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The Quality Changes of Rohu (*Labeo rohita*) treated with Lemon gelatin during frozen storage (-120C).

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Abstract

Presently an attempt was made to study the effect of low temperature on the quality changes in raw untreated muscle (RM) and lemon gelatin (LGT) treated fish muscle of rohu (*Labeo rohita*)) stored in freezer for a period of 30 days. In fresh (unfrozen) samples, protein(15.93±0.04%), fat (3.86±0.04%),moisture (84.74±0.1%.),and ash content (1.79±0.01%) were found to be the highest. A significant total percental decrease ($p \leq 0.05$) in protein, lipid, moisture and ash content was found in both the samples after 30 days of storage. It was 26.44%, 47,91%, 10.37%, 31% in raw samples and 20.17%, 25.63%, 7.21%, 14.4% in Lemon Gelatin treated samples respectively. Rancidity development was measured by several biochemical parameters including Free Fatty Acid (FFA), Extract Release Volume (ERV) and Thiobarbituric acid (TBA).Besides pH and quantitative microbial analysis was determined during 30 days of storage. The FFA,ERV, TBA and pH was 0.47, 37,0 & 6.4 in raw samples on day 0 where as it significantly changed to 3.67, 25, 3.17 & 7.8 in LGT and to 5.27, 12, 5.45 & 7.85 in RM respectively at the end of storage($P<0.05$).Results showed that microbial count of LGT samples was significantly lower than those in control samples.($p<0.05$).

Keywords: Rohu, Lemon Gelatin, frozen storage, microbial count, *Labeo rohita*

Contents

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1. Introduction

Fish is highly perishable, due to its high water activity (aw) and protein content, neutral pH and presence of autolytic enzymes which cause fish spoilage. The rate of fish spoilage is affected by species, fat content, fishing and slaughter method, hygiene manipulation, postmortem handling and many other factors (Huss, 1995). Postmortem fish undergoes four stages as rigor mortis, dissolution of rigor mortis, autolysis and bacterial spoilage. The oxidative rancidity of fish lipids is caused by the activity of tissue enzymes and the oxygen radical spices. For inhibition of the lipid oxidation in chilled fish it is necessary to limit or avoid the oxygen admission (Decker and Xu, 1998).

Different methods have been used for extending fish products shelf life such as low temperature storage, proper packaging and glazing with solution of protecting chemicals and antioxidants (Yildiz *et al.*, 2006). The use of antioxidants is emerging as an effective methodology for controlling rancidity in oils and food (Frankel, 1998, Pazos *et al*., 2005). Antioxidants block the formation of free radicals, stabilize hydroperoxides and thus slow down oxidation and rancidity development. Recently, the demand for novel natural antioxidants has increased; this is because of possible adverse side effects of synthetic antioxidants and beneficial effects of natural antioxidants (Benjakul *et al*., 2005; Sarkardei and Howel, 2006). Therefore, the present study aimed to improving the quality and extending shelf life of the frozen fish using antioxidants as citric acids and gelatin.

2. 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 were removed and the fish was washed with large amount of water. Analytical procedures for biochemical and microbiological changes were done on 0, 7th, 20th and 30th day of storage.

Fish Treatment

The fish were divided into two groups. One group was treated with lemon gelatin solution. The fish was cut in to pieces and these pieces were dipped in lemon-gelatin solution, immediately wrapped in aluminum foil, kept in air tight plastic container and stored at-12±2°C (frozen storage).The second group was treated as fresh (Control), freeze without pretreatment of lemon gelatin solution.

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). Thiobarbituric acid value of fish muscle during storage was determined using the method of Witte et al (1970). Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick) described by Koniecko (1979). Extract Release Volume (ERV) was determined as per the method of Strange *et al.* (1977). The pH of fish muscles was determined by the method of Keller *et al.* (1974) 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.

Statistical Analysis:

Means 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 ± SE. p values <0.05 were considered as significant and p values <0.001 were considered as highly significant.

3. Results and Discussion

The contents of FFA,TBA, ERV and pH was recorded 0.47, 37,0 & 6.4 as fresh (Day 0), while it significantly increased to 3.67, 25, 3.17 & 7.8 in LGT(fish muscle treated with lemon gelatin) and to 5.27, 12, 5.45 & 7.85 in RM(fish muscle without lemon gelatin) respectively at the end of storage(P<0.05) as shown in Table 1.

