E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2024; 12(3): 69-75

www.fisheriesjournal.com
Received: 09-02-2024
Accepted: 18-03-2024

Ester John Kapesa
Master in Fisheries Science, Department of Fish Processing Technology, College of Fisheries Science, Mangalore, Karnataka, India

Siddappaji
Assistance professor, Department of Fish Processing Technology, College of Fisheries Science, Mangalore, Karnataka, India

Hamis Miraji Simba
Istanbul Gelisim University, Department of Economics and Finance, Istanbul, Turkey

Corresponding Author:
Ester John Kapesa,
Master in Fisheries Science, Department of Fish Processing Technology, College of Fisheries Science, Mangalore, Karnataka, India

Impact of natural antioxidant in preserving the quality of mackerel fillets at frozen storage

Ester John Kapesa, Siddappaji and Hamis Miraji Simba

DOI: https://doi.org/10.22271/fish.2024.v12.i3b.2935

Abstract
With consumers demanding safe food, lipid oxidation has become a concern. This study looks into the use of artificial antioxidants such butylated hydroxy anisole (BHA) and butylated hydroxytoluene (BHT) to prevent or suppress oxidation. Fish was preserved naturally in this study using green tea extracts, or GTEs. GTEs are recognized for their antibacterial and antioxidant properties because they include a variety of polyphenols, especially catechin components. Ethanol and water were used to create the GTEs, which were subsequently concentrated. Fresh mackerel fillet pieces were dipped into a solution containing green tea ethanol and water extracts to conduct the experiments. The total plate count (TPC) method was utilized for the microbiological testing.
Results: The results showed that samples with GTEs of 4.57 and 3.95 may inhibit the growth of bacteria in fillets. Additionally, tests for FFA, TBARS, and PV showed lower results as compared to control samples, indicating that lipid oxidation was reduced during frozen storage. Biochemical parameters such as TVB-N and TMA were also tested to assess fillet freshness, and significant improvements in sample quality were observed. Sensory tests further indicated that GTEs could enhance the freshness of the fillet samples.
Conclusion: The study shows that even when used for commercial purposes, green tea extract can extend and safeguard the fish and fisheries products shelf life. The research found that green tea extract is a natural antioxidant that has no adverse impact on fish or fisheries management practices.

Keywords: Antioxidant, fish preservation, frozen storage, green tea extract, mackerel, oxidation

1. Introduction
Naturally occurring long-chain omega-3 polyunsaturated fatty acids (ω-3 PUFA) are abundant in marine fish (El-Hanafy *et al*., 2011) [8]. Mackerel fish (Restringer kanagulta) contributes significantly and steadily to global fish supplies. The primary issues with seafood's distribution and marketing plan are its perishability and low quality. These are due to spoilage by contamination and microorganisms. Low temperature such as frozen storage is an effective way for extending the fish and fishery products shelf life by reducing the spoilage and microorganisms' growth.
Lipid oxidation and the autolytic hydrolyzation of lipids by enzymes such lipases are the main causes of spoilage in fatty fish (Negi, 2012) [18]. Antioxidants are utilized to scavenge free radicals and stop free chain propagation in order to delay the development of breakdown and deteriorative changes connected to proteins and lipids (Mole and Waterman, 1994) [17]. Ascorbic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tert-butyhydroquinone (TBHQ) are examples of synthetic antioxidants. According to Shahidi *et al*. (1997) [25], the use of artificial antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) has been prohibited due to potential health issues. Because of their possible antibacterial and antioxidant properties, plant extracts have been suggested as substitutes. The primary barriers to the vital use of pelagic resources are their high fat content, seasonality, and significant change in proximate composition, which in particular cause many of the species' fat contents to fluctuate noticeably. Among pelagic resource in India, Indian Mackerel (*Rastrelliger kanagurta*) constitute a major part of the total fish catch. A good quantity of sardine and mackerel is consumed fresh along the coast and nearby places. During glut, they are challenging to handle as they are perishable and susceptible to rancidity and

spoilage. Fat oxidation is responsible aimed at the decrease in nutritive quality and changes in appearance, colour, taste and flavour (Wanasundara and Shahidi, 1998) [29]. Thus, valuable catch needs to be properly utilized to meet the growing demand. These food fishes can be processed into a valuable product named fish fillet, after separating them from non-edible parts. The marketing of fish fillet is very common and currently in high demand among developed countries (Sarah *et al*., 2010) [24]. Whereas in developing countries fish fillet production is likely gaining significant importance as an attractive alternative product (to whole fish) due to its convenience for easy use.

Tocopherols, phenolic compounds, flavonoids, phospholipids, and polyfunctional organic acids are a few examples of natural antioxidants that can be employed to preserve seafood (Wanasundara and Shahidi, 1998) [29]. The efficacy of rosemary extract for frozen fish storage of fillets and minced fish was investigated by Vareltzis *et al*. in 1997 [28]. Anthocyanins, which are antioxidants found in pomegranates, were described by Gil *et al*. (2000). Pomegranate peel extract's (PPE) antioxidant efficacy and phenolic content were found to be significantly correlated by Negi *et al*. (2003) [18]. Bioactive substances found in citrus peel extract include pectins, phenolic compounds, and flavonoids, which are antioxidants (Ebrahimzadeh *et al*., 2008) [9]. Thus, the industry can profitably use green tea's inherent antioxidant properties to preserve fish and fisheries products while preserving the items' quality. In this study, extracts from green tea were used as replace of synthetic antioxidant to preserve the mackerel fillets.

2. Materials and Methods

2.1 Green tea extract preparation

Commercially available green tea, obtained locally from Mangalore, was manufactured, packaged, and sold by Tata Global Beverages Limited under the "Tetley" label. It was created freshly from *Camellia sinensis* without any signs of oxidation or deterioration. Green tea leaves were pulverized and their extract was prepared in hot water (T3) and 95% ethanol (T4).

