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The Effect of Gaseous Ozone and Moringa Leaf–Carboxymethyl Cellulose Edible Coating on Antioxidant Activity and Biochemical Properties of 'Keitt' Mango Fruit

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Abstract: This study evaluated the effect of edible coating and gaseous ozone on the antioxidant activities and biochemical properties of mango fruit. Mango fruit (cv. Keitt) were coated with moringa leaf extract and carboxymethyl cellulose (EC) before exposure to ozone (0.25 ppm). Gaseous ozone (O_3) was administered intermittently for 24 or 36 h, and the control fruit were untreated. The fruit were stored at 10 °C for twenty-one days, then ripened at ambient temperature for seven days. The parameters measured were ascorbic acid, lipid peroxidation, phenolic content, total sugars, and antioxidant capacity (FRAP and DPPH). At the end of storage, the EC + O_3 (36 h) had high phenolic content: 175.02 µg GEA/g DM compared to 151.87 µg GEA/g DM and 138.98 µg GEA/g DM for the O_3 (24 h) and untreated fruit, respectively. Moreover, the combination of the EC and O_3 (36 h) had a higher effect ($p < 0.05$) on preserving the antioxidant capacity of the mangoes. The EC + O_3 (24 h) and EC significantly delayed fruit softening and maintained membrane integrity. Furthermore, the fruit treated with the EC reduced the accumulation of reducing (7.61 mg/mL) and total sugars (8.81 mg/mL) compared to the control treatment, which had a concentration of 12.74 mg/mL and 13.78 mg/mL, respectively. These findings demonstrate that EC combined with gaseous O_3 enhanced the antioxidants of mango fruit during storage.

Keywords: mango fruit; sugars; phenolics; antioxidants; edible coating; ozone

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1. Introduction

Mango (*Mangifera indica* L) is an excellent source of vitamins, carotenoids, antioxidants, including phenolics, and ascorbic acid. Recent studies have highlighted the nutritional value of fruit and vegetables and their effect on human health [1]. The high intake of fresh fruits that are rich in natural antioxidants is linked to a lower risk of diseases, such as cancer, Alzheimer's, cardiovascular diseases, and rheumatoid arthritis [2–4]. Antioxidants are reactive against reactive oxygen species, such as hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-) [3,5]. Reactive oxygen species (ROS) can cause oxidative stress in the fruit, resulting in decreased sensory quality, leading to off flavors [3,6]. In order to maintain the quality and high contents of antioxidants and bioactive compounds, postharvest chemical treatments are commonly used by the fresh produce industry [6,7]. Treatments such as nitric oxide, 1-Methylcyclopropene and salicylic acid are some of the commonly used chemicals for preserving the antioxidants and overall quality of various horticultural produce [6–8]. The increasing consumer demand for chemical-free food has necessitated searching for novel and environmentally friendly postharvest treatments.

Edible coatings and gaseous ozone have gained interest among food scientists and postharvest researchers in recent years. Edible coatings (EC) are produced from lipids,

proteins, resins, and polysaccharides [9,10]. Polysaccharide-based coatings include chitosan, cellulose, starch, pectin, and gums [11]. These coatings form a pervious sheet on the fruit surface, causing a modified atmosphere, thus reducing water loss and internal oxygen and increasing internal carbon dioxide [11,12]. The edible coatings have effectively decreased postharvest disease, extended shelf-life, and preserved antioxidants, such as carotenoids, phenolic compounds, flavonoids, and ascorbic acid [9,13]. For instance, Daisy et al. [14] reported that gum Arabic (15%) maintained the ascorbic acid and β-carotene in 'Apple' mango stored at ambient temperature for fifteen days. Likewise, a study by Ncama et al. [15] revealed that moringa leaf extract (MLE) (2%) and carboxymethyl cellulose (CMC) (1%) preserved the ascorbic acid in 'Marsh' grapefruit during storage at 5 °C for sixty-three days.

Ozone (O_3) is an unstable molecule with antimicrobial properties and high oxidation potential [5]. It decomposes to form radicals, such as superoxide, hydroxyl, and hydroperoxyl, leaving no chemical residues [16]. Numerous studies have been conducted to evaluate the effect of O_3 on maintaining the quality of various horticultural crops [2,17]. For instance, ozone effectively preserved the ascorbic acid, carotenoids in 'Palmer' mango, and flavonoids in 'Qiushui' pear during storage [18,19]. Ozone treatment (0.3 μL L^{-1}) enhanced both the ferric reducing antioxidant power assay (FRAP) and 1.1-diphenyl-2-picrylhydrazy (DPPH) antioxidant capacity in 'Hayward' kiwifruit stored at 0 °C for three months and ripened at 20 °C for twelve days [17]. Ozone reduces the O_2 accumulation in the fruit surroundings, thus decreasing the rate of respiration and resulting in biochemical changes that lead to enhanced fruit quality.

Firmness is an important quality indicator as consumers buy fresh fruit based on its appearance. Edible coatings enhance the fruit quality by preventing water loss and delaying the accumulation of electrolyte leakage and lipid peroxidation [20,21]. Kumar et al. [22] observed the delayed softening in 'Sefada' mango coated with chitosan 2% + pullulan 2% stored at 4 °C for fifteen days. Studies by Hmmam et al. [23] revealed that CMC and guar gum-based silver nanoparticles (AgNPs) coating retained the firmness in 'Seddik' mangoes during storage at 13 °C for thirty days. Edible coatings are linked to the modification of cell wall structures, which leads to fruit firmness retention [21].

Preserving the antioxidants and quality during the postharvest treatment is vital for maintaining the high nutritional value of the stored fruit. Although O_3 and edible coatings have been extensively studied in recent years, the combined effect of these treatments has not yet been investigated. Moreover, the infusion of moringa leaf extract into CMC has never been tested on mango fruit. Thus, the objective of this study was to investigate the effect of gaseous ozone and moringa leaf extract–CMC-based edible coating on the antioxidant activities and biochemical properties of 'Keitt' mango fruit.

