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Studies on the effect of different preservation methods on the biochemical, microbial and sensory profile of frozen *Tor tor*

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Abstract
The present study was aimed to investigate the effect of various preservation methods viz. brining, antioxidant (citric and ascorbic acid, sodium lactate) and antimicrobial (Potassium sorbate) treament on the rancidity development, microbial quality and sensory changes in the muscle of *Tor tor* under frozen storage (-12±2^{0}C) for a period of 40 days. Muscle samples of fish were divided into five groups; group A (Gp.A) without any treatment i.e control group, group B (Gp.B) included brined samples, group C (Gp.C) included citric and ascorbic acid treated samples, group D (Gp.D) included sodium lactate treated samples and group E (Gp.E) included potassium sorbate treated samples. After 40 days of storage, all the samples reported a significant ($p<0.05$) increase in the thiobarbituric acid (TBA), free fatty acid (FFA) and pH. The values for TBA, FFA and pH were 17.48 mg mal/kg, 9.7% and 7.8 in Gp.A, 11.79 mg mal/kg, 7.4% and 7.4 in Gp.B, 4.02 mg mal/kg, 2.3% and 6.9 in Gp.C, 4.80 mg mal/kg, 2.7% and 6.9 in Gp.D and 5.15 mg mal/kg, 4.8% and 6.9 in Gp.E respectively. Similarly, the bacteriological studies revealed that permissible limits for Total plate count (6 log cfu/g) Coliform count (2.69 log cfu/g) and Psychrophillic count (4.6 log cfu/g) was maintained by Gp.C, Gp.D and Gp.E upto the end of storage while control (Gp.A) and brined (Gp.B) samples crossed these limits on 10th and 20th day respectively. Sensory analysis revealed that Gp. C samples maintained the best scores for their appearance, consistency and rancid development till the end of 40 days of frozen storage period.

Keywords: biochemical, microbial, sensory, *Tor tor*, brined, citric and ascorbic acid, potassium sorbate, rancidity and frozen storage

Introduction
A major contribution to the protein requirement of the world population is fulfilled by fish and hence plays an important role in alleviating the prominent problems of malnutrition. Fish is also an excellent source of polyunsaturated fatty acids (PUFAs) of n-3 family, lipid soluble vitamins and minerals. Besides acting as a brilliant source of nutrition, it also provides substances necessary for the human body and also reduces the risk of various diseases. For example, the omega-3 acid it contains when consumed on a regular basis, reduce the risk of heart disease, strengthens the immune system, reduces blood pressure by small but significant amounts and improve blood clotting regulation (Nettleton J. A., 1995) [25]. But, however, fish is highly perishable commodity, more than cattle, sheep and poultry and hence needs to 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. A great number of preservation methods have been used to extend the shelf life of fish which include low temperature storage, icing, glazing, salting, brining, smoking, use of antimicrobials and anti-oxidants. Low temperature storage inhibits microbial deterioration but it is also associated with reactions like freezer burn, product dehydration, rancidity and drip loss. The use of salt and potassium sorbate in preservation is highly potent in controlling the bacterial growth but have a little effect on lipid oxidation and hydrolytic phenomena. The use of antioxidants is gaining pace these days due to their role in delay or prevention of oxidation process in fats and oils. Further, they also reduce the microbial growth by acidification of cell interior. Thus, keeping this in mind, the aim of present study is to observe the effect of different preservation methods on the biochemical and microbial profile of *Tor tor* and thus evaluate its shelf life.

Materials and Methods
Collection of fish samples
Fresh samples of *Tor tor* 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, 10th, 20th, 30th and 40th day of storage.

