

## INFLUENCE OF ICE GLAZING AND LONG TERM STORAGE ON SOME QUALITY PARAMETERS OF ROHU FILLETS DURING FROZEN STORAGE

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### ABSTRACT

This study was designed to investigate the influence of ice glazing on nutritional, chemical and microbial parameters of rohu fillets during frozen storage. Quality assessment of ice glazed rohu for up to 1 months at -12°C was done by the monitoring of nutritional quality, free fatty acids (FFA), thiobarbituric acid (TBA), pH and expressible moisture (EM). Results showed that free fatty acid, primary and secondary oxidation products, expressible moisture and pH value of ice glazed samples were significantly lower than those in control samples ( $p < 0.05$ ). Results indicated that ice glazing was effective in reduce lipid oxidation and increased shelf life of rohu frozen fillets. Similarly the microbial load of ice glazed samples was significantly lower as compared to control samples. Thus the employment of ice glazing alone or in combination with other protective strategies is recommended.

**Key words:** rohu, Frozen storage, Lipid oxidation, ice glazing.

### INTRODUCTION

Fish is a very perishable commodity, more than cattle, sheep and poultry. So, it must be properly preserved before it is disposed off. Major factors responsible for its perishable nature are the microbial growth and oxidation of lipids which influence the colour, texture, nutrition, safety along with flavour. All these negative changes limit the marketing process of fish products and hence to satisfy the consumer demand, it is necessary to produce good quality and safe fish. Delay in microbial contamination of fish may be achieved by different preservatives like potassium sorbate, citric acid, ascorbic acid and lactic acid etc. Potassium sorbate is considered to be an effective preservative against a wide spectrum of food spoilage microorganisms. They are among the safest, most efficient and versatile

preservatives used in the food industry today. Sorbates are tasteless and odourless (Omojowo *et al*, 2009). As they are non-toxic, they are used in a wide variety of foods, including cheese, yogurt, sour cream, bread, cakes, baking mixes, icing, beverages, margarine, fermented vegetables, fruit products, salad dressing, smoked and salted fish and mayonnaise. The antimicrobial activity of sorbates against molds, bacteria and fungi has been reported by researchers (Sofos, 2000). Considering the antimicrobial activity of Sorbate, the aim of present study was to determine the microbial and nutritional quality changes in the fillets of Silver Carp (*Hypophthalmichthys molitrix*) stored under frozen conditions ( $12 \pm 2^\circ\text{C}$ ) for 30 days. Therefore the this study was designed to investigate the influence of ice glazing on nutritional, chemical and microbial parameters of rohu fillets during frozen storage.

## MATERIALS AND METHODS

### Collection of fish samples:

Fresh samples of *Labeo rohita* were purchased from local market of Jammu city. They were immediately brought to the lab in polythene bags along with crushed ice. The viscera of fish was removed and the fish was washed with large amount of water. The fish was cut into pieces and these pieces were immediately wrapped in aluminum foil, kept in air tight plastic container and stored at  $-12\pm 2^{\circ}\text{C}$  (frozen storage). Analytical procedures for biochemical and microbiological changes were done on 0, 10th, 20th and 30th day of storage.

### Analysis:

The proximate composition (protein, lipid, ash and moisture) of the fish samples were evaluated using the standard AOAC procedure. The protein content was determined using the Lowry *et al* method. Fat content was determined using Folch *et al* method. Thiobarbituric acid value of fish muscle during frozen storage was determined by using the method of Witte *et al* (1970). Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick). Extract Release Volume (ERV) was determined as per the method of Strange *et al.* (1977). The pH of fish muscle was determined by the method of Keller *et al.* (1974). The microbiological profile was determined according to APHA method. Data was expressed as mean  $\pm$  SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

### Statistical Analysis:

Mean and standard errors were calculated for different parameters.

The data analyses were performed using SPSS software (12.0 for Windows). Differences between treatments were analyzed using independent-measures one-way ANOVA. Post-hoc comparisons were conducted using Duncan's test. The values were expressed as mean  $\pm$  SE. p values  $<0.05$  were considered as significant and p values  $<0.001$  were considered as highly significant.

## RESULTS AND DISCUSSION

### Proximate Composition:

#### Protein content:

In present investigation a decreasing trend was observed in Total protein content of both control and ice glazed samples for a period of 30 days.

