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Effect of Edible Coating Prepared from Aloe Vera Incorporated with Lemongrass Extract on the Antioxidant Properties of the Nile Tilapia Fish Fillets Under Refrigerated Storage

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ABSTRACT

The current investigation was set out to create an edible coat prepared from *Aloe vera* incorporated with ethanolic lemongrass extract (LGE) for improved lipid oxidative and antioxidant properties of refrigerated tilapia fish fillet LGE was characterized by measuring its total antioxidant capacity (TAC), total phenolic content (TP), 2,2-diphenyl-1-picrylhydrazyl (DPPH) minimum inhibition concentration of 50% (IC_{50}), total flavonoids content (TF), and ferric reducing power. LGE recorded 108380 mgAAE/kg for TAC, 76000 mgGAE/kg for TP, and 39705 mgQE/kg for TF. The IC_{50} of LGE was calculated as 0.044 mg/ml. As the base coat, *Aloe vera* gel was employed and two distinct coats were produced using LGE viz. 1000ppm and 2000ppm. The addition of LGE increased the antioxidant properties (TAC, DPPH radical scavenging activity, and TP). The highest scores were observed for *Aoe vera* incorporated with 2000 ppm LGE edible coats for all analyses attributes. The prepared edible coats were applied to Nile tilapia fish fillets. Four groups of fish samples were taken and consisted of (fish fillets only (control (-)), fish fillet and BHT antioxidant (control (+)), T1 (fish fillets and *Aloe vera* + 1000ppm LGE), and T2 (fish fillets and *Aloe vera* + 2000ppm LGE). The evaluation of antioxidant properties and TBRAS lipid oxidation of fish samples were investigated in 4-time intervals (1, 3, 6, and 9) days. The addition of T1 and T2 enhanced the fish fillets antioxidant capacity and storage quality. T2 fish fillets showed the significantly lowest mean values for TBARS (0.299mg MDA/kg), compared to other groups during storage. Also, it was detected the outperforming of T2 on control (+) indicating the advantage of using natural antioxidants instead of synthetic BHT preservatives. According to the study, natural, low-cost edible coatings might dramatically minimize fish waste in manufacturing processes.

INTRODUCTION

In recent years, a growing interest has emerged in finding natural methods to extend the shelf life and maintain the quality of various food products. One of the prominent techniques being explored is the use of edible coatings, which are thin films made from natural ingredients that can be applied to food surfaces. These coatings create a barrier between the food and its surroundings, thus protecting it from deterioration

caused by microbial growth, oxidation, and moisture loss (**Şimşek *et al.*, 2021; Anwar *et al.*, 2022).**

The Nile tilapia (*Oreochromis niloticus*) is one of the most commercially and economically important fish species worldwide **(Munguti *et al.*, 2022)**. However, due to its high perishability, maintaining its quality and freshness during storage remains a challenge. Therefore, there is a need to investigate novel ways to enhance the shelf life of the Nile tilapia fillets, without compromising its nutritional and sensory attributes.

Aloe vera and lemongrass (LG) are natural ingredients that have gained significant attention in recent years owing to their potential health benefits and antioxidant properties. Aloe vera is a succulent plant known for its gel-like substance containing bioactive compounds such as polysaccharides, phenolic, and flavonoids **(Birtane & Beyler Çiğil, 2022).** It was recorded as colorless, odorless, and not affecting the taste of the food, moreover, it was assesed as safe for the human health and ecofriendly **(Hassan *et al.*, 2022)**. Lemongrass, on the other hand, is a widely used herb known for its aromatic and medicinal properties, which are attributed to its high content of essential oils, phenolic, and terpenes **(Azizah *et al.*, 2023)**. It has been used in food as food additives and preservative. In addition, it is widely used in edible coating for different types of food **(Iftikhar *et al.*, 2022)**.

In a previous work of **Alkaabi *et al.* (2022)**, it was proved that, adding a mixture of *Aloe vera* and LG as an edible coating increased the shelf-life of some fruits. Incorporating Aloe vera and LGE into an edible coating could potentially provide a synergistic effect on the antioxidant properties of the Nile tilapia fish fillets. Antioxidants are substances that can scavenge or neutralize free radicals, preventing oxidative stress and lipid oxidation, which are common causes of food spoilage. Thus, by enhancing the antioxidant capacity of the edible coating, it may be possible to increase the shelf life and maintain the quality of the Nile tilapia fillets during refrigerated storage.

The objective of this study was to investigate the effect of an edible coating prepared from Aloe vera incorporated with LGE on the antioxidant properties of the Nile tilapia fish fillets under refrigerated storage conditions. Specifically, the changes in antioxidant activity and lipid oxidation of the coated fish fillets were evaluated compared to the control samples without the coating. Understanding the impact of this edible coating on the Nile tilapia fillets' antioxidant properties would provide valuable insights into the potential of natural ingredients to extend the shelf life of this fish species. This research contributes to the development of sustainable and natural preservation methods for the seafood industry, aligning with the growing consumer demand for safe and minimally processed food products.

