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Effects of packaging on Rohu (*Labeo rohita*) fillets during ice storage

M. Mehedi Raquib[1], Abu Rayhan[1]*, Md. Ismail Hossain[1], Sonia Sku[1], Shaheed Nasrullah Al Mamun[2]

[1]Department Fisheries Technology, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh
[2]Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh

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***Corresponding Author**

Abu Rayhan
rayhanbau@gmail.com

ABSTRACT

The effects of different types of packaging on the shelf life of Rohu fish (*Labeo rohita*) fillets in ice temperature were studies. Three types of packaging conditions were maintained those were non-pack, air-tight pack and vacuum pack conditions. The comparative study included determining of organoleptic, biochemical and bacteriological aspects. The organoleptic qualities of Rohu (*Labeo rohita*) fillets during ice storage were assessed on the basis of the sensory evaluation such as appearance, odour, texture and taste. On the basis of the score, the non-packed iced fillet samples were found in acceptable condition after 12 days in ice storage. In case of air-tight and vacuum packed fillets, the storage period extended over 18 days with acceptable scores in ice storage. The initial pH values of non-packed, air-tight packed and vacuum packed fillets were 6.98, 6.95 and 6.97, respectively in ice storage. The maximum pH value was 9.40, found in non-packed fillet after 18 days of ice storage. The final pH values of non-packed, air-tight packed and vacuum packed fillets were 9.4, 7.98 and 6.94, respectively in ice storage. The initial TVB-N values of non-packed, air-tight packed and vacuum packed fillets were 1.94, 1.93 and 1.94 mg/100g, respectively in ice storage. At the end of the experiment, the TVB-N values of non-packed, air-tight packed and vacuum packed fillets were 59.30, 38.70 and 24.25 mg/100g, respectively in ice storage. Here, vacuum packed fillets showed more acceptability than non-packed and air-tight packed fillets in ice storage. The initial peroxide value was 0.98 meq/kg of oil in all types of packaging conditions in ice storage. The final peroxide values were 10.17, 6.12 and 3.1 meq/100g of oil in non-packed, air-tight packed and vacuum packed fillets, respectively after 18 days in ice storage. The myofibrillar protein solubility gradually decreased in all types of packaging conditions. The initial myofibrillar protein solubility values were 90.12, 90.15 and 90.13% in non-packed, air-tight packed and vacuum packed fillets, respectively in ice storage. The final values were 20.50, 37 and 45.25% in non-packed, air-tight packed and vacuum packed fillets, respectively after 18 days of ice storage. Initially, the microbial loads in rohu fillets were 2.51×10^5, 2.7×10^5 and 2.66×10^5 CFU/g in non-packed, air-tight packed and vacuum packed fillets, respectively in ice storage finally, which were 2.82×10^5, 9.5×10^4 and 5.8×10^4 in non-packed, air-tight packed and vacuum packed fillets, respectively. In the present study vacuum packaging gave most acceptances than air-tight packaging and without packaging.

INTRODUCTION

Fish filleting is not a traditional technique but it is a modern approach of fish processing. By the filleting of fresh fish, fish becomes ready-made product to the consumers. Now a day, in all over the world, fish filleting is a new and appreciable technology which is spreading quickly from country to country.

In case of fish packaging, some distinct types of packaging are practiced all over the world. Vacuum packing is a method of storing food and presenting it for sale. Appropriate types of food are stored in an airless environment, usually in an air-tight pack or bottle to prevent the growth of microorganisms. The vacuum environment removes atmospheric oxygen, protecting the food from spoiling by limiting the growth of aerobic bacteria or fungi, and preventing the evaporation of volatile components. Vacuum packing is commonly used for long-term storage of dry foods such as cereals, nuts, cured meats, cheese, smoked fish, coffee, and crisps. It is also for storage of fresh foods such as fish meat, vegetables, meats, and liquids such as soups in a shorter term because

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vacuum condition cannot stop bacteria from getting water which can promote their growth. Vacuum packaging food can extend its life by up to 3-5 times (source: wikipedia). Vacuum packing can be used to reduce bulk of inflatable items as well. Vacuum packaging products using plastic bags, canisters, bottles, or mason jars are available for home use.