2. Materials and Methods

2.1. Fruit Material

Mango fruit (cv. 'Keitt') were harvested from Goedgelegen Farm of Westfalia (Pty) LTD, a commercial farm located in Tzaneen, South Africa. Fruit were transported within 24 h to Postharvest Research Laboratory of the University of KwaZulu-Natal (Pietermaritzburg Campus). The fruit used in the experiment were free from mechanical and physiological defects; they were also of the same size, color, and maturity. The experiment was replicated three times using three fruit per replicate. A total of 300 fruit were used for the experiment. The fruit were assigned to different treatments as follows:

Control: untreated
T1: O_3 (24 h)
T2: O_3 (36 h)
T3: EC (MLE 1% + CMC 1%)
T4: EC + O_3 (24 h)
T5: EC + O_3 (36 h)

2.2. Edible Coating

Moringa leaf extract was prepared as described by Tesfay and Magwaza [20], with some modification. Briefly, 100 g of moringa leaf powder was extracted with 1000 mL of 70% ethanol (v/v) for twelve hours at room temperature with constant agitation. The extract was evaporated at 37 °C using the Genevac (Genevac® EZ 2.3; Ipswich, UK). The crude extract was suspended with 1000 mL of distilled water and incorporated to 10 g of CMC.

2.3. Ozone Treatment

Gaseous ozone was generated using the corona discharge ozone generator (Ozone Purification Technology, Johannesburg, South Africa). Ozone treatment (0.25 ppm) of mango fruit was done in cold storage for twelve hours at a seven-day interval. For the 24 h treatment, fruit were exposed to O_3 at day zero and seven, whereas, for 36 h, it was day zero, seven, and fourteen. The O_3 times used in the current study were selected based on the physicochemical results a screening study conducted in 2018. Fruit were coated with moringa leaf extract–CMC before O_3 treatment. The control fruit were untreated, and all the fruit were stored at 10 °C and 90% relative humidity for twenty-one days, mimicking shipment from South Africa to the European Union markets. After cold storage, fruit were transferred to shelf-life at ambient temperature for seven days.

2.4. Sample Preparation

Mangoes were peeled, pulp removed from the kernel, and cut into small cubes. Afterward, the cubes were pureed using a blender (Bennett Read, Tornado Tech Cyclonic Action, Tevo, Durban, South Africa). The puree was filtered three times using a muslin cloth centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 1500 rpm for ten minutes to obtain a clear extract. The juice extract was stored in specimen bottles at −20 °C until used for analysis. The peel was freeze-dried using Vir Tis BenchTop Pro freeze drier (SP Scientific, Warminster, PA, USA). After that, samples were crushed into powder and stored at −20 °C until used. The experiment was replicated three times, with three fruit per replicate. Fruit firmness was measured as described by González-Aguilar et al. [24] using a hand-held tester (Bareiss, Baiersbronn, Germany) and expressed as Newton (N).

2.5. Antioxidant Activity

2.5.1. 1.1-Diphenyl-2-picrylhydrazy (DPPH)

The 1.1-diphenyl-2-picrylhydrazy (DPPH) radical scavenging assay was determined as described by Alothman et al. [2], with minor modifications. A 150 mg sample was added to 3 mL methanol 80% (v/v) and incubated for 1.5 h at 35 °C. After that, samples were cooled down and filtered with a 0.45 µm syringe filter (Merck, Warmstadt, Germany). In triplicates, 3.9 mL of methanolic DPPH (2.5 mg/100 mL) was added to the extract (100 µL) and incubated for 60 min at room temperature. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 515, using methanol as blank. The DPPH radical scavenging activity was calculated and plotted against Trolox standards and expressed as µM TE/g DM.

2.5.2. Ferric Reducing Antioxidant Power Assay (FRAP)

FRAP was determined as described by Alothman et al. [2], with modification. Briefly, 3 mL of methanol, water and hydrochloric acid (70:29.5:0.5 v/v) were added to 150 mg sample and incubated at 35 °C for 1.5 h. Samples were cooled down and centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) for ten minutes at 13,000 rpm and filtered with a 0.45 µm syringe filter (Merck, Warmstadt, Germany). In triplicates, extract (50 µL) was added to 3.6 mL of FRAP solution and incubated at 37 °C for ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 590 nm, using deionized water as a blank. FRAP was expressed as µmol Fe(II)/g dry mass.

2.6. Total Phenolic Content

Total phenolics were determined as described by Lamien-Meda et al. [25], with minor modification. Peel powder (1 g) was added to 10 mL acetone (80%) at room temperature for 30 min with constant agitation. After that, samples were centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) for ten minutes at 10,000 rpm (5 °C). Total phenolic compounds were determined using the Folin–Ciocalteau method. In triplicates, sample extract (0.1 mL) was added to 2.5 mL Folin–Ciocalteau reagent (2 N). After five minutes, 2 mL sodium carbonate (75 g/L) was added, and samples were incubated at 65 °C for 2 h. Thereafter, samples were cooled down, and absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 750 nm using acetone as blank. Total phenolic compounds were expressed as μg (GAE)/g dry matter.

2.7. Ascorbic Acid

Ascorbic acid was measured as described by More and Rao [26], with slight modification. Sample powder (1 g) was added to 9 mL of metaphosphoric acid (1%) and sonicated in ice for 3 min. Thereafter, samples were centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 10,000 rpm (4 °C) for five minutes. In triplicates, extract (1 mL) was added to 9 mL of 2,6-dichlorophenolindephenol dye (0.025%) and incubated in the dark at room temperature for ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 515 nm using 1% metaphosphoric acid as a blank. Ascorbic acid was expressed as mg/g dry mass.