MATERIALS AND METHODS

1- Materials

Lemongrass and Aloe vera were purchased from herbal store in Giza, Egypt. Aloe vera was obtained in fresh form, while lemongrass was obtained in dried form. Fish fillets from the Nile tilapia fishes were purchased fresh from local fish market and were directly transferred to the laboratory to be used.

All chemicals and reagents used were of an analytical grade and were obtained from Sigma-Aldrich.

2- Methods

A schematic diagram of the steps of work in this study is presented in Fig. (1)

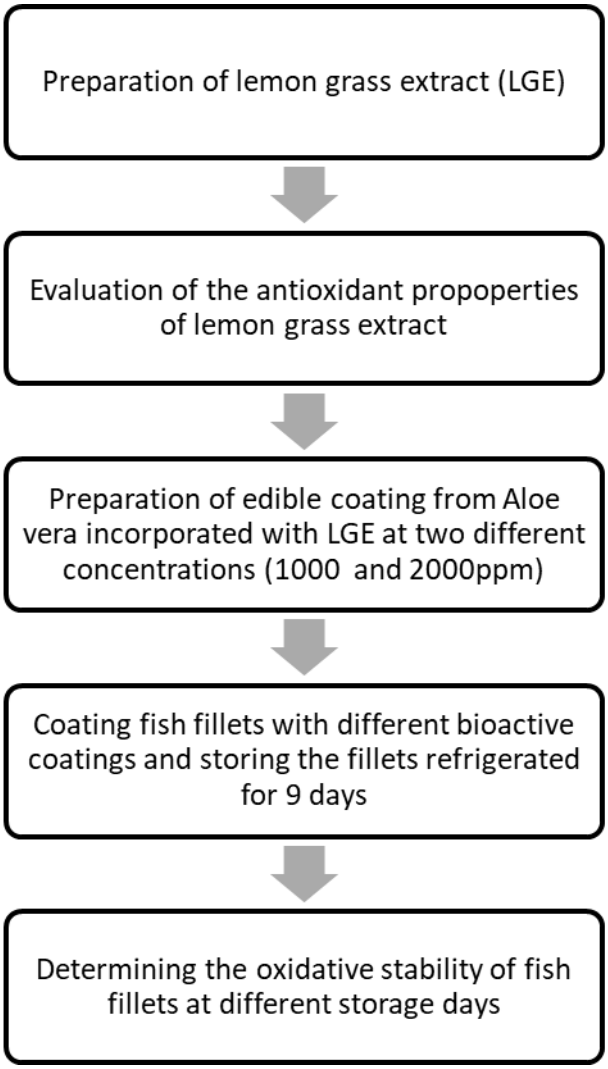

Fig. 1. Work scheme of method of preparation and analyses of studied samples

a- Preparation of lemongrass extract (LGE)

Fifty grams of lemongrass were soaked in 250ml of ethanol (70%). The mixture was stirred then subjected to sonication for one hour at room temperature. The solution obtained after filtering the mixture was dried to remove the solvent using a rotary evaporator, and the resulting extract was collected in a falcon tube that was tightly closed and kept in the freezer until used.

b- Determination of total antioxidant capacity of LGE

The phosphomolybdenum technique **(Prieto *et al.*, 1999)** was used to calculate the total antioxidant capacity (TAC) of different samples. The ascorbic acid reference antioxidant material was used to build a standard curve from which the results were calculated. The results were represented as mg ascorbic acid equivalent/kg of sample (mg AAE/Kg).

c- Determination of total phenolic contents of LGE

The Folin–Ciocalteu technique **(Turkmen *et al.*, 2006)** was used to calculate the samples' total phenol concentration (TPC). A calibration curve for gallic acid was created, and the results were represented as mg of gallic acid equivalents per kg of sample (mgGAE/Kg) based on the calibration curve's regression equation.

d- Determination of total flavonoids of LGE

Utilizing quercetin as a standard reference material, the total flavonoid content (TF) of the samples was determined using the aluminium chloride assay **(Mohdaly *et al.*, 2010)**. The results were expressed as mg of quercetin equivalent/Kg of sample (mgQE/Kg).

e- DPPH inhibitory activity of LGE

The free radical inhibitory or scavenging activity of LGE was determined using the DPPH assay, which was generally used for natural herbs and reported by the method of **Williams *et al.* (1995)**. Briefly, several dilutions of LGE (0.05; 0.075; 0.15 and 0.3 mg/ml) were prepared using methanol. A test tube containing each diluted sample was combined with 2 milliliters of 200mg/ L DPPH and left at room temperature for 20 minutes in the dark. Measurements of absorbance against methanol were made at 517nm using a spectrophotometer **(Wu *et al.*, 2022)**.