Vacuum packaging reduces product shrinkage. There is no moisture loss or evaporation in a sealed vacuum bag. Therefore, the weight you package will be the weight you buy or sell. It reduces trim losses by eliminating oxidation and freezer burn. It can enhance product quality. Vacuum packaged meat held at 32° to 35° F does not hinder "aging" or tenderizing. It allows more efficient use of time. Food can be prepared in advance without loss of freshness, so slack times are more productive and busy times are more manageable.

Rohu fish (*Labeo rohita*) is one of the most popular species of fish in Bangladesh. However, the market price of this fish is almost high compared to other cultured fishes. Reasons identified for such high interest to this species were attributed to the characteristic nature of the flesh, like nice expected flavor and texture of the meat. It is a delicious fish for the middle to rich peoples of Bangladesh. By means of packaging, the value of the rohu fillet would be raised and also would be acceptable to them.

Fish of any species generally undergo deterioration in muscle the same way, regardless of where they are caught, but there wide differences in the degree of deterioration in fish of different families, and even of the different species in same family. Cooling of fish in the ice or frozen is an important way of delaying biochemical changes or in other words preventing deterioration from spoilage. In the previous chapter, studies on the organoletpic changes in rohu fish (*Labeo rohita*) fillets during ice and frozen storage were described in details. The biochemical changes taking place in the post-mortem fish muscle are closely related to the organoleptic changes. These changes results in gradual accumulation of compounds in the flesh which therefore provides a measure of progress of changes that is independent of sensory assessment. There were a number of methods that had been widely used to measure the degree of biochemical changes in fish. With certain precautions these methods can be applied to chilled fish and other various products in order to provide a measure of the amount of spoilage that has occurred before processing. The most well known methods are measurement of muscle pH, TVB-N, Peroxide value, and protein solubility during ice and frozen storage.

In Bangladesh, fish processing plants are developing day by day along with the increasing production of fish. Still now, the peoples of Bangladesh are largely dependent upon open water fishery production. Day by day our inland fishery production is increasing due to proper management of closed water body in haor region and increasing of private hatcheries. All hatcheries lead to product fish fries especially freshwater fish fries that are the key features of increasing the inland fishery production. Indian major carp culture is practicing in Bangladesh widely. Those fishes are sold to nearby fish wholesaler markets or ready to go to processing plants for export purpose. To maintain the shelf life and quality fishes are kept into ice primarily and then transported by ice boxes. But fishes got stressed and deteriorate the keeping quality for rough handling. However, fish fillets may have a greater chance to be contaminated by microorganisms or animals but it reduces the gut content as well as natural and pathogenic microflora. Fish filleting is a labour intensive work but it improves the quality of fish and make it value added product. Appropriate filleting makes a fish meat more sterile and ensures a long shelf life. On the other hand, packaging also helps in increasing keeping quality of perishable items which were stated above. Bangladesh, there are many opportunities of having packaged fish fillets. The present study was undertaken to evaluate the keeping quality of the Rohu (*Labeo rohita*) fillets during ice storage with different types of packaging.

MATERIALS AND METHODS

Collection of fish species

Fish was collected from the fish market located in Mymensingh town. Immediately after collection, the fish was iced properly with crushed ice in an insulated box (Cosmos Ltd., Seoul, Korea, 20 kg capacity) and transported to the laboratory of the Faculty of Fisheries, Bangladesh Agricultural University. The average weight of the fish was 1.00 ± 0.35 kg.

Preparation of the fillets

The fishes were weighed and then washed with clean water. There were asbestos plates which were used for filleting and it was done by a sharp knife longitudinally from the head region to trunk region residing with the dorsal spines. All the utensils used in the experiment were cleaned with adequate washing and kept cool (5°C). The temperature was maintained lower to ambient temperature by using ice.

Packaging of fillets

In case of air-tight packaging of fillets, simple polyethylene packs were used and as possible, the air of the pack was removed by hand pressure and finally the mouth of the pack was sealed by candle heat. In case of vacuum packaging, special types of thick polyethylene packs were used where the packaging was done by the help of a vacuum pack machine in the laboratory.

