

Preservative Effect of Green Tea Extract on the Storage Stability of Mackerel Fillets Stored in Ice

¹Ester John, ²Siddappaji S.

¹Master of Fish Processing Technology, Department of Fish Processing Technology, College of Fisheries-Mangalore, Karnataka, India

²Associate Professor, Research supervisor, Department of Fish Processing Technology, College of Fisheries-Mangalore, Karnataka, India

¹Mobile: +918310061075; ¹Email: estajk26@gmail.com

ABSTRACT

Green tea contains polyphenolic compounds having antioxidant and antimicrobial properties that inhibit rancidity in fish and fishery products. Pelagic fish such as mackerel contain a high proportion of polyunsaturated fatty acid (PUFA) and are very prone to oxidation. In the present study, an attempt was made to investigate the potency of green tea extract as an antioxidant to extend the shelf life of the fillets. In this study, ethanol and water extracts of green tea were selected as natural antioxidants. Mackerel fillets were dipped into a different solutions that are GT aqua, GT alco, SA asco and CON. Mackerel fillets were evaluated by microbiological (TPC) biochemical (pH TVB-N, TMA-N, PV, TBA, and FFA) and sensory characteristics were studied for 8 days of storage in ice. Among the different treatments, T3 and T4 shown a higher shelf life compared to control. The PV value of T3 and T4 was 6.50 and 5.50 mEq/Kg of fat and control 11.50 mEq/Kg of fat. Based on the sensory score, samples treated with green tea extracts (T3 and T4) were more effective to improve attributive characteristics of mackerel fillets.

Keywords: Green tea, lipid oxidation, mackerel fish, natural antioxidants, preservative.

1. INTRODUCTION

Seafoods are highly nutritious but susceptible to spoilage if stored at ambient temperature. Therefore, fish is processed by different methods like icing freezing, canning, fermentation, smoking, use of preservatives and antioxidants as well as other methods. The catches from the sea are uncertain, the harvested catches are mostly marketed and consumed in fresh condition or iced condition. But, the shelf life of such a product is only 4 days [1]. In order to extend the shelf life, chemical preservatives and synthetic antioxidants are used. Many of these additives are suspected of their carcinogenic effects. Hence, health conscious people are demanding alternative sources from natural resources to keep the fish good condition for longer periods of time. Numerous plant extracts have been used for food preservation [2], [3], [4]. To fulfill these requirements an attempt was made in the present experiment to extract preserved compounds from green tea and to study its effect in fillets in extending shelf life; by preventing spoilage changes and to retard the development of oxidative rancidity.

Mackerel is preferred food fish in many parts of the World. Mackerel contribute 2.88 lakh tonnes [5] to the Indian marine catch. Mackerel is high seasonal and during glut, much of the catches are used for the production of fish meal. In order to satisfy the growing demand for high-quality protein food in the more convenience ready to cook products, these types of glut catches can be converted into fillets and then treated with natural extractants to extend the shelf life. An examples of the natural plants; rosemary, thyme, pomegranate peel, green tea, cloves, etc. Green tea contains catechins. Thus, crude tea extract may provide a viable alternative to synthetic antioxidants [6], [7], [8]. Fish amino acids, fish peptides, and polypeptides act as bioactive compounds [9], [10].

2. MATERIAL AND METHOD

2.1 Methods

2.1.1 Preparation of green tea extract in water/aqua extract

Green tea aqua extracts (GT aqua) was prepared using the hot water extraction method described by [3]. Tetley green tea packets commercially available tea leaves were grounded well into a fine powder, sieved and stored in a cool place until use. One hundred grams of ground tea powder was weighed and transferred into a 750ml conical flask into which 400mL of boiled distilled water was added, then the extraction was carried out with continuous shaking at 300rpm in a mechanical shaker for 45 min. The extraction temperature was set at 50°C. Then the filtration process was done to separate extract from residue using Whatman No.4 filter paper. After the first extraction, the used leaves were again added to boiled distilled water and by following the same procedure a second extract of green tea was prepared and mixed with the first extract and concentrated at 85°C. The concentrated soluble solid content was applied as green tea extract with water (GT aqua) in the experiment (T3).

2.1.2 Preparation of green tea extract in alcohol (Ethanol)

The warm ethanol extractions of the active ingredients of the leaves were carried out using the method as described by [11]. 100g of the leaves were ground extracted using 400mL of 95% ethanol in the soxhlet extraction unit. The extraction was carried out for 45 min at 300 rpm and 45°C. The extract was then separated from the residue by filtration through Whatman No.4 filter. After the first extraction, the used leaves were again added to 95% ethanol and by following the same procedure a second extract of green tea is prepared and mixed with the first extract and concentrated at 60°C to a powder. The concentrated soluble solid content was applied as green tea extract with ethanol (T4) in the experiment

2.1.3 Processing of fish

Fresh and prime quality mackerel fish (*Rastrelliger Kanagurta*) were obtained from purse seine boat in fish landing Centre, Mangalore. Soon after catching, during night fishing the fishes were washed in seawater and then packed in the ice in an insulated fish box and were brought to the landing Centre in a well-iced condition. The collected boxes containing fishes and ice were transported to the Fish Processing hall of the Department of fish processing Technology, College of Fisheries, Hoige Bazar.