The IC_{50} value (concentration of sample required to scavenge 50% of the DPPH free radical) was calculated from the curve of percentage of inhibition plotted against the respective sample concentration.

f- Ferric reducing power of LGE

One milliliter of each sample solution (0.15, 0.3, 0.6, and 0.9 mg/ml) was combined with phosphate buffer (2.5 mL, 0.2 mol/L, pH 6.6) to find the reducing power of LGE. Next, add 2.5 mL of potassium ferric cyanide (10 g/L). Following a 20-minute incubation period at 50°C, 2.5 mL of 10% tri-chloro acetic acid was added, and the mixture was

centrifuged for 10 minutes at 1207 XG using a Heraeus megafuge8, Thermo scientific rpm. The absorbance was measured at 700nm against a blank (methanol) after the upper layer of solution (2.5 mL) was combined with distilled water (2.5 mL) and ferric chloride (0.5mL, 1g/ L) **(El-Chaghaby *et al.*, 2014)**. A growing reducing power was indicated by the reaction mixture's increasing absorbance.

g- Preparation of edible coatings and assessment of their antioxidant properties

Aloe vera was extracted and incorporated with two different concentrations of LGE (1000 and 2000ppm). The antioxidant properties of the prepared coatings that were investigated included TAC, TP and DPPH (IC_{50}) according to the same methods described above.

h- Determining the oxidative stability of fish fillets at different storage days

Four different groups were tested; these included fish fillets only (control (-), fish fillets with BHT (control (+)), fish fillets coated with Aloe vera +1000ppm of LGE (T1) and fish fillets coated with Aloe vera +2000ppm of LGE (T2).

i- Determination of the total antioxidant properties of fish fillets after applying the edible coatings

In order to determine the effect of refrigerated storage on the antioxidant properties of the coatings, fish fillets were analyzed over different storage days (days 1, 3, 6, and 9).

The total antioxidant capacity, TP and the DPPH inhibition test of the fish fillets samples were determined. This test was frequently used to evaluate natural products' ability to scavenge free radicals, and it was thought to be a model compound for free radicals that originate in lipids found in food.

j- Test of thiobarbituric acid reacting substances (TBARS) of fish fillets

The TBARS test was a rapid and simple method described by **Castro-enr *et al*. (2023)** for determining the extent that a fat has degraded to non-metabolizable aldehydes, such as malondialdehyde (MDA). As fat content increases, the peroxide number or value in the TBARS test increases **(Elsherief *et al.*, 2019)**. Samples of fish fillets were weighed, and a solution of 2 M orthophosphoric acid was combined with 25ml of 20% trichloroacetic acid. After adding 25 milliliters of distilled water, the liquid was filtered. In test tubes, a predetermined amount of the filtrate was combined with 3ml of 2-TBA reagent (0.005 M), and the mixture was kept in the dark for 16 hours. At a constant wave length of 532nm, the absorbance was measured, and the results were given in mg MDA/kg.

3- Statistical analysis

All analysis were done in three replicates, and the mean data were presented. The data were statistically analyzed using one-way ANOVA test at a significance level ($P<0.05$). This analysis was performed using IBM SPSS Statistics 20.

RESULTS AND DISCUSSION

Antioxidant properties of lemongrass extracts

Table (1) presents the results of the antioxidant properties of the LGE. The total antioxidant capacity of LGE was 108380mgAAE/ kg. The total phenolic content was 76000mgGAE/ kg, and the total flavonoids content was 39705mgQE/ kg. Previous work carried out by **Kim *et al.* (2022)** assessed that lemongrass is a promising natural source of easily accessible, low-cost antioxidant compounds and the total phenolic and flavonoid concentrations in lemongrass ethyl acetate extract were 132.31 mg caffeic acid equivalent/g and 104.50mg naringin equivalent/ g, respectively,**() ()**.

Table 1. Antioxidant properties of LGE

Lemongrass extract		
Total antioxidant capacity mgAAE/ kg	**Total phenolic content mgGAE/Kg**	**Total flavonoids mgQE/Kg**
108380±577.3503	76000±513.8174	39705±504.7772

Data are given as mean values ±standards deviation.

DPPH scavenging activity of LGE

The DPPH scavenging activity of LGE was evaluated by using different concentrations (0.05; 0.075; 0.15 and 0.3 mg/ml). The inhibition percentages of DPPH increased with the increase in LGE concentration (Fig. 2). The IC_{50} of LGE was calculated as 0.044mg/ ml, indicating its strong antioxidant activity. Recently, lemongrass extracts were reported to be an antioxidant as determined by the DPPH scavenging test **(Kim *et al.*, 2022)**. Previous work done assessed that IC_{50} of LG methanolic extracts was 26µM **(Aboagye *et al.*, 2021)**, while IC_{50} of LG was 80.63mg/ ml in hot water extracts **(Muala *et al.*, 2021)**.