Three types of packaging conditions were used for Rohu fish (*Labeo rohita*) fillets during shelf-life study. The first one is non-packed fillets, the second one is air-tight poly-packed fillets and the third one is vacuum packed fillets.

The organoleptic characteristics, biochemical tests (pH, TVB-N, peroxide value and protein solubility test) and microbiological study were performed. For the ice stored samples, fish fillets were randomly taken out from storage container at selected time intervals (0, 3, 6, 9, 12, 15 and 18 days) used for the shelf-life study.

Organoleptic analysis

The organoleptic characteristics (appearance, odour, texture and taste) of fish was determined as described by EC freshness grade for fishery products (Howgate and Whittle, 1992).

Proximate analysis

Proximate composition of rohu fillets were determined as crude protein (AOAC, 1990), crude fat (Bligh and Dyer, 1959), crude ash (AOAC, 1990), and moisture (Ludorff and Meyer, 1973). Each determination was done three times.

Sensory analysis

A large number of schemes have been proposed for sensory evaluation of various types of fish. The evaluation method used in the study was based on the one currently in use in various institutes and industries of the world (Howgate and Whittle, 1992).

Table1
Grading of fresh fish.

Grade	Points	Degree of freshness
A	<2	Excellent/Acceptable
B	2 to <5	Good/Acceptable
C	5	Bad Rejected

Table 2
Determination of defect points.

Sl. No.	Characteristics of whole fish	Defect characteristics	Defect points	Grade
1.	Odour of neck when broken	Natural odour	2	Acceptable
		Faint or sour odour	5	Reject
2.	Odour of gills	Natural odour	1	Excellent
		Faint sour odour	2	Acceptable
		Slight moderate sour odour	3	Acceptable
		Moderate to strong sour odour	5	Reject
3.	Colour of gills	Slight pinkish red	1	Excellent
		Pinkish red or brownish red., some mucus may	2	Acceptable

		be present		
		Brown or gray colour covered with mucus	3	Acceptable
		Bleached; thick yellow slime	5	Reject
4.	General appearance	Full bloom; bright; shining; iridescent	1	Excellent
		Slight dullness and loss of bloom	2	Acceptable
		Definite dullness and loss of bloom	3	Acceptable
		Reddish lateral line; dull; no bloom	5	Reject
5.	Eyes	Bulging with protruding lens; transparent eye cap	1	Excellent
		Slight clouding of lens and sunken	2	Acceptable
		Dull, sunken, cloudy	3	Acceptable
		Sunken dye covered with yellow slime	5	Reject
6.	Slime	Usually clear, transparent and uniformly spread but occasionally may be slightly opaque or milky	1	Acceptable
		Becoming turbid opaque and milky, with marked increase in amount of slime present in skin	1	Acceptable
		Thick, sticky, yellowish greenish in colour	5	Reject
7.	Consistency of flesh	Firm and elastic	1	Acceptable
		Moderately soft and some loss of elasticity	2	Acceptable
		Some softening	3	Acceptable
		Limp and floppy	5	Reject

Calculation of organoleptic assessment ; Average grade points = $\frac{\text{Total grade points}}{\text{No. of characteristics}}$

Biochemical analysis

The pH value, Total Volatile Base-Nitrogen (TVB-N) value (AOAC, 1990), Peroxide value (Bligh and Dyer, 1959) and Myofibrillar protein solubility **(**Perry and Grey, 1956) were determined according to the methods described by referees.

Myofibrillar protein solubility

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 (4^0C) overnight. The suspension was centrifuged for 30 minutes at 400×g in cool condition. The protein in supernatant was determined by the Biuret method (Gornall et al. 1949).

Microbiological study

Plate count agar was a commercial preparation (Hi media, India) that was used for enumeration of viable bacterial count in experimental sample. Accurately weighed and suspended 23.5 g of media in 1000 ml distilled water and boiled to dissolve the ingredients completely. The media was then sterilized at 121˚C for 15 minutes under 15-lbs. /inch^2 pressure in an autoclave. Quantitative Bacteriological Analysis (APC) were done According to International Standard Organization (ISO, 1965) APC.