2.8. Electrical Conductivity

Electrical conductivity was determined as described by Jincy et al. [27] with modification. Mango cubes (10 mm) were rinsed in deionized water three times, then incubated at 25 °C in deionized water (10 mL) for four hours with constant shaking. Thereafter, samples were incubated for 60 min at 95 °C and cooled down to 25 °C. Electrical conductivity was measured with the Benchtop conductivity/TDS meter (Bante 510, Bante Instruments, Shanghai, China) before and after boiling. The relative electrolyte leakage (REL) was calculated using the following formula:

$$\text{REL } \% = E_i/E_f \times 100 \quad (1)$$

where E_i and E_f are the initial and final reading, respectively.

2.9. Lipid Peroxidation

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) as described by Diperierro and De Leonardis [28], with minor modification. Mango peel (1 g) was homogenized in 10 mL of 1% trichloroacetic acid (*w*/*v*). Thereafter, the sample was centrifuged (Avanti J-265 XP, Beckaman Coulter, Indianapolis, IN, USA) at 10,000 rpm at 4 °C for ten minutes. In triplicates, sample extract (1 mL) was added to 20% trichloroacetic acid (4 mL) containing 0.5% thiobarbituric acid (*v*/*v*) and boiled for thirty minutes at 95 °C. The samples were thereafter cooled down on the ice and centrifuged at 10,000 rpm for ten minutes. The absorbance was measured with a spectrophotometer (Shimadzu Scientific Instruments Inc., Columbia, MD, USA) at 532 nm and 600 nm. The MDA concentration was calculated using the equation 3 and expressed as $\text{ngmol}^{-1}\ \text{g}^{-1}$.

$$\text{Total MDA} = A_{532} - A_{600}/155 \times 10^3 \quad (2)$$

where A_{532} is the absorbance at 532 nm and A_{600} is the absorbance at 600 nm.

2.10. Total Sugars

For the analysis of total sugars, juice extract was filtered with a 0.45 μm filter (Merck, Warmstadt, Germany). The extract (200 μL) was diluted with 800 μL of ultrapure water and vortexed for 20 s. Sugars were analyzed as described by Tesfay and Magwaza [20] using an

isocratic HPLC. Sample extracts were infused into the Rezex RCM monosaccharide Ca^{+} (8%) of 300 mm × 7.8 mm column (Phenomenex, Torrance, CA, USA) with a Carbo-Ca^{2+} of 3 mm × 4 mm guard column (Phenomenex). The column temperature was 80 °C, and the mobile phase was ultra-pure water. The concentration of sugars was determined by comparing the peak areas of the standard curve with those of samples.

2.11. Statistical Analysis

Data were subjected to the analysis of variance (ANOVA) using GenStat statistical software (GenStat ®, 18 edition, VSN International, Hemel Hempstead, UK). Fischer's least significant difference (LSD) was used to separate means at 5% level of significance. Pearson correlation coefficient was used to determine relationships between lipid peroxidation, phenolic content, and antioxidants.

3. Results

3.1. Firmness

The untreated fruit showed a rapid decrease in firmness compared to the other treatments (Figure 1). There was a significant ($p < 0.05$) difference between the treatment means of the untreated fruit, O_3 (36 h), EC, EC + O_3 (24 h), and EC + O_3 (36 h), from day fourteen till the end of storage. High firmness was observed at the end of storage in the fruit treated with EC+ O_3 (36 h), O_3 (36 h), and EC.

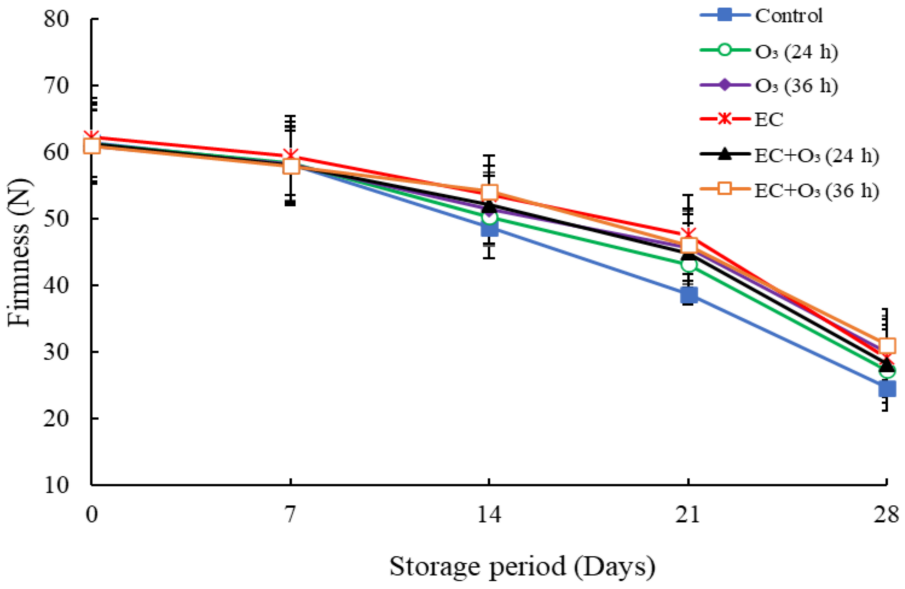

Figure 1. Effect of edible coating (EC) and gaseous ozone (O_3) on mango fruit firmness stored at 10 °C for twenty-one days and seven days shelf-life at ambient temperature (±SE, $n = 9$).

3.2. Antioxidant Activity

The DPPH scavenging activity in all the treatments increased up to day fourteen, then gradually decreased until the end of storage. The fruit coated with EC had significantly ($p < 0.05$) higher scavenging activity from day fourteen up to the end of storage compared to the other treatments (Figure 2). The DPPH activity of the untreated fruit was low compared to the other treatments, except at the end of storage when it was comparable to O_3 (24 h). At the end of storage, the DPPH activities of the EC + O_3 (24 h) and O_3 (36 h) were not significantly different.

