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

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

[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
E-mail: rayhanbau@gmail.com

ABSTRACT

This study was conducted to evaluate the effects of different types of packaging on the shelf life of Rohu fish (*Labeo rohita*) fillets. Shelf life of the Rohu (*Labeo rohita*) fillets was studied at frozen temperature in deep fridge. 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 and frozen storage were assessed on the basis of the sensory evaluation such as appearance, odour, texture and taste. In frozen storage, air-tight and vacuum packed fillets showed organoleptic scores in acceptable condition over 12 weeks of storage. The initial average pH value of fillets was 6.97 but the final pH values were 6.9, 6.83 and 6.78 in non-packed, air-tight and vacuum packed fillets, respectively in frozen storage. At the finishing day of the experiment the TVB-N values of non-packed, air-tight packed and vacuum packed fillets in frozen storage were 6.20, 5.34 and 5.20 mg/100g, respectively wherever the initial TVB-N values of non-packed, air-tight packed and vacuum packed fillets in frozen storage were 1.95, 1.97 and 1.96 mg/100g, respectively. Here, vacuum packed fillets showed more acceptability than non-packed and air-tight packed fillets. The initial peroxide values of non-packed, air-tight packed and vacuum packed fillets were 0.79, 0.78 and 0.79 m.eq./kg of oil, respectively in frozen storage. And the final peroxide values of non-packed, air-tight packed and vacuum packed fillets were 3.34, 2.25 and 1.84 m.eq./kg of oil, respectively after 12 weeks in frozen storage. The peroxide values were beyond the acceptable range in case of all types of packaging conditions after 12 weeks in frozen storage. The myofibrillar protein solubility gradually decreased in all types of packaging. The initial myofibrillar protein solubility values were 90.05, 90.18 and 90.12% in non-packed, air-tight packed and vacuum packed fillets, respectively in frozen storage. The final values were 71.50, 77.20 and 80.10% in non-packed, air-tight packed and vacuum packed fillets, respectively after 12 weeks of frozen storage. Initially the microbial load in non-packed, air-tight packed and vacuum packed fillets were 2.56×10^5, 2.67×10^5 and 2.59×10^5 CFU/g, respectively. Remarkable decrease in microbial count was found at 2 weeks of frozen storage, caused by the freezing effects. Finally they increased at the value of 8.2×10^3, 7.8×10^3 and 7.6×10^3 in non-packed, air-tight packed and vacuum packed fillets, respectively after 12 weeks of frozen storage. Finally, vacuum packaging gave most acceptance than air-tight packaging and obviously than without packaging.

Introduction

About 30% fish landed in Bangladesh are marketed fresh, about 40% iced, 20% sun dried and the rest is frozen, salted, smoked or made into small meal. The raw fishes are sold in different fish markets as whole primarily by icing. In some value shops, now a days fishes are sold in well packed and frozen whole. But, still now there is no practice of selling filleted fish. Fish fillet is a readymade processed fish where it is only remained for consumption after one processing step, viz. cooking, grilling, broiling, boiling etc and the supply of good quality raw materials is an essential prerequisite for either domestic consumption or value added activities like filleting for export in the international market.

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. Quality assurance is essential in each technological process, and suitable packaging materials and methods are of great importance. If these requirements are not met all efforts made during processing could be of little avail, which could lead to serious economic losses. 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. The advantages of frozen storage include many distinct criteria for the proper quality of fillets shelf life. An extreme decrease of temperature (-20ºC) in fish flesh makes it quite tolerant in autolysis and bacterial decomposition. The significance of frozen storage of rohu fillets may be considered followed by some special parameters like frozen temperature, duration of storage, frozen types etc. the present study was undertaken to observe the effects of different types of packaging of Rohu (*Labeo rohita*) fillets during frozen storage.

Materials and methods

The experiments were conducted in the laboratory of Fisheries Technology Department of the Faculty

of Fisheries, Bangladesh Agricultural University, Mymensingh during January 2009 to December 2009.

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 storage conditios considering the temperature frozen (-20°C). Three main categories of experiments were carried out to evaluate the shelf-life as well as the qualitative parameters. They qualitative evaluation included organoleptic evaluation (color, odor, taste, texture and general acceptability), biochemical tests (pH, TVB-N, peroxide value and protein solubility test) Microbiological study (Bacterial load (CFU/g) (Seely and Vandemark, 1972). Samples were frozen stored samples, they were randomly taken out from deep fridge at selected time intervals (0, 2, 4, 6, 8, 10 and 12 weeks) used for the shelf-life study.

Rohu fish (*Labeo rohita*) was selected for the study. Filleting was done by a sharp knife longitudinally from the head region to trunk region residing with the dorsal spines. In case of air-tight packaging of fillets, simple polyethylene packs were used 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.