Statistical analysis

One-way analysis of variance and the general linear model using Windows for SPSS 10.0 were used to analyze the data. The Duncan’s New Multiple Range Test (DMRT) was used to find the significant differences between storage periods.

RESULTS AND DISCUSSION

Proximate analysis

The proximate composition analyses of the fillets are Moisture 77.63%, Protein 17.12%, Lipid 1.8% and Ash 1.3% (Table 3). The moisture content in fillet was decreased slightly; this might be resulted from release of water-drip during filleting. Protein content was a slight high that might be due to avoiding the belly portion of fish during filleting. On the other hand, lipid contents were decreased for the previous reason. According to Saha and Guha (1939-1940), protein content of *Labeo rohita* was found to be 16.60% and these values were 19.50, 19.50, 22.80 and 17.70% respectively for *Catla catla*, *Cirrhiuns mirgala*, *Heteropneustes fossilis* and *Labeo calbasu*. Devadasan *et al.* (1978) determined the proximate composition of *Labeo rohita* and found 77.71% moisture content, 19.60% protein content, 1.83% lipid content and 1.31% ash content of the fishes. Chandrashekar and Deosthaley (1993) found a wide variation between species in protein content (13.5- 17.3%) and fat content (0.6-1.3%) was observed. The live weight of majority of fish usually consists of about 70-80% of water, 20-30% of protein and 2-12% of lipid (Love, 1980). However, the values of the present study varies in some cases with the published reports might be variation in size, sexual condition, feeding, time of the year and physical activity.

Table 3
Proximate composition of rohu fillet.

Parameters	% in rohu fillets
Protein	17.12 ± 0.13
Lipid	1.8 ± 0.13
Ash	1.3 ± 0.09
Moisture	77.63 ± 0.47

* mean value ± standard deviation of 3 individual measurements

Sensory analysis

The qualities of fillets were graded using the score from 1 to 5 (Table 2). On the basis of the defect scores the fillets were found in rejected conditions in 15 days of ice storage in case of non-packaged fillets where Faruk (1995) found 20 days acceptability for wholefish. This was caused by the exposure of flesh to the environments and led to be contaminated. The changes occurred in organoleptic quality during the storage period and this can roughly be divided into four phases corresponding to the periods of 0 to 4, 4 to 12, 12 to 15, and 15 to 18 days in ice. In phase 1 the fishes were varied with a species taste and natural flavour and odour. At this stage the fishes had the characteristics of excellent quality. In phase 2 there was little deterioration apart from some slight loss of natural flavour. At this stage there was little loss of the characteristic odour and the flesh was neutral but had no off-flavour. In phase 3 there were signs of early spoilage with sour of flavour. In the beginning of this phase the off-flavour was slightly sour, sickly sweet, fruity of like dried fish but the fishes were judged as acceptable quality. In phase 4, the fillets began to taste stale, its appearance and texture began to show obvious signs of spoilage with an unpleasant smell. The variation between wholefish and fillet may be cause for the exposure of flesh area to the environment. Microbial contamination might take place during the storage period. There was a great effect of temperature.

During ice storage, non-packed fillets showed a lower keeping quality compared to air-tight packed fillets and vacuum packed fillets. At 15 days of ice storage, the non-packed fillets reached at the defect point limit of 5, that means the fillets were not acceptable at 15 days of ice storage. On the other hand, air-tight packed fillets and vacuum packed fillets gave a defect score of 4.67 and 3.33 at the 18 days of ice storage respectively. This represented that the fillets of air-tight and vacuum packed were acceptable at the 18 days of ice storage. But the vacuum packed ice stored fillets were more acceptable than air-tight fillets considering the defect points (Table 4). Physically the fillets were changed, lose their good appearance and flavor in every types of packaging method. But, iced non-packed fillets were degraded more quickly (15 days) than the air-tight packed and vacuum packed fillets (Table 4). A decrease in sensory quality was observed for all the fish species during storage (Orak and Kayisoglus, 2002). The available reports suggest that 18 days shelf-life for *Catla catla* wholefish in ice storage with a score of 5 (Bandyopadhyay et al. 1986).

