THE EFFECT OF COMPOSITE EDIBLE COATING: CARBOXYMETHYL CELLULOSE AND MORINGA LEAF EXTRACT ON THE POSTHARVEST QUALITY OF 'GEM' AVOCADO FRUIT TREATED AT DIFFERENT HARVEST MATURITY STAGES

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ABSTRACT
Edible coatings play a critical role in reducing postharvest losses during storage and supply chain of horticultural commodities. The present study evaluated the efficacy of different concentrations of moringa leaf extract (MLE) combined with carboxymethyl cellulose (CMC) edible coating in preserving the quality and extending the shelf life of 'GEM' avocados. Fruit were harvested at different stages of maturity as evaluated by dry matter content. Different concentrations of moringa (8% and 16%) extracted with either chilled ethanol (100%) or nonchilled ethanol (50%) and functionalised with CMC (5%) were used to treat the fruit. Treated fruit were then stored at 5.5 ºC and 90% RH for 28 days plus an additional seven days at 23 ºC. The changes in physicochemical and biochemical fruit attributes were evaluated at weekly intervals. The application of moringa and CMC-based edible coatings maintained the quality of 'GEM' avocados by preserving phenolics, flavonoids, and antioxidant activity. The treatments significantly ($P < 0.001$) reduced the loss of mass and firmness. Furthermore, treated fruit were found to have delayed colour change and reductions in sugar concentrations, particularly mannoheptulose, compared to the control treatment. Therefore, edible coatings prepared by combining CMC and MLE could be the best alternative for substituting the currently used health-compromising synthetic chemicals.

Keywords: Postharvest losses; fruit quality; edible coatings; shelf life; nutritional compounds

INTRODUCTION
Avocado (*Persea americana* Mill.) is one of the most economically essential fruits from the *Lauraceae* plant family. This fruit is mainly produced in tropical and subtropical regions (Eça *et al*., 2014). The consumption of avocado is steadily increasing because of the health-related benefits it has, giving it status as a "super fruit" (Sivakumar *et al*., 2021). Its mesocarp tissue consists of bioactive phytochemicals, such as sterols, vitamin E, and carotenoids, that provide antioxidants and radical scavenging activities (Bill *et al*., 2014). A recent report by Sivakumar *et al*. (2021) highlighted that the world avocado market would reach a market value of about US$21.56 billion by 2026. However, the rate of postharvest losses remains a serious threat to this target.

Most avocados produced in countries such as Spain, Chile, Israel, and South Africa are exported to distant overseas markets, mainly Europe (Kassim *et al*., 2013). Usually, it takes about 21 or more days to transport fruit from South Africa to the target market. Generally, fruit maturity at harvest significantly influences its postharvest storage life and quality, impacting decisions around marketing, handling, and transport (Kader, 1997). Therefore, the decision to harvest must accommodate time to transport and make marketing flexible (Magwaza and Tesfay, 2015). Most commercial operations use dry matter content, moisture content, or mesocarp oil content to determine the maturity of avocados at harvest (Magwaza and Tesfay, 2015; Rivera *et al*., 2017). Amongst these maturity indices, dry matter is preferable due to its cost-efficacy and least amount of time required, making this technique convenient (Blakey *et al*., 2012).

Due to its climacteric nature, avocados produce more ethylene and continue to ripen during storage. This compromises the shelf life and makes it difficult to market this fruit, especially in international markets with a long transporting period. The avocado industry is highly dependent on various synthetic edible films and coatings after harvest and before storage to maintain the quality and extend the shelf life of this fruit (Liu *et al*., 2020). Most authors have highlighted the need to develop eco-friendly treatments to replace synthetic fungicides. This is due to health-related concerns caused by the application of chemical-based treatments. Besides their environmental unfriendliness and high residues left in the fruit's edible portion, most pathogens have also developed resistance against some of these fungicides (Sivakumar and Bautista-Baños, 2014; Romanazzi *et al*., 2018; Sivakumar *et al*., 2021).

