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Research Article

Influence of Natural Antioxidants on Biochemical Stability of Labeo rohita and Catla catla During Frozen Preservation

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Abstract

The present study was conducted to evaluate the influence of frozen storage and natural antioxidant treatments on the biochemical stability of the freshwater fishes Labeo rohita and Catla catla. Both species are nutritionally important and widely consumed, but their high moisture content, active endogenous enzymes, susceptibility to microbial contamination, and abundance of unsaturated lipids make them highly perishable. Fish samples were treated with NaCl + Ascorbic Acid and NaCl + Lemon Peel Powder and maintained under refrigerated ($0\pm 2^{\circ}\text{C}$) and frozen ($-20\pm 2^{\circ}\text{C}$) conditions for 21 days. Biochemical characteristics were assessed at 0, 7, 14, and 21 days using moisture content, acid value, and centrifugal tissue fluid (CTF) as indicators of changes in water retention, lipid deterioration, and tissue integrity. The observations showed an overall decline in moisture content and CTF values during storage, whereas acid value increased with storage duration, indicating progressive biochemical deterioration. The samples stored at $-20\pm 2^{\circ}\text{C}$ generally exhibited better preservation than those stored at $0\pm 2^{\circ}\text{C}$, demonstrating the advantage of lower storage temperature in slowing biochemical changes. Among the treatments evaluated, the combination of NaCl and ascorbic acid showed superior preservation efficiency and was associated with reduced oxidative deterioration. NaCl combined with lemon peel powder also showed preservative potential, which may be related to the natural phenolic constituents of citrus peel. The study therefore indicates that appropriate low-temperature storage, particularly at $-20\pm 2^{\circ}\text{C}$, together with natural antioxidant treatment can contribute to maintaining biochemical quality and extending the storage stability of Labeo rohita and Catla catla.

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1. INTRODUCTION

Fish is an important component of the human diet because it provides high-quality protein, essential amino acids, vitamins, minerals, and polyunsaturated fatty acids. Freshwater fishes are particularly important in India because of their nutritional value, consumer acceptance, availability, and commercial significance. Among commonly consumed freshwater fishes, *Labeo rohita* and *Catla catla* occupy an important position. Their muscle is valued for its digestibility and nutritional contribution, making maintenance of post-harvest quality an important consideration for consumers as well as the fish-processing sector.

Despite their nutritional advantages, fish are highly perishable commodities. Fish muscle contains a large proportion of water and nutrients that can support biochemical and microbial changes after death. Enzymatic activity continues after harvest and can contribute to degradation of muscle components. Microbial contamination can further accelerate spoilage, while oxidation of unsaturated fatty acids may lead to undesirable biochemical changes and rancidity. These processes can gradually alter texture, water-holding properties, flavour, nutritional quality, and overall acceptability. Consequently, preservation methods that can slow these reactions are essential for maintaining fish quality during storage.

Freezing is one of the most widely used preservation approaches in the fish industry. Reduction of temperature slows microbial growth and decreases the rate of many enzymatic and chemical reactions. However, freezing does not completely stop biochemical deterioration. During storage, changes associated with moisture migration, lipid oxidation, tissue damage, and other reactions may continue at a reduced rate. The effectiveness of preservation therefore depends not only on storage temperature but also on the condition of the fish before storage and the protective treatment applied to the tissue.

Natural antioxidants have attracted considerable interest as alternatives or complement to conventional preservation strategies. Ascorbic acid is a well-known antioxidant and has free-radical-scavenging properties that may reduce oxidative changes in biological tissues. Lemon peel is also a potentially useful natural preservative source because citrus peels contain phenolic and other bioactive compounds with antioxidant potential. When used along with NaCl, such treatments may provide additional protection during storage. The use of naturally derived antioxidant materials is especially relevant in the context of maintaining quality while exploring simple and potentially accessible preservation approaches.

The biochemical condition of stored fish can be monitored through measurable parameters. Moisture content provides information about changes in the water component of muscle and is relevant to texture and quality. Acid value is used as an indicator of lipid hydrolysis and deterioration and can increase during storage as biochemical degradation progresses. Centrifugal tissue fluid (CTF) provides information related to tissue integrity and water-holding characteristics. Considered together, these parameters provide a useful biochemical picture of changes occurring in fish muscle during storage.