Table 4
Changes in organoleptic qualities of rohu (*Labeo rohita*) fillets under different packaging conditions during ice storage

Days	Defect score		
	Non packed iced fillet	Air tight Packed iced fillet	Vacuum Packed iced fillet
0	1.00± 0.12	1.00± 0.14	1.00± 0.14
3	1.67 ± 0.15	1.33± 0.74	1.33± 0.41
6	3.00± 0.41	2.33± 0.45	2.00± 0.10
9	3.67± 0.54	2.67± 0.24	2.33± 0.12
12	4.33± 0.12	3.33± 0.14	2.67± 0.21
15	5.00± 0.14	4.00± 0.45	3.00± 0.21
18		4.67± 0.09	3.33± 0.42

* mean value ± standard deviation of 3 individual measurements

Table 5
Changes in pH of rohu (*Labeo rohita*) fillets under different packaging conditions during ice storage

Days	pH value		
	Non packed iced fillet	Air tight Packed iced fillet	Vacuum Packed iced fillet
0	6.98± 0.21	6.95± 0.15	6.97± 0.09
3	6.25± 0.14	6.36± 0.24	6.38± 0.11
6	6.75± 0.24	6.56± 0.45	6.34± 0.21
9	7.30± 0.09	7.00± 0.14	6.48± 0.20
12	8.05± 0.32	7.35± 0.19	6.67± 0.30
15	8.81± 0.23	7.77± 0.29	6.81± 0.24
18	9.40± 0.51	7.98± 0.27	6.94± 0.31

*mean value ± standard deviation of 3 individual measurements

Biochemical analysis

pH

Changes in muscle pH of ice stored fish are shown in (Table 5). Fish muscle pH immediately after death was around 7.0 which decreased gradually to 6.25 after 3 days in ice storage and finally it reached to 9.4 at 18 days when the fillets were organoleptically unacceptable for non-packed fillets. The muscle pH of fresh fillets was close to neutral. Due to the post-mortem anaerobic formation of lactic acid, pH decreased at the early days of storage. During the later postmortem-changes, pH increased at a high level due to formation of basic compounds within the fish fillets. The values of pH for the air-tight and vacuum packed fillets were not too high, they were 7.98 and 6.94 respectively in ice storage. The quality deterioration in vacuum packed fillets was lower compared to the other packaging conditions viewing the pH values. The available reports suggest that the quality of the fish fillets varies considerably depending on species, storage and packing conditions. Rohu chunks held in vacuum and air packs were examined during storage at refrigerated temperature (5±1°C) and the pH remained low in vacuum pack (Shakila et al., 2007).

Total Volatile base nitrogen (TVB-N)

In case of non-packed ice stored fish fillets, the initial TVB-N value was 1.94 mg/100g, which gradually increased with lapse of storage period (Table 6). At the end of the 12 days of ice storage TVB-N value increased 24.5 mg/100g, which is within the range of recommended value of 25 to 30 mg TVB-N/100g for fresh fish. However, at the end of 15 days, the TVB-N value was 40.25 mg/100g which exceeded the recommended value. The the upper limit of 30 mg TVB-N/100g is considered for finfish acceptability (Connell, 1975). The increase in TVB-N with the lapse of storage may be attributed to bacterial spoilage. However, TVB-N mainly accumulated in fresh fish fillets during the later phase of spoilage after the bacterial population has grown. Thus the TVB-N is low during the edible storage period and only when the fish is near rejection level increasing amount of TVB-N are found. Also there is a large species to species variation is in development of TVB-N. In the present study, at the end of 18 days the TVB-N value was 59.3 mg/100g, which conspicuously exceeded the range of acceptable value. For the air-tight and vacuum packed ice stored fillets, the values of TVB-N was 38.7 and 28.25 mg/100g at the end of the ice storage period (18 days) respectively. But Huang et al. (1992) found that vacuum-packaged catfish fillets have almost same TVB-N as air-tight fish fillets on the sampling days throughout three weeks of ice storage. On the other hand, Shakila et al. (2007) stated that the TVB-N values increased in their experiment but did not exceed the prescribed maximum permissible limits (40 mg/100g) in vacuum packaged rohu (*Labeo rohita*). Kinura and Kiamakura (1934) for salmon recommended TVB-N a level of 20 to 30 mg per 100 gm for salmon in the beginning of spoilage and over 30 mg for spoiled fish. Many authors suggested the upper limit of 30 mg TVB-N per 100 gm of fish at the onset of spoilage (Kawabata, 1953 and Connell, 1975).

