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Investigation of the Antimicrobial, Physico-Chemical, Sensory Qualities of Ascorbic Acid and Effect on the Shelf-Life of Hot Smoked Mackerel (*Scomber scombrus*)

Emmanuel Ude^a, Joshua Ekpenyong^b, Stanley Iheanacho^b, Irom Okey^c, and Benjamin Agada^a

^aDepartment of Fisheries and Aquaculture, Ebonyi State University, Abakaliki, Nigeria; ^bDepartment of Fisheries and Aquaculture, Alex Ekwueme Federal University Ndufu Alike, Ikwo, Nigeria; ^cDepartment of Fisheries and Aquatic Science, Cross River University of Technology, Obubra, Nigeria

ABSTRACT

The effectiveness of ascorbic acid in improving the quality and shelf life of processed (smoked) mackerel fillets was investigated. The fish were divided into four ascorbic acid (AA) treatment groups and smoked for 18 hours at a temperature between 120°C and 150°C. After 28 days, the fish were tested for proximate, biochemical, microbiological, and sensory status. Based on the tests conducted, fish treated with 15 mg L⁻¹ AA showed better nutrient composition, biochemical, and sensory qualities, while Enterobacteriaceae spp counts declined with higher AA concentrations. The study revealed that AA improved mackerel fish and extended their shelf life.

KEYWORDS

Natural antioxidants; processed fish; proximate; biochemistry; sensory evaluation

Introduction

Fish is an affordable source of animal protein that provides the body with essential nutrients for growth (Ekonomou et al. 2020; Iheanacho et al. 2018). It is recommended for human intake due to its important amino acid content, which can help to minimize the risk of chronic diseases like stroke, cancer, diabetes, and arthritis (Lund 2013; Noori et al. 2018; Suhem et al. 2022). Due to the high moisture content, availability of nutrients for microbial growth, enzymatic hydrolysis, and lipid oxidation, fish are very perishable, resulting in the rapid development of unpleasant flavor and odor (Ekelemu et al. 2021). This reduces the product's shelf life and makes it unsafe for human consumption, necessitating its preservation (Rathod et al. 2021).

Fish preservation is the process of preventing or reducing the deterioration of fish quality to prolong its shelf life (Ekonomou et al. 2022; Vijayan et al. 2021). From the time of harvest until the time the products are consumed, several preservation procedures have been used to retain the microbiological quality and safety of seafood. Freezing, salting, smoking, spicing, marinating, and adding antioxidants are a few examples (Ekelemu et al. 2021; Messina et al. 2015; Ngo et al. 2011; Rathod et al. 2021). Natural antimicrobial extracts from ginger (*Zingiber officinale* Roscoe), lime (*Citrus aurantifolia* Linn.), garlic (*Allium sativum* Linn.), rosemary, and basil essential oils have been shown to have effective fish preservation properties (Ahmed 2019; Hassoun and Coban 2017; Karoui and Hassoun 2017; Kumolu-Joh et al. 2015). Marinating, which is the practice of soaking fish flesh in acetic acid and salt before further processing, has been used to preserve anchovies, sardines, shad, codfish, and other fish in European countries (Hassoun and Karoui 2016; Poligne and Collignan 2000).

Antioxidants are synthetic or natural substances that can inhibit the process of oxidation by reacting with free radicals (Iheanacho et al. 2021; Nimse and Pal 2015). They control rancidity in