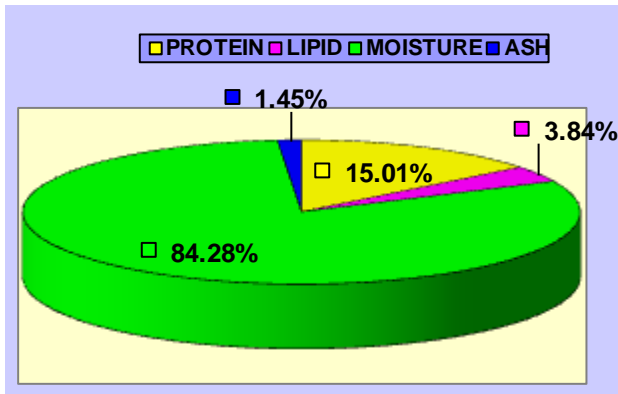
Perusals of table 1 & 2 depict that that minimum protein loss i.e. 16.52% occurred in processed ice glazed muscle and raw unprocessed muscles shows maximum loss i.e. 26.44%. This low protein content in unprocessed raw samples was perhaps mainly due to the increased microbial growth and higher water activity. Samples processed by thick ice glaze registered a comparatively less protein loss due to enzymatic activities of only psychotropic microbes. The ice layer sublimates instead of the fish below and it also excludes air from the surface of the fish and thereby reduces the rate of oxidation (Bogh-Sorensen 2002). In support with the present studies, Arannilewa *et al.* (2005) and Siddique *et al.* (2011), Keyvan *et al* (2008) 10 in Caspian white fish and Aberoumand (2013) in various Iranian fishes also found a protein loss during frozen storage. They suggested that the autolysis helps the bacteria to invade the tissue rapidly, the free amino acids and water soluble

**Table 1:-Changes in proximate and biochemical composition of frozen fish muscle**

Biochemical paramters	0	10th	20 <sup>th</sup>	30th
Protein (%)	15.01 $\pm$ 0.06%.	14.00 $\pm$ 0.02%	12.92 $\pm$ 0.03%.	11.04 $\pm$ 0.2% .
Lipid (%)	3.84 $\pm$ 0.014%	3.03 $\pm$ 0.025%	2.77 $\pm$ 0.03%	2.00 $\pm$ 0.03%
Moisture (%)	84.28 $\pm$ 0.1%.	82.01 $\pm$ 0.015%	79.56 $\pm$ 0.043%	75.54 $\pm$ 0.09%
Ash (%)	1.90 $\pm$ 0.12%..	1.66 $\pm$ 0.02%	1.13 $\pm$ 0.001%..	0.99 $\pm$ 0.04%.
TBAmg MA/kg	0	5.94 $\pm$ 0.06mg	9.16 $\pm$ 0.03	10.01 $\pm$ 0.02
FFA (%)	0.45 $\pm$ 0.024	4.14 $\pm$ 0.06%	8.26 $\pm$ 0.04%.	12.27 $\pm$ 0.07%.
Ph	6.32 $\pm$ 0.2.	7.0 $\pm$ 0.1	7.75 $\pm$ 0.5	7.85 $\pm$ 0.4

protein content of tissue serve as an excellent source for their growth and as a result not only the quality but also the quantity of protein decreases.

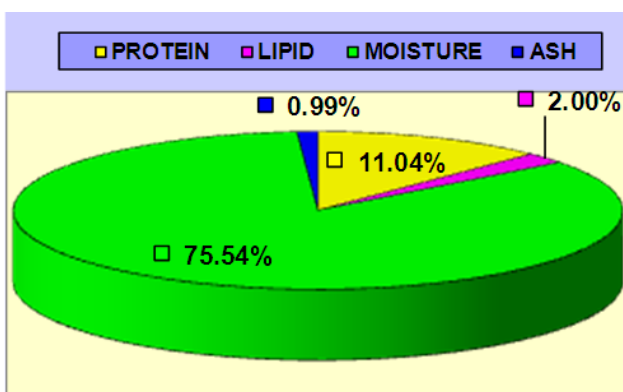
**Fig-1. Proximate composition of unprocessed raw muscle during frozen storage at -12±10c ON DAY 0.**