Peroxide value

The result of the changes in peroxide value of rohu fillets (*Labeo rohita*) during ice storage is shown in (Table 7). The initial value of non-packed ice stored fillets was below 0.78 meq/kg of oil, which increased gradually with the lapse of storage period. At the end of 18 days of storage, the peroxide value was 10.17 meq/kg of oil, which was within recommended value of 10 to 20 meq/kg of oil. According to Connell (1980) the recommended value of peroxide for fresh finfish was 10-20 meq/kg of oil. The value above 20, the fish were found to be emitting smell and taste rancid. In case of air-tight and vacuum packed ice stored fillets the initial values were 0.78 and 0.78 meq/kg of oil respectively. At the 18 days of ice storage period, the values were 6.12 and 3.1 meq/kg of oil respectively. The peroxides were presumed to be eventually further oxidized to aldehydes and ketones which had a very disagreeable “fishy” or rancid odour and taste. However, depending on the fish species and storage condition a good correlation between peroxide value and organoleptic quality was found. According to Hossain et al. (2005), the initial peroxide value was 1.1 meq/kg of oil which continuously increased with the lapse of storage period. At the end of 20 days of ice storage peroxide value was 16.64 meq/kg of oil for non-packed Thai pangas (*Pangasius sutchi*). In case of *Labeo rohita* fillet, the PV of non-pack, air-tight and vacuum packed were initially 1.12, 0.91 and 0.80 meq/kg of oil respectively and at the finishing date of the experiment (21th day) the value was 12.33, 7.55 and 4.25 meq/kg of oil respectively (Huang et al., 1992).

Table 6
Changes in TVB-N (mg/100g) of Rohu (*Labeo rohita*) fillets under different packaging conditions during ice storage.

Days	TVB-N (mg/100g)		
	Non packed iced fillet	Air tight Packed iced fillet	Vacuum Packed iced fillet
0	1.94± 0.11	1.93± 0.21	1.94± 0.22
3	2.95± 0.20	2.68± 0.24	2.67± 0.24
6	6.23± 0.82	3.95± 0.79	3.32± 0.49
9	13.72± 1.25	10.00± 1.10	7.98± 0.98
12	24.50± 0.39	17.27± 1.24	14.28± 1.05
15	40.25± 2.50	28.00± 3.08	19.32± 2.23
18	59.30± 3.32	38.70± 2.23	24.25± 1.78

*mean value ± standard deviation of 3 individual measurements

Table 7
Changes in peroxide value (meq/kg of oil) of rohu (*Labeo rohita*) fillets under different packaging conditions during ice storage.

Days	Peroxide value (meq/kg of oil)		
	Non packed iced fillet	Air tight Packed iced fillet	Vacuum Packed iced fillet
0	0.78± 0.24	0.78± 0.20	0.78± 0.24
3	0.89± 0.21	0.81± 0.21	0.82± 0.25
6	1.45± 0.10	1.12± 0.10	1.05± 0.21
9	2.79± 0.15	1.99± 0.09	1.72± 0.31
12	4.89± 0.85	3.34± 0.38	2.15± 0.92
15	7.46± 1.13	4.56± 1.25	2.75± 0.15
18	10.17± 0.46	6.12± 0.58	3.10± 0.25

* mean value ± standard deviation of 3 individual measurements

Table 8
Changes in myofibrillar protein solubility (%) of rohu (*Labeo rohita*) fillets under different packaging conditions during ice storage.