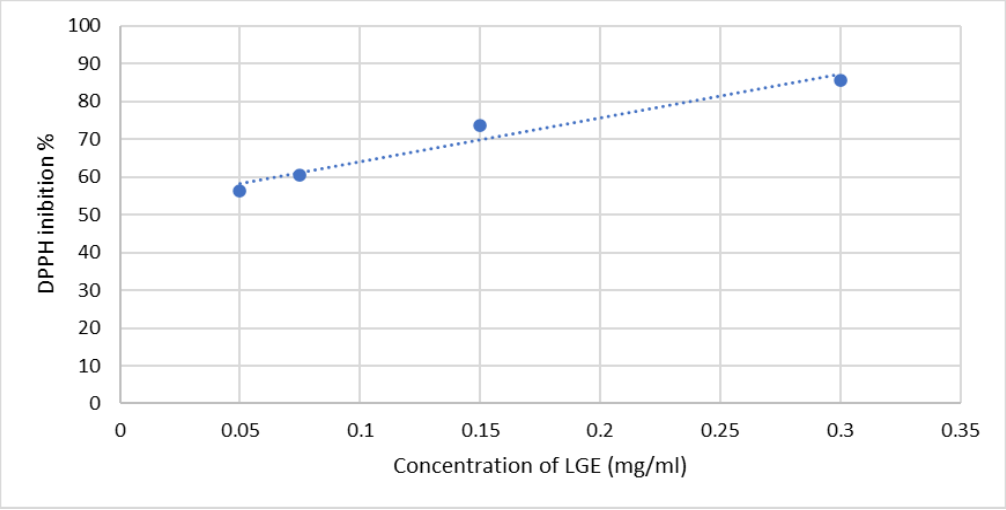

Fig. 2. DPPH inhibition % of LGE

Ferric reducing power of LGE

The ferric reducing power of LGE was also determined using different concentrations (0.15; 0.3; 0.6 and 0.9mg/ ml). The reducing power increased with the increase in extract concentration (Fig. 3), indicating the ability of LGE to donate electrons and act as a reducing agent. An aqueous ethanol extract of lemongrass was previously reported to decrease reactive oxygen species production and lipid peroxidation **(Aboagye *et al.*, 2021).**

As mentioned above, the depicted results of LGE declared that the phenolic and flavonoid contents of LGE are correlated to the total antioxidant capacity, DPPH scavenging, and ferric reducing power activity **(Kim *et al.*, 2022)**.

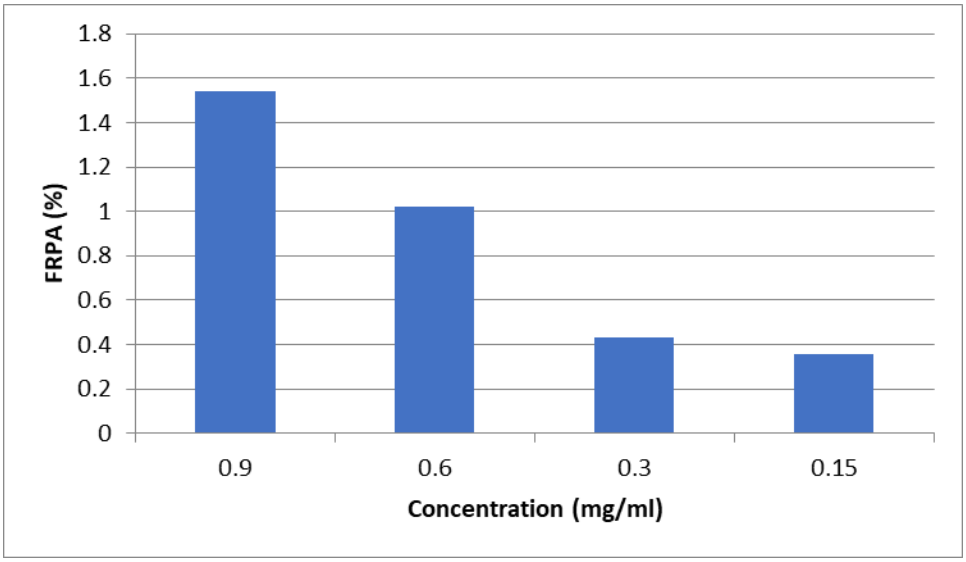

Fig. 3. Ferric reducing power of LGE

Antioxidant properties of the prepared coatings

Next, the antioxidant properties of the prepared coatings were evaluated. Three different treatments were applied: Aloe vera alone and Aloe vera with two different concentrations of LGE 1000ppm (T1) and 2000ppm for (T2), respectively, were addressed. TAC, DPPH IC_{50}, and TP were determined for each coating (Table 2). The results showed that the addition of LGE to the Aloe vera coating increased significantly (P<0.05) the antioxidant properties of the prepared coating. From the experimental values displayed in Table (2), a positive correlation could be observed among TAC, DPPH IC_{50} value, and TP results. The TAC was at its highest value in T2 coating (1680.98 ppm), followed by T1 coating (982.58 ppm) and Aloe vera (509.75 ppm).