Table 1. Free Fatty Acid (FFA), TBA (mg malonaldehyde/kg), Extract release volume (ERV) & pH in LGT and RM during frozen storage

	FFA(%)		**TBA(mg ma/kg),**		**ERV(ml)**		**pH**	
Days of storage	LGT	RM	LGT	RM	LGT	RM	LGT	RM
0	0.47±0.014	0.47±0.014	0.10	0.10	37±0.4	37±0.	6.4±0.1	6.4±0.1
10th	2.00±0.025	3.00±0.06	0.45±0.13	1.96±0.06	35±0.03	34±0.19	6.9±0.03	7.0±0.1
20th	2.52±0.03	4.14±0.04	1.45±0.1	3.16±0.0	31±0.06	24±0.14	7.01±0.0	7.2±0.5
30th	3.67±0.03	5.27±0.07	3.17±0.13	5.45±0.02	25±0.02	12±0.12	7.8±0.3	7.85±0.4

Free Fatty Acid

Results shown in table 2 clearly indicated that the total percent increase was 87.19% in LGT and 91.08% in RM samples during 30 days of storage . The present results are supported by the findings of Aubourg *et al*., (2004) who reported a positive effect of Citric acid in delaying lipid oxidation during frozen storage of mackerel. A gradual increase in FFA formation was observed in all fish samples there by indicating that hydrolytic activity of lipid constituents was occurring over time but by the end of experiment a lower lipid hydrolysis development (7.7%) in Citric acid treated samples compared to that of control (11.8%).The positive role of CA can be explained by chelation of heavy metal ions so that catalytic effect of these ions on peroxide decomposition during frozen storage

would be diminished.(Bratkowska et al.,1986). Similar results were reported by Badii &Howell (2002) ,Aubourg *et al*. (2004) and Pourashouri *et al*. (2009).Citric acid have been reported to act as synergist of primary oxidants in fish processing because of their oxygen scavenger and reducing roles.(Stodolnik *et al*.,1992).In present study the deceleration in FFA release in lemon gelatine treated samples may be due to the delay in lipid oxidation by the lemon juice containing citric acid. Moreover a glaze of lemon gelatine makes a thick layer on the samples preventing it to come in direct contact with air which possibly resulted in delayed lipid hydrolysis.

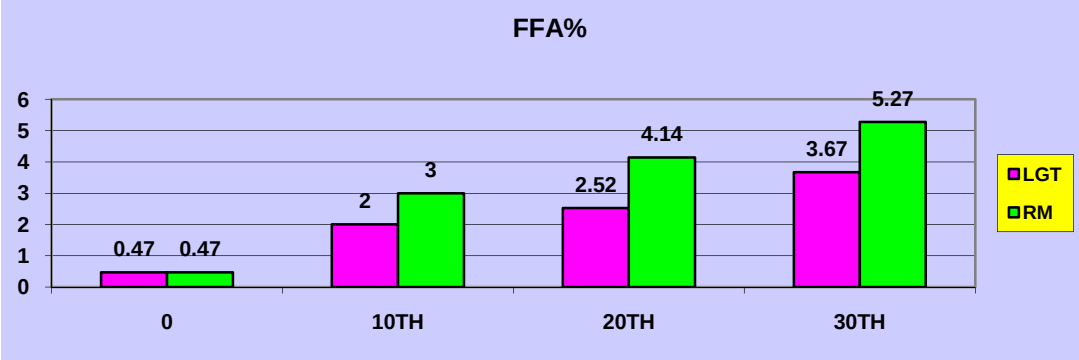

Figure.1

1.4.2 TBA:-Perusals of Table 2 depicted that percent increase in TBA during 21 days of frozen storage was 3.17 in LGT samples and 5.45 in RM samples. The antioxidants had positive effect on delaying lipid oxidation and increasing shelf life of fish.(Rostamzad *et al*.2011).Lower TBA values in samples treated by antioxidants in comparison to control samples were found by Benjakul *et al.*(2005), Sarkadei and Howell (2008), and Pourashouri *et al*.(2009).