Organoleptic evaluation

The organoleptic evaluation of the fish represented post harvest quality loss and studies on the physical or organoleptic changes in fish and their relationship with the freshness of Rohu fish fillets under frozen storage. Post-mortem changes begin to set in immediately after death and some physical changes take place in fish subsequently. The earliest changes are, in particular, those which concern with appearance and texture and rigor-mortis. The latter changes are related with the senses, i.e., appearance, odour, texture and taste. These changes in organoleptic characteristics are mostly related to the freshness of a fish. Many methods have been proposed or tested for measuring the fish quality in industry. Many have been shown to have shortcoming for practical use. The organoleptic assessment is a simple and widely used method in selecting quality of fish in the industry. In this section, studies on the physical or organoleptic changes and their relationship with the freshness of fish have been presented.

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. The guidelines has been prepared to get maximum value from them by being able to compare the results. The guidelines and methods given here using score on the organoleptic characteristics of fish as described by EC freshness grade for fishery products (Howgate and Whittle, 1992).

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

The studies on the physical or organoleptic changes and their relationship with the freshness of frozen fillets have been presented. The results of the organoleptic quality assessment of rohu fish (*Labeo rohita*) fillets during frozen in a deep fridge are presented in Figure 1. The qualities of fillets were graded using the score from 1 to 5. The grades were defined in terms of the total number of defects or demerit points. The score points less than 2 were considered as excellent. The points from 2 to less than 5 were judged as good or acceptable conditions, while 5 and above considered as bad or rejected. During frozen storage, the quality deterioration occurred very slowly because of having very low temperature, where the enzymatic degradation took place in a very low scale and the bacterial growth rate was too low. But, in case of non-pack frozen fillets the defect score reached at 5 at the time of 10 weeks of frozen storage. For the air-tight and vacuum frozen fillets, defect scores were 4.67 and 4.33 at the 12 weeks of frozen storage. Vacuum packed fillets showed more acceptability than air-tight packed fillet considering the organoleptic assessment (Fig. 1, plate 1-3). A decrease in sensory quality was observed for all the fish species during storage (Orak and Kayisoglus, 2002). Valls et al. (2004) evaluated the physical, chemical and sensorial changes in sardine (*Sardinella aurita*) fillets under frozen storage at -18°C for 6 months. The results showed that storage time significantly changes organoleptic properties of the sardine fillets. The organoleptic property was the most suitable parameter in the measurement of the stability of frozen fillets. The fillets showed an excellent grade of freshness until the 2nd month of frozen storage. After that, its quality decreased, but still had good organoleptic properties until the 5th month of storage. On the 6th month, the muscle had yellow spots, which could be due to lipid oxidation.

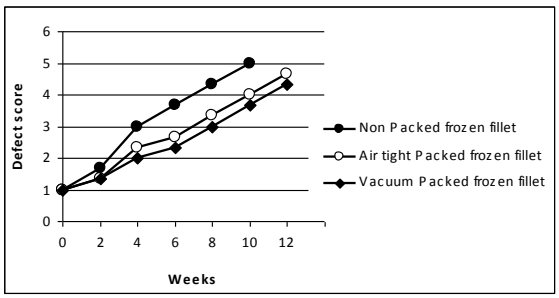

Fig. 1. Changes in organoleptic qualities of rohu (*Labeo rohita*) fillets under different packaging conditions during frozen storage.