Polysaccharide coating materials have gained popularity for their application in fresh produce because of their characteristics, such as exceptionally high stability and solubility (Panahirad *et al*., 2021). Coatings from polysaccharides are the most convenient ones due to their easy accessibility, non-toxicity, and cost effectiveness (Singh *et al*., 2019). Amongst cellulose derivatives, hydropropyl cellulose (HPC), methylcellulose (MC), hydropropylmethyl cellulose (HPMC), and carboxymethyl cellulose (CMC) have been extensively used for coating most fruit and vegetables (Maftoonazad and Ramaswamy, 2005; Malmiri *et al*., 2011). However, CMC is the most commonly used commercial derivative, with greater production and applications in the food sector (Dhall, 2016). This is due to its easily accessibility because of its reasonable price and its nontoxicity as a polysaccharide, thus making it safe for human consumption.

Moringa oleifera Lam has recently drawn more research attention for its use in postharvest quality preservation. This is due to the exceptional performance of edible coatings containing moringa extract in suppressing fruit postharvest diseases, thereby preserving the fruit quality and extending its shelf life (Tesfay and Magwaza, 2017; Tesfay *et al*., 2017). Most developed countries have opted to use fresh organic products in food preservatives, which necessitates continued research aiming to develop or improve organic postharvest treatments. This study therefore evaluated the effect of moringa leaf extract and carboxymethyl cellulose edible coating on the quality and shelf life of 'GEM' avocados.

MATERIALS AND METHODS

1. Preparation of moringa leaf extracts

Fresh moringa leaf powder was obtained from the Agricultural Research Council (ARC), located in Pretoria, South Africa. Moringa extracts were prepared following a modified method previously described by Addo *et al*. (2022), using chilled 100% ethanol (ETH 1), which was firstly refrigerated at -20 ºC overnight before use, and non-chilled 50% ethanol (ETH 2). Different moringa extracts were prepared, specifically 8% (g/v) and 16% (g/v).

2. Preparation of coating solution

To prepare the coating solution, 50 g of CMC powder was dissolved in 1 L of the prepared moringa solution to obtain 5% CMC.

3. Application of treatments and storage

The 'GEM' avocados used in this study were supplied by Westfalia Fruit (Pty) Ltd commercial farm located in Howick, South Africa. The fruit were harvested at different maturity stages, as determined by dry matter content (DM) (25, 27, and 30% DM for fruit harvested – maturity stages M1, M2, and M3 respectively). From each maturity stage, a total of 250 fresh avocado fruit, free from mechanical damage and diseases, were assigned to five treatments: Control (T1), 5% CMC + 8% MLE/ETH 1 (T2), 5% CMC + 16% MLE/ETH 1 (T3), 5% CMC + 8% MLE/ETH 2 (T4), and 5% CMC + 16% MLE/ETH 2 (T5). Each treatment was assigned 50 fruit and replicated five times, with each replicate having 10 fruit. Just before cold storage, five fruit were sampled to assess the fruit status at harvest and as a reference. Before the application of treatments, all fruit were first washed with distilled water to avoid any potential contamination. The fruit were dipped into their assigned treatment for one minute, whereas the control was only washed with distilled water and no treatment was applied. Following treatments, fruit were allowed to dry at room temperature, placed in labelled open boxes, and kept at 5.5 ºC and 90% relative humidity (RH) for 28 days. After 28 days of cold storage, the fruit were transferred to room temperature (23 ºC) at the laboratory shelf life benches for seven days. The changes in fruit quality were observed at weekly intervals throughout the 35-day storage period.

4. Evaluation of postharvest fruit quality

4.1 Fruit firmness

Fruit firmness was measured using a whole-fruit compression analysis described by Jeong and Huber (2004).