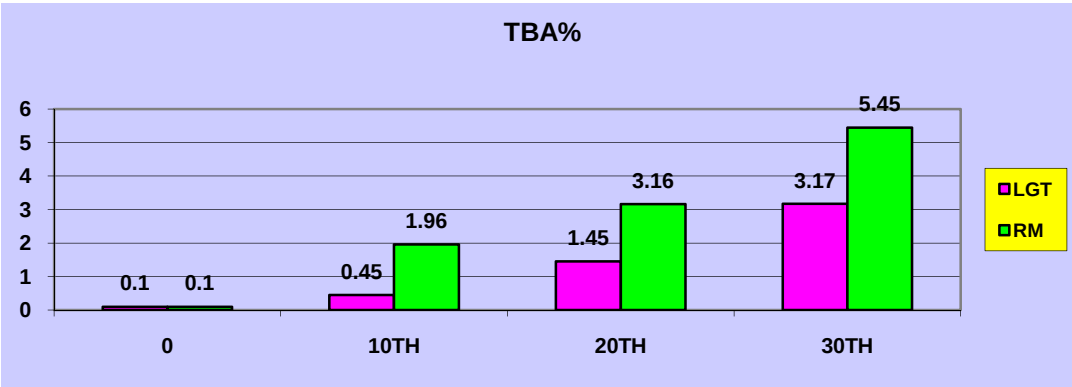

Figure.2

Sarkardei & Howell,2006 found a significant increase ($P<0.01$) in TBA value in untreated samples as compared to those treated with a combination of (Vit.A +Vit.C +Citric acid) during frozen storage of 16 weeks in Horse Mackerel (*Trachurus trachurus*).They suggested that a combination of proved to be an effective combination of (Vit.A +Vit.C +Citric acid)antioxidants because of the presence of metal chelating acids (ascorbic & citric acid) which isolate and inhibit the ability of metals to initiate or catalyse the oxidation reaction. The antioxidants had positive effect on delaying lipid oxidation and increasing the shelf life of fish.(Rostamzad *et al*.,2011).

Tokur *et al.(*2006) while studying the quality changes in trout with vegetable topping during frozen storage observed that increase in TBA of control samples(1.30) was higher than in samples treated with vegetable toppings(0.50).Their results showed that topping of trout fillets with vegetable topping may be protecting the lipid oxidation because of preventing it to be exposed to air. Treatment of fish muscle with lemon gelatin may be having a significant positive effect on decreasing the lipid oxidation and production of metabolites of fat deterioration due to the antioxidant properties of lemon gelatine . The antioxidants had positive effect on delaying lipid oxidation and increasing shelf life of fish.(Rostamzad *et al.*2011).

ERV: The decrease in extract release volume in LGT and RM samples after 30 days of frozen storage was 48% and 67% respectively.(Table 2). Rostamzad *et al*.,(2011) while studying the effect of ascorbic acid (AA) on surgeon

reported that expressible moisture (EM) content showed a gradual increase for all the samples during cold storage. comparison of two samples showed that EM of AA treated samples (29ml) was lower than control samples(58ml).

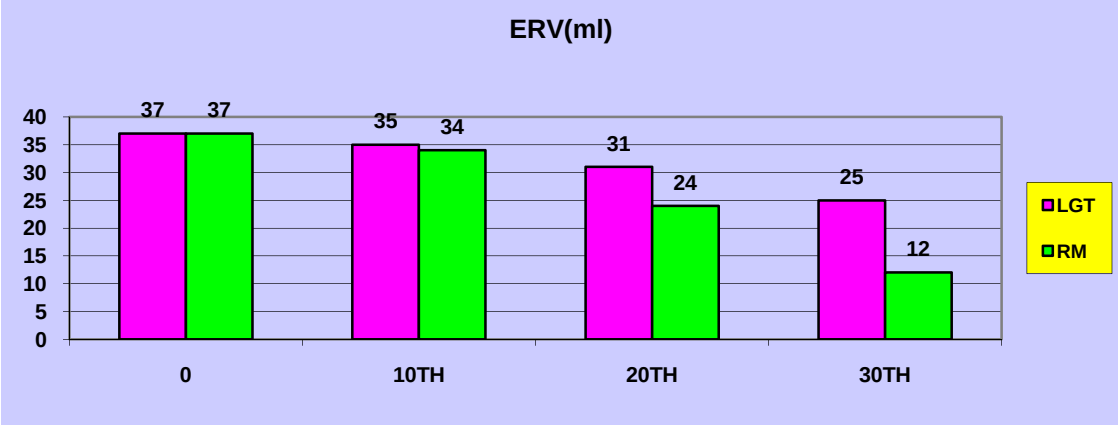

Figure.3

Water holding capacity in meat tissue is strongly related to myofibril proteins. Increase of expressible moisture is a sign of reduction of water holding capacity due to denaturising of proteins (Suvanich *et al*., 2000). This phenomenon leads to reduction of flavour agents and nutrition value (Reddy and Srikar,1991). The change in water holding capacity might have resulted from freezing process and length of time these fillets remained in cold storage.

pH:

Mean pH measurements over the period of storage at -12^{0}C in LGT and RM samples are shown in table 1.The initial pH values of 6.4 significantly increased to 7.4 in LGT & 7.85 in RM samples .Post mortem pH has been reported to vary from 6.0 to 7.1 depending on season, species and other factors (Simenidou *et al.*,1998).However Aubourg *et al*.,2004 found no statistical difference in pH values arising from the presence of antioxidants(Citric acid) on storage to all antioxidant treated samples as well as control samples .The muscle of living fish in general is approximately neutral in reaction and after catching, the pH value decreases, due to formation of lactic acid from glycogen by series of reaction.(Tarr *et al*.,1954). As spoilage advances ,the pH value of the muscle rises first slowly and later quite rapidly. This is due to accumulation of the basic end product of bacterial spoilage and pH rises up to 7.5 to 8.0.(Wood *et al*.,1942)The increased surface pH in spoiled fillets may be partly attributed to the production of alkaline compounds such as ammonia by spoilage bacteria (Cann *et al.*, 1983; Stammen *et al.*, 1990).