The DPPH IC_{50} value, which indicates the concentration of the coating required to scavenge 50% of DPPH radicals, was significantly (P<0.05) the lowest in T2 coating (58.12 mg/ml). DPPH IC_{50} values of prepared coats were ascending as T2, T1 (66.69

mg/ml) and Aloe vera (181.80 mg/ml). The highest (TP) was detected in T2 coating (1098mgGAE/ Kg), followed by T1 (813 mgGAE/Kg) and Aloe vera (456.9mgGAE/ Kg). Many studies previously published assessed the antioxidant activity of Aloe vera edible coats like the study of **Gel *et al*. (2020)** who proved that Aloe vera gel had 201mgGAE/ Kg TP content and 0.73mg/ l of DPPH IC_{50} value.

As a whole, it was remarkable that an augmentation of the bioactive compounds from LGE caused a significant (P<0.05) increase in the antioxidant properties of the prepared edible coatings. These results are attributed to the effectiveness of LGE as a rich source of bioactive phytochemicals, such as essential oils, phenolics, and terpenes, which enhanced the antioxidant properties of the prepared edible coats **(Hassan *et al.*, 2022)**.

Table 2. Antioxidant properties of prepared coatings

Prepared coatings	**TAA mgAAE/ kg**	**DPPH IC_{50} (mg/ml)**	**TP mgGAE/Kg**
Aloe vera	509.75^{c}±3.16	181.80^{a}±1.51	456.9^{c}±3.00
Aloe vera **+ 1000 ppm Lemongrass**	982.58^{b}±2.50	66.69^{b}±2.01	813^{b}±5.13
Aloe vera **+ 2000 ppm Lemongrass**	1680.98^{a}±20.22	58.12^{c}±1.01	1098^{a}±17.50

Data are given as mean values ±standards deviation. Letters in the superscript (a,b,c) signify statistical differences (P<0.05) within same column.

Effect of coating and storage time on the antioxidant properties fish fillets

In order to determine the effect of the prepared edible coatings on the antioxidant properties during refrigerated storage, fish fillets were analyzed over different storage days (days 1, 3, 6 and 9). The control group without any additive (control (-)), and the positive control group with BHT (control (+)) were also included for comparison. TAC, TP and the DPPH inhibition of the fish fillets samples were determined, and the results are depicted in Table (3).