4.2 Fruit mass loss percentage

The fruit mass was measured using a digital weighing scale (RADWAG Wagi Electronic Inc., Poland) and determined as a percentage of mass loss using Eq. 3.1:

$$\% \text{ Mass loss (ML)} = \frac{IM - FM}{IM} \times 100 \quad (1)$$

Where ML = mass loss (%),
IM = initial mass of fruit (g),
and FM = final mass of fruit (g).

4.3 Fruit colour
Avocado fruit colour was determined on five fruit per treatment using a CR 400 Chromameter (Minolta Co. Ltd., Osaka, Japan). The values for L^*, a^*, and b^* were recorded. The value for Hue angle (H^*) was also recorded.

4.4 Total Phenolic Content (TPC)
The determination of phenolic compounds was performed following a slightly modified FolinCiocalteau method previously described by Milbury *et al*. (2006).

4.5 Total Flavonoid Content (TFC)
The total flavonoid (TF) concentration was determined following the method previously described by Obeng *et al*. (2020), with slight modifications.

4.6 2,2' Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay
The DPPH assay was used to estimate avocado mesocarp tissue's free radical scavenging ability following the modified method previously described by Fan *et al*. (2022). The DPPH scavenging capability was calculated using Eq. 2:

$$\text{Inhibition (\%)} = \frac{Ac - At}{Ac} \times 100 \quad (2)$$

Where Ac = absorbance of control;
At = absorbance of the extract.

4.7 Determination of C7 sugars (Mannoheptulose and Perseitol)
Determination and quantification of soluble sugars were based on the slightly modified method previously described by Tesfay and Magwaza (2017).

Statistical analysis
The collected data were subjected to analysis of variance (ANOVA) using GenStat statistical software (GenStat 20th Edition, VSN International Ltd, UK). The mean separation was performed using Duncan's Multiple Range Test (DMRT) at 5% significance level.

RESULTS AND DISCUSSION

Fruit firmness
Fruit firmness is one of the key quality attributes influencing consumer purchase decisions and determining the shelf life and market value of most fresh fruits. The firmness of fruit is mainly affected by different factors such as harvest maturity, relative humidity, and storage temperature. This study showed a significant change in fruit firmness for both the treated and untreated avocado fruit during the 28 days of cold storage (5 ºC) and 7 days of shelf life at room temperature (23 ºC) (Table 1). As expected, a significant firmness loss was observed after 28 days of storage when the fruit were transferred to 23 ºC. Generally, firmness loss occurs due to water loss, mainly regulated by temperature (Paniagua *et al*., 2013). The results demonstrated a significant effect of the interaction between harvest time and treatments ($p < 0.05$) on fruit firmness loss during the storage period. As usual, all fruit showed firmness loss during the storage period regardless of the harvest time and treatments; however, the loss was severe in untreated fruit (Table 1). At the end of the storage period, the untreated fruit recorded lower firmness than all the treated fruit which were 6.59, 7.91, and 6.81 N for maturity stages M1, M2, and M3, respectively. According to the ripening standards described by Jeong and Huber (2004) for avocado fruit, based on the whole fruit compression analysis, the untreated fruit were overripe (<10 N) and no longer suitable for markets. Briefly, these standards classify fruit as ripe and ready for consumption when the whole fruit compression attains values ranging between 10 and 20 N. The firmness declines to below 10 N on overripe fruit (Jeong and Huber, 2004). Although no sensory evaluations were conducted, the results from this study are aligned with these classifications based on the observations and statistical analysis.

Different concentrations of MLE in combination with CMC, delayed firmness loss depending on the harvest time; however, the chilled treatments were more effective than non-chilled. Given that all the untreated fruit had compression values of less than 10 N at the end of the storage period, this indicated that the MLE and CMC composite coating could delay fruit ripening, thereby delaying the rate of fruit softening. Based on these results, the different concentrations (8% and 16%) of chilled MLE and CMC used in this study potentially delayed changes that take place in different components, such as cell wall structure weakening, hydrolysis of cellulose and hemicellulose, loss of membrane integrity, and depolymerization of pectin and starch, thereby delaying firmness loss (Yaman and Bayoındırlı, 2002). Combining 8% chilled MLE and CMC delayed firmness loss on fruit harvested at maturity stages 1 and 3, whereas increasing the concentration to 16% resulted in reduced firmness loss on fruit harvested at maturity stage 2. The efficacy of these treatments could be due to the presence of CMC.