aquatic products (Taheri et al. 2012), making them necessary for improved sensory quality, storage stability, and nutritional value of fish (Kazimierczak et al. 2008; Sampels 2013). Due to the carcinogenic and mutating effect of synthetic antioxidants like tertiary butylhydroquinone, butylated hydroxyanisole, and butylated hydroxytoluene on fish and other aquatic products (Taheri et al. 2012), natural antioxidants such as ascorbic acids (AA), citric acid, vegetable extracts, various spices, and herbs are preferred by the food industry (Nirmala et al. 2018) because of their phenolic contents. Natural, clean label antimicrobial, antioxidants, texturizers, and colors have been used in meat products as a better alternative to synthetic additives (Delgado-Pando et al. 2021). The use of AA as antioxidant to extend shelf life of Persian sturgeon (*Acipenser persicus*) (Rostamzad et al. 2010, 2011), cobia (*Rachycentron canadum*) (Taheri et al. 2012), tilapia (*Oreochromis niloticus*) (Lee et al. 2019), silver carp (*Hypophthalmichthys molitrix*) (Monirul et al. 2019), and rainbow trout (*Oncorhynchus mykiss*) (Kilic and Oztan 2013) fillets has been reported to reduce lipid oxidation on treated fish.

Mackerel of the species *S. scombrus* belongs to the family Scombridae. It is an important commercial species and highly traded in commercial quantities in the Nigerian market. Though not native to the Nigerian waters, the fish is highly relished in restaurants, bars, relaxation centers, and hotels. Unfortunately, the transportation time, lack of suitable storage facilities, and shortage of electricity are only a few factors affecting the economics of the fish (mackerel) product. Furthermore, fish vendors lack the necessary technical expertise for processing and preserving the fish, resulting in spoilage caused by autolysis or microbial activities, leading to huge economic loss for the fish dealers (Kruijssen et al. 2020; Onyeneke et al. 2020). Therefore, there is a need for suitable preservatives such as ascorbic acid as an alternative to enhance the shelf life of mackerel fish.

Materials and methods

Sample preparation, treatment and storage

Thirty-six adult mackerel samples of 1 kg average weight were obtained from fish vendors at Abakaliki, Ebonyi State and conveyed to the fish processing unit of Fisheries and Aquaculture Department, Ebonyi State University, in sterile polythene bags. Fish samples were rinsed with tap water, eviscerated, and filleted without skin removal. The filleted fish were sliced into three homogenous sizes and then thoroughly rinsed again with clean water. The samples were separated into four groups of twenty-seven fillets per group. Three groups were given a dip treatment of 5 mg L⁻¹, 10 mg L⁻¹, and 15 mg L⁻¹ AA extracts for two hours, and the remaining group received no AA treatment, as a control. Afterwards, the treated fish were withdrawn from the solution and allowed to drain for 30 minutes (Ekelemu et al. 2021). The processed fish fillets were then smoked using a locally fabricated smoking kiln and wood charcoal as fuel, while the temperature was set and controlled between 120°C and 150°C for 18 hours. The dried fish were allowed to cool and stored in perforated experimental plastic containers for 28 days at ambient temperature (28.00 ± 02°C), after which random samples were taken for analysis. Each analysis was performed in triplicate.

Microbiological analysis

Microbial study was examined after 28 days of preservation. Representative samples per group were aseptically weighed (about 10 g) and blended in a Stomacher (HiMedia, Mumbai, India) with 90 mL of Ringer solution. The spread plate method was used to determine total viable counts (TVC) in plate count agar, while Enterobacteriaceae *spp* were cultured using red bile glucose agar (HiMedia). More decimal dilutions were prepared, and then 0.1 mL of each dilution that was anticipated to fall within the range for counting was pipetted in triplicate onto the surface of the plate count agar plate (Kuleya et al. 2017). Following a 16–24-hour incubation period at 37°C, the plates were examined for red colonies and Enterobacteriaceae *spp* counts. Subsequently, the incubation of the plates was done for 48 hours at 35°C.

