Quality changes in the raw and brined muscle (20%) of *Labeo rohita* stored under frozen storage conditions (-12^{0}C).

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ABSTRACT: *The aim of present study was to investigate the effect of 20% brine treatment on the fillets of Labeo rohita stored at -12^{0}C for 30 days. The fillets were divided into two groups viz. Gp. A (raw untreated, control) and Gp. B (treated with 20% brine solution) and were analyzed for proximate and microbial parameters. The total percental decrease in protein, lipid, ash and moisture was .95%, 27.5%, 2.61%, 35% in brined samples (Gp. B) and 6.69%, 13.26% 4.30 %, 20% in raw samples (Gp. A) repectively. Also, the Total plate count (TPC), Coliform count (CC) and Psychrophillic count (PC) showed a comparative low counts in Gp. B (brined samples). Thus, 20% brine treatment is effective in extending the shelf life of frozen fish fillets of Labeo rohita.*

Key Words: *Brined, Labeo rohita, Proximate, Microbial and Sensory*

INTRODUCTION

A mass contribution to the survival and health of a significant portion of world's population, particularly to the developing nations has been done by the fish. It is a good source of proteins, poly-unsaturated fatty acids, micronutrients like vitamins (A, D, E, K, B_{12} etc) and minerals (iron, calcium, iodine, potassium etc). However, the high water content in fish makes it highly perishable commodity, as water acts a good medium for bacterial growth. Therefore, there is a need of good preservation method so as to control if not totally arresting the process of spoilage. Various preservation methods applied for enhancing the shelf life of fish include chilling, freezing, drying, use of antioxidants and brining etc.

Brining is a method of salting where the fish is kept immersed in brine of desired concentration for the required time. The properties of fish muscle vary due to changes in water and salt content: the muscle gains salt, whereas water is lost or gained depending on the salting procedure (Thorarinsdottir *et al.* 2002 and Thorarinsdottir *et al.* 2004). Since brine becomes progressively diluted, hence it must be replaced with strong one after the initial brine becomes diluted. The main advantage of storing the brined fish under freezing is the rapidity with which heat is removed from the fish because of intimate contact of fish with brine from all sides. Under this background, an attempt was undertaken to assess the quality changes through proximate and microbial evaluation in muscle of *Labeo rohita* available in the market of Jammu.

MATERIALS AND METHODS

Sample collection

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.

Sample processing

The viscera of fish were removed and the fish was washed with large amount of water. The fish was cut into pieces and then,

- To prepare raw control sample (Gp. A), these pieces were washed and immediately wrapped in aluminium foil, kept in air tight plastic container and stored at -12±2°C (frozen storage).
- To prepare the brined samples (Gp. B), these pieces were immersed in 20% brine solution in ratio 1:2 for 2 hours. Brining was conducted in glass containers at 4±1°C. After 2 hours, the fish pieces were removed from the brine solution and left as such for half an hour for extract release. Finally the fish pieces were washed and immediately wrapped in aluminium foil, kept in air tight plastic container and stored at -12±2°C (frozen storage). Analytical procedures for proximate and microbial changes were done on 0, 10th, 20th and 30th day of storage

Sensory Analysis

Sensory analyses were conducted by a taste panel consisting of five to seven semi experienced judges, as per the guidelines presented in Table-1 (DOCE, 1989). Four categories were observed viz. highest quality (E), good quality (A), fair quality (B) and rejectable quality (C). the fish samples under different treatments were analyzed for appearance, rancid odour and flesh consistency. At each sampling time, the fish samples of Gp. A and Gp. B were thawed and then presented to the panel members in the individual polyethylene bags and the scores were recorded.

Table 1: Scale employed for evaluating sensory quality (Adapted from DOCE, 1989)

Attribute	E(Highest quality)	A (Good quality)	B (Fair quality)	C (poor quality)
Flesh appearance	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated
Rancid odor	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharp, sour and rancid
Flesh consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes as a result of mechanical factors

Analyses

The proximate composition (ash and moisture) of the fish samples were evaluated using the standard AOAC procedure (AOAC, 1995). The protein content was determined using the Lowry et al. (1951). Fat content was determined using Folch et al. (1957). The microbiological profile was determined according to APHA method (1984). Data were expressed as mean ± SD and were analyzed by one-way ANOVA test using SPSS statistical programme.

RESULTS AND DISCUSSIONS

Proximate composition

The proximate composition and the percental decrease in Gp. A and Gp. B samples stored at -12±2°C for 30 days is shown in Figure1 and 2 and Table- 1.