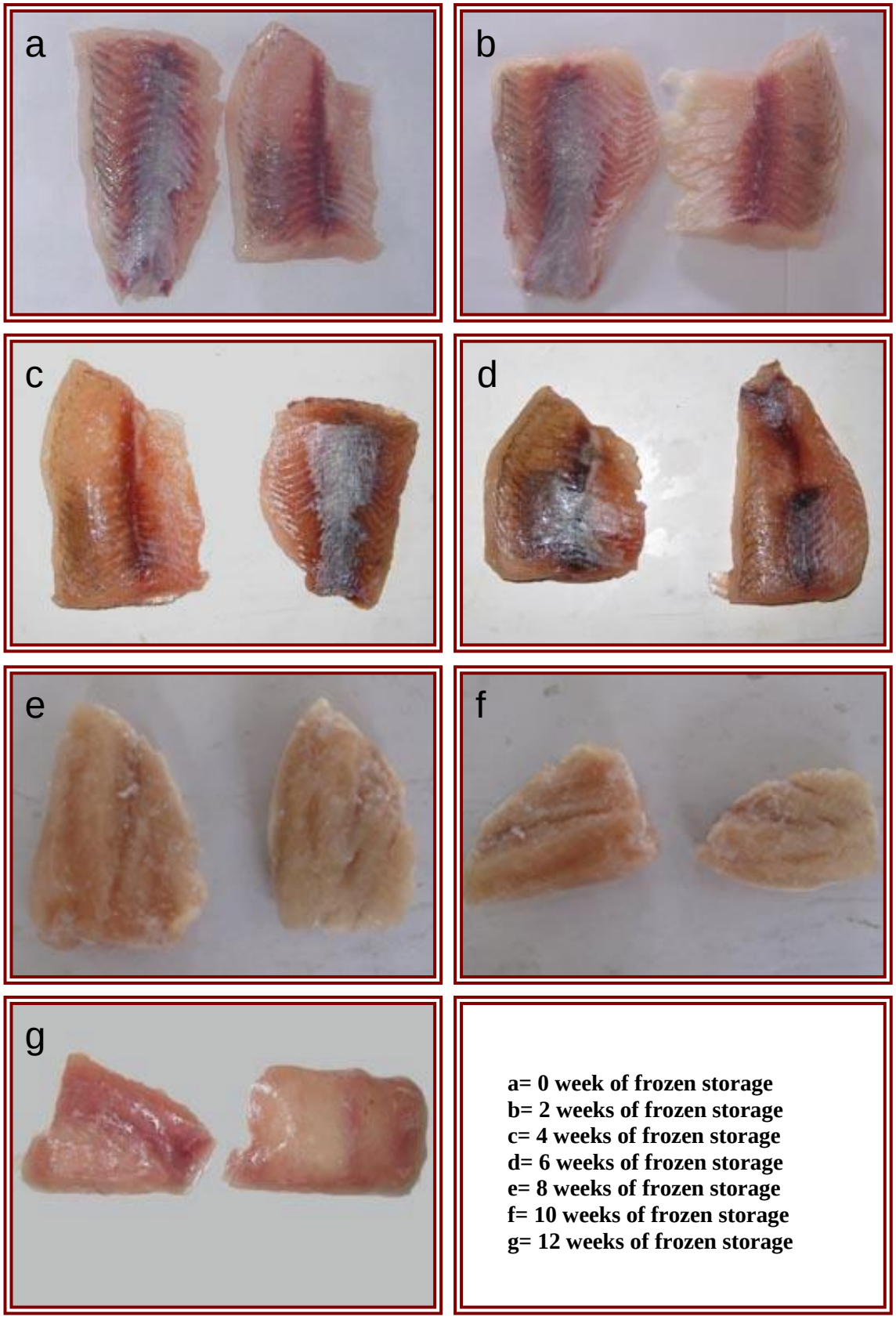

Plate 1. Changes in organoleptic qualities of rohu (*Labeo rohita*) fillets under non-packaging conditions during frozen storage