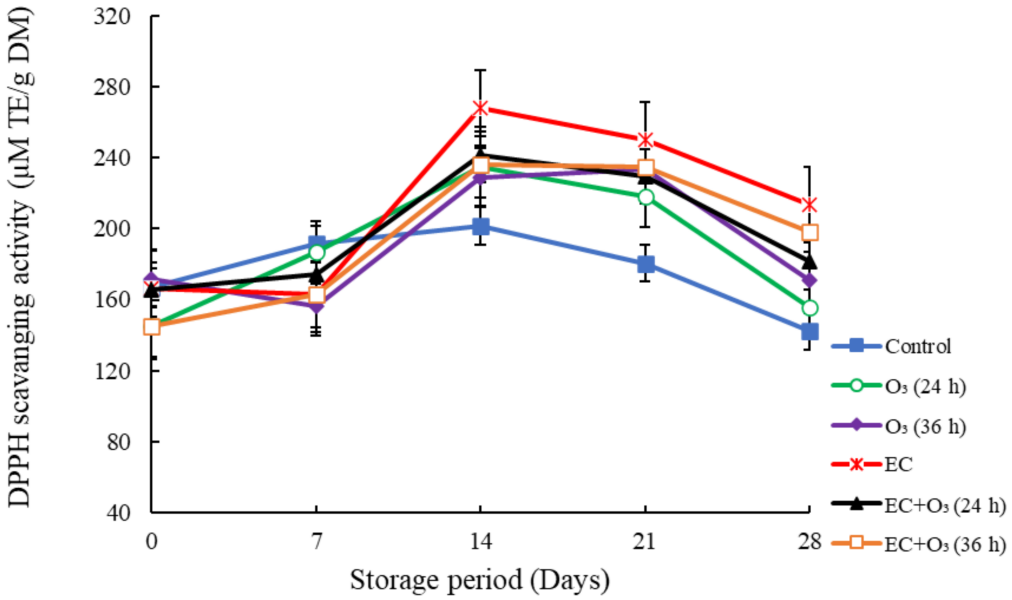

Figure 2. Effect of edible coating (EC) and gaseous ozone (O_3) on DPPH scavenging activity of mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (± SE, n = 9).

The antioxidant activity measured by the FRAP of the mango fruit decreased in all the treatments during storage (Figure 3). The FRAP activity of the O_3 (24 h) was low compared to the other treatments throughout the storage period. At the end of storage, the EC + O_3 (36 h) had a high FRAP antioxidant compared to the control, O_3 (24 h), and EC + O_3 (24 h). However, there was no significant difference between the treatment means of O_3 (24 h), EC, EC + O_3 (24 h), and the control at the end of storage.

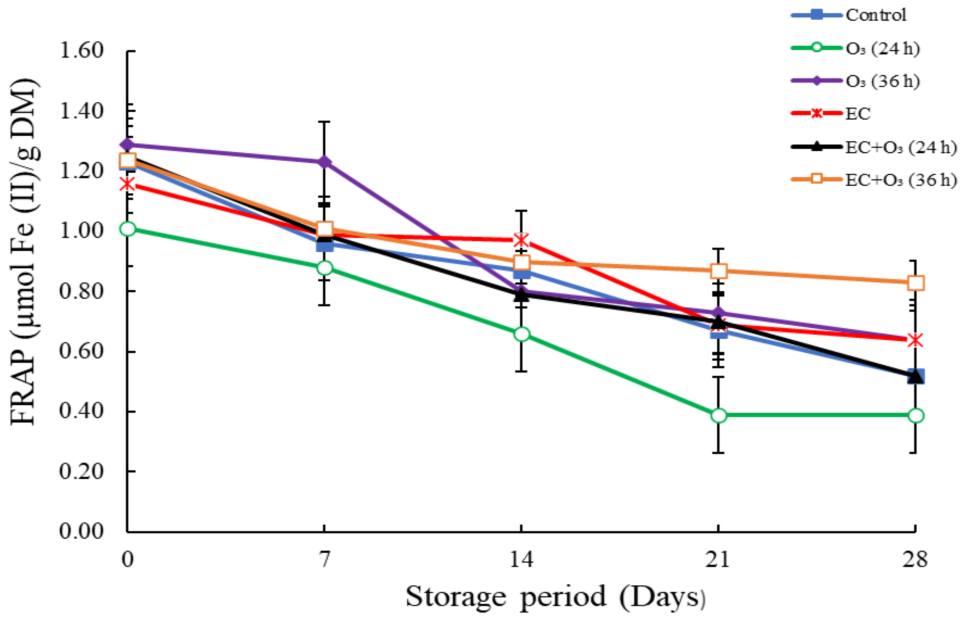

Figure 3. Effect of edible coating (EC) and gaseous ozone (O_3) on FRAP activity in mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, n = 9).

3.3. Total Phenolics

The total phenolic compounds in the mango fruit significantly decreased during storage. However, the phenolic content of the treated fruit decreased gradually compared to the rapid reduction in the untreated fruit (Figure 4). The phenolic content of the mango fruit was significantly ($p < 0.05$) affected by the different treatments. The phenolic content in the EC and EC+ O_3 (36 h) was higher than the other treatments from day fourteen until the end of storage. At the end of storage, low phenolic content was observed in the O_3 (24 h), EC+ O_3 (24 h), and untreated fruit.