Chemical analysis

According to the method described by Lee and Shin (2019), fish samples' pH was assessed by blending each sample (5 g) with 20 ml distilled water for 1 minute. A laboratory pH meter (Knick, 765 SET) was then used to measure pH. Peroxide value (PV) and free fatty acid (FFA) in the lipid extract were determined following the procedures of Egan et al. (1997). The method described by Kilic and Richards (2003) was used to measure the thiobarbituric acid (TBA) value. The proximate parameters were assessed using AOAC standard procedure (AOAC 2005). Moisture content was evaluated by drying group samples to constant weight (g) between 20 and 24 h at 105°C. The sample was heated overnight at 550°C to quantify the amount of crude ash. The Kjeldahl method was used to calculate the crude protein content with a 6.25 factor. Furthermore, the same method of solvent extraction that was used to determine the crude fat of meat was used to assess the fat content of group samples.

Sensory evaluation

The treated and untreated smoked fish fillets were randomly selected and given to a 10-member trained panel to assess the sensory quality characteristics after 28 days of preservation. Sensory analysis was assessed using the methodologies of Baten et al. (2020) and Ekonomou et al. (2022). The panelists evaluated the overall acceptability, odor, taste, texture, flavor, and appearance using a 9-point hedonic scale approach (1 for extremely dislike, < 5: dislike, > 5: like, and 9 for extremely like).

Statistical analysis

IBM's Statistical Package for the Social Sciences (SPSS) version 21 was used to carry out analysis of variance (ANOVA) on the data from various quality measurements. Results obtained were presented as mean $\pm$ standard deviation. When differences in means occurred, the Tukey method was performed and significance declared at $p = 0.05$.

Results

Proximate composition

The effects of AA as a preservative on the nutrient composition of smoked mackerel fish was assessed (Figure 1). In comparison to the treated groups, the control recorded the highest moisture content (Figure 1a, $p < .05$); however, there was no significant change in crude fat (Figure 1d, $p > .05$) between the two groups. Fish fillets treated with AA at 10 and 15 mg L⁻¹ concentrations recorded the highest content of crude protein (Figure 1b, $p < .05$), followed by 5 mg L⁻¹ AA treated group, while the control had the lowest crude protein content. Crude ash content was significantly higher in AA treated groups (Figure 1c, $p < .05$) with the maximum value recorded at 15 mg L⁻¹.

Biochemical quality

The effect of AA on the chemical properties of mackerel fillets (*S scombrus*) after 28 days of preservation was studied (Figure 2). Significant changes ($p < .05$) were noted between the AA treated groups and control with regards to TBA (Figure 2a), PV (Figure 2b), and FFA (Figure 2c), but the pH value of the control did not differ substantially (Figure 2d, $p > .05$) from the AA treated groups. The highest values of TBA (1.79 ± 0.01), PV (34.13 ± 0.01), and FFA (0.88 ± 0.01) were recorded in the control, while 15 mg L⁻¹ AA treated group recorded the lowest values for the same parameters (TBA: 0.95 ± 0.05 , PV: 18.33 ± 0.01 , and FFA: 0.14 ± 0.01).

