Studies on Iced Storage of Cultured Rohu *(Labeo rohita)*

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The biochemical, bacteriological and organoleptic changes in cultured rohu *(Labeo rohita)* during iced storage have been studied. Non-protein nitrogen decreased and water soluble nitrogen remained almost same during storage in ice. Initially, when the fish was in pre-rigor and rigor conditions, the extractibility of protein was low (45 to 50%) which increased after the resolution of the rigor and the decrease in extractability towards the end of storage was insignificant. The total volatile base nitrogen remained steady upto 7 days in ice and showed slight decrease on further storage. During iced storage the bacterial count increased from 10^3/g to 10^6/g by the 11th day of storage. Nearly 80–90% of the total bacterial population in fresh fish was constituted by mesophiles which decreased gradually (decreased to 1% by 13th day of iced storage). Organoleptically the fish was acceptable upto 15 days in ice.

The iced storage changes in most of the commercially important marine fishes in India have been studied (Velankar & Kamasastri, 1956; Kamasastri *et al.*, 1967; Venkataraman *et al.*, 1967; Govindan, 1971; Shenoy & James, 1974; Banck, *et al.*, 1976; Surendran & Iyer, 1976; Joseph *et al.*, 1980). The changes associated with the iced storage of freshwater fish and its shelf-life in ice have not been studied extensively. Nair *et al.* (1971), Nair & Dani (1975) and Bandhyopadhyay *et al.* (1985) studied the iced storage shelf-life of some freshwater fishes. For the freshwater fish mrigal *(Cirrhina mrigala)*, Nair *et al.* (1971) reported an iced storage life of 36 days while Bandhyopadhyay *et al.* (1985) reported an iced shelf-life of 13 days for the same fish of size range 30–33 cm and 17 days for size range 36–40 cm. Disney *et al.* (1974), Nair *et al.* (1971) and Nair & Dani (1975) are of opinion that tropical fishes have longer iced shelf life than temperate or cold water species. Because of the variations in shelf life of iced freshwater fishes obtained by different workers, it is necessary to study in detail the changes in iced storage of freshwater fish before arriving at a conclusion.

In this study the iced storage shelf-life of cultured rohu *(Labeo rohita)* was determined after making detailed investigations on the changes in biochemical, bacteriological and sensory qualities.

Materials and Methods

Fresh rohu *(Labeo rohita)* was procured from a freshwater pond near Cochin. Soon after catch the fish was killed and kept under crushed ice in an insulated box and brought to the laboratory. Ice loss was made up by the addition of crushed ice at intervals of 2 days. The samples were taken at regular intervals for biochemical, bacteriological and sensory studies.

Moisture, fat, total nitrogen and non-protein nitrogen (NPN) in the muscle of the fish were determined by the methods of AOAC (1975). The changes in salt soluble nitrogen (SSN) and water soluble nitrogen (WSN) were followed by the method of Dyer *et al.* (1950) and the total volatile basic nitrogen (TVBN) by the micro-diffusion method of Conway (1947) from the trichloracetic acid extract of the muscle. Alpha amino nitrogen was determined by the method of Pope and Stevens (1939). Organoleptic studies were conducted as described by Joseph and Perigreen (1983).

Total aerobic bacterial count (TPC) of the muscle with skin of rohu was determined by the pour plate method using trypton glucose beef extract agar (TGA). The plates were incubated at room temperature (RT 28 $\pm$ 2°C) and 37°C for 48 h and counts taken. Total coliforms were determined

by the most probable number (MPN) method using the three dilution three tube technique, with lactose broth (LB) as the medium, at an incubation temperature of 37°C. Confirmation of total coliforms was done by MPN method, using brilliant green bile broth (2%, BGLB) incubated at 37°C. Loop fulls of culture from positive BGLB tubes were transferred to EC broth tubes (FDA, 1978) and incubated for 48 h at 45 ± 0.5°C. Tubes with positive growth and gas production showed the presence of faecal coliforms. Tests for *Escherichia coli* were done for culture from positive EC tubes by first transferring to eosine methylene blue agar (EMB) followed by IMVIC tests (FDA, 1978). Faecal streptococci were determined using KF streptococct agar. Plates were incubated at 37°C for 48 h and dark red colonies and colonies with red or pink were counted as faecal streptococci. Coagulase positive staphylococci were determined using Baird-Parker agar (FDA, 1978).