Fish Treatment
The fish was cut in to pieces and these pieces were divided into five groups viz. Gp.A, Gp.B, Gp.C, Gp.D and Gp.E. Gp.A samples were kept as control samples (without any treatment), Gp.B samples were given 20% brine treatment for 15 minutes, Gp.C samples were dipped in the solution of 0.5% Ascorbic acid and Citric acid (1:1) for 15 minutes, Gp.D were dipped in 2.5% sodium lactate solution and Gp.E were given

5% Potassium sorbate dip treatment for 15 minutes. The samples taken out and immediately wrapped in aluminum foil, kept in air tight plastic container and stored at- 12±2°C (frozen 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) [11]. 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 different fish fillets 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) [11]

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	Sharply 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

Thiobarbituric acid value of fish muscle during storage was determined using the method of Witte *et al* (1970) [38]. Free Fatty Acid (FFA) was determined by method of US Army laboratories (Natick) described by Koniecko (1979) [21]. Extract Release Volume (ERV) was determined as per the method of Strange *et al.* (1977) [36]. The pH of fish muscles was determined by the method of Keller *et al*. (1974) [20] the microbiological profile was determined according to APHA method (1984) [4]. Data were expressed as mean ± SD and were analyzed by one- way ANOVA test using SPSS statistical programme.

Results and Discussions

Chemical Changes

Thiobarbituric Acid TBA

The TBA value is an index of lipid oxidation measuring malonaldehyde (MDA) content and widely used for assessment of degree of lipid oxidation. MDA is formed through hydroperoxides, which are the initial reaction product of polyunsaturated fatty acids with oxygen (Sallam, 2007) [35]. As observed in Table-2, the TBA values reported an increase with the increasing storage period. On day 0, the values for TBA were 0.95 mg mal/kg in Gp. A,1.03 mg mal/kg in Gp. B, 0.48 mg mal/kg in Gp. C, 0.72 mg mal/kg in Gp. D and 0.84 mg mal/kg in Gp. E. These values rose to 17.88 mg mal/kg in Gp. A,11.79 mg mal/kg in Gp. B, 4.02 mg mal/kg in Gp. C, 4.80 mg mal/kg in Gp. D and 5.15 mg mal/kg in Gp. E on 40th day of frozen storage. It is clear that Gp. A samples crossed the permissible limits of 8 mg mal/kg on day 20th while Gp. B crossed the same on day 30th. On the other hand, Gp. C, D and E samples were found to be within the acceptable limits till the end of frozen storage period of 40 days. Increase in TBA in the present results is due to the lipid oxidation process going on in fish muscle along with the destruction of hydroperoxides into secondary oxidation products as suggested by Subbaiah *et al.* (2015) [37] in frozen Tilapia (*Oreochromis niloticus*), Aydin and Gokoglu (2014) [7] in frozen Anchovy (*Engraulis encrasicholus*), Al-Jasser and Al-Jasass, F.M. (2014) [2] in Spanish mackerel (*Scomberomorous commerson*). However, the lower increase in TBA values of Gp. C, D and E is in line with the studies of Cheng (2016) [10] in meat, Rayeni (2016) [31] in seafood, and Djenane (2015) in *Sardina pilchardus*, Gandotra *et al.* (2013 and 2014 in *Hypophthalm ichthys molitrix*) [15, 16], Rostamzad *et al., (*2011a and 2011b) [33, 4] in Persian Sturgeon fillets and Sallam (2007) [35] in refrigerated Salmon. They attributed this lower TBA value to the antioxidant effect of these preservatives which blocks the formation of free radicals, stabilize hydroperoxides and thus slow down oxidation and rancidity development in fish meat.

Free fatty acids (FFA)

FFA are formed when the fats are deteriorated due to their hydrolysis. Present results (Table-2) reveal that FFA content increased in all the fish samples with the increasing storage period. However, on 40th day, the highest FFA values were observed in Gp. A (9.76%) followed by Gp. B (7.44%), Gp E (4.82%), Gp D (2.77%) and Gp C (2.32%). It is observed that Gp. A crossed the acceptable limits for FFA (5%) on day 20th while the brined samples i.e. Gp. B crossed the same on day 30th. However, the Gp. C and Gp. D samples were well within the acceptable limits till the end of storage of 40 days while Gp. E samples were approaching the acceptable limits on day 40th.The present results are in concordance with the studies of Okeyo *et al*. (2009) [26] in Nile Perch, Pourashouri (2009) [30] in Wels Catfish, Gandotra *et al.* (2013) [15] in *Wallago attu*, Subbaiah *et al.*, (2015) [37] in fresh Nile tilapia. They attributed it to the lipases and phospholipase activity occurring in digestive organs and muscles of fish. Further, Nayak *et al*. (2003) [24] forthput that extracellular lipases produced by certain micro-organisms during storage result in increased lipolytic changes in fish muscle and hence increase the free fatty acids.