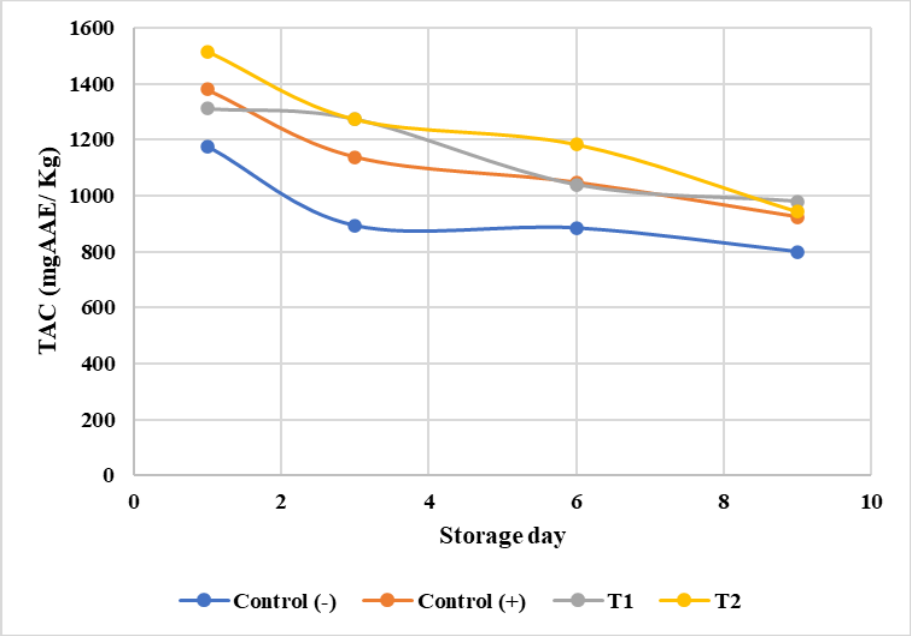

Fig. 4. Total antioxidant capacity of fish over different storage days

From Table (3) and Fig. (4) it was noticed that, there is a significant (P<0.05) decrease in TAC values of each of the treatments as a general trend from the beginning to the end of the storage time. These results could be attributed to the damage mechanism like lipid oxidation, which happened in fish tissue during storage **(Castro-enr *et al.*, 2023)**. The results also revealed that control (-) had a TAC (1175.87mgAAE/Kg) at the beginning of the storage time. The fish antioxidant properties came from its rich content of unsaturated fatty acids and vitamin E **(Kheiri *et al.*, 2022)**; however, there was a remarkable significant (P<0.05) decrease in TAC at the ninth day (799.5mgAAE/ Kg). Additionally, significant differences (P<0.05) were recorded for TAC among the control (+), T1 and T2 treatments, with T2 having the highest value for TAC, followed by the control (+) and T1.

TP analyses are graphically displayed in Fig. (5). It appeared that, at the beginning of the storage, all tested samples contained high TP values. The lowest sample in TP content (238.04 mgGAE/Kg) was the control (-). Samples are arranged in an ascending order as follows: control (-), control (+), T1 and T2. The highest values were T2 during any time of storage. During the nine days of storage, a depression was detected in TP content for all tested samples.

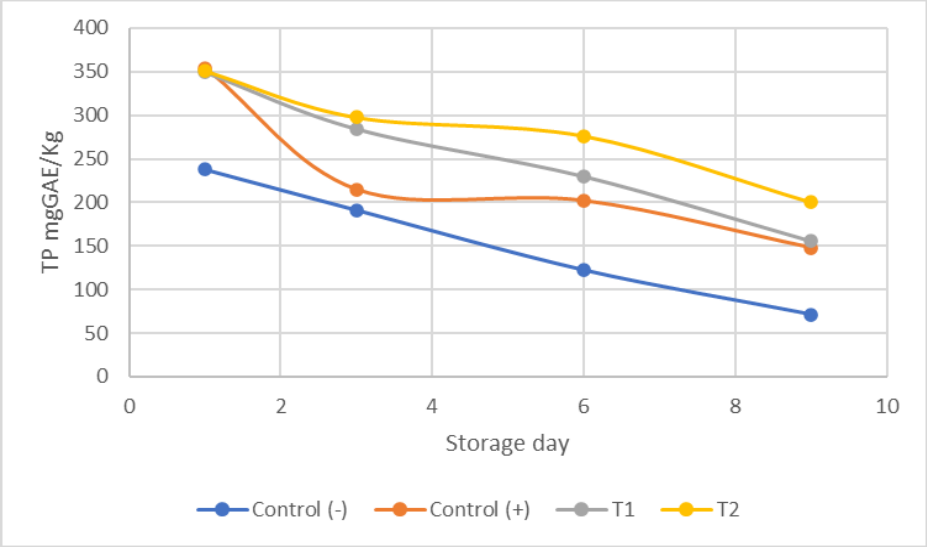

Fig. 5. Total phenolic contents of fish over different storage days

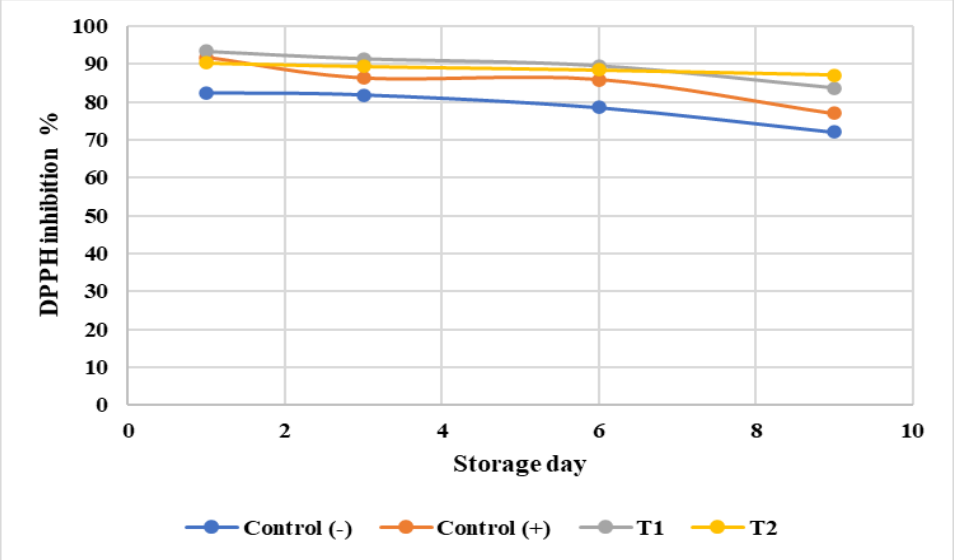

Fig. 6. DPPH inhibition for fish fillet samples (concentration of 200mg/ml extracted in ethanol; DPPH 0.4mg/ml) over different storage days