Mackerel fish with an average weight of 166.80 ± 25.70 g and the total length of 23.83 ± 1.17 cm were used for the study.

2.1.4 Preparation of mackerel fillets

Partially frozen fish was dipped in iced water for some time so as to enable the handling of mackerel without causing bruising, tearing and over softening. The fillets were made from the partially frozen fishes, fillets were rinsed in chilled water at 3 to 4°C to remove adhered blood and drained, divided into four batches for different dip treatment. T1 – fillets dipped in chilled distilled water (control), T2 – fillets dipped in standard Ascorbic acid at a concentration (SA asco) of 500 ppm, T3 - fillets dipped in green tea water extract at a concentration (GT aqua) of 2000 ppm, T4 - fillets dipped in 95% ethanol extract solution at a concentration (GT alco) of 2000 ppm. During the storage of mackerel fillets, ice was added to the insulated boxes as required. All analyses were performed in triplicate on days 0, 2, 4, 6, and 8.

2.2 Methods

2.2.1 Sensory analysis

Sensory analysis was performed by 10 panelists. Both raw and cooked fillets were evaluated using 9 hedonic scale. They were required to evaluate the raw fillets samples as per 9 points hedonic scale; 0 = dislike extremely, 1 = dislike very much, 2 = dislike moderately, 3 = dislike slightly, 4 = neither like nor dislike, 5 = light slightly, 6 = like moderately, 7 = like very much, 8 = like extremely, 9 = excellent [12]. Panellists evaluated the sensory characteristics based on the appearance, colour, odour, taste, flavour, texture and overall acceptability of treated and untreated fish fillets. Water was provided to panelists for washing their mouth before and after the evaluation of each sample, to restore their taste sensitivity.

2.2.2 Microbial analysis

Samples were drawn to analyses total bacterial count (TPC) once in two days. 1g of fish fillets was mixed with 9ml of physiological saline as describe by [13]. Further dilutions were made and then 0.1ml of dilution was pipetted onto the surface of

the plate count plates uniformly spread and then, the plates were incubated at 37°C for 24h.

2.2.3 Biochemical analysis

2.2.3.1 Measurement of pH

The pH value was measured using pH meter (Eutech Instruments pH-510, Malaysia) as described by [14]. Fish sample 5g was macerated with 45mL of distilled water (1:9). Prior to pH measurement, the pH meter was calibrated with a standard buffer solution of pH 4.0 and 9.0 (Merck Specialities Pvt. Ltd., Mumbai, India).

2.2.3.2 Total Volatile Base Nitrogen and Trimethylamine Nitrogen Analysis

Total volatile base-nitrogen (TVB-N) and Trimethylamine Nitrogen (TMA-N) were measured by the procedure of [15] using Conway's micro-diffusion units.

2.2.3.3 Lipid analysis

The peroxide value (PV), thiobarbituric acid (TBA) and free fatty acid (FFA) contents were determined in the extracted lipid. PV and FFA were determined according to the method of [16] and expressed in milliequivalents (mEq) peroxide per kg of lipid and percent of oleic acid, respectively. According to the method of [17] TBA showed absorbance at 530nm and results expressed as mg malonaldehyde (MDA) per kg of fat.

Lipid oxidation leading to rancid odour and flavour is the main type of deterioration occurring in fish [18]. Rancidity occurs as a result of auto-oxidation, which is measured in terms of malonaldehyde content. TBA is an indicator of the quality of the fish whether it was iced, chilled or stored in frozen condition [19], [20]. TBARs is the secondary breakdown of lipid peroxidation [4]. It is expressed as malonaldehyde that is a degradation of the product.

3. RESULTS AND DISCUSSION

Ready to cook products from fish have become prevalent increasingly but due to its perishability, the shelf life is limited because of lipid oxidation and rapid microbial growth [21], [22].

3.1 Sensory valuation

The total scores of mackerel fillets stored in ice are presented in Table 1 as shown in appendix. The initial scores for treated and untreated fillets were found to be the same on the 0day. The acceptable scores of treated mackerel fillets were found to be more than 8 days whereas for untreated fillets was less than 8 days. The results of the sensory analysis indicated that the storage lives of fish fillets to maintain quality and nutrients can be achieved when fillets are preserved with green tea aqua extract.