Results and Discussion

Table 1 gives the size and weight range of the fish used for the studies and its proximate composition. The composition is comparable to that of lean fish from marine environments.

Tables 2 gives the changes in the chemical parameters during iced storage of rohu. Moisture showed an increase during iced storage. The same trend was noticed in all fishes and shell fishes. In the case of whole fish with thick skin and scales, the moisture increase in iced storage was 2 to 3% (Joseph *et al.*, 1980; Perigreen *et al.*, 1987) while it was more than 3% in prawn (Govindan & Perigreen, 1972). WSN remained same except a slight decrease after 11 days of storage. The salt extractability of nitrogen was around 50% of total nitrogen during pre-rigor and rigor period, but increased after the resolution of rigor. The same phenomenon was noticed by Nikkila and Linka (1954) in Baltic herring *(Clupea herengus)* and Perigreen *et al.* (1987) in common murrel *(Channa striatus)*. The decrease in extractability on further iced storage was insignificant. The NPN values after an increase in the beginning showed a decrease. It was noticed that the TVBN value did not increase during iced storage of rohu, but showed a decrease after 11 days of storage. This strongly indicated that the spoilage pattern and bacterial flora associated with spoilage was entirely different from that of marine fishes which generally showed an increase in TVBN values during iced storage. The alpha amino nitrogen content was 70 mg/100 g initially which decreased during storage.

Table 1. *Proximate composition of rohu (Size range* 41–44 cm, weight range 650–850 g*)*

Moisture %	76.45
Protein %	20.95
Fat %	0.62
Ash %	0.95

The changes in the bacteriological quality during iced storage of rohu are given in Table 3. The initial bacterial load of the muscle with the skin of rohu was 9.35 x 10^3/g. The fish harboured total coliform in the order of 10^2/g of which *E. colt* formed nearly

Table 2. *Chemical changes during iced storage of rohu*

Days of storage	Moisture %	WSN % of TN	SSN % of TN	NPN mg/100g	∝-amino nitrogen mg/100g	TVBN mg/100g
0	76.45	23.6	45.4	350	70.4	11.2
1	77.39	25.2	50.1	406	77.3	11.2
5	78.00	24.7	70.8	392	77.4	11.2
7	78.45	24.3	71.2	364	72.8	11.2
11	78.32	25.3	72.1	356	68.6	10.6
13	78.09	21.2	69.0	364	67.2	9.8
15	78.41	23.2	65.4	352	65.4	8.4
18	78.40	23.1	61.2	322	67.6	7.0
20	78.14	22.8	66.1	336	93.8	8.4

Table 3. *Bacteriological changes during iced storage of rohu*

Days of storage	TPC/g RT 28 ± 2°C	TPC/g 37°C	Total coliform/g	E. coli/g	Staphylococci/g	Streptococci/g
1	9.35×10^3	7.66×10^3	5.06×10^2	Present	66	9.3×10^2
5	9.38×10^4	6.86×10^4	3.11×10^1	1.6×10^1	Nil	8.84×10^1
7	1.82×10^5	5.10×10^4	—	Nil	Nil	6.06×10^1
11	3.36×10^5	6.20×10^4	8.71×10^1	Nil	Nil	8.0×10^1
13	6.98×10^6	1.72×10^4	38	Nil	Nil	1.2×10^1
15	6.93×10^6	7.52×10^4	30	Nil	Nil	1.8×10^1
18	7.50×10^6	3.06×10^5	28	Nil	Nil	7.0×10^1
20	6.09×10^7	6.51×10^5	35	Nil	Nil	2.2×10^2

50%. Faecal streptococci count was also in the range of 10^2/g. The incidence of faecal indicator bacteria was a reflection of the bacterial profile of the culture ponds.