The carboxylic group in CMC's chemical structure results in hydrogen atoms bonding inside the coating matrix and between the coating and the fruit peel, resulting in preserved firmness (Panahirad *et al*., 2019). This positive effect may also be attributed to reduced enzyme activity, including pectin-methylesterase, which contributed to delayed fruit ripening. Pectin methylesterase is a major enzyme that depolymerizes pectin substances (Payasi *et al*., 2009). This also implies that the coatings could serve as a gas barrier, as the enzymatic activities are reduced by low oxygen and high carbon dioxide concentrations, which ultimately help maintain fruit firmness (Payasi *et al*., 2009). Similarly, Kubheka *et al*. (2019) reported a reduced firmness loss in 'Maluma Hass' avocado treated with 1% CMC and moringa leaf extract.

Fruit mass loss (%)

Mass loss is mainly caused by the water lost during metabolic processes such as transpiration and respiration, and its rate depends on the storage environment (Abebe *et al*., 2017). This loss of water takes place through stomatal openings and skin cracks. The storage temperature and relative humidity impact the fruit mass loss due to the effect caused by the differences in vapour pressure between the fruit and the atmosphere (Wróblewska-Krepsztul *et al*., 2018). This was evident when the fruit from all the treatments showed the highest mass loss during the last week, from days 28 to 35, when the fruit were transferred to ambient conditions (23 ºC), compared to cold storage (Fig. 1). The interaction between coatings, storage period, and harvest time significantly affected the fruit mass ($P < 0.05$). All fruit suffered a weight loss throughout the storage time; however, the untreated fruit suffered the most, especially after cold storage, at ambient temperature. All the evaluated edible coating treatments resulted in a lower mass loss percentage than the control for all harvests. The CMC and MLE treatments preserved the fresh mass of treated fruit throughout the evaluated 28 days of cold storage at 5 ºC and 7 days of shelf life at 23 ºC. However, 16% chilled MLE was more effective than all other treatments. This can be attributed to the hydrophilic nature of these treatments. It can be argued that the treatments inhibited the transfer of water between the fruit and the atmosphere by forming a semipermeable layer that acted as a barrier between the fruit and the environment, covering the fruit surface and protecting it from mechanical injury and thereby reducing desiccation (Khorram *et al*., 2017). These results agree with those of Tesfay *et al*. (2017) who reported a reduced mass loss in avocado fruit treated with CMC combined with moringa leaf or seed extract. Similar results were also reported by Kubheka *et al*. (2019) where the CMC (1%) incorporated with moringa reduced the avocado mass loss throughout the 21 days of cold storage and 7 days of shelf life. Another study conducted by Zhang *et al*. (2019) reported that *Osmunda japonica*-CMC coatings significantly reduced the water loss in tomato fruit compared to untreated fruit. The fruit mass loss, caused by water loss, may also result in changes in the whole fruit texture and flavour (Ballesteros *et al*., 2022), and eventually the fruit starts to decay as the loss gets severe, which was evident in this study.

Fruit colour

Fruit colour is the best indicator for the ripening stage in avocados, particularly the 'GEM' cultivar. This cultivar is characterised by a ripening process that is accompanied by the colour change from green to purple or black. This study showed a decrease in yellowness (b*), lightness (L*), and hue angle (hº) values (Fig. 3, Fig. 4, and Fig. 5). In contrast, the greenness (a*) values increased during storage, as the fruit ripens regardless of the treatments and harvest time (Fig. 2). There was negligible colour change during the cold storage period, with significant changes observed between days 28 and 35. These observations agree with Mwelase *et al*. (2022) who also reported the influence of temperature on avocado fruit colour. Similarly, the higher temperature accelerated the avocado colour change compared to cold storage. There was a significant effect ($p < 0.001$) of treatments and storage time on the decrease in hº and L* values. Inversely, no statistical difference ($p > 0.05$) existed for the increase in a* and decrease in b* values. This observed increase in a* value from negative to positive indicates colour reduction from greener to red with fruit ripening, which was clearly expected in this study. This could indicate that the