3.2 Microbiological assessment

Total plate count (TPC) on the mackerel fillets stored in ice are presented in Table 2 and Figure 1 as shown in appendix. There were increases in the TPC over the storage period. The maximum acceptable TPC count for fresh fish is 10^7 CFU/g (7 log CFU/g) as recommended by the International Commission on Microbiological Specification for Foods (ICMSF). The Bacteria grow more quickly in the sample without preservative than the sample with preservative during the storage period. In the present study, the average initial counts of T1 was 3.31 ± 0.08 which has increased to 6.17 ± 0.17 log cfu/g, after 8 days. Among the treated sample T3 sample recorded lower values 4.25 ± 0.11 log cfu/g at the end of the storage period of 8 days. Other samples showed a gradual increase in TPC with the increase of storage days. This conclusion indicated that sensory analysis correlated well with the microbiological analysis of the fish fillets.

3.3 Changes in pH

Changes in the pH value of mackerel fillets are shown in table 3 and figure 2 as shown in appendix. In the present study, a gradual increase in the pH value of fish fillets was observed for 8 days. Initially the pH value for T1, T2, T3 and T4 were found to be 6.80 ± 0.02 , 6.49 ± 0.04 , 6.71 ± 0.05 and 6.78 ± 0.04 respectively. During the storage, the pH value showed the fluctuation trend

means increasing and decreasing and at the end of storage time the values of all samples were found to be 7.09 ± 0.13 , 6.92 ± 0.05 , 6.81 ± 0.06 and 6.84 ± 0.02 respectively. The increase in pH was postulated to be due to an increase in volatile base compounds produced by endogenous or microbial enzymes and decomposition of nitrogenous components [21]. According to [23], the enzymatic degradation of ATP caused the liberation of inorganic phosphate and ammonia, resulting in the changes in pH value.

3.4 Changes in TVB-N

The values of TVB-N is used as an indicator to measure the freshness quality of fish and fishery products [24]. The change in TVB-N content of fillets treated with green tea aqua extracts during storage at ice is shown in Table 4 and Figure 3 as shown in appendix. Mackerel fillets T3 and T4 showed a lower increase in TVB-N compared to fillets T1 and T2. The initial value of TVB-N in treated fillets stored at ice was recorded as 2.97 ± 0.74 which increased slowly to 14.87 ± 1.23 at the end of the 6th day in T3, whereas it was 20.30 ± 0.00 for fillets T4 at the end of 8 days. In the case of T2 and T1 showed higher values such as 28.87 ± 1.23 and $25.37 \pm 1.23\text{mg}\%$ respectively at the end of 8th days of storage. The acceptability limit of total volatile base nitrogen compound for flesh fish is between the ranges of 30-40 mg N/100 g for flesh [25]. According to [26] and [27], a level of 30mg N/100g is well thought-out to be the upper limit, above which fish and fishery products are considered unfit for human consumption in the present study, the TVB-N values of all the samples were found to be within the acceptable limit of 30mg-N/100g.

3.5 Change in Trimethylamine Nitrogen (TMA-N)

The changes in the TMA-N content of mackerel fillets stored in ice are presented in Table 5 and Figure 4 as shown in appendix. The initial TMA-N content of T1(fresh fillets) was found to be $1.75 \pm 0.00\text{mg}\%$. TMA-N content of mackerel fillets with T2, T3 and T4 were found to be lesser than was 0.87 ± 0.24 , 0.70 ± 0.00 and $0.52 \pm 0.24\text{mg}\%$ respectively. During ice storage, TMA-N values for the T1 sample gradually increased to 3.32 ± 0.74 and $5.07 \pm 0.24\text{mg}\%$ on the 4th and 6th day respectively and $4.72 \pm 0.74\text{mg}\%$ in T2 on the 6th day. T3 and T4 treated fillets showed a

very marginal rise in TMA-N content which was found to be 2.97 ± 0.24 and $2.45 \pm 0.99\text{mg}\%$ on the 6th day, indicating that green tea extracts (T3 and T4) are highly effective in controlling the rise in TMA-N values when products were stored in ice.

3.6 Changes in Alpha amino nitrogen (AAN)

Free amino acid contents in shellfish are greater than fish. In crustaceans, it is over 300mg/100g of muscle, while the fish contains only 1/10th of this amount [28]. Amino acid occurring in fish tissue is estimated in addition to protein. Addition of TCA solution at 10% concentration, protein can be precipitated leaving free amino acids in the extract. When treated with copper salt like copper phosphate, cupric chloride it can solubilize amino acids to form copper-amino complex compound which has a dark colour depending upon the concentration of amino acids. The decrease in AA-N of the investigational samples during ice, storage could be attributed to the deamination of amino acids and probable loss through drip. A decrease in AA-N content during ice storage has been reported by different investigators such as, in storage of ribbon fish [29], cuttle fish [30] and clam meat [31].