Days	Myofibrillar protein solubility (%)		
	Non packed iced fillet	Air tight Packed iced fillet	Vacuum Packed iced fillet
0	90.12± 3.12	90.15± 3.05	90.13± 2.89
3	88.20± 2.03	88.35± 2.56	88.52± 1.28
6	81.20± 1.27	84.00± 1.11	84.15± 1.10
9	69.20± 1.23	77.30± 1.25	78.88± 0.99
12	55.90± 0.98	63.24± 0.91	68.20± 0.78
15	37.00± 0.85	48.80± 0.24	57.00± 0.58
18	20.50± 1.12	37.00± 0.74	45.25± 0.55

* mean value ± standard deviation of 3 individual measurements

Myofibrillar protein solubility

The, the initial myofibrillar protein solubility of non-packed ice stored fish fillets was 90.12%, which gradually decreased with lapse of storage period (Table 8). However, at the end of 18 days, the myofibrillar protein solubility was 20.5%. The decrease in TVB-N with the lapse of storage may be attributed to bacterial spoilage as well as bacterial decomposition. For the air-tight and vacuum packed ice stored fillets, the myofibrillar protein solubility was 37 and 45.25% at the end of the ice storage period (18 days) respectively. Myofibrillar protein solubility of muscle proteins from fresh water fish rohu (*Labeo rohita*) showed an increasing trend up to 11 days of storage followed by a decrease (Mukund et al. (2006) Solubility of sarcoplasmic protein showed a decreasing trend throughout the storage period. Chakrabarti and Madhusudana (2008) observed that, *Labeo rohita* (rohu), *Catla catla* (catla), *Cirrhinus mrigala* (mrigala) and *Cyprinus carpio* (common carp) were stored both in ice and freezer and the results showed gradual reduction in the protein solubility during the storage. Vacuum-packaged rohu (*Labeo rohita*) fillets had almost same pH, TVB-N, and Protein solubility values as air-tight fish fillets on the sampling days throughout three weeks of ice storage (Huang et al., 1992). Seki et al. (1979) reported that solubility of carp myofibril decreased from 95 to 20% during ice storage within 2-3 weeks.

Microbiological study

The initial microbial load was 2.51×10^5 cfu/g, which gradually decreased to 3.1×10^4 cfu/g at 6^{th} day of storage period (Table 9). However, at the end of 18 days, the myofibrillar protein solubility was 20.5%. The microbial load started to increase and finally at the 18^{th} day of storage it was 2.82×10^5 cfu/g. The increase of microbial load with the lapse of storage may be attributed to spoilage as well as decomposition. For the air-tight and vacuum packed ice stored fillets, the microbial loads were 9.5×10^4 cfu/g and 5.8×10^4 cfu/g at the end of the ice storage period (18 days) respectively. Microbial changes during iced storage of fillets from Rohu fish (*Labeo rohita*) with non-packed, air-tight packed, vacuum packed was determined by biochemical and microbial plate counts. Vacuum-packaged fish had significantly lower ($p < 0.05$) microbial load than overwrapped throughout three weeks of ice storage (Huang et al., 1992). Psychrotrophic bacteria count was above 1×10^7 cfu/g on the 12th day in 100% CO_2. However, in rainbow trout (*Oncorynchus mykiss*) mesophilic bacteria count was below 1×10^6 cfu/g at the end of the 14-day storage period.

Table 9
Microbial study of iced fillet.

Days	Non packed iced fillet (log CFU/g)	Air tight Packed iced fillet (log CFU/g)	Vacuum Packed iced fillet (log CFU/g)
0	5.40± 0.25	5.43± 0.13	5.42± 0.45
3	4.40± 0.24	4.37± 0.24	4.39± 0.24
6	4.50± 0.42	4.48± 0.21	4.45± 0.61
9	4.72± 0.12	4.62± 0.05	4.51± 0.28
12	4.93± 0.09	4.79± 0.12	4.61± 0.42
15	5.29± 0.25	4.87± 0.34	4.69± 0.42
18	5.45± 0.31	4.98± 0.54	4.76± 0.13

*mean value ± standard deviation of 3 individual measurements.

Garg and Stephen (1982) reported that in ice-stored kati fish (*Pellona* sp.) initial bacterial load of 4.18×104/g was decreased to 1.15×103/g by the 5th day of storage. Therefore it gradually increased to 5.4×105/g by 13th day of storage.

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