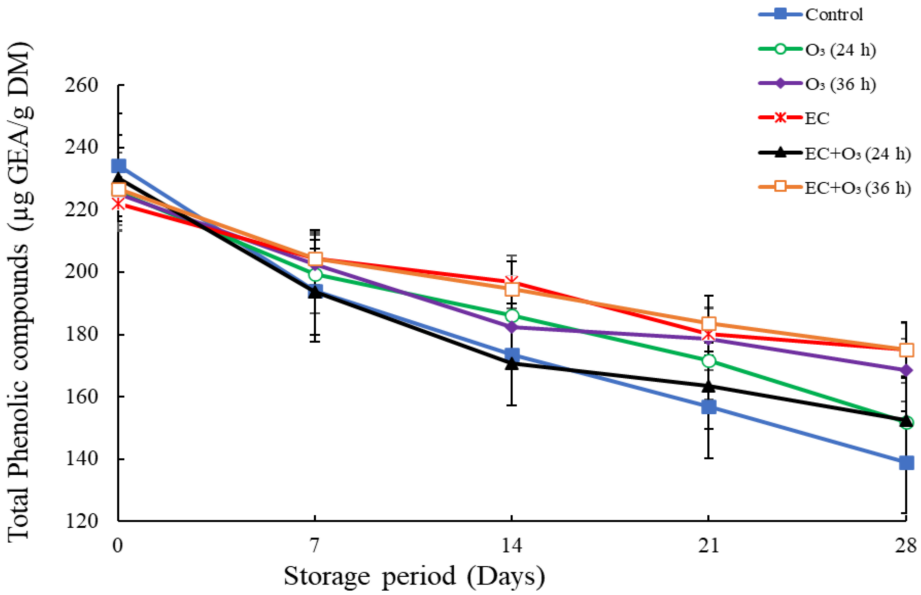

Figure 4. Effect of edible coating (EC) and gaseous ozone (O_3) on total phenolics in mango peel during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, $n = 9$).

3.4. Ascorbic Acid

There was a significant ($p < 0.05$) difference between the treatment means over storage time (Figure 5). The fruit coated with the EC had the highest AA compared to the other treatments from day fourteen till the end of storage. However, there was no significant difference between the treatment means of the EC and EC + O_3 (24 h) at the end of storage. The fruit treated with the O_3 (36 h) inhibited the decrease in AA compared to the O_3 (24 h) during storage. The AA in the EC + O_3 (24 h) and EC + O_3 (36 h) was similar throughout the storage period. At the end of storage, the highest AA was observed in the EC (98.70 mg/g DM) compared to the EC + O_3 (24 h), O_3 (36 h), EC + O_3 (36 h), O_3 (24 h), and untreated fruit, which had 87.30 mg/g DM, 77.10 mg/g DM, 70.20 mg/g DM, 50.70 mg/g DM, and 37.41 mg/g DM, respectively.

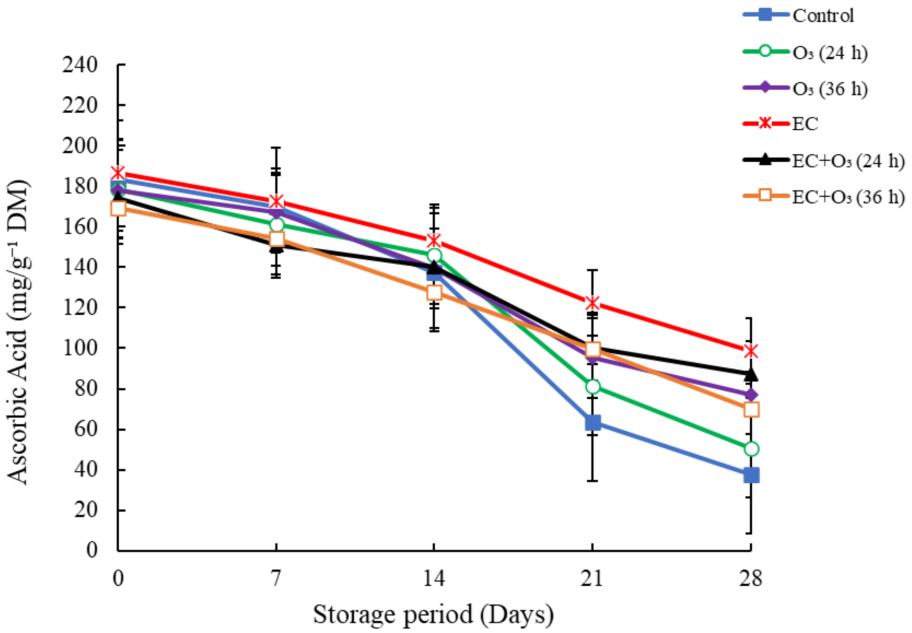

Figure 5. Effect of edible coating (EC) and gaseous ozone (O_3) on AA in mango fruit during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature (± SE, n = 9).

3.5. Electrical Conductivity

The results of the relative electrolyte leakage (REL) are shown in Table 1. The REL significantly ($p < 0.05$) increased in all the treatments throughout storage. However, a sharp increase was observed in the untreated fruit compared to the other treatments. The low percentage of REL was observed in the EC, EC + O_3 (24 h), and EC + O_3 (36 h) from day fourteen till the end of storage. The fruit treated with the EC + O_3 (24 h) (22.52%) maintained membrane integrity compared to the EC (24.40%), O_3 (36 h) (26. 46%), and the untreated fruit (39.77%) at the end of storage.

Table 1. Effect of edible coating (EC) and gaseous ozone (O_3) on relative electrolyte leakage (REL) (%) of mango fruit during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature. Data represent means (LSD = 2.00, n = 9).

Treatments	Day 0	Day 7	Day 14	Day 21	Day 28
Control	4.13a	9.57bc	18.11ij	27.79l	39.77m
O_3 (24 h)	4.10a	9.47bc	14.25efg	20.11j	27.94l
O_3 (36 h)	4.47a	9.45bc	15.05fg	19.29ij	26.46l
EC	3.99a	9.94bc	13.51def	18.52ij	24.40k
EC + O_3 (24 h)	4.14a	8.00b	11.51cd	16.19gh	22.52k
EC + O_3 (36 h)	3.92a	7.81b	12.19de	17.37hi	23.74k

Averages with common superscript letters within a column and between columns were not significantly different ($p < 0.05$).