Table 3. TAC, TP, DPPH and TBARS for fish fillets samples under different treatments over storage days

Storage day / Treatment	Day 1	Day 3	Day 6	Day 9
TAC (mg AAE/Kg)				
Control	$1175.9^{d,A}$ ±20.61	$892.87^{c,B}$ ±2.06	$884.12^{c,B}$ ±5.03	$799.5^{d,C}$ ±10.00
BHT	$1380.4^{b,A}$ ±20.00	$1138.8^{b,B}$ ±1.81	$1047.5^{b,C}$ ±1.32	$923.12^{c,D}$ ±10.06
T1	$1312.5^{c,A}$ ±3.97	$1275.3^{a,B}$ ±5.00	$1040^{b,C}$ ±9.02	$943.75^{b,D}$ ±5.05
T2	$1514.9^{a,A}$ ±2.93	$1272.8^{a,B}$ ±2.50	$1183.4^{a,C}$ ±1.50	$980^{a,D}$ ±10.11
TP (mg GAE/Kg)				
Control	$238.04^{c,A}$ ±1.02	$191.12^{d,B}$ ±1.022	$122.17^{c,C}$ ±1.040	$71.12^{d,D}$ ±0.82
BHT	$354.00^{a,A}$ ±1.04	$214.13^{c,B}$ ±0.82	$200.87^{b,C}$ ±1.51	$146.87^{c,D}$ ±0.66
T1	$349.17^{b,A}$ ±1.02	$284.17^{b,B}$ ±1.06	$221.00^{a,C}$ ±1.02	$155.27^{b,D}$ ±0.37
T2	$350.45^{b,A}$ ±16.46	$297.35^{a,B}$ ±1.04	$230.00^{a,C}$ ±0.37	$199.75^{a,D}$ ±0.46
DPPH (%)				
Control	$82.44^{c,A}$ ±1.50	$81.85^{c,AB}$ ±3.01	$78.50^{c,B}$ ±1.02	$72.00^{d,C}$ ±0.51
BHT	$91.65^{ab,A}$ ±3.00	$86.30^{b,B}$ ±3.74	$85.89^{b,B}$ ±2.01	$77.00^{c,C}$ ±2.00
T1	$90.36^{b,A}$ ±0.52	$91.41^{a,AB}$ ±1.51	$89.55^{a,B}$ ±1.50	$83.59^{b,C}$ ±1.52
T2	$93.38^{a,A}$ ±0.51	$89.41^{ab,A}$ ±1.00	$88.40^{a,AB}$ ±1.51	$87.04^{ab,B}$ ±0.52
TBARS (mg MDA/Kg)				
Control	$0.956^{b,D}$ ±0.025	$2.14^{a,C}$ ±0.203	$2.501^{a,B}$ ±0.096	$3.447^{a,A}$ ±0.250
BHT	$1.775^{a,C}$ ±0.050	$1.961^{b,C}$ ±0.020	$2.218^{b,B}$ ±0.155	$2.505^{b,A}$ ±0.190
T1	$0.120^{c,D}$ ±0.025	$0.563^{c,C}$ ±0.010	$1.296^{c,B}$ ±0.012	$1.706^{c,A}$ ±0.045
T2	$0.009^{d,D}$ ±0.001	$0.094^{d,C}$ ±0.003	$0.133^{d,B}$ ±0.008	$0.299^{d,A}$ ±0.006

All data are presented as mean value ± standard deviation. Small letters (a,b,c,d) in the superscript signifies statistical differences between values in the same column. Capital letters (A,B,C,D) signifies statistical differences between values in the same row. Statistical differences are computed at $P<0.05$.

The DPPH % values were computed for different samples during the storage as given in Fig. (6). At the first day of storage, T2 samples showed the highest DPPH % values in comparison to other samples, followed by T1. The DPPH % values were gradually decreased by increasing the storage time. The incremental pattern in DPPH % values arranged at any time of storage in an ascending order as follows T2, T1, control

(+) and control (-). These different analyses provided an insight view into the changes in antioxidant properties of extracted samples because of the free radicals originating in food lipids during refrigerated storage. The use of antioxidants was very effective in reducing lipid oxidation in fish fillets because of its phenolic coated layer which acted as inhibitors for radical reactions on autoxidation **(Kasprzak & Grzebieniarz, 2023)**.