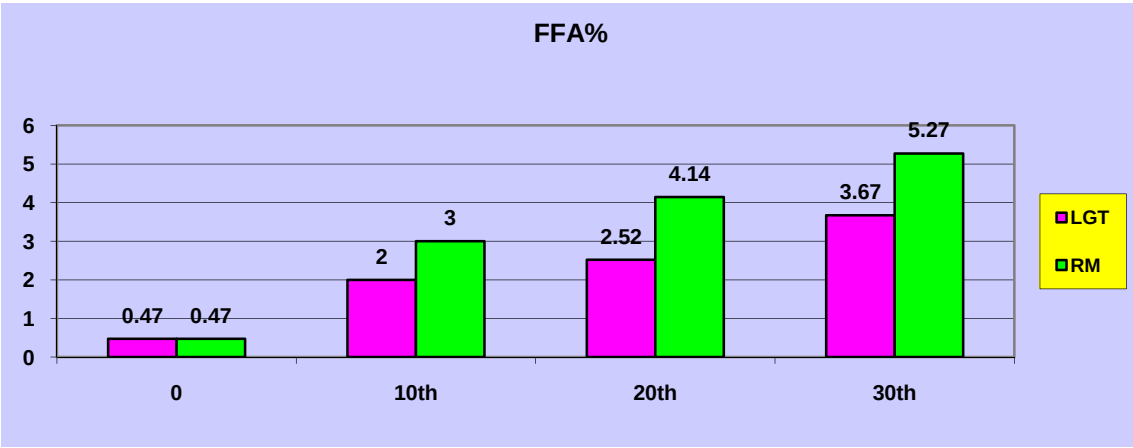

Figure.4

Microbial analysis:

As per results shown in Table 3 the total Plate Count (TPC) in raw fish muscle on day zero was rather low i.e. 2.0 4±0.2 log cfu/g however, on 21st day of storage, the TPC was found to increase further to the final value of 7.65±0.02 log cfu/g in RM and 4.88±0.04 log cfu/g in LGT samples. The Psychrophillic count increases exponentially with the increase in storage time. In 21 days of storage, the Psychrophilic count increased from 2.00±0.2 log cfu/g to 5.96±005 log cfu/g and 5.72±0.4 log cfu/g in RM & LGT samples respectively.

Table 2. Change in microbial count of fish muscle of *Labeo rohita* from 0 day to 21st day

Days of storage	TPC log cfu/g		PC log cfu/g	
	RM	**LGT**	**RM**	**LGT**
0	2.04^{a}±0.2	2.04^{a}±0.2	2.10^{a}±0.2	2.00^{a}±0.2
7th	4.01^{b}±0.11	3.17^{b}±0.03	3.30^{b}±0.04	2.99^{b}±0.04
14th	5.10^{c}±0.07	3.98^{c}±0.012	4.45^{c}±0.1	3.15^{c}±0.1
21st	7.65^{d}±0.02	4.88^{d}±0.04	5.96^{d}±0.05	4.06^{d}±0.05

--Mean±SD with different superscripts in a row differs significantly (P<0.05)

The above results (Table 3)clearly depicted that the microbial growth was more rapid with increasing storage temperature. It has been reported that in lemon glazed muscle samples, the TPC (5.37±0.2 log cfu/g) was found to be within the permissible limit on 21st day of storage whereas in raw muscle samples, the TPC crossed the permissible limit on 14th day of storage. The results of the present study confirm the earlier findings of Castell *et al.* (1948) and Liston (1957). The inhibiting effect of citric acid on bacteria was reported by (Santos & Zarzo, 1996). Likewise, lower degree of spoilage was reported among samples stored with citric acid (Schirmer *et al*., 2009). The effect of citric acid and sodium sulfite on Aerobic Plate Count was confirmed by Gokogen 2004 who stated that citric acid exhibit certain antimicrobial properties against bacteria and that citric acid dipping alone or in combination with sodium metabisulfite extended the shelf life of shrimp for two days longer. Similarly Cosansu *et al*.(2011), reported lower values of psychrophilic count in lemon juice treated samples of bonito as comapared to control samples.

4. Conclusion

LGT Samples were found to be within the limits proposed for FFA, TBA, TPC and Psychrophillic count while as RM Samples exceeded the proposed limits after 14th day of storage. This result shows that shelf life of rohu treated with lemon gelatine was one week longer than control samples.

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