3.6. Lipid Peroxidation

The MDA content in the mango fruit significantly ($p < 0.05$) increased during storage. The fruit treated with the EC and O_3 (24 h) inhibited the accumulation of the MDA content during cold storage (Table 2). At the end of storage, high MDA content was observed in the untreated fruit compared to the EC + O_3 (24 h), EC + O_3 (36 h), and O_3 (24 h). The MDA content of the EC + O_3 (24 h) and EC + O_3 (36 h) was not significantly different at the end of storage.

Table 2. Effect of edible coating (EC) and gaseous ozone (O_3) on mango peel lipid peroxidation (MDA ngmol^{-1} g^{-1}) during storage for twenty-one days at 10 °C and shelf-life for seven days at ambient temperature. Data represent means (LSD = 0.50, n = 9).

Treatments	Day 0	Day 7	Day 14	Day 21	Day 28
Control	0.47ab	0.65abc	1.68e	0.65f	5.12j
O_3 (24 h)	0.42a	0.66abc	1.43de	2.68f	4.03gh
O_3 (36 h)	0.42a	0.65abc	1.49de	2.37f	3.51g
EC	0.49ab	0.73abc	1.04bcd	2.54f	3.87gh
EC+ O_3 (24 h)	0.43a	0.73abc	1.09cd	2.26f	4.64ij
EC+ O_3 (36 h)	0.44a	0.70abc	1.04bcd	2.44f	4.39hi

Averages with common letters within a column and between columns were not significantly different ($p < 0.05$).

3.7. Soluble Sugars

The treatments significantly ($p < 0.05$) affected the sugar content of the mango fruit. The reducing sugars (RS) increased in all the treatments during storage (Figure 6). A rapid increase in the RS content was observed in the untreated fruit compared to the other treatments. At the end of storage, the low RS content was observed in the fruit treated with the EC and EC + O_3 (36 h), whereas it was notably high in the untreated fruit.

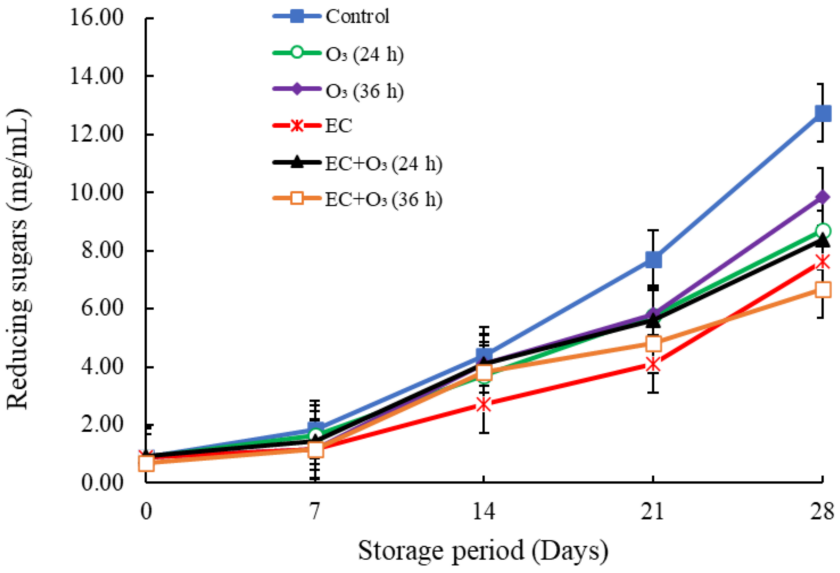

Figure 6. Effect of edible coating (EC) and gaseous ozone (O_3) on reducing sugars of mango fruit during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, n = 9).

The non-reducing sugars (NRS) increased in all the treatments from day zero till day fourteen, then slowly decreased (Figure 7). At the end of storage, there was no significant difference between the treatment means of the O_3 (24 h), O_3 (36 h), and EC + O_3 (24 h). The total sugars (TS) significantly increased in all the treatments during storage (Figure 8).

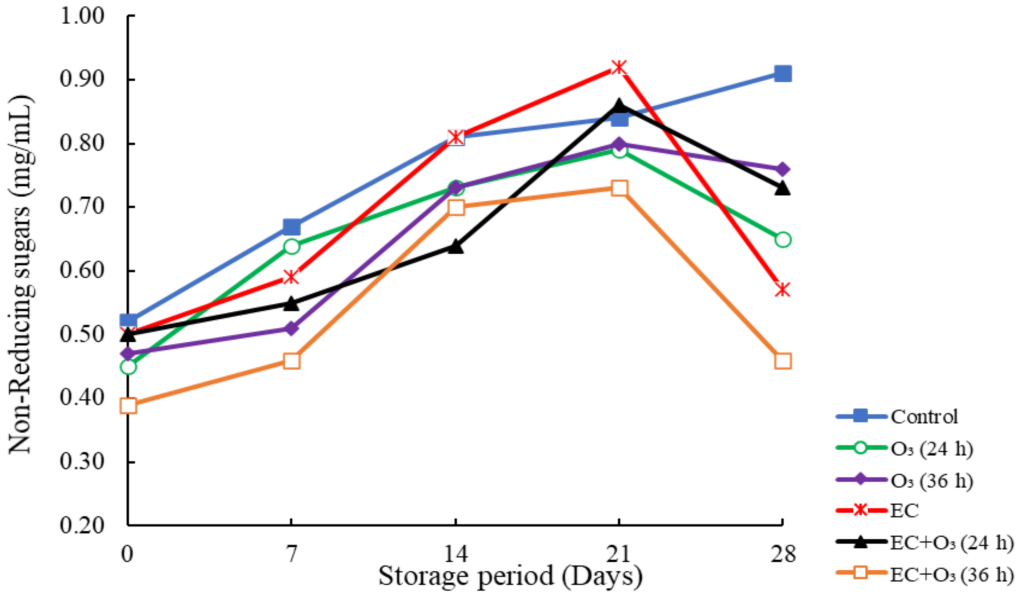

Figure 7. Effect of edible coating (EC) and gaseous ozone (O_3) on non-reducing sugars of mango fruit during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, n = 9).