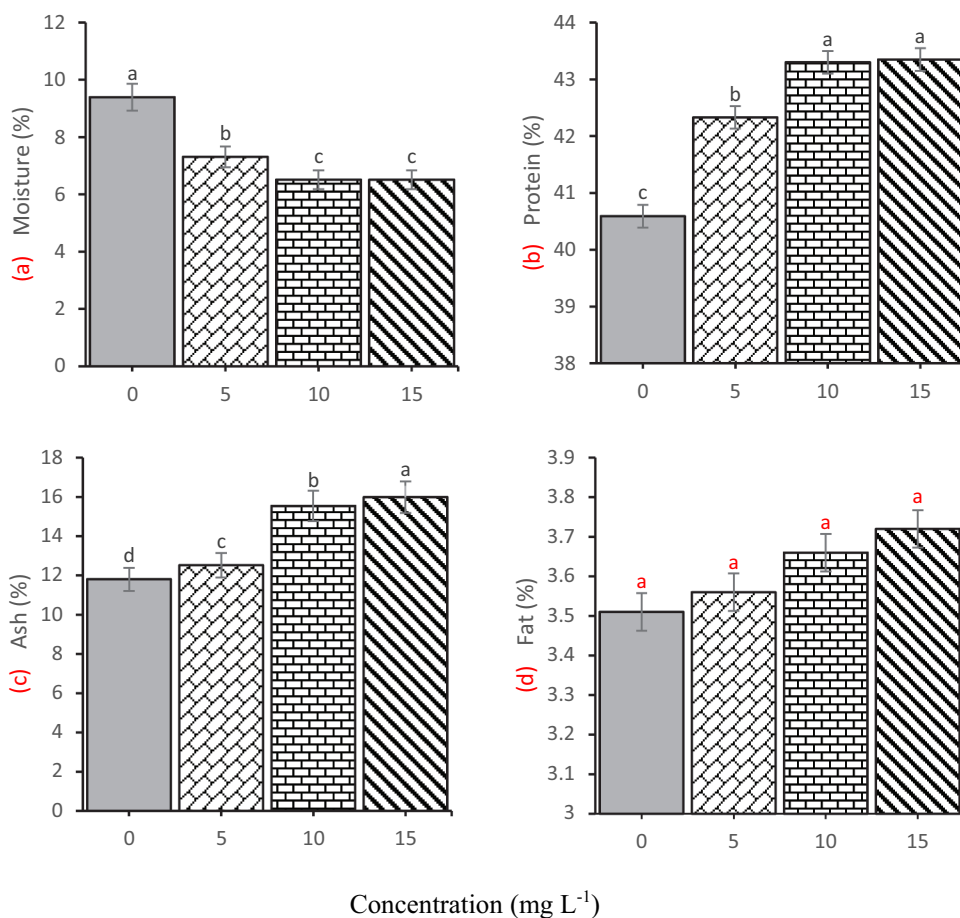


Figure 1. Changes in proximate composition of mackerel fillets (a-d). All data are expressed as mean $\pm$ standard deviation (SD). Different lowercase letters above the bars denote significant differences ($p < .05$).

Microbiological quality

The microbial properties, Enterobacteriaceae *spp* counts (Figure 3a), and TVC (Figure 3b) of fish fillets treated with AA were assessed after 28 days of preservation. The control groups had significantly higher Enterobacteriaceae *spp* counts (6.35 ± 0.01 log cfu/g) and TVC (6.42 ± 0.01 log cfu/g) than the AA treated groups. The lowest Enterobacteriaceae *spp* (6.10 ± 0.1 log cfu/g) and total viable (6.23 ± 0.05 log cfu/g) counts were seen in fish treated with the highest concentration of AA.

Sensory quality

The influence of AA on the physical properties of fish were assessed using hedonic scale measurement (Figure 4). With the exception of appearance, which showed no significant change (Figure 4e, $p > .05$), the panelists concurred that all sensory attributes considered in the present study varied considerably (Figure 4a–d,f, $p < .05$) across treatments. It was observed that the highest concentration (15 mg L⁻¹) recorded a higher mean value for odor (5.80 ± 0.20), flavor (5.90 ± 1.70), texture (6.90 ± 1.35), taste (6.00 ± 1.85), and overall acceptability (6.00 ± 1.30), while the control recorded the least values for the same parameters: odor (2.80 ± 0.50), flavor (1.50 ± 0.90), texture (2.80 ± 2.25), taste (0.30 ± 0.00), and overall acceptability (3.00 ± 0.20).