It is quite evident from the Fig. 1 and 2 that proximate composition of both raw and brined samples revealed a decreasing trend. The protein, fat, ash and moisture decreased from (16.52%, 2.18%, 1.10% and 81.20%) to (11.4%, 0.81%,. 0.88% and 74.8%) in raw Gp.A samples while from (15.9%, 1.92%, 3.1% and 80.42%) to (14.9%, 1.5%, 2.9% and 76.42%) in Gp.B i.e brined samples respectively. However, the total percental decrease of proximate composition after 30 days of frozen storage was 30.99 and 6.28% for protein, 62.84 and 21.87% for lipid 20.00 and 6.84% for ash and 4.97% and 4.97% for moisture in Gp. A and Gp. B.samples respectively.

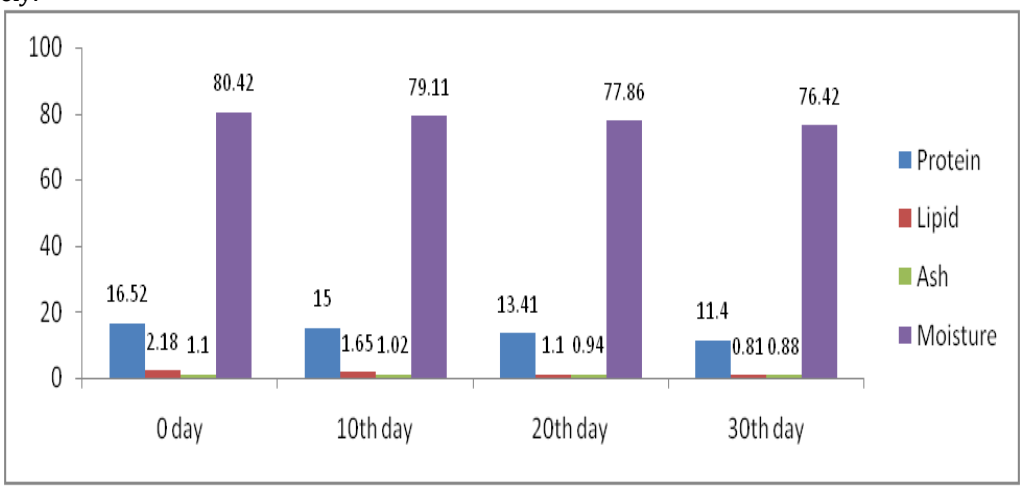

Fig. 1. Proximate composition changes in Gp. A samples stored at -12°C for 30 days.

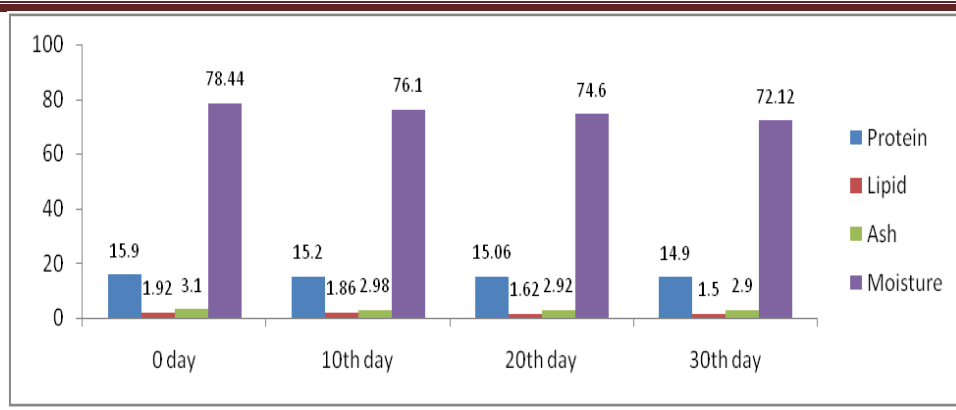

Fig. 1. Proximate composition changes in Gp. B samples stored at -12°C for 30 days

Table 2. Percental decrease in proximate composition of Gp. A and Gp. B samples of *Labeo rohita* stored at -12±2°C for 30 days.

Days	Protein (%)		Lipid (%)		Ash (%)		Moisture (%)	
	Gp. A	Gp. B	Gp. A	Gp. B	Gp. A	Gp. B	Gp. A	Gp. B
0-10	9.20	4.40	24.31	3.12	7.27	3.87	1.62	2.98
0-20	18.82	5.28	49.54	15.62	14.54	5.80	3.18	4.89
0-30	30.99	6.28	62.84	21.87	20.00	6.45	4.97	8.05

Presently, the decrease in protein content during storage in both the samples might be due to the denaturation of fish protein i.e. due to the changes in the proportion of chemical composition and protein breakdown. This is in accordance with Thorarinsdottir *et al* (2002) in salted cod (*Gadus morhua*), Gandotra *et al.* (2013) in *Cyprinus carpio* who found that the denaturation of protein leads to increased leaching effect of amino acids and hence decreased protein content. Initial lower values of protein in 20% brined samples is due to the large uptake of salt (NaCl) by fish muscle which resulted in competition with muscle protein for water molecule and denaturation and aggregation of proteins by a process of salting out (Unlusayin *et al.*, 2010). Similarly, Nketsia-Tabiri and Sefa-Dedah (1995) reported that salting of Tilapia caused losses in protein due to a reduction in moisture that has denaturing effect of salt on fish protein However, the lower decrease in protein content of Gp. B samples after 30 days may be attributed to the fact that NaCl concetntartion slowed down autolysis in fish muscle which consequently slowed down the protein breakdown (Sameul *et al*, 2010).