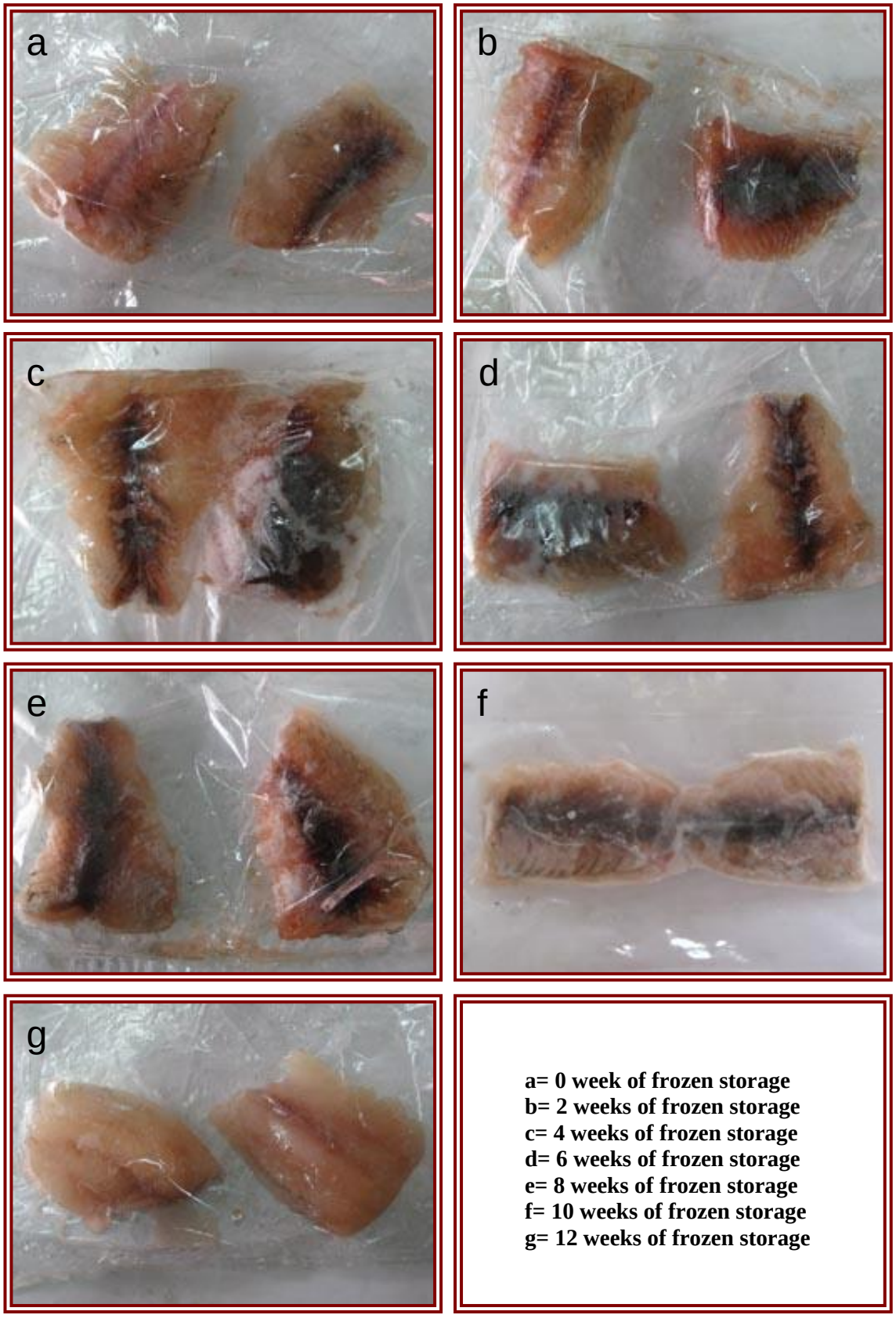

Plate 2. Changes in organoleptic qualities of rohu (*Labeo rohita*) fillets under air-tight packaging conditions during frozen storage.