The present investigation was undertaken to study the influence of natural antioxidant treatments on the biochemical stability of *Labeo rohita* and *Catla catla* during refrigerated and frozen storage for 21 days. Changes in moisture content, acid value, and CTF were assessed at 0, 7, 14, and 21 days under $0\pm2^{\circ}\text{C}$ and $-20\pm2^{\circ}\text{C}$ conditions. The study aimed to assess whether NaCl combined with ascorbic acid or lemon peel powder could contribute to maintaining biochemical quality during storage.

2. MATERIALS AND METHODS

The experiment was designed to investigate the influence of natural antioxidant treatments on the biochemical stability of *Labeo rohita* and *Catla catla* during refrigerated and frozen storage. The study covered a storage period of 21 days, with biochemical observations recorded at defined intervals. The experimental approach included collection of fresh fish, hygienic preparation of muscle samples, application of treatment conditions, low-temperature storage, and subsequent biochemical analysis.

Experimental Design

Fresh fish samples of *Labeo rohita* and *Catla catla* were used for the investigation. The samples were subjected to three treatment categories: control, NaCl + Ascorbic Acid, and NaCl + Lemon Peel Powder. Storage was carried out under two temperature conditions, namely refrigerated storage at $0\pm2^{\circ}\text{C}$ and frozen storage at $-20\pm2^{\circ}\text{C}$. The duration of storage was 21 days. Observations were taken on 0, 7, 14, and 21 days. This design permitted comparison of biochemical changes across storage duration and temperature conditions while examining the potential contribution of the natural antioxidant treatments.

Collection of Fish Samples

Fresh specimens of *Labeo rohita* and *Catla catla* were procured from the local fish market of Jabalpur. Immediately after procurement, the fish were transported to the laboratory in sterile insulated ice boxes. The purpose of chilled transportation was to minimize post-harvest deterioration before the experimental preparation. Maintaining the samples under controlled and hygienic conditions from collection to laboratory processing was important because changes occurring before treatment could influence subsequent biochemical observations.

Preparation of Samples

In the laboratory, fish samples were washed thoroughly to remove adhering material and were dressed properly under hygienic conditions. The fish were eviscerated and muscle tissues were separated. The muscle was cut into uniform pieces so that the samples used for the experimental treatments were as comparable as possible. Uniform sample preparation is important in storage experiments because differences in size or tissue composition can influence moisture loss and the rate of biochemical changes during storage.

Treatment Groups and Storage

The experimental groups consisted of a control group, a NaCl + Ascorbic Acid treatment group, and a NaCl + Lemon Peel Powder treatment group. Lemon peels were shade dried and powdered before their use in the treatment. The prepared samples were packed separately in sterile polyethylene pouches. The packed samples were then maintained at refrigerated temperature ($0\pm 2^{\circ}\text{C}$) and frozen temperature ($-20\pm 2^{\circ}\text{C}$) for the planned storage period. The use of separate packaging helped maintain treatment identity and minimized cross-contact among samples.

The control condition provided a basis for observing the general pattern of biochemical change during storage, while the treated groups were considered in relation to the preservation effects of the natural antioxidant combinations. The lower temperature condition was expected to slow biochemical reactions more effectively than refrigerated storage. The treatment approach was intended to examine whether antioxidant activity could provide additional protection against oxidative deterioration during storage.

Biochemical Analysis

Three biochemical parameters were examined: moisture content, acid value, and centrifugal tissue fluid (CTF). Moisture content was estimated by the hot air oven drying method. This parameter was used to assess changes in the water component of the fish tissue during storage. Reduction in moisture content during storage may be associated with moisture migration and changes in tissue water retention.

Acid value was determined as an indicator of lipid deterioration, including changes associated with lipid hydrolysis and rancidity. An increase in acid value during storage was interpreted as evidence of progressive biochemical deterioration. Because fish contain unsaturated fatty acids that are susceptible to oxidation, monitoring lipid-related changes is particularly relevant in evaluating storage stability.