2.1.1 Green tea extraction preparation in aqua/water

Hot water extraction was used to create green tea aqua extract, or GT aqua. After finely grinding the green tea leaves into a powder, they were sieved and kept in a cool, airtight container until needed. In a 750 mL conical flask, hot water was combined with weighed green tea powder at 1:4 (tea powder to water). A centrifuge was used to perform the extraction, spinning at 300 rpm for 45 minutes at 50 °C. After filtering through the Whatman No. 4 filter paper to separate the extract from the residue, the residues were combined with hot water for a second extraction. To turn the combined extract into a powder, it was dried at 85°C. Following the application of the concentrated soluble solid substance in the experiment green tea extract with water after dissolution.

3. 2.1.2 Green tea extract preparation in Ethanol

The method described by Grager and Harbone (1994) was utilized to extract the active components from the leaves using pure ethanol. Using a 1:4 ratio of 95% ethanol, ground tea leaves were extracted in 45 minutes at 45°C and 300 rpm in a mechanical shaker. Whatman No. 4 filter paper was then used to filter the extract and separate it from the residue. The residues were subjected to a second extraction with 95% ethanol. The first and second extracts were then combined and concentrated. A soxhlet extraction apparatus was utilized to concentrate the two extracts at 60^0C to obtain a powder. The concentrated soluble solid content was used to administer the green tea extract with ethanol in the study.

2.2 Sample Preparation and Storage of mackerel fillets

The mackerel fish caught by purse-seiners along the coast of Karnataka was obtained from the Kadala Jothi purse-seine fishing vessel at Mangalore's main fish landing place. After being caught during night fishing, the fish were promptly transported to the landing station in a well-iced state and delivered to the fish processing facility right away. The mackerel fish measured 23.83±1.17 cm in total length and weighed, on average, 166.80±25.70 g. This information was gathered at the College of Fisheries in Mangalore. The fish were cleaned, partially dressed, and stored in a deep freezer to make filleting easier. The partially prepared and frozen fish were then turned into fillets. These fillets were treated with different solutions, including distilled water (T1), ascorbic acid (500 ppm) (T2), green tea water extract (2000 ppm) (T3), and green tea ethanol extract (2000 ppm) (T4), before being kept at 4 ± 1^0C in a refrigerator. The quality of the fillets during storage was assessed at 30-day intervals using biochemical, microbiological, and sensory markers.

2.3 Biochemical parameters

The pH of a homogenate of 5 g of fish fillets in 50 mL of distilled water was measured using a pH meter in accordance with Carballo *et al*. (1995). The AMC (1979) method was used to determine the amounts of total volatile basic nitrogen (TVB-N), alpha-amino nitrogen (AA-N), and trimethylamine nitrogen (TMA-N). Using the Tarladgis *et al*. (1960) method, we measured free fatty acid (FFA), thiobarbituric acid (TBA), and peroxide value (PV).

2.4 Microbiological Analysis

The APHA-recommended plate count agar medium sing spread plate method was used to determine the total plate count (TPC).

2.5 Sensory Analysis

A group of eight seasoned panelists used a nine-point hedonic scale to rate the overall acceptability and sensory characteristics of mackerel fillets. The color, texture, aroma, and general appearance of the fish were among the sensory attributes that were being assessed. The guidelines provided by Santos *et al*. (1981) served as the basis for the development of the sensory quality evaluation scale.

2.6 Statistical Analysis

On the data, a two-way analysis of variance was used to look at the effects of GTE levels and time intervals. Applying a post-hoc test of the Tukey test, at the significance threshold of $p< 0.05$, the mean changes were significant. SAS (SAS 2000, Version 21) was the computer program used to run all of the statistics.

3. Results and discussion

The effects of preservation on the biochemical characteristics of frozen fish

3.1 Changes in pH

The pH of mackerel fillets has been suggested as a useful

indicator of freshness (Chung and Latin, 1979). The pH of the samples increased slightly during the storage period (see Table 1 and Figure 1 in the appendix). The initial pH values for T1, T2, T3, and T4 were 6.80, 6.49, 6.71, and 6.78 during frozen storage. For T1, the pH rose to 6.79; for T2, T3, and T4, it increased to 6.71, 6.68, and 6.69, respectively. Similar results were found by Pal (2016) [21], who observed that the application of black and green tea to pangasius sutchi fillets during storage raised their pH. The pH value increased to 7.12 in the control, and to 6.77 and 6.82 in the green and black tea samples, respectively, according to the author's notes. Several studies have shown that the acceptable rang of pH is between 6.2 and 7.0 (Pal, 2016) [21]. The pH values of the research treatments remained within this range for the entire 120 days of storage period. All over the storage period, the treated samples (T3 and T4) had a meaningfully lower pH ($p< 0.05$) related to T1 sample. This could be due to the antimicrobial properties of tea polyphenols, or tea catechins. The phenolic components of green tea extracts may enhance microbial suppression, protecting fillets from internal protease and preventing protein degradation and amine formation.

3.2 Change in total volatile base Nitrogen (TVB-N)

The TVB-N levels are a result of microorganism's breakdown and enzyme activity. Table 2 and Figure 2 in the appendix illustrate the changes in TVB-N content due to different treatments during frozen storage. Initially, all samples had TVB-N contents ranging from 2.62 to 6.47 mg%. After 120 days, the samples showed an increase, with final values of 24.50, 17.50, 10.67, and 10.32 mg% for T1, T2, T3, and T4 samples, correspondingly. This indicates that the treated samples were successful in delaying the increase in TVB-N levels throughout storage. According to Connell (1990), the acceptable maximum TVB-N for fish muscles is 35–40 mg-N/100 g of meat. Özoğul *et al*. (2011) [20] found that after six months of storage, the TVB-N concentration increased for both the control and natural antioxidant-treated samples, rising from an initial value of 20.90 mg% to 24.24, 23.16, and 21.89 mg%. The increase in TVB-N levels may be attributed to fish endogenous enzymes and bacterial activity (Vareltzis, Koufidis, Gavriilidou, Papavergou, and Vasiliadou, 1997) [28]. The sample containing T3 and T4 in this study showed a greater preservation effect, possibly due to the stronger inhibitory impact of tea polyphenolic compounds on bacteria and enzymatic activity involved in the generation of volatile bases during decomposition.