Lipid content decreased with storage time in both Gp. A and Gp. B samples due to the ongoing lipid hydrolysis and oxidation processes (Gandotra *et al*., 2013 in *Wallago attu*). Further, Unlusayin *et al*. (2010), Ahmed *et al*. (2010) in salted kass (*Hydrocynus forskalii*) observed that the total lipid content decreased after brining and stated that a part of fat in fish muscle was oozed out as extract release volume during salt treatment and there existed a correlation between transfer rate of lipid from muscle and salt concentration in muscle. Also, the lower percental reduction in Gp. B (brined) samples might be due to the water loss from the muscles which reduces the lipid hydrolytic phenomena.

The decrease in moisture content of both Gp. A and Gp. B samples was associated to the drip loss during thawing process. Findings of Emire *et al.* (2009) in Nile Tilapia fish (*Oreochromis niloticus*), Akter *et al*. (2012) in tilapia fish (*Oreochromis niloticus*) and bele fish (*Glossogobius giuris*), Gandotra *et al.* (2013) in *Wallago attu* supported the present results.

The higher ash content in salted samples was due to the water losses associated in brining and salt penetration in to fish flesh during salt curing process (Jittinandana *et al.* 2002, Ahmed *et al.* 2010 and Unlusayin *et al.* 2010).

MICROBIAL CHANGES

Perusals of Table-2 revealed the changes in Total plate count (TPC), coliform count (CC) and Psychrophillic count (PC) of both Gp. A and Gp. B samples. The bacterial count increased with the increase in storage period but the higher increase was observed in Gp.A samples.

Table-3. Changes in the microbial count in Gp. A and Gp. B samples of *Labeo rohita* stored at -12±2°C for 30 days.

Days	TPC (log cfu/g)		CC (log cfu/g)		PC (log cfu/g)	
	Gp. A	Gp. B	Gp. A	Gp. B	Gp. A	Gp. B
0 day	2.78	1.54	1.66	1.02	1.96	1.29
10th day	4.44	2.15	2.24	1.34	3.09	2.54
20th day	6.73	3.67	3.92	1.93	4.25	3.41
30th day	8.12	4.98	6.32	2.42	5.02	4.14

The permissible values for TPC (6log cfu/g) and CC (2.69log cfu/g) was crossed on day 10th of storage while that of PC (4.6log cfu/g) were crossed on 20th day of storage. However, the brined samples (Gp. B) maintained the permissible values upto the end of storage period. The present results are in accordance with Tsai *et al*. 2005, Gumus *et al*. 2008 and Khanipour *et al.* 2014) who advocated that low rate of bacterial growth in salted products is due to the fact that free water bound by the sodium chloride is not readily available for bacterial growth. Further, Balachandran, K.K. (2016) reported that when concentration of salt is 20% or more in flesh, the decomposition process in the fish proceeds very slowly due to prevention of bacterial spoilage.

SENSORY ANALYSIS

The food quality is generally assessed through sensory evaluation. Sensory analysis or sensory evaluation is a scientific discipline that evokes, measures, analyses and interprets reaction to those characteristics of food that are perceived by senses of sight, smell, taste, touch and hearing (Stone and Sidel, 1993). Perusals of Table-3 reveal that progressive scores for the three attributes viz. flesh appearance, rancid odour and flesh consistency decreased with the increasing frozen storage period. Gp. A (raw, controlled) samples were rejected after 10th day of storage while Gp. B samples were acceptable upto 30th day of frozen storage.

Table- 4 Sensory changes in the Gp. A and Gp. B muscle samples of *Labeo rohita* stored under frozen conditions at -12±2 ˚C.

Frozen storage time	Flesh appearance		Rancid odour		Flesh consistency	
	Gp. A	Gp. B	Gp. A	Gp. B	Gp. A	Gp. B
0th day	A	E	A	E	A	E
10th day	B	A	C	A	B	A
20th day	C	B	C	A	C	B
30th day	C	B	C	B	C	B

The higher acceptability of Gp. B samples might be due to bacteriostatic effect of salt and lower lipid hydrolysis which contributes to higher acceptability by sensory panel.

CONCLUSION

Thus, the present study clearly indicated that brining has a positive effect on nutritional quality and shelf life of *Labeo rohita*. When introduced in sufficient quantities in fish flesh, salt can delay the activity of bacteria or even inactivate them by reducing the water activity. This forms the basis of preservation by brining. Higher level of salt may be required in the preservation of fatty fish species.

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