Table 6 and Figure 5 present AA-N for control and treated samples as shown in appendix. For ice storage, the initial values of AA-N were 29.63 ± 0.33 , 30.80 ± 2.64 , 34.06 ± 0.66 , $34.53 \pm 0.00\text{mg}\%$ for T1, T2, T3 and T4 respectively. At the end of storage period the AA-N content was reached to 14.80 ± 1.10 , 15.19 ± 0.55 , 17.92 ± 0.00 and $17.53 \pm 0.55\text{mg}\%$ for T1, T2, T3 and T4 respectively. The value of AA-N was decreased more in the untreated sample than treated samples. This shows the effectiveness of the green tea extract in increasing the shelf life and maintaining the nutritive compounds. Also the decreasing trend of alpha amino in fish during ice storage is reported by [32], they found that initially AA-N content was 70.4mg% which decreased to 67.6mg% during 20 days of ice storage. [33] reported the decrease of AA-N in common murrel (*channa striatus*) from 44mg% to 25mg% for batch I and 34mg% to 13mg% for batch II during 10 and 13 day of ice storage respectively. In the present study, more decrease of AA-N was observed in T1 sample compared to T3 and T4.

3.7 Changes in Peroxide value (PV)

PV is a measure of the primary degree of oxidation in the fish muscle. Peroxides are the main initial products of autoxidation during the lipid oxidation process. PV is one of the most significant aspects responsible for fish putrefaction during the storage period. Hydroperoxide is a primary oxidation product of polyunsaturated fatty acid [34]. It is an unstable compound which breakdown to low molecular weight products such as ketones, acids, aldehyde, and alcohol, which are volatile and causes off-flavor and off-odor in products [4]. According to [35] the acceptable limit of PV without objectionable off-taste and odour is up to 30mEq of O₂/kg lipids. Other reviews found that the acceptability limit of PV of crude fish oil is between 3 and 20mEq O₂/Kg [36]. In the present study, changes in PV of the mackerel fillets stored at ice throughout the storage period are presented in Table 7 and figure 6 as shown in appendix.

During ice storage, the initial value of PV was not recorded in all samples, this may be due to the absence of hydroperoxide formation which may result to increase of PV. At the 4th day of storage, there was a high increase of T1 sample and T2 than T3 and T4. At the 6th day of storage, the T1 PV was 6.50 ± 0.70mEq of O₂/Kg of fat and at end of storage (8th day), the PV increased to 11.50 ± 0.70mEq of O₂/Kg of fat which shows the more formation of hydroperoxide in the fish sample. On the other hand T3 and T4, at the 6th day the values were 3.50 ± 0.70 and 2.50 ± 0.70mEq of O₂/Kg of fat respectively then gradually increased to 6.50 ± 0.70 and 5.50 ± 0.70mEq of O₂/Kg of fat respectively at the end of storage 8th day which shows lesser values compared to T1 sample. During ice storage, the values of PV were within the acceptable limit (3-20mEq of O₂/Kg). From this context, it shows the effects of green tea extracts in keeping the good quality of the fish fillets.

3.8 Changes in Thiobarbituric acid (TBA)

TBA is an indicator of the quality of the fish and fishery products whether it was iced, chilled or stored in frozen condition [19], [20]. TBARS is the secondary breakdown product of lipid peroxidation [4]. Fish and fishery products of good quality will have the limit of acceptability of TBA value ranging from 1-2mg MDA/Kg of fat in the fish sample [37]. Also, it is recommended that a level of 7 – 8 mg MDA/Kg as the upper limit of acceptable

freshness [38]. In the present study, the changes of the TBA value of mackerel fillets both treated and control stored in ice are presented in the Table 8 and Figures 7 as shown in appendix.

During ice storage studies, the level of malondialdehyde of fresh fish samples for T1, T2, T3, and T4 were found to be 0.37 ± 0.02, 0.36 ± 0.02, 0.34 ± 0.01 and 0.33 ± 0.01mg MDA/Kg of fat in fish sample respectively, which shows almost same lower values of fresh mackerel fillets. In the present study TBA values of all samples increased with time during the storage period. According to the statistical analysis of variance, this increase was significant (p<0.05). The value of the TBA increase was more rapid in the T1 and T2 samples compared to the T3, and T4 samples. The maximum value of TBA was found in the T1 samples 2.01 ± 0.03mg MDA/Kg of fat in the fish sample on the 8th day, it was higher than fillets treated with ascorbic acid at 500ppm (1.91 ± 0.02mg MDA/Kg of fat in the fish sample). T3 and T4 samples also showed a slow rise in malonaldehyde content to an average value of 1.74 ± 0.09mg MDA/Kg of fat in fillets at the end of the storage period of 8 days. This finding showed that T3 and T4 compared to T2 and T4 were effective in delaying or inhibiting the increase of TBA levels of mackerel fillets during ice storage.

According to [39] TBA value of the control has increased and reached to 3.99mg MDA/Kg of sample after 6 days of storage while TBA value of sample stored on frozen green tea extract (GTE) were 2.98, 1.72 and 1.167mg MDA/Kg of sample for 2, 4, 6% GTE treatment after 10, 14 and 14 days of storage respectively. Therefore, the application of green tea extracts in the form of dip treatment has an important antioxidant effect.