3.3 Change in Trimethyamine Nitrogen (TMA-N)

Trimethylamine oxide is the source of TMA-N (TMAO), which is responsible for the fishy flavor and odor in marine fish. The level of TMA in fish is an important factor in assessing fish quality. When fish fillets were kept at a temperature of -20 ± 2^0C over a 120 days storage period, those treated with T4 and T3 showed a gradual rise in TMA-N content, reaching a maximum value of 2.88 mg% (see Figure 3 and Table 3 in the appendix). On the other hand, samples treated with T2 showed a slightly larger steady rise in TMA-N compared to T1, with both products reaching an average value of 5.16 mg% on the 120th day of storage. The outcomes clearly prove that the polyphenolic chemicals in green tea, present in both T4 and T3, were extremely active in controlling the formation and growth of TMA-N in mackerel fillet tissues throughout storage. In the current investigation, TMA-N readings for all products kept in frozen storage conditions were found to be within the acceptable range of 10-15 mg per 100 g (Connell, 1975). According to Estévez *et al*. (2009) [10], the generation of ammonia from amino acids and higher pH inhibits TMAO reductase and decreases its contribution to spoilage. TMA-N production may increase due to TMAO reductases from certain spoilage microorganisms. Over the 15-day storage period, the production of TMA-N in hake fillets was reduced by the crude polyphenols present in green tea extract, which also exhibited antibacterial activity.

3.4 Changes in Alpha Amino Nitrogen (AA-N)

Table 4 and Figure 4 indicate that the AA-N content decreased in both the preserved and control samples. Over the course of 120 days, the T1 and T2 samples exhibited a greater overall percentage decline in AA-N compared to the T3 and T4 samples. This variance in total percentage decrease suggests that the T1 and T2 samples lost more free amino groups due to potential reactions that could denature the protein. Regier *et al*. (2009) also supported the greater decrease in AA-N. The initial values of AA-N were 28.93±1.32, 30.80±2.64, 34.53±1.32, and 34.53±0.00. The final values for T1, T2, T3, and T4 were 15.58±0.00, 16.75±0.55, 18.70±0.00, and 18.09±0.55, respectively. Basu and Chouksey (2001) [3] have also described a reduction in AA-N levels during frozen storage, which they attribute to potential bacterial consumption of nitrogenous compounds. The thawing of samples in our current investigation may have led to the loss of some water-soluble amino acids, which could explain the decrease in AA-N levels.

3.5 Change in free fatty acid (FFA)

The breakdown of phospholipids by fish-derived lipases and phospholipases is indicated by the increase in FFA concentration (Gopakumar, 2002) [12]. The acceptable level of FFA (as oleic acid) is 15 mg/g. After 30 days of storage, the FFA levels in the Mackerel fillets of treatments T3 and T4 under frozen storage were slightly lower than those in the T1 and T2 samples. The recorded values were 1.00% for T3, 0.99% for T4, 1.84% for T1, and 1.41% for T2 (Table 5 and Figure 5) (appendix). Enzymatic breakdown of fish products' lipids results in FFA. The decrease in FFA levels observed in frozen items may be attributed to cold storage-induced volatilization. The slight growth in FFA through storage was caused by the enzymatic deprivation of triglycerides and phospholipids (Bilinski *et al*., 1981). Pal (2016) [21] discovered comparable outcomes of an increase in FFA when pangasius sutchi treated with green and black tea were frozen. In this study, the rate at which FFA generation increased during frozen storage was slowed down by the use of T3 and T4 serve as antioxidants. Additionally, Shinde and Patange (2015) [26] discovered that during the course of the storage period, the amount of FFA created more than doubled. A noteworthy distinction was observed between the GTE control and treated samples that were kept in frozen storage.

3.6 Change in peroxide value (PV)

During frozen storage, one common occurrence is lipid oxidation, which can cause off-flavors in fish and fishery products. This oxidation of lipids/fat has been associated with changes in texture and odor. The interaction of proteins with oxidized lipids forms lipo-protein complexes, altering the functional and nutritional characteristics of the protein and causing rancidity. Regardless of the treatment used, the

peroxide value (PV) was originally non-existent in all frozen samples, according to the current study's findings. But then there was a noticeable upward trend in PV numbers. According to Ramadan and Mörsel (2004) [22] and Ozen *et al*. (2017), the primary product of lipid oxidation, hydroperoxide, may be responsible for the initial increase in PV. The values of samples T1 and T2 on the 90th day were 6.50 ±0.07 and 5.50±0.70 meq of O_2/kg of fat, correspondingly, which was significantly different from the values of treated samples T3 and T4 (p<0.05) (see Table 6 and Figure 6). By the 120th day of frozen storage, the peroxide value (PV) had increased in all samples, likely due to fat oxidation. This led to an increase in PV for all samples T1, T2, T3, and T4. However, throughout the storage period, the values for treated and untreated samples remained within the acceptable range. The reduction in PV values for the treated samples associated to T1 indicates a important antioxidant effect of green tea extracts.