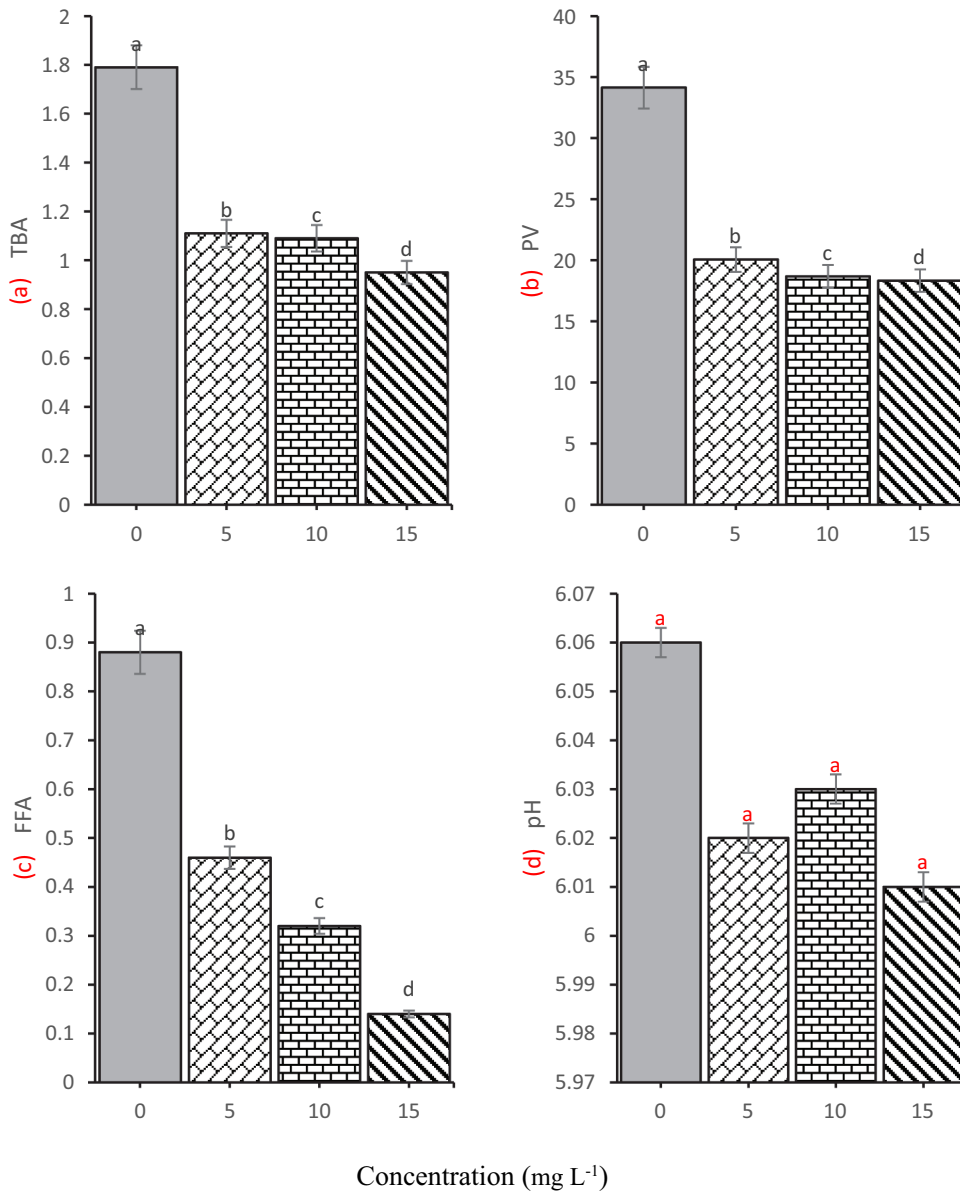


Figure 2. Effects of ascorbic acid on chemical changes of mackerel fillets (a-d). All data are expressed as mean $\pm$ standard deviation (SD). Different lowercase letters above the bars denote significant differences ($p < .05$). TBA – Thiobarbituric acid, PV – Peroxide value, FFA – Free fatty acid, pH – Hydroxide ions.