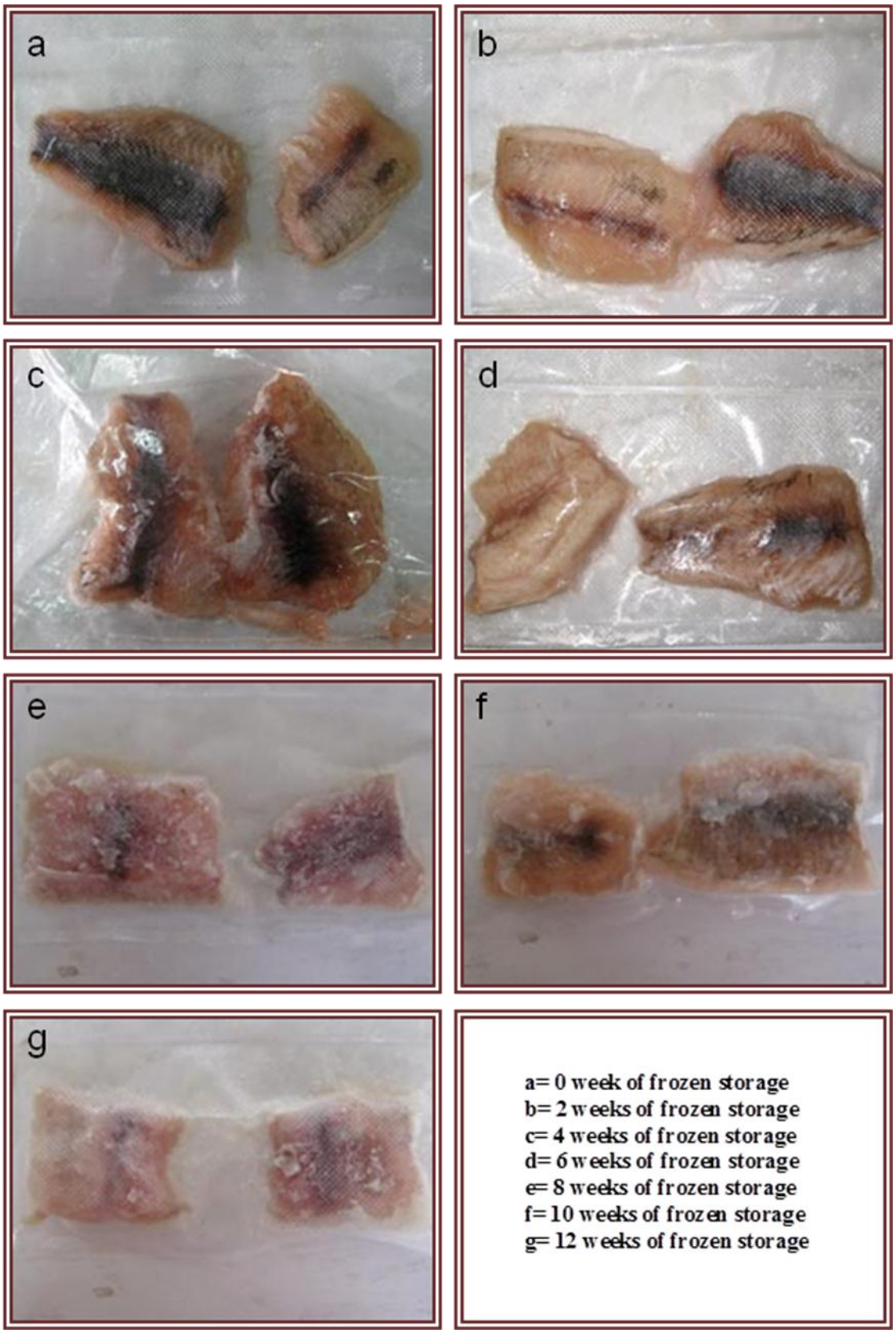

Plate 3. Changes in organoleptic qualities of rohu (*Labeo rohita*) fillets under vacuum packaging conditions during frozen storage.

Biochemical analysis

The biochemical changes taking place in the post-mortem fish muscle are closely related to the organoleptic changes as before.

Changes in pH

In case of frozen storage, initially the pH value was around 7.0 in all three samples, non-packed fillets, air-tight packed fillets and vacuum packed fillets. For non-packed fillets, pH value was decreased at the level of 6.32 during 6 weeks of frozen storage and this value was again raised up at the level of 6.9 at the 12 weeks of frozen storage. The values of pH for the air-tight and vacuum packed fillets were not too high in level, they were 6.83 and 6.78 respectively in frozen storage. The quality deterioration in vacuum packed fillets was much minimum compared to the other packaging conditions considering the pH values (Fig. 2). The quality deterioration in vacuum packed fillets was lower compared to the other packaging conditions viewing the pH values. The degradation of myofibrillar proteins of rohu carp (*Labeo rohita*) muscle was analysed at -8°C or -20°C for up to 6 months. Where Jasra et al. (2000) found a lower change in pH in first month of their study and the pH level gradually decreased and again rose at a basic level. Fillets of cod (*Gadus morhua*) were vacuum packed, frozen in an air-blast freezer and stored at -28°C. After 1 week, 3, 6 and 12 months, samples were thawed. The increase of pH was significantly retarded in the thawed fish and, initial and final pH value scored as 7.0 and 8.2, respectively (Vyncke, 1983). Simeonidou et al. (1997) studied on whole fish and fillets of horse mackerel (*Trachurus trachurus*) and mediterranean hake (*Merluccius mediterraneus*) which were assessed for quality (physical, chemical and sensory attributes) changes throughout 12 months of frozen storage at -18°C. The pH increased, while sensory attributes decreased during the frozen storage period. There were significant differences ($P < 0.05$) in pH. In case of frozen storage, pH had fallen down after 6 weeks (Fig. 2). The main reasons behind that fall were the onset of rigor mortis which was very slow in frozen storage and the low rate of quality deterioration by enzymes (both self and bacterial). The rigor came at the 6 weeks of frozen storage in every type of packaged samples. Valls et al. (2004) evaluated the physical, chemical and sensorial changes in sardine (*Sardinella aurita*) fillets under frozen storage at -18°C for 6 months. They also got the same causes and results for the sardine fillets.

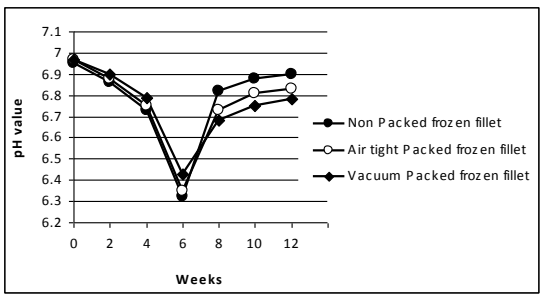

Fig. 2. Changes in pH of rohu (*Labeo rohita*) fillets under different packaging conditions during frozen storage.