Table 2: Chemical changes in the muscle samples of *Tor tor* treated with different preservatives and stored under frozen conditions at -12±2 ˚C

Days	TBA (mg mal/kg)					FFA (%)					pH				
	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E
0 day	0.95^{a}±0.02	1.03^{a} ±0.05	0.48^{a} ±0.03	0.72^{a} ±0.01	0.84^{a} ±0.05	1.12^{a} ±0.02	0.88^{a} ±0.01	0.68^{a} ±0.05	0.70^{a} ±0.01	0.81±0.04	6.8^{a} ±0.01	6.0^{a} ±0.05	6.2^{a} ±0.02	6.3^{a} ±0.05	6.3^{a} ±0.03
10^{th} day	6.88^{b}±0.03	4.15^{b} ±0.01	2.01^{b} ±0.01	1.67^{b} ±0.03	2.81^{b} ±0.01	4.32^{b} ±0.01	2.12^{b} ±0.05	0.90^{a} ±0.01	0.97^{a} ±0.05	2.08±0.02	7.3^{b} ±0.05	6.6^{b} ±0.01	6.3^{a} ±0.05	6.4^{a} ±0.03	6.5^{a} ±0.02
20^{th} day	13.02^{c} ±0.03	6.05^{c} ±0.04	2.74^{b} ±0.02	3.50^{c}±0.01	3.87^{c} ±0.02	6.90^{c}±0.05	4.88^{c} ±0.02	1.64^{a} ±0.04	1.88^{b} ±0.01	4.10±0.03	7.7^{b} ±0.02	7.0^{c} ±0.02	6.5^{b} ±0.05	6.4^{a} ±0.04	6.6^{a} ±0.01
30^{th} day	15.97^{d} ±0.05	9.12^{d} ±0.05	3.88^{c} ±0.02	4.32^{cd} ±0.05	4.76^{d} ±0.05	8.44^{d} ±0.02	6.92^{d} ±0.02	1.96^{b} ±0.02	2.08^{b} ±0.04	4.50±0.03	7.8^{b} ±0.06	7.2^{c} ±0.03	6.7^{b} ±0.01	6.8^{b} ±0.01	6.8^{b} ±0.02
40^{th} day	17.88^{e}±0.01	11.79^{e} ±0.02	4.02^{cd} ±0.04	4.80^{d} ±0.01	5.15^{de} ±0.02	9.76^{de} ±0.04	7.44^{de} ±0.05	2.32^{c} ±0.03	2.77^{bc} ±0.01	4.82±0.03	7.8^{b} ±0.05	7.4^{c} ±0.02	6.9^{c} ±0.03	6.9^{b} ±0.05	6.9^{b} ±0.02

pH

The values for pH on day 0 were found to be 6.8 in Gp. A, 6.0 in Gp. B, 6.2 in Gp. C, 6.3 in Gp. D and 6.3 in Gp. E. Further, on day 40th, these values increased to 7.8 in Gp. A and 7.4 in Gp. B while Gp. C, Gp. D and Gp. E reported the same value of 6.9. pH increase as observed in present studies could be correlated with the findings of Zhang *etal.* (2016) in chicken, Karami *et al.,* (2012) [19] in Red tilapia (*Oreochromis niloticus* and *Tilapia mosambicus*) fillets, Ozogul *et al.,* (2011) [28] in Common Sole (*Solea solea*)**,** Indira *et al.,* (2010) [17] in *Cyprinus carpio* L. var. *communis*, Emire and Gebremariam (2009) [12] in Nile Tilapia fish and Arannilewa *et al*. (2005) [5]. They associated it with the formation of volatile bases from the decomposition of nitrogenous compounds by endogenous or microbial enzymes.