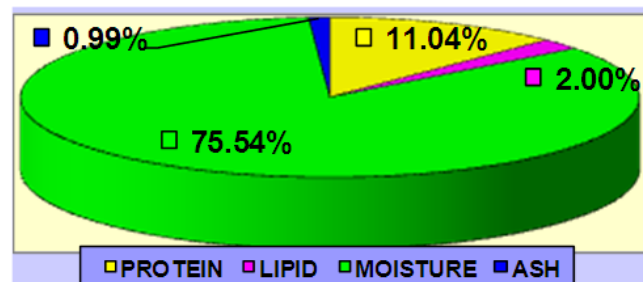
**Lipid content:**

The results shown in table-1 & 2 show that the lipid content decreased significantly ( $p \leq 0.05$ ) from day 0 i.e.  $3.84 \pm 0.014\%$  to  $2 \pm 0.03\%$  in control and  $3 \pm 0.03\%$  in ice glazed on day 30 th .Arannilewa et al in *Tilapia*, Keyvan et al in Caspian sea white, Siddique et al in *Puntius sps.* and Aberoumand (2013) in various Iranian fishes also found a decrease in total lipid content during frozen storage. They opined that this protein loss must be due to oxidation and hydrolysis of lipids during frozen storage.

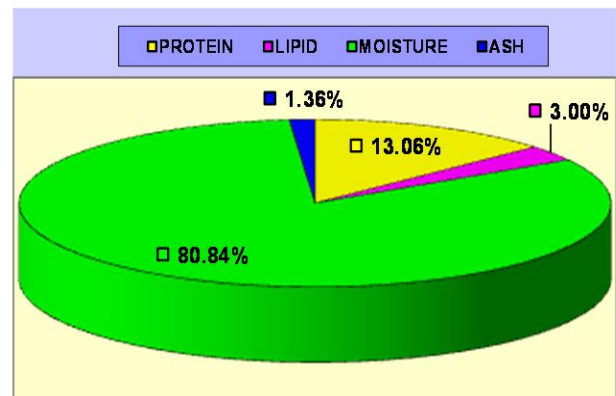
**Fig 2: Proximate composition of unprocessed raw muscle during frozen storage at -12±10c ON DAY 30th**



**Fig-3. Proximate composition of ice glazed fish muscle during frozen storage at -12±10c ON DAY 30th**



**Fig-4: Proximate composition of ice glazed fish muscle during frozen storage at -12±10c ON DAY 30<sup>th</sup>.**



**Moisture:**

The total moisture content of the fish sample decreased from  $84.28 \pm 0.1\%$  on day 0 to  $75.54 \pm 0.09\%$  in control and  $80.84 \pm 0.09\%$  in ice glazed on day 30th . Total percent decrease was 5.34% and 11.63% in ice glazed and control samples respectively. These results are favoured by the findings of Gandotra et al 2012 Rostamzad et al,(2011) Bhat et al., 2010, Arannilewa et al(2005) who proposed that Ice glazing fish prior to storage, is the commercial way of affording protection against dehydration and to some extent against the development of rancidity .

**Biochemical Composition:**

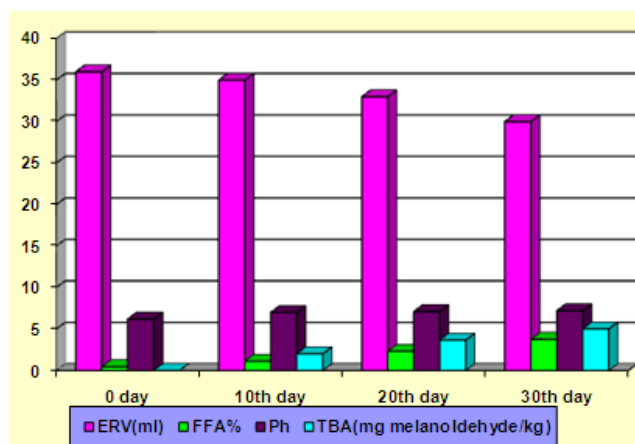
**Thiobarbituric acid (TBA):**

The TBA value is an index which measures the malondialdehyde (MDA) content and is a widely used method for assessment of degree of lipid oxidation. MDA is formed through