3.9 Changes in Free fatty acid (FFA)

During storage, fats may be hydrolyzed by microorganisms with the liberation of FFA. The FFA is the effects of enzymatic decomposition (lipases and phospholipases) of lipids in fish and fishery products [4]. The acceptability limit of free fatty acid is 10% of oleic acid. Changes in the FFA content of different samples stored in ice temperatures throughout the storage period are presented in Table 9 and Figure 8 as shown in appendix.

In the present study, the average initial content of FFA in T3 and T4 stored in ice was found to be 0.49 ± 0.00 and $0.20 \pm 0.00\%$ of oleic acid respectively. The FFA content of both T3 and T4 showed a marked increase in its value with an increase of storage period. The initial values of FFA for T1 and T2 have increased from 0.99 and 0.61% of oleic acid to 7.87 and 6.60% of oleic acid on the 6th day of storage in ice. The FFA content of both T1 and T2 showed faster hydrolysis than fillets treated with extracts of green tea (T3 and T4). Compared to T1, the other values were significant ($p < 0.05$) and the green tea extracts (T3 and T4) have shown their effectiveness in reducing FFA formation.

4. CONCLUSION

The results have proved that crude green tea extracts at 2000ppm (with water extract and ethanol extract) can be used as a safe natural antioxidant in place of synthetic antioxidants. The green tea extracts contain compounds that have even controlled the growth of microorganisms better than standard ascorbic acid use at 500ppm. All the green tea samples were well accepted by the consumers.

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APPENDIX

List of tables

Table 1. Changes in organoleptic sensory evaluation of mackerel fillet for control and treated samples stored in ice

Storage days	Treatment	Colour	Odour	Taste	Texture	General appearance	Overall acceptability
0	T1	9.25 ± 0.70 ^a	9.00 ± 0.70 ^a	9.30 ± 0.70 ^a	9.00 ± 0.00 ^a	9.50 ± 0.70 ^a	9.46 ± 0.05 ^a
	T2	9.00 ± 0.00 ^a	9.20 ± 0.70 ^a	8.50 ± 0.70 ^a	9.00 ± 0.00 ^a	9.40 ± 0.70 ^a	9.40 ± 0.17 ^b
	T3	9.50 ± 0.00 ^b	9.00 ± 0.00 ^{ac}	9.25 ± 0.35 ^b	9.50 ± 0.00 ^b	9.00 ± 0.00 ^b	9.86 ± 0.11 ^{cd}
	T4	9.50 ± 0.35 ^b	9.25 ± 0.35 ^b	9.25 ± 0.35 ^c	9.25 ± 0.35 ^b	9.00 ± 0.00 ^b	9.93 ± 0.11 ^{cd}
2	T1	7.60 ± 0.41 ^a	8.10 ± 0.22 ^a	7.70 ± 0.24 ^a	7.40 ± 0.41 ^a	7.60 ± 0.22 ^a	8.50 ± 0.20 ^a
	T2	7.50 ± 0.50 ^a	7.50 ± 0.50 ^a	7.90 ± 0.22 ^a	7.40 ± 0.41 ^a	7.60 ± 0.54 ^a	8.86 ± 0.15 ^b
	T3	8.40 ± 0.41 ^b	7.70 ± 0.44 ^{ac}	7.92 ± 0.63 ^b	8.20 ± 0.08 ^b	8.06 ± 0.13 ^b	9.10 ± 0.10 ^{cd}
	T4	8.64 ± 0.41 ^b	8.28 ± 0.27 ^b	8.30 ± 0.27 ^c	8.18 ± 0.24 ^b	8.24 ± 0.32 ^b	9.20 ± 0.17 ^{cd}
4	T1	7.30 ± 0.83 ^a	8.00 ± 0.00 ^a	7.50 ± 0.50 ^a	7.24 ± 0.33 ^a	7.50 ± 0.35 ^a	7.53 ± 0.08 ^a
	T2	7.20 ± 0.44 ^a	7.40 ± 0.54 ^a	7.80 ± 0.44 ^a	7.20 ± 0.44 ^a	7.40 ± 0.54 ^a	8.10 ± 0.10 ^b
	T3	8.10 ± 0.22 ^b	7.60 ± 0.54 ^{ac}	7.80 ± 0.75 ^b	7.80 ± 0.44 ^b	7.80 ± 0.44 ^b	8.13 ± 0.11 ^{cd}
	T4	8.30 ± 0.44 ^b	8.10 ± 0.22 ^b	8.20 ± 0.61 ^c	8.00 ± 0.00 ^b	8.00 ± 0.00 ^b	8.33 ± 0.28 ^{cd}
6	T1	7.00 ± 0.00 ^a	7.20 ± 0.27 ^a	7.50 ± 0.00 ^a	7.10 ± 0.22 ^a	7.20 ± 0.27 ^a	6.53 ± 0.15 ^a
	T2	7.20 ± 0.44 ^a	7.00 ± 0.00 ^a	7.40 ± 0.54 ^a	7.10 ± 0.22 ^a	7.26 ± 0.43 ^a	7.00 ± 0.10 ^b
	T3	7.80 ± 0.44 ^b	7.50 ± 0.50 ^{ac}	7.70 ± 0.67 ^b	7.60 ± 0.54 ^b	7.80 ± 0.44 ^b	7.33 ± 0.15 ^{cd}
	T4	8.00 ± 0.00 ^b	8.00 ± 0.00 ^b	7.80 ± 0.44 ^c	7.80 ± 0.44 ^b	7.70 ± 0.44 ^b	7.46 ± 0.45 ^{cd}
8	T1	6.60 ± 0.41 ^a	6.00 ± 0.41 ^a	6.50 ± 0.50 ^a	6.60 ± 0.54 ^a	6.40 ± 0.54 ^a	6.10 ± 0.10 ^a
	T2	6.80 ± 0.44 ^a	6.70 ± 0.44 ^a	6.90 ± 0.22 ^a	7.00 ± 0.35 ^a	7.00 ± 0.00 ^a	6.66 ± 0.28 ^b
	T3	7.70 ± 0.44 ^b	7.50 ± 0.50 ^{ac}	7.60 ± 0.65 ^b	7.60 ± 0.54 ^b	7.70 ± 0.43 ^b	7.55 ± 0.05 ^{cd}
	T4	7.84 ± 0.23 ^b	7.82 ± 0.24 ^b	7.74 ± 0.43 ^c	7.70 ± 0.44 ^b	7.76 ± 0.43 ^b	7.60 ± 0.00 ^{cd}