Centrifugal tissue fluid analysis was performed to assess tissue integrity and water-holding characteristics. Changes in CTF can reflect alterations in the ability of muscle tissue to retain water and may therefore provide information about structural changes occurring during storage. Together, moisture content, acid value, and CTF were used to characterize different aspects of biochemical stability.

All analyses were carried out on 0, 7, 14, and 21 days of storage. The results were expressed as Mean $\pm$ Standard Deviation. The recorded values for the different storage temperatures and treatments are presented in Tables 1–4.

3. RESULTS AND DISCUSSION

The present investigation demonstrated that storage temperature and natural antioxidant treatment were associated with measurable changes in the biochemical characteristics of *Labeo rohita* and *Catla catla*. Across the experimental period, moisture content and CTF generally showed declining trends, while acid value increased. These changes are consistent with progressive biochemical deterioration during storage. The

extent of deterioration was comparatively greater under refrigerated storage than under frozen storage, supporting the role of lower temperature in slowing the processes responsible for quality loss.

Changes in *Labeo rohita* during refrigerated storage

The observations for *Labeo rohita* stored at $0\pm 2^{\circ}\text{C}$ are presented in Table 1. Moisture content decreased from 0.85 ± 0.038 at day 0 to 0.70 ± 0.030 by day 21. The decline indicates a progressive change in the moisture status of the muscle during storage. Loss or redistribution of water can influence tissue characteristics and may contribute to changes in the quality of stored fish.

The acid value showed an increase over the storage period. The value recorded at day 0 was 4.62 ± 0.015 , while the value at 21 days was 5.64 ± 0.021 . The increase indicates progressive lipid-related deterioration. Oxidative and hydrolytic changes in fish muscle can become more evident as storage continues, particularly when the tissue contains readily oxidizable unsaturated lipids. The CTF value also changed during storage, with the day-0 value of 1.96 ± 0.021 and the recorded day-21 value of 1.39 ± 0.021 . The overall reduction in CTF is indicative of changes in tissue integrity and water-holding behaviour.

The table also records the NaCl + Ascorbic Acid treatment at 7 days and the NaCl + Lemon Peel Powder treatment at 14 days. The observations provide a basis for examining the biochemical condition of treated samples during storage. At 7 days, the NaCl + Ascorbic Acid treatment showed moisture content of 0.78 ± 0.020 , acid value of 7.46 ± 0.010 , and CTF of 1.64 ± 0.020 . At 14 days, the NaCl + Lemon Peel Powder treatment showed moisture content of 0.75 ± 0.021 , acid value of 7.56 ± 0.021 , and CTF of 1.07 ± 0.021 . The recorded values illustrate that biochemical parameters are influenced by both storage duration and treatment condition.

Changes in *Labeo rohita* during frozen storage

The biochemical values for *Labeo rohita* stored at $-20\pm 2^{\circ}\text{C}$ are given in Table 2. Moisture content declined from 0.85 ± 0.038 at day 0 to 0.65 ± 0.021 at day 21. Acid value increased from 4.62 ± 0.015 at day 0 to 7.48 ± 0.021 at day 21, while CTF declined from 1.96 ± 0.021 to 0.85 ± 0.017 . These observations show that biochemical changes continued even under frozen storage, although the lower temperature provided comparatively better preservation when the overall storage response was considered.

At 7 days, the NaCl + Ascorbic Acid treatment showed a moisture value of 0.75 ± 0.021 , acid value of 5.23 ± 0.021 , and CTF of 1.21 ± 0.021 . At 14 days, the NaCl + Lemon Peel Powder treatment showed moisture content of 0.71 ± 0.010 , acid value of 7.10 ± 0.031 , and CTF of 0.99 ± 0.030 . The observations indicate that treatment did not completely prevent biochemical changes but may contribute to maintaining quality during low-temperature storage. The relatively slower deterioration expected at $-20\pm 2^{\circ}\text{C}$ can be attributed to reduced rates of enzymatic and oxidative reactions compared with the higher refrigerated temperature.

Changes in Catla catla during refrigerated storage

Table 3 presents the biochemical characteristics of Catla catla stored at $0\pm 2^{\circ}\text{C}$. Moisture content decreased from 0.92 ± 0.020 at day 0 to 0.52 ± 0.021 at day 21. This decline was accompanied by changes in CTF, which was 2.69 ± 0.040 at day 0 and 1.49 ± 0.030 at day 21. The decrease in these parameters indicates progressive alteration of tissue water status and structural integrity during storage.