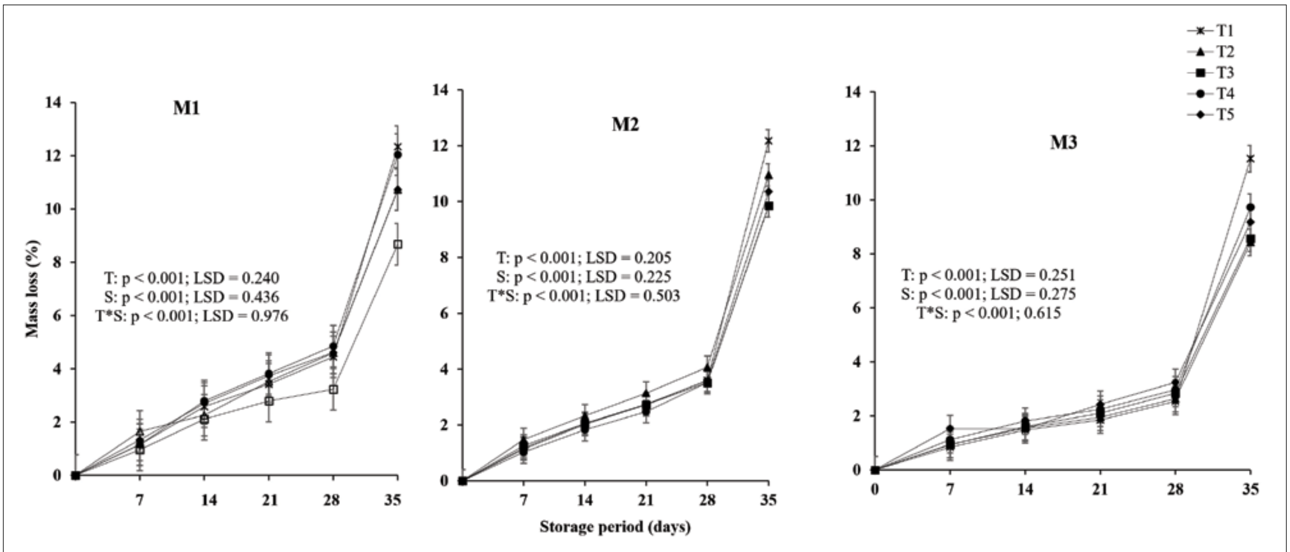

Figure 1: Mass loss of 'GEM' avocado fruit harvested at different maturity stages (M1, M2, and M3) as influenced by CMC and different MLE concentrations during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

Table 1: The effect of CMC and different MLE concentrations on the firmness (N) of 'GEM' avocados harvested at different maturity stages during 28 days cold storage at ±5 ºC followed by seven days shelf life at ±23 ºC