Lipid stability fish fillets after applying edible coatings

TBARS is a significant quality index for fish containing fat. The larger fish lipids content has larger TBARS value in fish **(Elsherief *et al.*, 2019)**. The TBARS test was conducted to evaluate the lipid oxidation in the fish fillets. The mean values of various lipid stability parameters of coated fish fillet samples with edible film and control samples are presented in Table (3) and Fig. (7). From the displayed data, and by considering the permissible limit of TBARS value in fish and fish products (4.5 mg MDA/kg) recommended by **EOS (2005)**, neither treated samples nor the control group exceeded such limit in any step during the examination. Results showed that the TBARS values of fish fillets coated with T2 had the significantly (P<0.05) lowest mean values during the entire storage time. Moreover, TBARS value of the control (+) was 2.5mg MDA/kg on the ninth day, whereas values of T1 and T2 were 1.706 and 0.299mg MDA/kg, respectively, indicating the outperforming the effect of the tested coats T1 and T2 on the synthetic antioxidant BHT in suppressing lipids peroxidation.

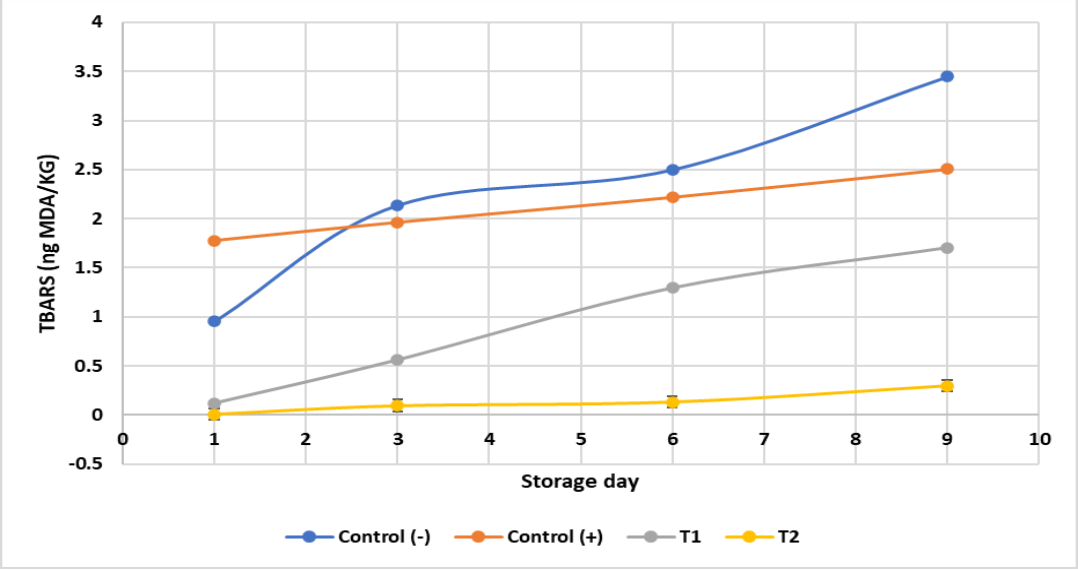

Fig. 7. TBRAS values of fish samples over different storage days

This result could help in avoiding the drawbacks of BHT (synthetic preservative), which caused diseases leading to liver damage cytotoxicity and carcinogenesis (**Netam *et al.*, 2018)**. On the ninth day, the TBARS value of the T2 coated fish fillet samples was 0.299mg MDA/kg.

This reduction in lipid oxidation would be ascribed to a number of phytochemicals found in LGE and *Aloe vera*, including flavonoids, phenolic compounds, and vitamins C and E, which have the ability to directly combat free radicals and stop the chain processes causing lipid oxidation **(Şimşek *et al.*, 2021)**. Therefore, based on these results, *Aloe vera* and LGE+2000ppm coated film helped maintaining the quality of fish fillet during storage for 9 days.

Several works clarified that there was a significant elongation of the food storage period with an assist of using *Aloe vera* and LGE as an edible coating film. This finding has been previously reported **(Alkaabi *et al.*, 2022; Iftikhar *et al.*, 2022; Marzanna & Dziedzic, 2019; Socaciu *et al.*, 2018)**. A recently published research carried out by **Sarnes *et al*. (2020)** assessed that the addition of 2000ppm of LGE as soaking solution showed 7 days extension of storage time. Another work carried out by **Socaciu *et al.*, (2018)** used 1% of LGE on fish fillets with 15 days storage period **()**.

CONCLUSION

Given the high importance of Nile tilapia fish fillets as one of the most popular fish products preferred by consumers, the present study gives an effective solution for preserving fish fillets by a natural edible coating material. The edible coat prepared from *Aloe vera* incorporated with lemongrass extract showed excellent results in delaying the lipid oxidation of fish fillets and enhancing their antioxidant capacity. This suggested that the *Aloe vera* and LGE coating may have potential as a natural antioxidant agent to preserve the quality and extend the shelf life of fish fillets during refrigeration storage. Furthermore, this could fulfill the rising public demand for healthier products that does not contain synthetic additives.

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