The current investigation supports the findings of Lin & Lin (2005). It suggests that using ice, green tea, and pouching tea can prevent additional hydroperoxide from being produced in bonito fillets during a seven-week storage period. However, treatments involving glazing with ice or tea extracts do not significantly alter PV. Alghazeer *et al*. (2008) [2] found that, adding instant green tea (250 ppm and 500 ppm) to Atlantic mackerel (*Scomber scombrus*) fillets and storing them at -10 °C for eight weeks resulted in decreased peroxide and hydroperoxide formation compared to the control sample. Alghazeer *et al*., (2008) [2] found that the PV rose gradually until 16 weeks and then histrionically reduced in the treated (green tea) and control samples during the frozen storage. Pal (2016) [21] described the increase of PV upto 29.94±l.80 for control, l8.77±1.70 and 22.01±1.60meq of O2/Kg of fat for green tea and black tea samples respectively. Green tea treated samples showed lesser value when compared to other groups.

3.7 Change in thiobarbituric acid (TBARs)

One of the main degradation processes in frozen fish is lipid oxidation, which results in rancidity, odor, and flavor (Connell and Shewan, 1980) [7]. Auto-oxidation, which is quantified by the amount of malonaldehyde present, leads to rancidity. After 60 days of frozen storage, the TBARS values of all the samples increased to 1.34±0.08, 1.29±0.16, 0.97±0.02, and 0.94±0.03 mg MDA/kg of samples for T1, T2, T3, and T4, correspondingly, from initial values of 0.37±0.02, 0.36±0.02, 0.34±0.01 and 0.33±0.01 mg MDA/kg of samples (Table 7 and Figure 7) (appendix). The data from this investigation shows that throughout the storage period, TBARs values of the sample containing T3 and T4 were within the acceptable range (1-2 mg MDA/kg of lipid). Through the 120 days storage period, all treated samples showed a considerably reduced TBARS (p< 0.05) than T1, indicating a significant impact of green tea extracts as an antioxidant. El-Hanafy *et al*. (2011b) report that the TBARs value of the samples stored on frozen green tea extract (GTE) was 2.98, 1.72, and 1.167 mg MDA/kg of sample for 2, 4, and 6% GTE treatment after 10, 14, and 14 days of storage, correspondingly, whereas the TBARs value of untreated sample increased to 3.99 mg MDA/kg of sample after 6 days of storage. As per Pal's (2016) [21] findings, the TBARs values for the control sample climbed to 1.21±0.07 mg MDA/kg, the green tea sample to 0.89±0.06 mg MDA/kg, and the black tea sample to 0.83±0.05 mg MDA/kg. After 180 days of freezer storage, the levels of TBARS in the treated (green and black tea) samples remained within the acceptable range (1-2 mg MDA/kg). Consequently, using green tea extracts as dip treatment has an significant antioxidant consequence.

3.8 Change in total plate count (TPC)

The freezing process can help prevent fish and shellfish from spoiling, as it reduces the quantity of bacteria initially and slows down their growth during storage. While some bacteria may die off during freezing and storage, the rate of destruction can vary. A maximum allowable bacterial count of 10^7 cfu/g is suggested for fresh fish by the International Commission on Microbiological Specifications for Foods (ICMSF).

At first, TPC did not significantly differ between the treated and untreated samples. After 30 days of storage, TPC did, however, gradually rise (Table 8 and Figure 8; appendix). Regarding T3, the TPC saw a gradual increase in all samples after 30 days, rising to 4.20±0.03 log cfu/g from 3.86±0.02 log cfu/g. The TPC values for T1, T2, T3, and T4 were 5.94±0.01, 5.75±0.02, 4.57±0.04, and 3.95±0.03 log cfu/g, respectively, after the 120-day storage period. The T1 sample had higher numbers than the treated samples. This difference in microbial counts could be attributed to the tea polyphenol effect, which inhibits the growth of microbial counts. According to El-Hanafy *et al*. (2011b), the control sample's TPC started at 4.36 log cfu/g and grew to 7.8 log cfu/g after six days of cold storage. Furthermore, compared to T1 under frozen storage, all treated samples with green tea extracts showed a significant (p<0.05) suppression of the TPC. Green tea extracts polyphenol content is credited with reducing the number of bacteria.

3.9 Changes in organoleptic characteristics

The current investigation on mackerel fillets found that a sensory score of more than "6" was considered "acceptable". If any characteristic scored lower than "6", the sample would be rejected. Table 9 and Figure 9 present the mean scores for various attributes of the frozen sample. In both the control and treatment samples, the mean scores for all qualities decreased as the storage period increased. Throughout storage, the mackerel fillets remained in a satisfactory state. Compared to T1 frozen mackerel fillets, the treated samples (T3 and T4) received higher scores from the sensory evaluation for a longer duration.

Table 1: Changes in pH of mackerel fillets for control and treated samples stored in frozen condition. All values are expressed as mean ± standard deviation.

Days	pH			
	T1	T2	T3	T4
0	6.80±0.02	6.49±0.04	6.71±0.05	6.78±0.04
30	6.61±0.01	6.65±0.01	6.58±0.03	6.60±0.04
60	6.71±0.02	6.62±0.05	6.65±0.04	6.66±0.04
90	6.74±0.05	6.69±0.07	6.71±0.04	6.71±0.03
120	6.79±0.05	6.71±0.08	6.68±0.01	6.69±0.01

Table 2: Changes in TVB-N

Days	TVB-N (mg %)			
	T1	T2	T3	T4
0	6.47±0.74	5.25±0.00	2.97±0.74	2.62±1.23
30	8.75±0.00	6.12±1.23	4.02±0.74	3.15±0.49
60	9.62±1.23	7.00±0.00	5.25±0.00	3.50±0.00
90	16.62±1.23	10.50±2.47	6.47±0.74	6.30±0.49
120	24.50±0.49	17.50±2.47	10.67±0.24	10.32±0.24