		Storage period (days)					
Harvest time	**Treatment**	**0**	**7**	**14**	**21**	**28**	**35**
Maturity Stage 1 (25% DM)	Control	222.42 ± 23.82^{a}	198.96 ± 19.62^{bc}	174.26 ± 28.45^{bcd}	130.54 ± 40.28^{ab}	40.94 ± 22.73^{a}	6.59 ± 0.98^{a}
	CMC + 8% chilled MLE	222.42 ± 23.82^{a}	202.85 ± 13.62^{c}	181.55 ± 21.37^{cd}	125.99 ± 38.32^{a}	81.39 ± 22.27^{bc}	18.58 ± 2.80^{d}
	CMC + 16% chilled MLE	222.42 ± 23.82^{a}	192.85 ± 20.65^{bc}	186.64 ± 23.75^{d}	162.13 ± 19.06^{b}	39.45 ± 17.98^{a}	15.14 ± 2.71^{cd}
	CMC + 8% nonchilled MLE	222.42 ± 23.82^{a}	188.84 ± 18.14b^{c}	160.29 ± 21.53^{abc}	146.64 ± 13.10^{ab}	110.63 ± 18.27^{de}	14.32 ± 0.67^{cd}
	CMC + 16% nonchilled MLE	222.42 ± 23.82^{a}	190.50 ± 22.57^{bc}	167.99 ± 4.53^{abcd}	162.23 ± 3,40^{b}	58.82 ± 19.62^{ab}	16.84 ± 3.92^{d}
Maturity Stage 2 (27% DM)	Control	211.97 ± 24.02^{a}	191.29 ± 26.63^{bc}	153.30 ± 2.37^{ab}	133.30 ± 11.11^{ab}	122.34 ± 16.28^{de}	7.91 ± 0.79^{ab}
	CMC + 8% chilled MLE	211.97 ± 24.02^{a}	195.21 ± 18.84^{bc}	166.74 ± 7.03^{abcd}	152.91 ± 11.76^{ab}	96.56 ± 30.79^{cd}	16.3 ± 2.93^{d}
	CMC + 16% chilled MLE	211.97 ± 24.02^{a}	194.98 ± 13.80^{bc}	159.79 ± 5.21^{abc}	152.08 ± 3.50^{ab}	118.63 ± 5.74^{de}	18.17 ± 4.7^{d}
	CMC + 8% nonchilled MLE	211.97 ± 24.02^{a}	186.27 ± 12.69^{bc}	159.15 ± 7.04^{abc}	157.12 ± 7.93^{ab}	119.89 ± 2.08^{de}	9.71 ± 0.98^{ab}
	CMC + 16% nonchilled MLE	211.97 ± 24.02^{a}	185.29 ± 7.18^{bc}	166.21 ± 9.26^{abcd}	143.66 ± 6.58^{ab}	81.69 ± 9.66^{bc}	14.45 ± 2.65^{cd}
Maturity Stage 3 (30% DM)	Control	194.41 ± 12.71^{a}	156.58 ± 7.80^{a}	144.99 ± 3.44^{a}	129.33 ± 3.73^{ab}	115.26 ± 6.38^{de}	6.81 ± 0.88^{a}
	CMC + 8% chilled MLE	194.41 ± 12.71^{a}	181.02 ± 8.92^{bc}	173.50 ± 7.33^{bcd}	163.28 ± 13.77^{b}	125.60 ± 9.42^{de}	11.89 ± 2.40^{bc}
	CMC + 16% chilled MLE	194.41 ± 12.71^{a}	187.54 ± 10.63^{bc}	165.64 ± 12.04^{abcd}	152.09 ± 16.20^{ab}	136.21 ± 8.06^{e}	9.56 ± 1.96^{ab}
	CMC + 8% nonchilled MLE	194.41 ± 12.71^{a}	174.64 ± 12.65^{ab}	160.91 ± 4.56^{abc}	140.44 ± 13.42^{ab}	129.40 ± 9.02^{e}	11.58 ± 1.34^{bc}
	CMC + 16% nonchilled MLE	194.41 ± 12.71^{a}	187.96 ± 3.05^{bc}	150.34 ± 7.61^{ab}	138.50 ± 12.36^{ab}	129.31 ± 6.25^{e}	11.55 ± 0.90^{bc}
l.s.d (H*T)		**28.98**	**20.78**	**20.49**	**28.94**	**26.60**	**4.041**
P-value							
Harvest time (H)		**< 0.001**	**0.002**	**0.004**	**0.879**	**< 0.001**	**< 0.001**
Treatment (T)		**1.000**	**0.268**	**0.033**	**0.071**	**0.004**	**< 0.001**
*** Harvest time**	**1.000 0.206 0.331 0.185 <0.001 0.021 Treatment**						