Changes in Total Volatile base nitrogen (TVB-N)

The results of the changes in TVB-N (mg/100g) are presented in the (Fig. 3). Here, non-packed frozen stored fish fillets showed the initial TVB-N value, 1.95 mg/100g, which gradually increased with lapse of storage period beyond the level of acceptance. At the end of the 12 weeks in frozen storage TVB-N value increased 6.2 mg/100g, which was within the range of recommended value of 25 to 30 mg TVB-N/100g for fresh fish. On the other hand, air-tight packed fillets and vacuum packed fillets also resulted acceptable values those were 5.34 and 5.2 mg TVB-N/100g respectively. Three types of packaging conditions in frozen storage showed that those three values were beyond the recommended value and they are so far from the rejection limit. The available report suggests that 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, the available information indicates that 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. Simeonidou et al. (1997) studied on whole fish and fillets of horse mackerel (*Trachurus trachurus*) and mediterranean hake (*Merluccius mediterraneus*) which were assessed for quality (physical, chemical and sensory attributes) changes throughout 12 months of frozen storage at -18°C. The pH, the total volatile base nitrogen (TVB-N) the thiobarbituric acid number (TBA), peroxide value (PV) and amount of free fatty acids (FFA) increased, while TVB-N value increased very slowly during the frozen storage period. In the present study, at the end of 12 months the TVB-N value was 6.2 mg/100g, which conspicuously beyond the range of acceptable value (30 mg/100g according to Connell, 1975). For the air-tight and vacuum packed ice stored fillets, the values of TVB-N was 5.34 and 5.2 mg/100g at the end of the ice storage period (12 months) respectively. Ozogul et al. (2007) worked on the quality assessment of the wild European eel (*Anguilla anguilla*) stored at -20°C, The level of TVB-N showed fluctuations (7.09-14.72 mg TVB-N/100 g) during frozen storage period, thus TVB-N could not be used as an indicator of frozen eel quality. This study showed that off-flavour and off-odour was not detected and frozen European eels were still acceptable by panellists and could be stored for more than 48 weeks at -20°C. So, obviously, TVB-N value would not be an indicator of quality assessment for frozen fillets with a 12 weeks limit of storage period.

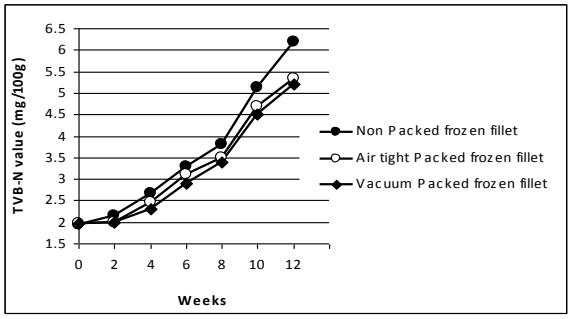

Fig. 3. Changes in TVB-N (mg/100g) of rohu (*Labeo rohita*) fillets under different packaging conditions during frozen storage

Changes in peroxide value

The Peroxide value of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during frozen storage. Peroxide value is the milliequalivalants of peroxidises per kilo gram of sample. It is titrimetric determination. In this experiment the peroxide values for frozen storage were quite different compared to ice storage. The result of the changes in peroxide value of rohu fillets (*Labeo rohita*) during frozen storage is shown in (Fig. 4). The initial value of non-pack frozen stored fillets was 0.79 meq/kg of oil, which increased gradually with the lapse of frozen storage period. At the end of 12 weeks of storage, the peroxide value was 3.34 meq/kg of oil, which was within recommended value of 10 to 20 meq/kg of oil. In case of air-tight and vacuum packed frozen stored fillets the initial values were 0.78 and 0.79 meq/kg of oil respectively. At the 12 weeks of frozen storage period, the values were 2.25 and 1.84 meq/kg of oil respectively.

According to Connell (1980) the recommended value of peroxide for fresh finfish were 10-20 meq/kg of oil. The value above 20, the fish were found to be emitting smell and taste rancid. 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, Simeonidou et al. (1997) reported that salmon did not show significant lipid oxidation during frozen storage, although it was a fatty fish. Various factors, such as the freezing temperature, the rate of freezing, vacuum packaging or packaging materials, can affect frozen fish quality, and these had been studied. Frozen fish were often stored in the form of fillets; however, filleting operations can affect frozen fish quality, as reported for hake (*Merluccius hubbsi*) during long-term frozen storage. Frozen storage of oil sardine mince stored for 150 days at -20°C resulted in an increase in peroxide value (PV) and free fatty acids (FFA). Ozogul *et al.* (2007) found peroxide value (PV) of the wild European eel (*Anguilla anguilla*) stored at -20°C of the maximum level of 13.20±1.73 meq/kg, which did not exceed the maximum recommended value for human consumption (20 meq/kg). But, Muslemuddin *et al.* (1984) reported that the peroxide value of hilsa increased from 3.5 to 35.0 in 75 days of storage at -7^{0}C to -10^{0}C. Aubourg et al. (2005) studied on frozen mackerel (*Scomber scombrus*) fillet that was increased in a minute value for lipid oxidation (peroxide value and thiobarbituric acid index) during the frozen storage.