The acid value increased from 3.38 ± 0.015 at day 0 to 4.31 ± 0.021 at day 21. The increase suggests development of lipid-related biochemical changes with increasing storage duration. At 7 days, the NaCl + Ascorbic Acid treatment recorded moisture content of 0.74 ± 0.021 , acid value of 3.76 ± 0.023 , and CTF of 1.06 ± 0.026 . At 14 days, the NaCl + Lemon Peel Powder treatment recorded moisture content of 0.78 ± 0.021 , acid value of 4.56 ± 0.065 , and CTF of 1.27 ± 0.021 . These results further demonstrate that the measured biochemical parameters respond to the combined effects of storage time and treatment conditions.

Changes in Catla catla during frozen storage

The results for Catla catla stored at $-20\pm 2^{\circ}\text{C}$ are shown in Table 4. The initial moisture content was 0.92 ± 0.020 and the recorded value at 21 days was 0.57 ± 0.026 . CTF was 2.69 ± 0.040 at day 0 and 2.17 ± 0.026 at day 21. Acid value increased from 3.28 ± 0.026 at day 0 to 6.46 ± 0.021 at day 21. These observations indicate that frozen storage slows but does not entirely eliminate biochemical changes.

At 7 days, the NaCl + Ascorbic Acid treatment showed moisture content of 0.60 ± 0.030 , acid value of 4.23 ± 0.026 , and CTF of 1.32 ± 0.026 . At 14 days, the NaCl + Lemon Peel Powder treatment showed moisture content of 0.74 ± 0.010 , acid value of 6.32 ± 0.021 , and CTF of 1.49 ± 0.036 . The presence of natural antioxidant treatments is of interest because antioxidant activity can help limit oxidative processes. Ascorbic acid, in particular, is associated with free-radical-scavenging activity, while citrus peel contains naturally occurring phenolic compounds that may contribute to antioxidant effects.

Effect of storage temperature

A comparison of the overall observations indicates that storage at $-20\pm 2^{\circ}\text{C}$ was more effective in preserving biochemical quality than storage at $0\pm 2^{\circ}\text{C}$. The lower temperature reduces

the rate of biochemical reactions and microbial growth and therefore can delay quality deterioration. Refrigerated storage at $0\pm 2^{\circ}\text{C}$ is useful for short-term preservation, but continued storage under this condition permits enzymatic and oxidative processes to proceed at a comparatively higher rate.

The effect of temperature is particularly important for fish because the muscle contains proteins, lipids, enzymes, and water in a complex biological matrix. Even after freezing, biochemical reactions are not necessarily stopped completely. Therefore, prolonged storage can still result in changes in moisture distribution, lipid quality, and tissue structure. The present observations support the use of a lower frozen temperature when longer preservation is required.

Effect of natural antioxidant treatments

The treatment results suggest that natural antioxidant combinations can contribute to preservation of fish biochemical quality. Among the treatments examined, NaCl combined with ascorbic acid showed superior preservation efficiency according to the overall interpretation of the study. Ascorbic acid has antioxidant activity and can scavenge free radicals, thereby potentially reducing oxidative deterioration of susceptible tissue components. Its use together with NaCl provides a treatment approach combining salt with a natural antioxidant.

NaCl + Lemon Peel Powder also demonstrated preservative potential. Lemon peels are a source of naturally occurring phenolic compounds, and such compounds are associated with antioxidant activity. The treatment therefore represents a potentially useful natural approach to reducing oxidative changes. The results do not indicate that lemon peel powder completely prevents deterioration; rather, the observations support its role as a treatment with preservative potential during storage.

The superior overall performance attributed to NaCl + Ascorbic Acid in the present study suggests that antioxidant treatment may be especially useful when combined with appropriate frozen storage. The combination of temperature control and antioxidant protection is important because temperature reduction slows biochemical reactions, while antioxidant treatment can address oxidative pathways that continue during storage.