Table 3: Change in TMA-N

Days	TMA-N (mg %)			
	T1	T2	T3	T4
0	4.20±0.99	3.50±0.00	2.24±0.99	1.75±0.99
30	3.32±1.23	2.45±2.47	1.57±1.23	0.70±0.00
60	3.67±1.23	2.80±0.00	1.92±1.23	1.57±0.74
90	4.20±0.99	3.50±0.00	2.45±0.99	1.75±0.99
120	5.25±0.00	5.07±0.24	2.97±0.74	2.80±0.99

Table 4: Changes in AA-N

Days	AA- N (mg %)			
	T1	T2	T3	T4
0	28.93±1.32	30.80±2.64	34.53±1.32	34.53±0.00
30	26.13±0.00	28.00±0.00	30.33±0.66	30.33±0.66
60	22.40±0.00	26.13±1.32	27.06±1.32	27.53±0.66
90	17.14±0.00	19.09±0.55	21.43±0.55	22.21±0.55
120	15.58±0.00	16.75±0.55	18.70±0.00	18.09±0.55

Table 5: Changes in FFA

Days	FFA (% of oleic acid)			
	T1	T2	T3	T4
0	0.99±0.07	0.85±0.01	0.62±0.00	0.41±0.06
30	1.84±0.29	1.41±0.00	1.00±0.28	0.99±0.28
60	4.20±0.10	3.43±0.44	2.77±0.09	2.33±0.17
90	6.28±0.53	5.22±0.00	4.76±0.25	4.28±0.25
120	8.68±0.41	7.49±1.13	6.43±0.16	6.31±0.33

Table 6: Changes in PV

Days	PV (meq of O2/Kg fat)			
	T1	T2	T3	T4
0	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
30	3.50±0.70	2.75±0.35	1.15±0.21	1.60±0.56
60	4.00±0.00	3.00±0.00	1.95±0.07	2.25±0.35
90	6.50±0.70	5.50±0.70	4.25±0.35	3.50±0.70
120	10.50±2.12	7.50±0.70	6.00±1.41	5.50±0.70

Table 7: Changes in TBARs

Days	TBARs (mg MDA/ Kg of the fish sample)			
	T1	T2	T3	T4
0	0.37±0.02	0.36±0.02	0.34±0.01	0.33±0.01
30	0.97±0.02	0.96±0.01	0.88±0.01	0.87±0.01
60	1.34±0.08	1.29±0.16	0.97±0.02	0.94±0.03
90	1.58±0.11	1.38±0.05	1.00±0.06	0.97±0.01
120	2.02±0.11	2.00±0.05	1.07±0.07	1.21±0.17

Table 8: Changes in TPC

Days	TPC (log cfu/g)			
	T1	T2	T3	T4
0	3.31±0.08	3.78±0.02	3.87±0.00	3.20±0.03
30	4.31±0.06	3.84±0.04	4.20±0.03	3.67±0.03
60	4.59±0.07	4.42±0.17	3.86±0.02	3.98±0.01
90	5.81±0.00	5.65±0.06	3.96±0.01	3.90±0.03
120	5.94±0.01	5.75±0.02	4.57±0.04	3.95±0.03

Table 9: Changes in organoleptic evaluation

Storage Days	Treatment	Colour	Odour	Taste	Texture	General appearance	Overall Acceptability
0	T1	9.25±0.70a	9.00±0.70a	9.30±0.70a	9.00±0.00a	9.50±0.70a	9.46±0.05a
	T2	9.00±0.00b	9.20±0.70a	8.50±0.70b	9.00±0.00b	9.40±0.70b	9.40±0.17a
	T3	9.50±0.00cd	9.00±0.00b	9.25±0.35cd	9.50±0.00c	9.00±0.00cd	9.86±0.11b
	T4	9.50±0.35cd	9.25±0.35b	9.25±0.35cd	9.25±0.35d	9.00±0.00cd	9.93±0.11b
30	T1	8.10±0.22a	8.20±0.27a	8.10±0.22a	8.20±0.27a	8.20±0.27a	8.16±0.28a
	T2	8.26±0.25b	8.10±0.22a	8.30±0.27b	8.16±0.23b	8.10±0.224b	8.23±0.25a
	T3	8.60±0.22cd	8.60±0.41b	8.60±0.22cd	8.50±0.00c	8.50±0.00cd	9.40±0.10b
	T4	8.70±0.27cd	8.60±0.22b	8.66±0.23cd	8.60±0.22d	8.46±0.36cd	9.50±0.10b
60	T1	7.40±0.41a	7.60±0.22a	7.30±0.27a	7.20±0.27a	7.30±0.27a	7.50±0.50a
	T2	7.80±0.27b	7.70±0.44a	7.70±0.27b	7.80±0.44b	7.70±0.44b	7.50±0.50a
	T3	8.10±0.22cd	8.20±0.27b	8.30±0.44cd	8.10±0.22c	8.200±0.274cd	8.73±0.25b
	T4	8.20 ± 0.27^{cd}	8.30 ± 0.27^{b}	8.20 ± 0.274^{cd}	8.20 ± 0.27^{d}	8.360 ± 0.416^{cd}	8.86 ± 0.23^{b}
90	T1	6.80 ± 0.44^{a}	7.00 ± 0.35^{a}	6.60 ± 0.41^{a}	6.90 ± 0.22^{a}	6.800 ± 0.274^{a}	6.90 ± 0.17^{a}
	T2	7.10 ± 0.22^{b}	7.40 ± 0.41^{a}	7.30 ± 0.27^{b}	7.40 ± 0.54^{b}	7.30 ± 0.44^{b}	7.00 ± 0.00^{a}
	T3	8.00 ± 0.00^{cd}	7.90 ± 0.22^{b}	8.00 ± 0.35^{cd}	7.90 ± 0.22^{c}	8.10 ± 0.22^{cd}	8.33 ± 0.28^{b}
	T4	7.80 ± 0.27^{cd}	8.10 ± 0.22^{b}	8.00 ± 0.35^{cd}	8.00 ± 0.00^{d}	8.06 ± 0.13^{cd}	8.63 ± 0.15^{b}
120	T1	6.30 ± 0.44^{a}	6.40 ± 0.41^{a}	6.20 ± 0.27^{a}	6.30 ± 0.44^{a}	6.50 ± 0.35^{a}	6.66 ± 0.28^{a}
	T2	6.70 ± 0.44^{b}	6.90 ± 0.54^{a}	7.10 ± 0.22^{b}	7.10 ± 0.54^{b}	6.80 ± 0.44^{b}	6.80 ± 0.50^{a}
	T3	7.60 ± 0.41^{cd}	7.50 ± 0.50^{b}	7.60 ± 0.54^{cd}	7.60 ± 0.41^{c}	7.70 ± 0.44^{cd}	8.06 ± 0.11^{b}
	T4	7.50 ± 0.35^{cd}	7.60 ± 0.41^{b}	7.60 ± 0.41^{cd}	7.80 ± 0.44^{d}	7.90 ± 0.22^{cd}	8.36 ± 0.32^{b}