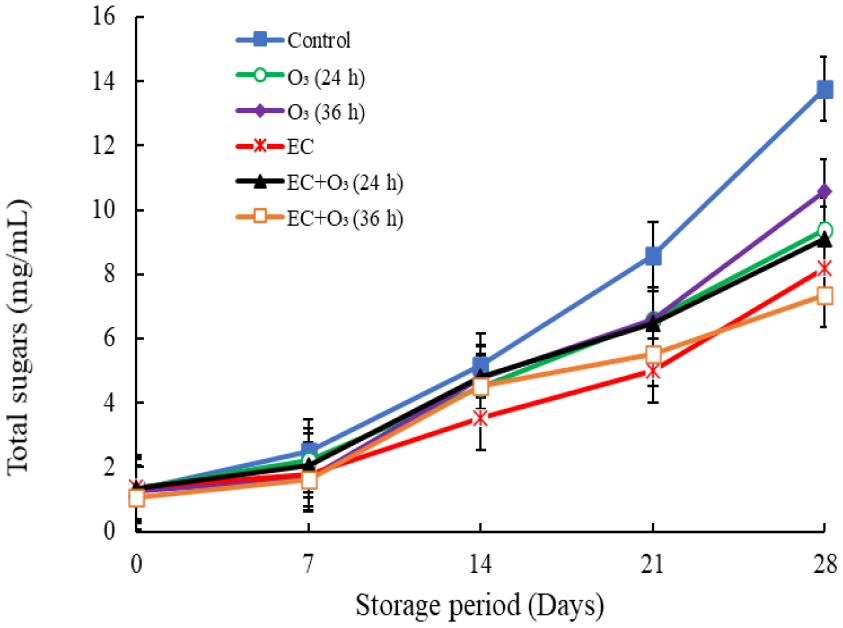

Figure 8. Effect of edible coating (EC) and gaseous ozone (O_3) on total sugars of mango fruit during storage at 10 °C for twenty-one days and shelf-life at ambient temperature for seven days (±SE, n = 9).

3.8. Correlations of Mango Firmness and Biochemical Parameters

The fruit firmness was positively correlated to the FRAP (R^2 = 0.51) and had a strong negative correlation with the MDA (R^2 = −0.77), REL (R^2 = −0.75), and RS (R^2 = −70). A positive correlation (R^2 = 0.61) was observed between the FRAP and phenolic content (Table 3). The ascorbic acid was negatively correlated with all the parameters measured. The DPPH had a positive correlation to the MDA (R^2 = 0.64), RS (R^2 = 0.59), and REL (R^2 = 0.59). A medium to strong positive correlation was observed between the MDA, RS (R^2 = 0.72), NRS (R^2 = 0.51), TS (R^2 = 0.73), and REL (R^2 = 0.89).

Table 3. Pearson correlation coefficient of AA, FRAP, DPPH, phenolics, MDA, RS, NRS, TS, REL in mango fruit.

	Firmness	AA	FRAP	DPPH	Phenolics	MDA	REL	RS	NRS
AA	0.25								
FRAP	**0.51**	0.17							
DPPH	**−0.60**	−0.20	−0.34						
Phenolics	0.48	0.15	**0.61**	−0.38					
MDA	**−0.77**	−0.26	−0.45	**0.64**	**−0.60**				
REL	**−0.75**	−0.18	−0.36	**0.59**	**−0.63**	**0.89**			
RS	**−0.70**	−0.16	−0.35	**0.59**	−0.47	**0.72**	**0.72**		
NRS	−0.43	0.05	−0.17	0.37	−0.32	**0.51**	**0.53**	**0.54**	
TS	**−0.70**	−0.15	−0.34	**0.59**	−0.47	**0.73**	**0.72**	**1.00**	**0.58**

4. Discussion

The antioxidant activities of the mango fruit in various treatments were measured by FRAP and DPPH. Sousa et al. [29] observed an increased DPPH activity of 'Palmer' mango fruit treated with hydroxypropyl methylcellulose and beeswax (10%) during storage at 21 °C for fifteen days. To the best of our knowledge, there are no data on the effect of O_3 on DPPH scavenging activity in mango fruit. This study is the first to seek an understanding of the ozone and antioxidant activity of mango fruit. Ozone is reported to enhance the antioxidant activity of mango fruit during storage [19]. The current results are comparable to those reported by minas et al. [17] in 'Hayward' kiwifruit, where O_3 increased the DPPH scavenging activity during storage. Alothman et al. [2] reported that O_3 (8 mL/s, 30 min) reduced the DPPH scavenging activity of fresh-cut guava fruit during storage. The increased DPPH scavenging activity in the treated fruit could be attributed to the O_3 acting as a signaling molecule in inducing an antioxidant stress response in mango fruit through oxidative stress during cold storage. The FRAP assay of the O_3 treated fruit increased with the exposure time. Similar results have been reported by minas et al. [30] in 'Hayward' kiwifruit treated with O_3 (0.3 µL L^{-1}). De Almeida et al. [19] observed a decrease in the FRAP of 'Palmer' mango treated with ozonated water (1 mg $L^{-1}S^{-1}$) for 20 min.

Phenolic compounds are secondary plant metabolites that are produced through the shikimic acid pathway. The current findings confirm Awad et al. [31], who reported that chitosan coating decreased the total phenolic content in 'Hindi-Besennara' mangoes during storage. Similarly, More and Rao [26] reported that ozonated water (400 mg h^{-1}) decreased the phenolic content of 'Alphanso' mango stored at ambient temperature for fifteen days. The higher phenolic content observed in the EC could be attributed to the mLE antioxidants infusing into the mango fruit. The EC forms a semipermeable barrier on the fruit surface, allowing the infusion of antioxidants into the fruit [10,32]. Furthermore, a modified atmosphere caused by the EC in the fruit could decrease the respiration rate, leading to delayed biochemical processes associated with phenolic compound synthesis.