The values are expressed as mean ± standard deviation, **Different superscripts in the same column indicates significant difference (p<0.05)**

Table 2. Changes in TPC of mackerel fillet for control and treated samples stored in ice

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
2	4.15 ± 0.21	3.90 ± 0.03	4.02 ± 0.29	3.73 ± 0.05
4	4.69 ± 0.06	4.28 ± 0.15	3.82 ± 0.06	3.96 ± 0.01
6	5.82 ± 0.02	5.81 ± 0.04	3.92 ± 0.03	3.98 ± 0.02
8	6.17 ± 0.17	5.93 ± 0.08	4.25 ± 0.11	4.30 ± 0.06

Table 3. Changes in pH of mackerel fillet for control and treated samples stored in ice

Days	pH value			
	T1	T2	T3	T4
0	6.80 ± 0.02	6.49 ± 0.04	6.71 ± 0.05	6.78 ± 0.04
2	6.61 ± 0.02	6.89 ± 0.01	6.65 ± 0.01	6.61 ± 0.03
4	6.83 ± 0.01	6.54 ± 0.02	6.52 ± 0.03	6.67 ± 0.02
6	6.90 ± 0.01	6.87 ± 0.02	6.80 ± 0.02	6.80 ± 0.01
8	7.09 ± 0.13	6.92 ± 0.05	6.81 ± 0.06	6.84 ± 0.02

Table 4. Changes in TVB-N of mackerel fillet for control and treated samples stored in ice

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
2	11.37 ± 1.23	10.50 ± 0.00	6.30 ± 0.99	5.25 ± 0.00
4	19.25 ± 2.47	14.87 ± 1.23	9.62 ± 1.23	7.87 ± 1.23
6	24.50 ± 0.00	19.25 ± 0.00	14.87 ± 1.23	14.00 ± 0.00
8	28.87 ± 1.23	25.37 ± 1.23	20.47 ± 0.74	20.30 ± 0.00

Table 5. Changes in TMA-N of mackerel fillet for control and treated samples stored in ice

Days	TMA-N (mg %)			
	T1	T2	T3	T4
0	1.75 ± 0.00	0.87 ± 0.24	0.70 ± 0.00	0.52 ± 0.24
2	2.97 ± 0.24	1.57 ± 0.24	1.40 ± 0.49	1.22 ± 0.24
4	3.32 ± 0.74	2.45 ± 0.49	1.57 ± 0.24	1.22 ± 0.24
6	5.07 ± 0.24	4.72 ± 0.74	2.97 ± 0.24	2.45 ± 0.99
8	5.42 ± 0.24	5.25 ± 0.00	3.50 ± 0.00	3.85 ± 0.49

Table 6. Changes in AA-N of mackerel fillet for control and treated samples stored in ice

Days	AA-N (mg %)			
	T1	T2	T3	T4
0	29.63 ± 0.33	30.80 ± 2.64	34.06 ± 0.66	34.53 ± 0.00
2	23.80 ± 0.66	26.60 ± 1.98	28.46 ± 0.66	28.93 ± 1.32
4	18.31 ± 0.55	20.65 ± 0.55	22.60 ± 0.00	22.21 ± 1.65
6	16.75 ± 0.55	17.53 ± 0.55	19.87 ± 0.55	19.09 ± 1.65
8	14.80 ± 1.10	15.19 ± 0.55	17.92 ± 0.00	17.53 ± 0.55

