68)	4.9x10^{2}(2.69)
	48h	8.8x10^{1}(1.94)	0.8x10^{1}(0.90)	5.0x10^{5}(5.70)	3.7x10^{2}(2.56)	4.9x10^{2}(2.69)	4.9x10^{2}(2.69)
18	24h	7.4x10^{1}(1.86)	0.4x10^{1}(0.60)	8.0x10^{5}(5.91)	3.9x10^{2}(2.59)	5.9x10^{2}(2.77)	5.6x10^{2}(2.74)
	48h	8.2x10^{1}(1.91)	0.5x10^{1}(0.69)	8.0x10^{5}(5.91)	4.0x10^{2}(2.61)	6.0x10^{2}(2.78)	5.6x10^{2}(2.74)

Period of incubation; Figures in parenthesis indicate Log bacterial counts;

$ Not enumerated; cfu: colony forming units