Bacteriological Changes

Total Plate Count

The Total Plate Count (TPC**)** represents the bacterial populations in a sample. The initial values for TPC on 0 day were found to be 2.60 log cfu/g in Gp. A, 1.90 log cfu/g in Gp. B, 1.28 log cfu/g in Gp. C, 1.41log cfu/g in Gp. D and 1.11log cfu/g in Gp. E. Progressively, these values increased to 11.1 log cfu/g in Gp. A, 8.1log cfu/g in Gp. B, 4.12log cfu/g in Gp. C, 4.40 log cfu/g in Gp. D and 3.90±0.05 log cfu/g in Gp. E on 40th day of frozen storage. The permissible limits of 6 log cfu/g were crossed by Gp. A on 10th day while Gp. B on 20th day while samples under Gp. C, D and E were acceptable till the end of storage period of 40 days (Table-3)

Table 3: Microbial changes in the muscle samples of *Tor tor* treated with different preservatives and stored under frozen conditions at -12±2 ˚C

Days	TPC (log cfu/g)					CC (log cfu/g)					PC (log cfu/g)				
	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E
0 day	2.60^{a} ±0.05	1.90^{a} ±0.01	1.28±0.03	1.41^{a} ±0.02	1.11^{a} ±0.03	2.40^{a} ±0.02	1.83 ^{a}±0.02	0.90 ^{a}±0.04	1.07 ^{a}±0.08	0.81 ^{a}±0.06	2.34 ^{a}±0.05	2.13 ^{a}±0.02	0.81 ^{a}±0.03	0.96 ^{a}±0.01	0.74 ^{a}±0.05
10^{th} day	6.18^{b} ±0.01	4.12^{a} ±0.03	2.31±0.05	2.52^{b} ±0.05	2.1^{b} ±0.04	5.0^{b} ±0.05	4.71 ^{b} ±0.03	1.20 ^{b} ±0.02	1.28 ^{a}±0.06	1.13 ^{b} ±0.05	3.91 ^{b} ±0.02	2.91 ^{b} ±0.04	1.41 ^{b} ±0.02	1.58 ^{b}±0.05	1.28 ^{b} ±0.05
20^{th} day	7.88^{bc} ±0.02	6.84^{c} ±0.02	2.90±0.01	3.04^{c} ±0.05	2.72^{b} ±0.02	6.52^{c}±0.03	5.26 ^{c}±0.08	1.88 ^{c} ±0.01	1.91 ^{b} ±0.02	1.67 ^{b} ±0.05	5.01 ^{c}±0.06	3.64 ^{c}±0.05	2.06 ^{c}±0.0 2	2.2^{c} ±0.03	1.91 ^{c}±0.04
30^{th} day	9.16^{c} ±0.03	7.49^{d} ±0.05	3.66±0.03	3.89^{c} ±0.06	3.42^{c} ±0.05	7.97^{d}±0.02	7.31 ^{d}±0.03	1.97 ^{c}±0.02	2.09 ^{c}±0.03	1.78 ^{b} ±0.02	6.65 ^{d}±0.04	5.91 ^{d}±0.05	2.68 ^{d}±0.02	2.91^{d} ±0.03	2.37 ^{d}±0.04
40^{th} day	11.1^{d} ±0.04	8.1^{de} ±0.01	4.12±0.05	4.40^{d} ±0.01	3.90^{d} ±0.04	8.44 ^{e}±0.05	7.95 ^{d}±0.02	2.26 ^{d}±0.05	2.47 ^{c}±0.03	2.05 ^{c}±0.02	7.81 ^{e}±0.04	6.41 ^{e}±0.05	3.09 ^{e}±0.03	3.27 ^{e}±0.02	2.93^{de}±0.05

Coliform Count

Coliform are not the normal flora of fish muscle and their presence suggests the fecal contamination. The coliform count increased from 2.40 log cfu/g in Gp. A, 1.80 log cfu/g in Gp. B, 0.9 log cfu/g in Gp. C, 1.0 log cfu/g in Gp. D and 0.8 og cfu/g in Gp. E on day 0 to 8.44 log cfu/g in Gp. A, 7.95 log cfu/g in Gp. B, 2.26 log cfu/g in Gp. C, 2.47 log cfu/g in Gp. D and 2.05 log cfu/g in Gp. E on day 40. The permissible values of 2.69 log cfu/g were crossed by Gp. A and B on day 10th while other groups were within the permissible limits till the end of frozen storage of 40 days (Table-3).