The current results of the O_3 treatment could be attributed to the phenylalanine ammonialyase enzyme (PAL) synthesizing phenolic compounds [5]. Ozone could induce the synthesis of phenolic compounds by the PAL enzyme, thus alleviating oxidation stress [2]. minas et al. [17] reported that kiwifruit phenolic extracts treated with ozone (0.3 µL L^{-1}) suppressed the damage caused by peroxynitrite and hydroxyl radicals. The ozone could activate the antioxidant defense mechanism through the biosynthesis of the phenolic compounds linked to the shikimate pathway. Therefore, the high phenolic content in the O_3 treated fruit could be responsible for suppressing the DNA damage caused by peroxynitrite and hydroxyl radicals during oxidative stress. Our results show that incorporating the EC with O_3 maintains the phenolic content in mango fruit during storage.

The ascorbic acid content of mangoes decreases during storage due to the oxidation process [26]. In the current study, the EC preserved the AA content of mango fruit during storage. Sausa et al. [29] reported that HPMC and 10% beeswax maintained the AA content in 'Palmer' mangoes during storage at 21 °C for fifteen days. The retention of AA content

in coated fruit could be attributed to EC concentration and reduced oxygen permeability, which can prevent the oxidation of AA. Abd El-Razek et al. [33] observed that moringa coating (10%) decreased the oxygen permeability and preserved the AA content of 'Zelda' mangoes during storage at 10 °C for forty-two days. The mangoes treated with ozone (36 h) had a high content of AA compared to the untreated fruit. The current results are similar to de Almeida [19] in 'Palmer' mangoes soaked in ozonated water for twenty minutes. The ozone and EC maintained the AA, which could be scavenging reactive oxygen species, thus reducing the oxidative stress and delaying senescence.

Ozone decreases the respiration rate and ethylene production, delaying all the biochemical processes involved in fruit ripening [34]. Studies by Aday and Caner [35] revealed that O_3 (0.07 mg/L) treatment maintained the textural properties of strawberries stored at 4 °C for thirty-five days. The current results of the EC are comparable to those of Escamilla-García et al. [21]. The authors reported that chitosan-starch coating (1%) maintained the firmness of 'Maradol' papaya during storage at ambient temperature for fifteen days. Tesfay and Magwaza [20] reported that moringa 2% + CMC 1% delayed the electrolyte leakage in 'Hass' avocado fruit during storage at 5.5 °C for twenty-one days. It could be hypothesized that EC creates a modified atmosphere within the fruit, leading to biochemical changes of the cell membrane, thus decreasing electrolyte leakage and fruit softening. In the current study, the increasing REL during storage could be an indication of the modification of the cell wall structure and decreased fruit texture.

The MDA is the end product of unsaturated fatty acids peroxidation and a crucial measure of fruit quality [33]. The current results are comparable to Tesfay and Magwaza [20], where moringa 2% and CMC 1% decreased the MDA content in 'Hass' avocados stored at 5.5 °C for twenty-one days. The oxidative stress causes excessive oxygen reactive species production, which leads to cell membrane injury [33]. Similar results have been reported in 'Soreli' kiwifruit treated with O_3 (300 ppb) stored at 2 °C for sixty days. Ozone reacts with fatty acids to form ozonides, which affects lipid fluidity [36]. The current results indicate that preserving the membrane integrity in mango fruit is associated with delaying the accumulation of MDA and REL and reduces the rate at which phenolics are used. Additionally, these results indicate that O_3 modifies the lipid membrane, which inhibits oxidative stress and prevents membrane damage.

During mango fruit ripening, the NRS decreases and the RS increases due to starch hydrolysis. The current results of the NRS contradict those of Ncama et al. [15], who reported that mLE + CMC (2%) had no significant effect in 'Marsh' grapefruit. Barboni et al. [37] observed an increase in the RS in 'Hayward' kiwifruit treated with O_3 (4 mg/h) and stored at 0 °C for twenty-three days. Studies by Shalluf et al. [38] reported a decline in the sucrose and increased fructose and glucose content of 'Elegance' tomatoes treated with O_3 (0.5 mg O_3/g) and stored at 15 °C for five days. The increase in the soluble sugars could be attributed to the biosynthesis of sucrose by the sucrose phosphate synthase enzyme (SPS). Sucrose synthase (SS), SPS, acid invertase, and neutral invertases affect fruit's glucose, sucrose, and fructose ratio [39]. The SPS and SS are the key enzymes involved in sucrose metabolism in mango fruit during fruit ripening [40]. Tanou et al. [41] reported a low level of sucrose phosphate synthase expression in 'Hayward' kiwifruit treated with SNP (100 µM) and ozone (0.3 µL L^{-1}) during storage (0 °C for two months, 20 °C for eight days). In mango fruit, the increase in the soluble sugars is associated with fruit ripening and the accumulation of sweetness taste.

5. Conclusions

This is the first study to present findings on the combined effect of moringa leaf extract–CMC coating and gaseous O_3 on the postharvest treatment of mango fruit. The results indicated that the combination of the EC and gaseous O3 (36 h) effectively maintained the firmness and delayed electrolyte leakage and MDA accumulation. These treatments also decreased the accumulation of sugars, delaying ripening in mango fruit. The enhanced phenolic content was associated with an increased FRAP. Moreover, the EC effectively

maintained the AA content of the mango fruit. The current results suggest that increasing the O_3 exposure time does not negatively affect the total phenolic content and soluble sugars during the storage of mango fruit. The present results provide the mechanism of action and application time of ozone in maintaining the antioxidant activity and quality of mango fruit. These results indicate that the postharvest treatment combination of EC and O_3 can preserve the membrane integrity, antioxidants, and enhance the fruit quality.

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