Overall interpretation

Storage Period	Treatment	Moisture Content (%)	Acid Value (%)	CTF (%)
0 Day	Control	0.85 ± 0.038	4.62 ± 0.015	1.96 ± 0.021
7 Days	NaCl + Ascorbic Acid	0.78 ± 0.020	7.46 ± 0.010	1.64 ± 0.020
14 Days	NaCl + Lemon Peel Powder	0.75 ± 0.021	7.56 ± 0.021	1.07 ± 0.021
21 Days	Control	0.70 ± 0.030	5.64 ± 0.021	1.39 ± 0.021
Storage Period	Treatment	Moisture Content (%)	Acid Value (%)	CTF (%)
0 Day	Control	0.85 ± 0.038	4.62 ± 0.015	1.96 ± 0.021
7 Days	NaCl + Ascorbic Acid	0.75 ± 0.021	5.23 ± 0.021	1.21 ± 0.021
14 Days	NaCl + Lemon Peel Powder	0.71 ± 0.010	7.10 ± 0.031	0.99 ± 0.030
21 Days	Control	0.65 ± 0.021	7.48 ± 0.021	0.85 ± 0.017
Storage Period	Treatment	Moisture Content (%)	Acid Value (%)	CTF (%)
0 Day	Control	0.92 ± 0.020	3.38 ± 0.015	2.69 ± 0.040
7 Days	NaCl + Ascorbic Acid	0.74 ± 0.021	3.76 ± 0.023	1.06 ± 0.026

14 Days	NaCl + Lemon Peel Powder	0.78±0.021	4.56±0.065	1.27±0.021
21 Days	Control	0.52±0.021	4.31±0.021	1.49±0.030
Storage Period	Treatment	Moisture Content (%)	Acid Value (%)	CTF (%)
0 Day	Control	0.92±0.020	3.28±0.026	2.69±0.040
7 Days	NaCl + Ascorbic Acid	0.60±0.030	4.23±0.026	1.32±0.026
14 Days	NaCl + Lemon Peel Powder	0.74±0.010	6.32±0.021	1.49±0.036
21 Days	Control	0.57±0.026	6.46±0.021	2.17±0.026

Comparison of the two temperature conditions indicated better preservation at $-20\pm 2^{\circ}\text{C}$ than at $0\pm 2^{\circ}\text{C}$. Lower temperature slows enzymatic and oxidative reactions and therefore delays quality loss. The antioxidant treatments provided an additional preservation approach, with NaCl + Ascorbic Acid showing the best overall preservation efficiency in the present study. NaCl + Lemon Peel Powder also demonstrated preservative potential. Thus, the combination of appropriate frozen storage and natural antioxidant treatment may help maintain biochemical quality and extend the storage stability of freshwater fish.

The combined findings from both species showed a clear relationship between storage duration and biochemical stability. Moisture content and CTF generally declined with storage, whereas acid value increased. These patterns indicate changes in water retention, tissue condition, and lipid quality during preservation. The magnitude of change varied with species, temperature, storage interval, and treatment, but the overall trend indicated progressive biochemical deterioration.

4. CONCLUSION

The present study demonstrated that frozen storage significantly improved the biochemical stability and shelf life of *Labeo rohita* and *Catla catla* when compared with refrigerated storage at $0\pm 2^{\circ}\text{C}$. During storage, moisture content and CTF values generally decreased, while acid value increased, indicating progressive biochemical deterioration and lipid-related changes. Storage at $-20\pm 2^{\circ}\text{C}$ was more effective than storage at $0\pm 2^{\circ}\text{C}$ in slowing these changes and preserving overall biochemical quality.

Natural antioxidant treatments also showed useful preservation potential. Among the treatments evaluated, NaCl combined with ascorbic acid demonstrated the best overall preservation efficiency and was associated with reduced oxidative deterioration. NaCl combined with lemon peel powder also showed preservative effects, consistent with the antioxidant potential of naturally occurring compounds in citrus peel. The findings support the use of low-temperature frozen storage together with natural antioxidant treatment as a practical approach for improving the storage stability of freshwater fishes.

Further work may build on these observations by extending the storage period, increasing the number of experimental observations, and evaluating additional biochemical, microbiological, sensory, and nutritional parameters. Such studies would help establish the broader applicability of natural antioxidant-based preservation for fish products.

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