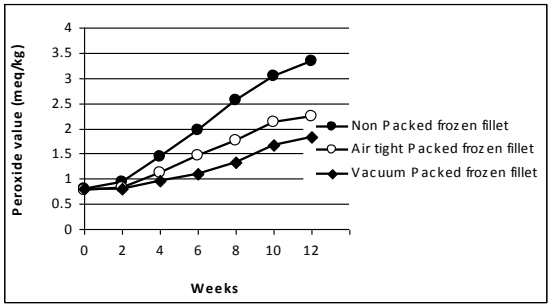

Fig. 4. Changes in peroxide value (meq/kg of oil) of rohu (*Labeo rohita*) fillets under different packaging conditions during frozen storage.

Changes in myofibrillar protein solubility

Protein solubility is one of the most important parameter to assess the fish quality during storage. Proteins are soluble in their natural environments. A given natural environment provides all materials and tools necessary and sufficient for a particular protein expression. Multiple factors may be important for protein solubility. Combining special medium, cell strains with molecular chaperones, and low protein synthesis rate (at low temperature up to 10^{0}C), many proteins will become soluble and functional. Those could be easily analyzed to get an experimental result. In case of frozen stored fillets, the results of the myofibrillar protein solubility are presented in the (Fig. 5). Here, non-packed frozen stored fish fillets showed the initial myofibrillar protein solubility, 90.05% which gradually decreased with lapse of storage period. At the end of the 12 weeks in frozen storage myofibrillar protein solubility decreased at 71.5%. On the other hand, air-tight packed fillets and vacuum packed fillets also gave the results of myofibrillar protein solubility and those were initially 90.18 and 90.12% respectively. After 12 weeks of frozen storage, the myofibrillar protein solubility percentages were 77.2 and 80.1% respectively.

Three types of packaging conditions in frozen storage showed that those three values were beyond the recommended value and they were so far from the rejection limit. So, myofibrillar protein solubility would not be an indicator of quality assessment for frozen fillets with a 12 weeks limit of storage period. Chakrabarti and Madhusudana (2008) found that, *Labeo rohita* (rohu), *Catla catla* (catla), *Cirrhinus mrigala* (mrigala) and *Cyprinus carpio* (common carp) were dressed, frozen and stored at -20°C in commercial deep freezer for 10 months. The results showed gradual reduction (92.2~92.5% to 70.23~70.05%) in the protein solubility during the storage. It was also reported that frozen storage of oil sardine mince stored for 150 days at -20°C resulted in a decrease in protein solubility (Namulema *et al.,* 1999). Valls et al.

(2006) evaluated the physical, chemical and sensorial changes in sardine (*Sardinella aurita*) fillets under frozen storage at -18°C for 6 months. Samples were taken monthly to evaluate solubility of proteins. The results showed that storage time significantly reduced the protein solubility of sardine.

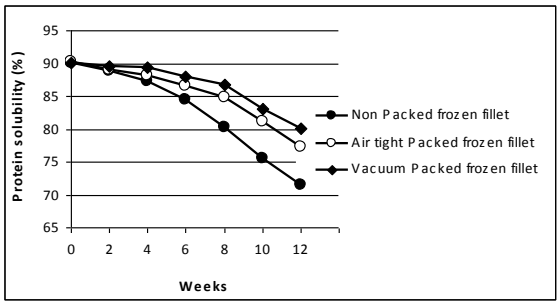

Fig. 5. Changes in myofibrillar protein solubility (%) of rohu (*Labeo rohita*) fillets under different packaging conditions during frozen storage

Microbiological study

Changes in microbial load

In case of frozen stored fillets, the results of the microbial load are presented in the (Fig. 6). Here, non-packed frozen stored fish fillets showed the initial microbial load, 2.56×10^5 cfu/g which suddenly decreased to 1.9×10^3 cfu/g within the first days of storage period. This may be caused by cold shock and effects of freezing on bacterial cells. But the survivals were mostly spore-formers and highly psychrophilic bacteria. At the end of the 12 weeks in frozen storage microbial load turned into 8.2×10^3 cfu/g. On the other hand, air-tight packed fillets and vacuum packed fillets showed initially microbial loads of 2.67×10^5 cfu/g and 2.59×10^5 cfu/g respectively. After 12 weeks of frozen storage, the microbial loads were 7.8×10^3 cfu/g and 7.6×10^3 cfu/g respectively. The declination of microbial load in second weeks of frozen storage was caused by cold shock and deactivation of bacterial cells. About to all mesophillic and thermophillic bacterial populations were completely died except spore-formers. However, psycrophillic populations increased over the time of storage period but the rate was very slowly.