Discussion

Processing, preservation, and value addition to improve the quality and shelf life of food products is vital for food sufficiency, nutritional health, and economic development. This aligns with the United Nations' mandates of the sustainable development goals (SDGs, 1, 2, 3 and 8). The present study reveals that AA is an important preservative that enhanced the quality of smoked mackerel fish, which is evident in the findings of the parameters considered. The concentration-dependent increases of crude protein and ash levels of fishes cured with AA indicate the potential of AA to promote the nutritional values of food products (Gallie 2013). This observation is in agreement with the findings of

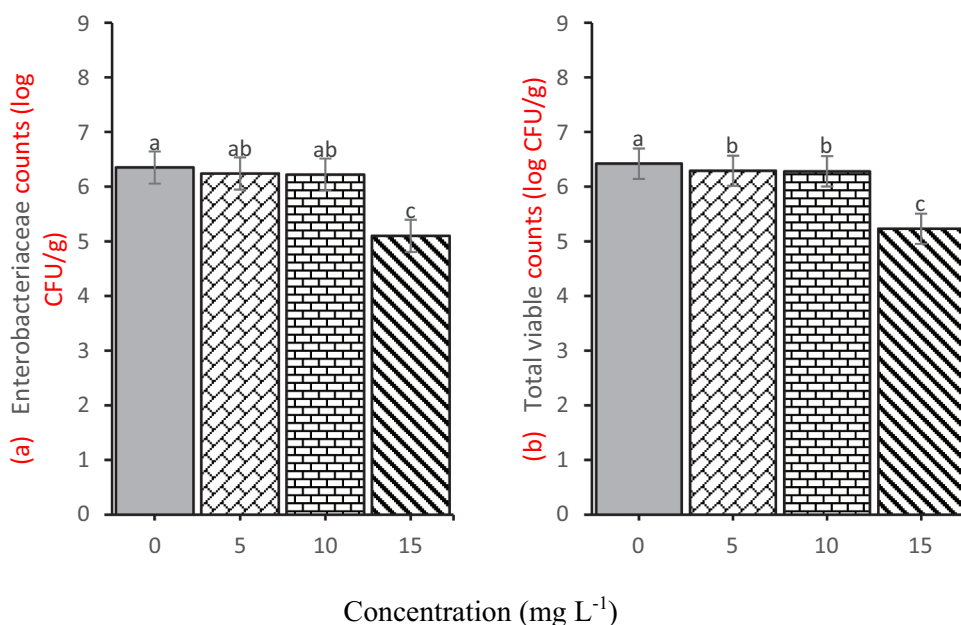


Figure 3. Enterobacteriaceae and total viable counts in mackerel fillets (a-b). All data are expressed as means $\pm$ standard deviation (SD). Different lowercase letters above the bars denote significant differences ($p < .05$).

Iheagwara (2013), who investigated the stability and sensorial quality of mackerel fish (*S. scombrus*) treated (soaked) with ginger antioxidants. Alexandre et al. (2021) also suggested that the incorporation of both natural and synthetic antioxidants to food products and promote their nutrient values. The present study revealed a significant reduction of moisture content in AA-treated groups than in the control. Smoking (heat treatment) of the fish samples before storage and application of AA in treated groups could be responsible for the significant reduction in moisture content (Iheanacho et al. 2017). Our finding is in agreement with reports of Iheagwara (2013), Kilic and Oztan (2013), and Messina et al. (2018), which showed that different fish species treated with AA and other antioxidants had lower moisture content. This study found an inverse relationship between the moisture content and other proximate components examined. The pH values obtained across treatments remained significantly unchanged, indicating that the application of AA had no measurable negative effect on the pH of the smoked mackerel. This was contrary to the findings of Rostamzad et al. (2011), who recorded a significant reduction in pH value of Persian sturgeon (*Acipenser persicus*) treated with AA solution. Additionally, Lee et al. (2019) observed that the pH value of chilled tilapia fish (*Oreochromis niloticus*) decreased significantly based on AA treatment. The authors further stated that the significant reduction in pH value could have contributed to the enhanced shelf life of treated fish. Therefore, maintaining fish fillet pH at a lower level will boost the long-term preservation of the product (Lee et al. 2019). PV was highest in the control and significantly decreased in treated groups, which could be attributed to the antioxidant capacity of AA to degrade peroxides and further inhibit oxidative rancidity. This agrees with the findings of Monirul et al. (2019) and Rostamzad et al. (2011), who noted significant reduction of peroxide contents and the deterrence of oxidative rancidity to cause spoilage as a result of AA treatment of silver carp and Persian sturgeon fillets, respectively. Given that peroxides are directly associated with the development of rancidity, the results of the current study show that the group treated with the highest concentration (15 mg L⁻¹) of AA was the most effective in preventing primary peroxidation when compared to the other groups. Contrary to the control, the decreased TBA values in AA treated groups indicate reduced/negative lipid peroxidation levels credited to the antioxidant capacity of AA. The outcome of the current study agrees with the result