Note: Different superscripts in the same column indicates significant difference ($p<0.05$)

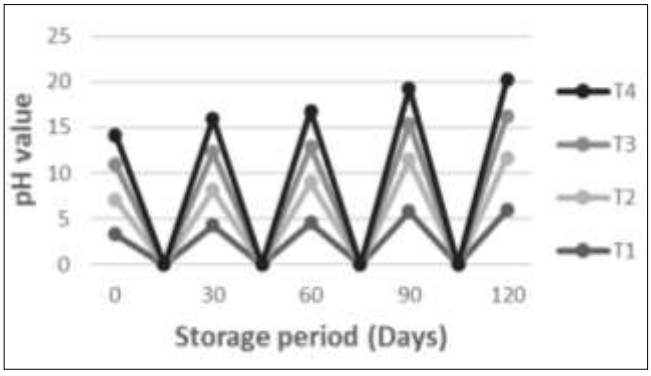

Fig 1: Changes in pH

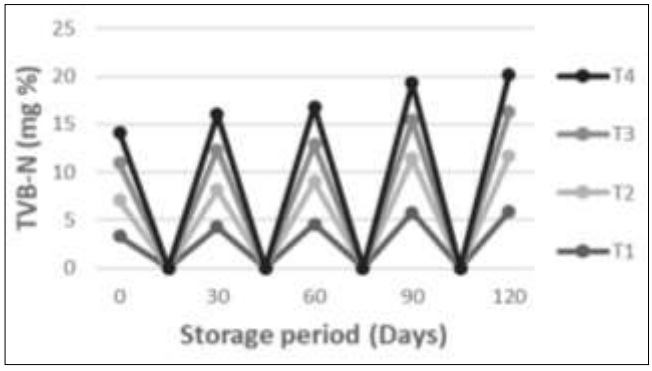

Fig 2: Changes in TVB-N

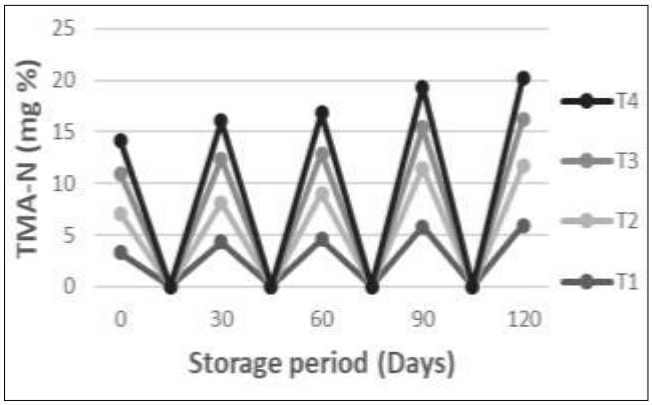

Fig 3: Changes in TMA-N

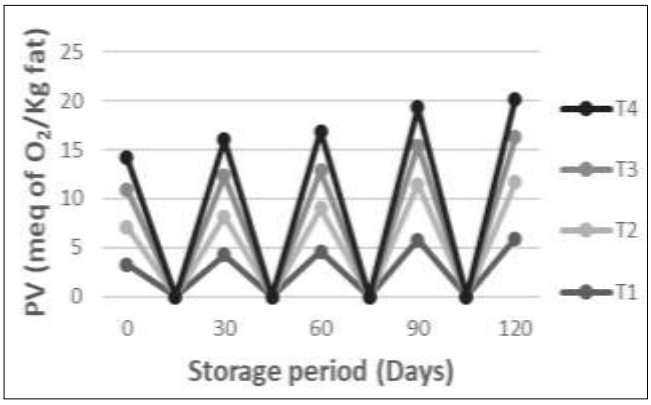

Fig 6: Changes in PV

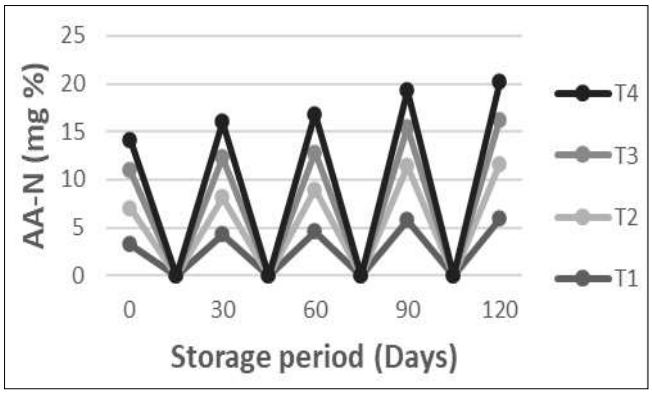

Fig 4: Changes in AA-N

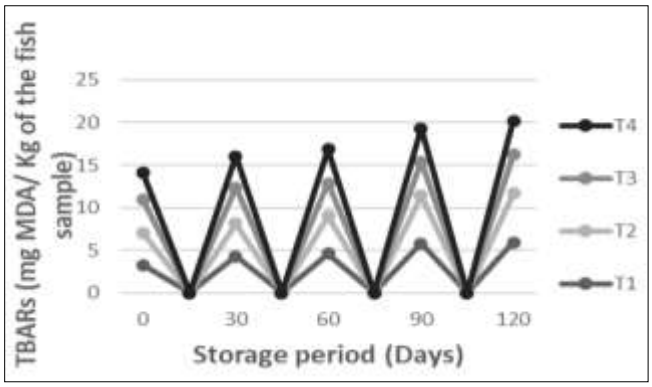

Fig 7: Changes in TBARs

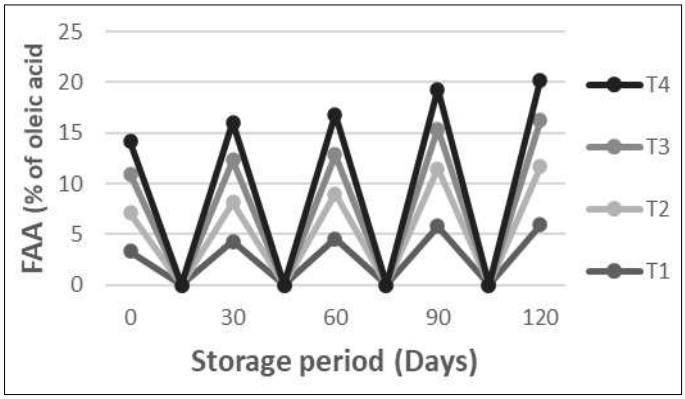

Fig 5: Changes in FAA

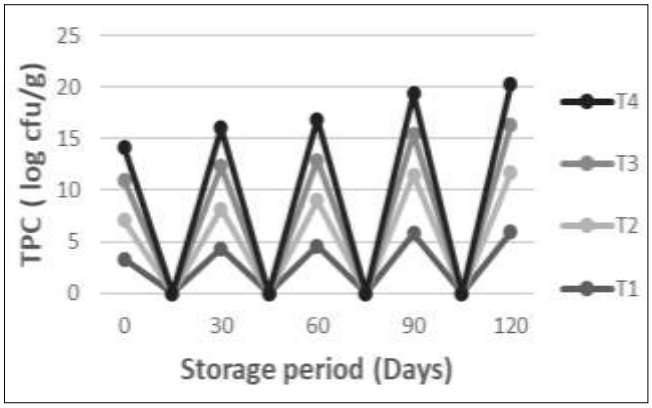

Fig 8: Changes in TPC

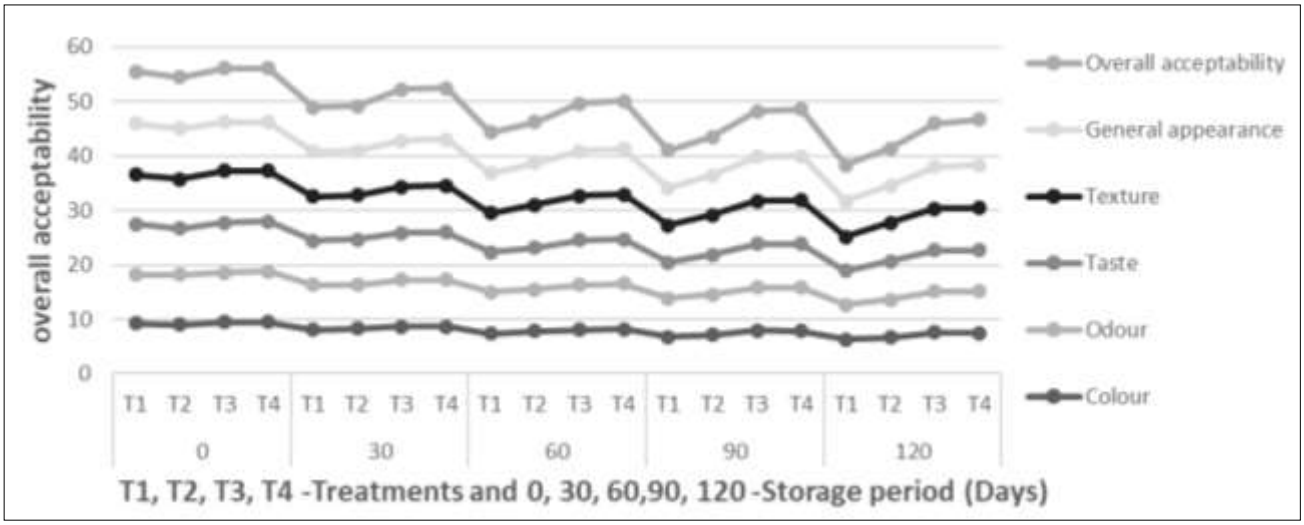

Fig 9: Changes in organoleptic evaluation

4. Conclusion

Foods with a longer shelf life are demanded by end users. Natural preservation methods for inactivating microbes and enzymes in fish and fishery products are getting more attention as a means of guaranteeing food security and satisfying requirements for the preservation of nutrition and quality qualities. Green tea consumption is popular and linked to benefits for health since it is employed as a plant-based ingredient in functional meals and supplements. The current investigation has unequivocally demonstrated that mackerel fillets' shelf life has been increased by green tea extract preservation. Additionally, the research study suggests employing fish green tea extract to extend shelf life, and fisheries products may be used for commercial purposes.