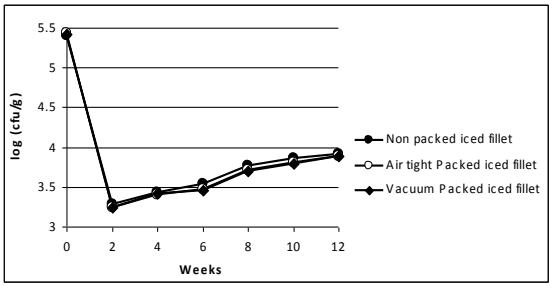

Fig. 6. Bacterial study of frozen fillet.

Information about the microbiology of ice and frozen stored Rohu fillet was scanty. However, handful information was available on the quality and quantitative aspects of bacteriology change in variety of chilled fish. The storage quality of some underutilized fishes was evaluated during storage at −20°C for 3 months. Quality and storage stability were evaluated through total aerobic and coliform bacterial count, peroxide value, protein solubility, and color. Total aerobic bacteria were reduced significantly (P<0.05) by 84% of the initial load, whereas coliforms were completely destroyed at the end of storage. Fishes were acceptable for 3 months at −20°C. Storage stability was rationalized by the effectiveness of freezing (Ismail et al., 2009). The experiment was carried out in a summer season (July-October) where the temperature remained on an average 34°C and which temperature is very susceptible for microbial growth. Obviously environment contained a huge mesophilic bacterial population. So, may be, the unwanted contamination of mesophilic bacteria increased the microbial counts.

However, the quality of fillets remained as Vacuum packed fillets > Air-tight packed fillets > Non-packed fillets of rohu (*L. rohita*).

References

Aubourg, S. P., Rodriguez A. and Gallardo J. M. (2005). Rancidity development during frozen storage of mackerel (*Scomber scombrus*): Effect of catching season and commercial presentation. *European Journal of Lipid Science and Technology.* 107(5): 316-323.

Chakrabarti, R. and Madhusudana R. B. (2008). Effect of storage in commercial deep freezer (-20°C) on quality of four tropical freshwater carp meats. *Journal of Food Science and Technology, Mysore*. 45(2): 157-160.

Connell, J.J. (1975). Quality deterioration and extrinsic quality defects in raw materials. In: Control of fish quality. 2nd. Ed. Fishing New Books Ltd. Surrey, England. pp. 31-35.

Howgate, P. A. J. and Whittle K. J. (1992). Multilingual Guide to EC Freshness Grades for Fishery Products. Torry Research Station, Food safety Directorate, Ministry of Agriculture, Fisheries and Food, Aberdeen, Scotland.

Ismail, M., Al-Bulushi, Stefan, K., Hamed A. O. and Sultan A. Mamari (2009). Evaluating the quality and storage stability of some underutilized fish during frozen storage. *Fisheries Science*, 71 (3): 648-654.

Jasra, S. K., Jasra P. K. and Talesara C. L. (2000). Myofibrillar protein degradation of carp (*Labeo rohita* (Hamilton)) muscle after post-mortem unfrozen and frozen storage, Muscle Histophysiology Unit, Department of Zoology, University of Delhi, Delhi, India.

Namulema, A., Muyonga J. H. and Kaaya A. N. (1999). Quality deterioration in frozen Nile perch (Lates niloticus) stored at ÿ13 and ÿ27_C, *Food Research International* 32: 151-156.

Orak, H. H. and Kayisoglu S. (2002). Quality changes of whiting (*Gadus euxinus*), gray mulet (*Mugil cephalus*) anchovy (*Engraulis encrasicholus*) fish species under iced storage. *Bulgarian Journal of Agricultural Science.* 8(5/6): 587-596.

Ozogul, F., Gokbulut, C. G., Ozyurt, Ozogul, Y. and Dural M. (2005). Quality assessment of gutted wild seas

bass (*Dicentrarchus labrax*) stored in ice, cling film and aluminium foil. *European Food Research and Technology.* 220(3/4): 292-298.

Simeonidou S., Govaris A. and Vareltzis K. (1997). Effect of frozen storage on the quality of whole fish and fillets of horse mackerel (*Trachurus trachurus*) and mediterranean hake (*Merluccius mediterraneus*). Zeitschrift fur Lebensmittel Untersuchung und Forschung. A, *European food research and technology*. 204(6): 405-410.

Valls, J., A. Paredes and Gonzalez D. (2006). Stability of sardine fillets (*Sardinella aurita*) under frozen storage at -18°C. *Revista Cientifica, Facultad-de-Ciencias Veterinarias, Universidad del Zulia.* 16(2): 176-185.

Vyncke, W. (1983). Shelf life of thawed cod fillets kept in ice. *Zeitschrift für Lebensmitteluntersuchung und -Forschung*. 177(1): 19-21.