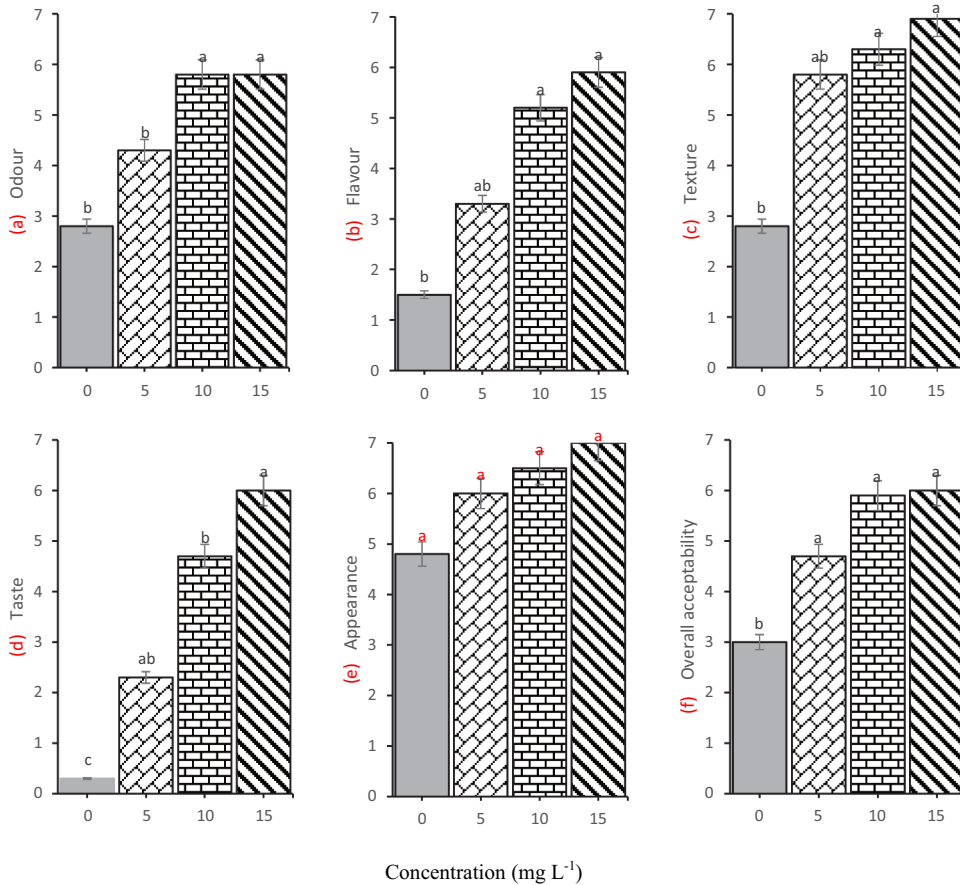


Figure 4. Effects of ascorbic acid on sensory attributes of mackerel fillets (a-f). All data are expressed as means $\pm$ standard deviation (SD). Different lowercase letters above the bars denote significant differences ($p < .05$).