5. References

1. Al-Bulushi IM, Kasapis S, Al-Oufi H, Al-Mamari S. Evaluating the quality and storage stability of fish burgers during frozen storage. Fisheries Science. 2005;71:648-654.
2. Alghazeer R, Saeed S, Howell NK. Aldehyde formation in frozen mackerel (*Scomber scombrus*) in the presence and absence of instant green tea. Food Chemistry. 2008;108(3):801-810.
3. Basu S, Chouksey MK. Frozen storage characteristics of cultured *Macrobrachium rosenbergii* (de Man); c2001.
4. Bilinski E, Jonas REE, Peters MD. Treatments affecting the degradation of lipids in frozen Pacific herring, *Clupea harengus pallasi*. Canadian Institute of Food Science and Technology Journal. 1981;14(2):123-127.
5. Connell JJ. Control of fish quality. Surrey. Fishing News; c1975. p. 127-139.
6. Connell JJ. Control of fish quality. Vol. 179. London: Fishing News Books; c1975.
7. Connell JJ, Shewan JM. Sensory and non-sensory assessment of fish. In: Advances in fish science and technology: papers presented at the Jubilee conference of the Torry Research Station, Aberdeen, Scotland, 23-27 July 1979, edited by JJ Connell and staff of Torry Research Station; c1980.
8. EL-Hanafy AEA, Shawky HA, Ramadan MF. Preservation of *Oreochromis niloticus* fish using frozen green tea extract: Impact on biochemical, microbiological and sensory characteristics. Journal of Food Processing and Preservation. 2011;35(5):639-646.
9. Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR. Iron chelating activity, phenol and flavonoid content of some medicinal plants from Iran. African journal of Biotechnology, 2008, 7(18).
10. Estévez M, Ollilainen V, Heinonen M. Analysis of protein oxidation markers α-aminoadipic and γ-glutamic semialdehydes in food proteins using Liquid Chromatography (LC)- Electrospray Ionization (ESI)-Multistage Tandem Mass Spectrometry (MS). Journal of Agricultural and Food Chemistry. 2009;57(9):3901-3910.
11. Flick Jr GJ, Martin RE, Correa AI. Processing and Presentation. In: Marine and Freshwater Products Handbook, 2000, 289.
12. Gopakumar K. Textbook of fish processing technology; c2002.
13. Green M. Quantitative studies on freshly caught and iced shrimp. Food Research. 1949;14(5):372-383.
14. Green M. Quantitative studies on freshly caught and iced shrimp. Food Research. 1949;14(5):372-383.
15. Lin CC, Lin CS. Enhancement of the storage quality of frozen bonito fillets by glazing with tea extracts. Food control. 2005;16(2):169-175.
16. De Lacey AL, López-Caballero ME, Montero P. Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life. LWT-Food Science and Technology. 2014;55(2):559-564.
17. Mole S, Waterman S. Analysis of phenolic plant metabolites. Blackwell Sci. Publ; c1994.
18. Negi PS. Plant extracts for the control of bacterial growth: Efficacy, stability and safety issues for food application. International journal of food microbiology. 2012;156(1):7-17.
19. Ozen A, Comrie WA, Ardy RC, Domínguez Conde C, Dalgic B, Beser ÖF, *et al*. CD55 deficiency, early-onset protein-losing enteropathy, and thrombosis. New England Journal of Medicine. 2017;377(1):52-61.
20. Özoğul Y, Boğa EK, Tokur B, Özoğul F. Changes in Biochemical, sensory and microbiological quality indices of common sole (*Solea solea*) from the Mediterranean Sea, during ice storage. Turkish Journal of Fisheries and Aquatic Sciences, 2011, 11(2).
21. Pal D. Effect of tea extract on the shelf life of *Pangasius sutchi* fillets during frozen storage. Kolkata: Dissertation; c2016.
22. Ramadan MF, Mörsel JT. Oxidative stability of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils upon stripping. European Journal of Lipid Science and Technology. 2004;106(1):35-43.
23. Regier M, Rother M, Schuchmann HP. Alternative heating technologies. In: Processing Effects on Safety and Quality of Foods; c2010. p. 187-229.
24. Sarah H, Hadiseh K, Gholamhossein A, Bahareh S. Effect of green tea (*Camellia sinenses*) extract and onion (*Allium cepa*) juice on lipid degradation and sensory acceptance of Persian sturgeon (*Acipenser persicus*) fillets. International Food Research Journal. 2010;17(3):751-761.
25. Shahidi F, Amarowicz R, HE Y, Wettasinghe M. Antioxidant activity of phenolic extracts of evening primrose (*Oenothera biennis*): A preliminary study. J Food Lipids. 1997;4(2):75-86.
26. Shinde PA, Reddy SVK, Patange SB. Quality of Indian mackerel as affected by pomegranate peel and tea leaf extracts during ice storage; c2015.
27. Stewart MM. The effect of exposure to low temperatures on the number of bacteria in fish's muscle; c1934.
28. Vareltzis K, Koufidis D, Gavriilidou E, Papavergou E, Vasiliadou S. Effectiveness of a natural rosemary (*Rosmarinus officinalis*) extract on the stability of filleted and minced fish during frozen storage. Zeitschrift für Lebensmitteluntersuchung und-Forschung A. 1997;205:93-96.
29. Wanasundara UN, Shahidi F. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chem. 1998;63(3):335-342.
30. Yerlikaya P, Gokoglu N. Inhibition effects of green tea and grape seed extracts on lipid oxidation in bonito fillets during frozen storage. Int J Food Sci Technol. 2010;45(2):252-257.