Table 7. Changes in PV of mackerel fillet for control and treated samples stored in ice

Days	PV (mEq of O ₂ /Kg)			
	T1	T2	T3	T4
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
2	4.25 ± 0.35	2.50 ± 0.70	1.75 ± 0.35	1.50 ± 0.70
4	5.80 ± 0.70	3.80 ± 0.70	2.80 ± 0.70	2.15 ± 0.21
6	6.50 ± 0.70	4.50 ± 0.70	3.50 ± 0.70	2.50 ± 0.70
8	11.50 ± 0.70	7.85 ± 0.21	6.50 ± 0.70	5.50 ± 0.70

Table 8. Changes in TBARs of mackerel fillet for control and treated samples stored in ice

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
2	1.10 ± 0.01	1.02 ± 0.09	0.84 ± 0.02	0.86 ± 0.02
4	1.22 ± 0.07	1.20 ± 0.10	0.86 ± 0.11	0.88 ± 0.02
6	1.38 ± 0.04	1.31 ± 0.19	1.11 ± 0.05	1.01 ± 0.05
8	2.01 ± 0.03	1.91 ± 0.02	1.77 ± 0.08	1.70 ± 0.09

Table 9. Changes in FFA of mackerel fillet for control and treated samples stored in ice

Days	FFA (% of oleic acid)			
	T1	T2	T3	T4
0	0.99 ± 0.00	0.61 ± 0.06	0.49 ± 0.00	0.20 ± 0.00
2	2.00 ± 0.00	1.62 ± 0.00	1.16 ± 0.06	0.95 ± 0.15
4	3.21 ± 0.41	2.19 ± 0.00	1.30 ± 0.20	1.11 ± 0.24
6	7.87 ± 0.58	6.60 ± 1.03	4.06 ± 0.03	3.82 ± 0.07
8	10.14 ± 0.35	9.83 ± 0.00	8.13 ± 0.00	7.05 ± 0.90

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Figure 1. Changes in TPC of mackerel fillet for control and treated samples stored in ice

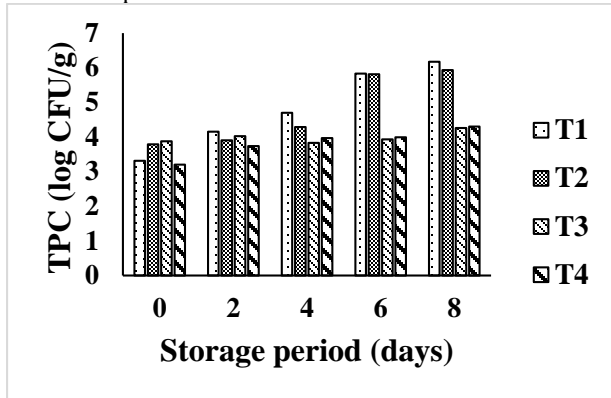


Figure 2. Changes in pH of mackerel fillet for control and treated samples stored in ice

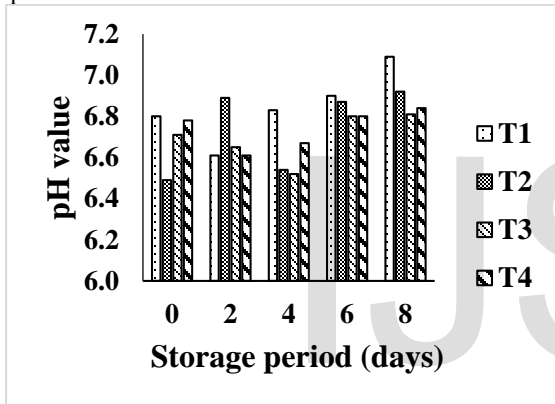


Figure 3. Changes in TVB-N of mackerel fillet for control and treated samples stored in ice

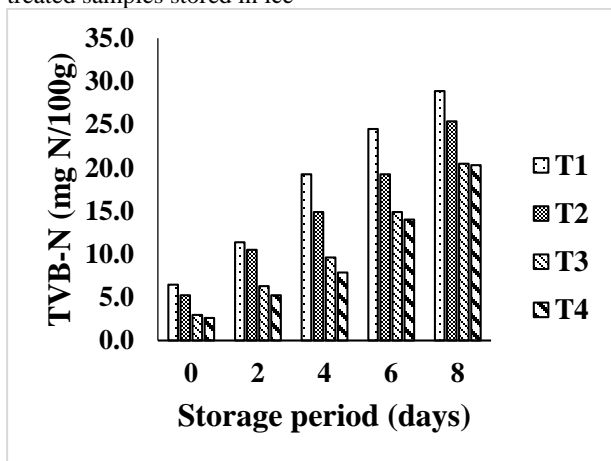


Figure 4. Changes in TMA-N of mackerel fillet for control and treated samples stored in ice