**Table 2:- Changes in proximate and biochemical composition of ice glazed fish muscle**

Biochemical paramters	0	7th	14 <sup>th</sup>	21 <sup>st</sup>
Protein	15.93±0.04%	15.01±0.02%	14.14±0.03%.	13.06±0.04%.
Lipid	3.86±0.04%	3.65±0.02%	3.25±0.04%	3.00±0.03%
Moisture	84.74±0.1%.	83.82±0.015%.	82.45±0.02%.	80.84±0.09%
Ash	1.79±0.01%	1.69±0.012%	1.57±0.02%	1.36±0.03%
TBA	0.16±0.04	2.01±0.04	3.67±0.13	5.99 ±0.01
FFA	0.45±0.04%	1.12±0.02%	2.32±0.03%.	3.76±0.04%.
Ph	6.2±0.2	7.0±0.02	7.1±0.15	7.2±0.4.

hydroperoxides, which are the initial reaction products of polyunsaturated fatty acids with oxygen. The present study showed a progressive increase in TBA value (secondary oxidation product) with increase in storage period under frozen conditions. The values rose from 0.16±0.2 on day 0 to 10.01±0.05 in control and 5.99 mg MA/kg in ice glazed on 30th day of frozen storage period. The above results are in accordance with those of Ryder *et al.* (1984), Zamir *et al.* (1998), Mazonra Manzano *et al.* (2000), Chijan *et al.* (2006) and Kandeepan and Biswas (2007). The increase in TBA is attributed to the fact that freezing and thawing accelerates the accumulation of secondary oxidative products released due to the destruction of cell membrane by crystals of ice and release of pro-oxidants, especially haem-iron.

**Figure 5: Changes in biochemical composition Of ice glazed muscle of *Labeo rohita*****Free fatty acids (FFA) :**

The values for Free Fatty Acids (FFA) were 0.45±0.02 on day 0 and it rose to 12.27 in

control and 3.76 in ice glazed samples on 30 day of frozen storage respectively. The results thus clearly depicts, that there was a gradual increase in the FFA content with increasing storage time. The levels had also direct correlation with pH (Table) showing that it could act as a good indicator for the assessment of the freshness of all the three forms of stored fish muscles. These results are in accordance with Ozogul *et al* (2011) in *Solea solea*, Peter *et al* (2010), in (*Theragra chalcogramma*) and Gandotra *et al* in *Mystus*. These findings are devoted to the glazing of fish fillets that had a significant positive effect on decreasing the lipid oxidation and production of metabolites of fat deterioration.

**pH:** The pH values also showed an increasing trend with increase in frozen period. The pH values ranged from 6.32±0.2. on day 0 to 7.85±0.4 in control and 7.2±0.4. in ice glazed on 30th day. These results are in line with Arannilewa *et al* (2005), Bao *et al* (2007) in Arctic Charr in frozen Tilapia (*Sarotherodon galiaenus*), Jezek and Buchtova (2011) in Common carp (*Cyprinus carpio* L.) and Silver carp (*Hypophthalmichthys molitrix* V.) and Erkan and Ozden (2007) in Guttred sardines (*Sardina pilchardus*) who attributed this increase to the production of basic components induced by bacterial growth.

**Microbial quality+:**

The quality of fish meat is largely dependent on its microbial contamination. Inquitive study of table- 4 shows an increasing trend for TPC, CC and PC during the frozen storage period. Initially the values for TPC were 2.44±0.2 log cfu/g and

increased to  $9.10 \pm 0.02$  log cfu/g in control and  $5.10 \pm 0.02$  log cfu/g in ice glazed samples at the end of storage, thus crossing the permissible limits of 6 log cfu/g on 10th day of storage in control samples. Similarly, CC and PC also showed an increasing trend in both control and ice glazed samples on last day of storage. Likewise, Arannilewa *et al* found an increase in Coliform count with the increasing storage period in frozen Tilapia. Ozogul *et al* also reported a significant statistical increase in total viable counts of whole gutted common sole (*Solea solea*) over the storage period of 24 days. Similarly, Ola and Oladipo and Liu found an increasing trend for psychrotrophs during storage period. This increase in microbial count is attributed to growth promoting effect of moisture during frozen storage.

### CONCLUSION

The main objective of this study was to observe nutritional, biochemical and microbial changes in *Labeo rohita* during frozen conditions. The freezing of fish at low temperature makes it less prone to spoilage by decreasing the bacterial activity. However, it was observed that there was a decrease in the nutritional parameters while an increase was observed in biochemical composition and microbial count during frozen storage. Therefore, it could be concluded that we should try to consume fish while it is fresh only. Since, all fishes are not available throughout the year; hence, freezing is best preferred when preservation of such fish species is of priority.

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