Psychrophillic Count

The present study reported an increase in psychrotrophic count from 2.30 log cfu/g in Gp. A, 2.10 log cfu/g in Gp. B, 0.8 log cfu/g in Gp. C, 0.9 log cfu/g in Gp. D and 0.74 log cfu/g in Gp. E on day 0 to 7.81 log cfu/g in Gp. A, 6.41 log cfu/g in Gp. B, 3.09 log cfu/g in Gp. C, 3.27 log cfu/g in Gp. D and 2.93 log cfu/g in Gp. E on day 40. The permissible values of 4.6 log cfu/g were crossed by Gp. A on 20th day and Gp. B on day 30th while other groups were within the permissible limits till the end of frozen storage of 40 days (Table-3)

The present results clearly revealed that lowest bacterial counts were observed in potassium sorbate treated samples i.e Gp.E followed by Gp.C (treated with citric acid and ascorbic acid) and Gp.D (treated with sodium lactate). Similar view has been put forward by Chellaram (2015) [9] in *Pleuroploca trapezium*, Petrus (2015) [29] in *Anabas testudineus*, Djenane (2015) in *Sardina pilchardus*, Al hajj (2014) [1] in *Caranx sexfasciatis* fillets and *Penaeus semisulcatus.* They related it to the ability of these organic acids in diffusing along the bacterial cell wall, acidifying the cell interior and hence disrupting the cellular metabolism of microbes. Further, the studies of Can (2011) in Sardine (*Sardina pilchardus*), Omojowo *et al*. (2009b) [27] in catfish, Remisha *et al.* (2016) in

Indian Mackerel (*Rastrelliger kanagurta*) reported the antibacterial activity of potassium sorbate. They associated it with the bactericidal and sporostatic activitiy of Potassium sorbate against bacteria.

Sensory Analysis

Perusals of Table-4 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 samples were rejected after 10th day of storage and Gp. B crossed the rejectibility score on day 20th. However, the best scores for the given attributes were observed for Gp. C followed by Gp. D and Gp. E which may be attributed to the role in inhibiting the lipid oxidation and microbial spoilage occurring in fish muscle. Attala (2012) [6] in unpeeled shrimps, Rostamzed *et al.* (2009) in Persian sturgeon fillets, Pourashouri in Wels Catfish, Leaflet (2004) [22] and Fagan and Gormley (2004) [13] while favouring the similar view suggested that these preservatives can slow down improper changes during frozen storage.

Table 4: Sensory changes in the muscle samples of *Tor tor* treated with different preservatives and stored under frozen conditions at -12±2 ˚C

Frozen storage time	Flesh appearance					Rancid odor					Flesh consistency				
	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E	Gp. A	Gp. B	Gp. C	Gp. D	Gp. E
0TH DAY	E	E	E	E	E	E	E	E	E	E	A	A	A	A	A
10TH DAY	C	B	A	A	A	C	B	E	A	A	C	B	B	B	B
20TH DAY	C	C	A	A	B	C	C	A	A	B	C	C	B	B	B
30TH DAY	C	C	B	B	B	C	C	A	B	B	C	C	B	B	B
40th DAY	C	C	B	B	C	C	C	B	B	C	C	C	B	C	C

Where E (excellent), A (good), B (fair) and C (rejectable).

Conclusion

The results of this study showed that the proliferation of bacteria, protein denaturation, lipid hydrolysis and oxidation increase as the storage period increase. The strongest rancidity inhibition was found in Gp.C (treated with 0.5% citric acid and ascorbic acid treated) samples while the highest antimicrobial activity was observed in Gp. E (Potassium sorbate treated) samples. Further, the sensory attributes revealed the efficiency of organic acids viz. citric and ascorbic acid, sodium lactate and potassium sorbate in maintaining the sensory acceptability till the end of 40 days of storage period. Therefore, research in this area should be focused on the optimization these preservatives so as to obtain effective antioxidant and antimicrobial activity at sufficiently low concentrations having no adverse influence on the organoleptic acceptability of the foods.

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