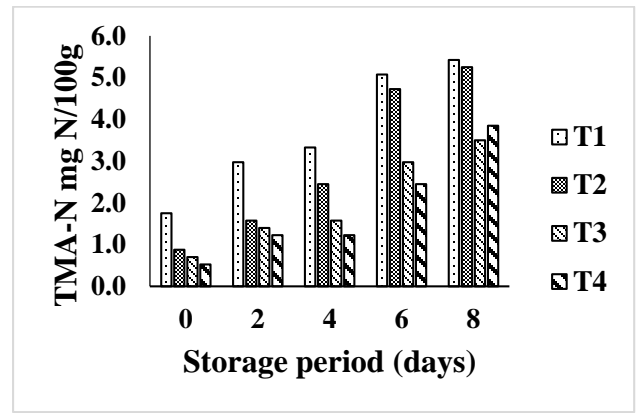


Figure 5. Changes in AA-N of mackerel fillet for control and treated samples stored in ice

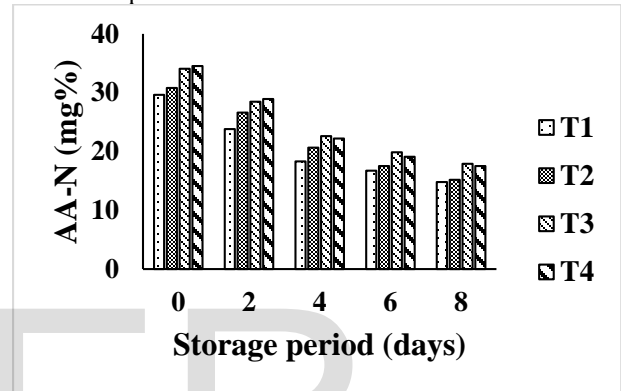


Figure 6. Changes in PV of mackerel fillet for control and treated samples stored in ice

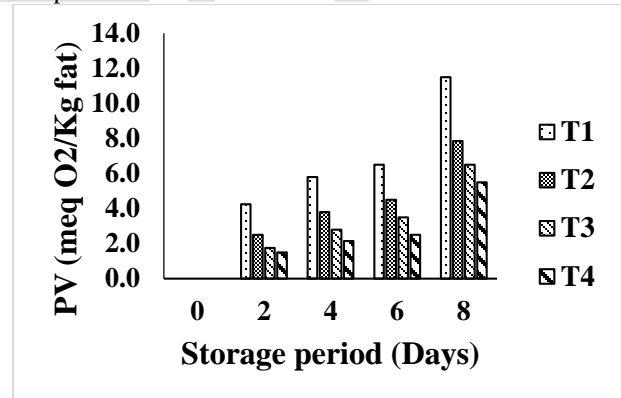
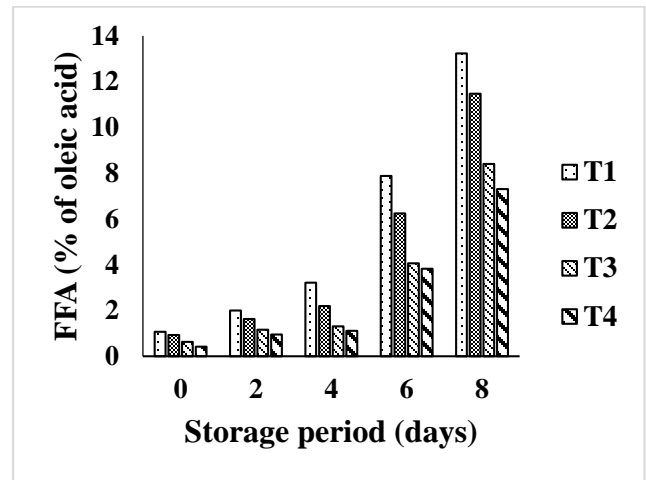
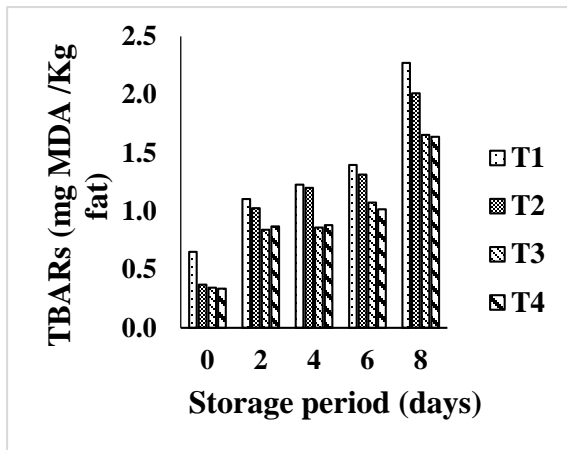


Figure 7. Changes in TBARs of mackerel fillet for control and treated samples stored in ice



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Figure 8. Changes in FFA of mackerel fillet for control and treated samples stored in ice