*The results were presented as mean ± standard deviation (SD). Mean values in the same column followed by the same letter(s) shows no significant difference according to Duncan's Multiple Range Test (DMRT) Test (P = 0.05); H, harvest time; T, treatment; CMC, carboxymethyl cellulose; MLE, moringa leaf extract.

composite edible coating of MLE and CMC potentially delays the transition of chloroplasts into chromoplasts that contain yellow and red pigments, thereby inhibiting colour change and enzymatic browning (Sharma *et al*., 2019).

The correlation between the a*, b*, L*, and h⁰ values in this study is in line with Handayani *et al*. (2018) who reported an inverse relationship between the a* and b* values on avocados treated with cassava peel edible coating. There was a highly significant effect ($p < 0.001$) of treatments and storage time on L* and h*; however, a sharp decline was observed between days 28 and 35 at room temperature. This delayed colour change observed in coated fruit could be linked to the effect of coatings in modifying the fruit's atmosphere. Edible coatings slow the respiration rate and ethylene accumulation (the ripening hormone) (Ali *et al*., 2011). The results for the L* values and visual judgments also indicated that the temperature, especially in cold storage, was suitable for storing avocado without causing chilling injury, which causes the darkening of fruit pulp (Careli-Gondim *et al*., 2020).

Total phenolics

Phenolics are produced in fruit tissues as secondary metabolites that activate antioxidants against oxidative stress (Peretto *et al*., 2017). These phyto-

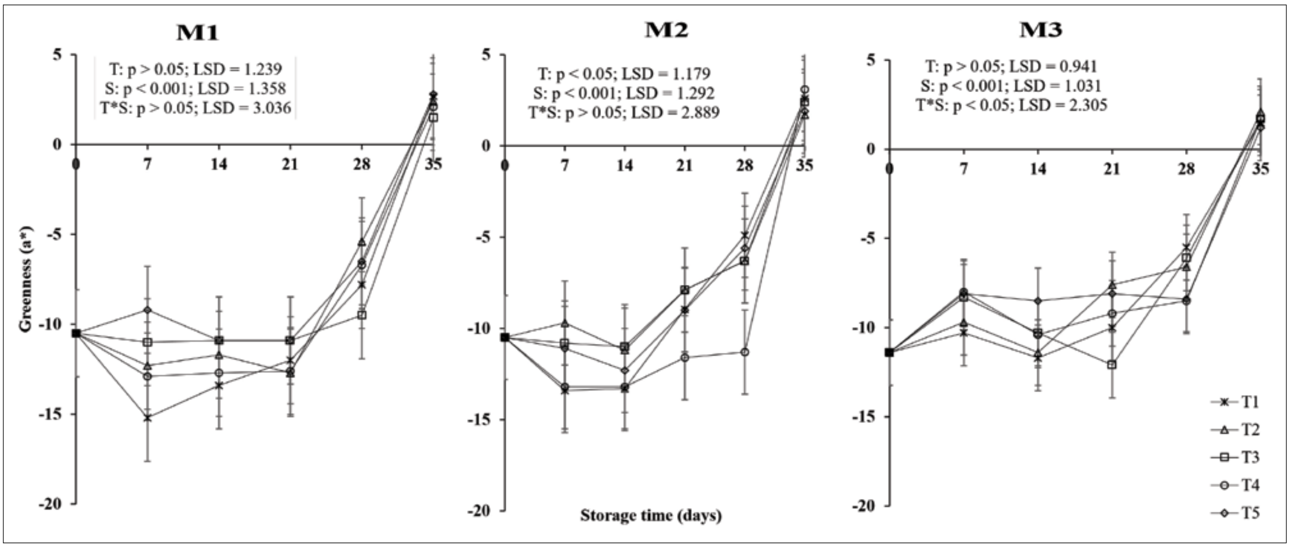

Figure 2: The effect of CMC and MLE composite coating on the exocarp colour (a*) of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