of Lee et al. (2019), who observed a similar trend in tilapia fish (*Oreochromis niloticus*) fillet treated with AA coating. FFA was assessed to investigate the breakdown of fats. They are known to go through further oxidation (Pourashouri et al. 2009), resulting in compounds with reduced molecular weight that give fish and fish products an unpleasant flavor and taste (Refsgaard et al. 2000; Rostamzad et al. 2011). Evident reduction of FFA value in AA treated groups might be connected to the antioxidant activity of AA to prevent oxidation of fatty acids. Taheri et al. (2012) observed a similar pattern in cobia (*Rachycentron canadum*) fillets exposed to various levels of AA during frozen storage.

The activities of microorganisms (bacteria) are well-known to facilitate spoilage and decomposition of food materials, among other factors (environmental, inadequate processing) (Lorenzo et al. 2018). The microbial load (*E. cloacae*, *S. Typhimurium*, *C. koseri*) was lower in 15 mg L⁻¹ AA treated group, which confirms the antibacterial effect of AA against the proliferation of bacterial contamination in food products (In et al. 2013). According to Monirul et al. (2019), 2% AA spray treatment in combination with 1% acetic acid spray lowered the bacterial load and prevented bacterial growth in fresh silver carp (*Hypophthalmichthys molitrix*). Other antioxidants including lactic acid, citric acid, acetic acid, ginger, and garlic oil have also been reported to inhibit microbial loads on processed fish products (Ekelemu et al. 2021; Monirul et al. 2019; Murhekar et al. 2017; Noori et al. 2018), which is consistent with the findings of the current study.

Sensory evaluation is considered one of the effective methods for assessing the quality and acceptability of processed fish products among consumers (Ekonomou et al. 2022; Loutfi et al.

2015; Messina et al. 2018). Both the control and samples treated with 5 and 10 mg L⁻¹ experienced sensory deterioration. The sensory assessment at these concentrations did not pass the threshold limit of 5.0 due to incidence of unpleasant odor, flavor, and taste, making the products less attractive. However, the application of AA treatments at 15 mg L⁻¹ was beneficial in improving the quality, deferring spoiling indices, and extending the shelf life of mackerel fish, since the assessment reached the acceptability score. The rapid degradation of untreated samples (control) could be attributed to the presence of bacteria that affect the structure of fish protein (Rathod et al. 2021). Our result aligns with previous studies by Monirul et al. (2019), Rostamzad et al. (2011), and Taheri et al. (2012), who reported that fresh silver carp (*Hypophthalmichthys molitrix*), Persian sturgeon (*Acipenser persicus*), cobia (*Rachycentron canadum*) samples treated with AA had better aroma, texture, and taste than the control. Kilic and Oztan (2013) suggested that cold-smoked trout (*Oncorhynchus mykiss*) treated with AA had improved sensory characteristics including aroma and texture. Other antioxidants used on fish products, such as ginger extract and citric acid have been shown to improve sensory properties when compared to untreated samples (Iheagwara 2013; Rostamzad et al. 2011).

Conclusion

The expansion of the food and agricultural industries, and consequently, the global economy is significantly hampered by food spoilage. Improvement in preservation and value addition using natural products/supplements has become imperative to enhance the shelf life and qualities (physico-chemical/organoleptic) of food products amid the current global food crisis. The current study reveals that the use of AA can impede undesirable chemical changes, improve sensory attributes, inhibit microbial growth, reduce lipid oxidation, and prolong the shelf life of mackerel fish fillets at the end of their storage temperature. It was also observed that none of the samples was above the spoilage limit of 7 logs, and the addition of AA at higher concentrations (15 mg L⁻¹) could inhibit the microbial growth of TVC during the period of storage. The use of AA as a natural antioxidant in the food sector is therefore highly recommended to producers and researchers for the lengthy preservation of fish products and promotion of food safety and security.

Disclosure statement

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