During iced storage the bacterial count gradually increased from 10^3/g to 10^5/g by the 11th day of storage. Nearly 80–90% of the total bacterial population in the fresh fish was constituted by mesophiles as indicated by the bacterial count at 37°C. However, during iced storage the population of the mesophiles gradually decreased which by the 13th day iced storage was only 1% of the total bacterial count. When the total count of 10^6/g muscle was attained in marine fishes incipient spoilage might have set in, but in the case of cultured rohu even after the total count had reached 10^6/g mark, the fish was organoleptically acceptable.

Table 4 shows the changes in the sensory characteristics and gives the average score of the cooked fish in the hedonic scale. The meat had sweet taste and muddy odour. The muscle was soft. The muddy odour was noticed in many fishes collected from ponds. The soft texture is characteristic of freshwater fishes. The changes in the gills, eyes and muscle gave an indication of the extent of changes and the condition of the fish. On storage in ice the muddy odour decreased and spoiled weedy odour took its place. The gills changed from bright red to pink, bleached, brown and finally to dark colour. After 5 days storage the gills became slimy, colour change was noticed in the muscle by 7 days storage and yellow discolouration developed at the belly portion on 11th day. By this time separation of the muscle from the bones was noticed at the belly flap. Organoleptically the muscle was in acceptable condition upto 15 days.

It is not possible to assess the iced shelf life of fish by following the chemical changes alone. Since the TVBN values decreased after a period of storage it could not give any indication of spoilage except the difference in the spoilage pattern from marine fish. The SSN values were low in pre-rigor and rigor conditions which may be due to the effect of rigor on the myofibrillar proteins. Total plate count reached the value of 10^6/g muscle by 13 days storage when the plates were kept at room temperature while at 37°C the value did not reach 10^6/g muscle even after 20 days storage. The high values of faecal indicator bacteria initially indicated that the pond from where the fish was collected was contaminated with faecal material. The total plate count could provide an indication of spoilage, but many times it was not possible to correlate between the number of bacteria and stage of spoilage. Since the native bacterial flora associated with fish changes a lot with the environment where it lives, it is necessary

Table 4. *Sensory characteristics of rohu during iced storage*

Days of storage	Fresh fish	Cooked fish	Score
0	Pre-rigor, muddy odour	Sweet, soft, slight muddy taste, white meat	8.5 ± 0.4
1	Resolving stage of rigor, muscle soft, slight muddy odour, gills bright red, eyes transparent	Sweet, soft, slight muddy taste, white meat	8.2 ± 0.5
5	Post-rigor, muscle soft, eyes slightly opaque, gills slightly bleached and slimy, no off odour	Soft, slight muddy odour, sweet taste, no off odour	7.5 ± 0.55
7	Muscle soft, mixed odour of seaweed and mud, gills brown with mucous, colour of meat changed to dull gray	Slight muddy odour, soft and slightly pasty, slightly sweet, white colour	6.0 ± 0.72
11	Eyes sunken and opaque, red colour around the eyes, gills brown, decayed odour of weed at the gills, soft muscle, yellowish and dark colour at the belly flap, separation of the muscle and bone at the belly flap	Dull colour to the muscle, no off odour, texture soft and moderately pasty, slightsweet taste	5.05 ± 0.72
13	Eyes sunken, opaque and red colour around the eyes, soft muscle, gills brown, yellow discolouration at the belly portion. Muscle colour turned slightly brown	Dull colour, no off odour, soft and moderately pasty	5.2 ± 0.65
15	Eyes opaque and sunken, red colour around the eyes, brown gills, soft muscle, scales intact, overall appearance satisfactory. Yellow discolouration in the belly portion.	Muscle soft and moderately pasty, dull colour, slight muddy taste	4.6 ± 0.55
18	Gills, dark, slimy, eyes opaque sunken and pink colour around the eyes. Muscle soft, and had decayed weedy odour, slight pink colour to the minced fish.	Slight decayed odour and off taste, dull colour, not acceptable	3.7 ± 0.82
20	Dark gills, decayed odour, sunken and opaque eyes, pink colour around the eyes, soft and pasty muscle.	Pasty, decayed odour, spoiled	2.0 ± 0.75

to study the flora also in detail and then correlate the spoilage. It is seen from this study that detailed study of the sensory parameters by a trained taste panel could only give a reliable result on the shelf-life of iced fish.

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