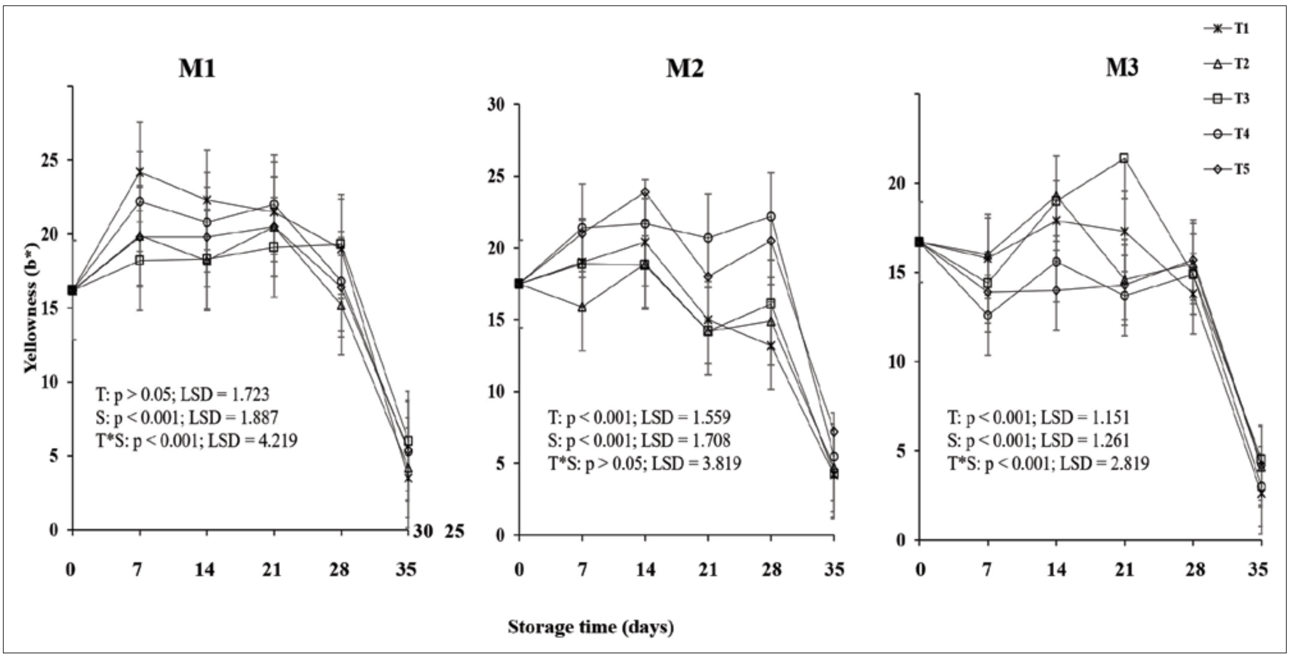

Figure 3: The effect of CMC and MLE composite coating on the exocarp colour (b*) of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days shelf life storage. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

chemicals have a crucial role in the sensory and nutritional properties of the produce. The storage time, conditions, and stress severity affect the secondary metabolites in fruit. While storage period and harvest time showed a significant effect ($p < 0.001$), no statistically significant difference ($p > 0.05$) existed between treatments on the total phenolic compounds produced. The changes in total phenolics followed the same trend for all harvest times (Fig. 6). Early harvested fruit showed a decline in phenolic content for the first seven days of cold storage; after that, slight changes occurred depending on treatments. The decline in phenolics could be caused by the stress induced by the cold storage. These observed changes in phenolics may also result from applying edible coatings: edible coatings have previously been reported to influence the production of phenolic compounds by modifying its metabolism, resulting in abiotic stress (Dávila-Aviña *et al*., 2014). There were no remarkable differences in the fruit's phenolic content throughout the storage period between the treatments, especially in mid- and late harvested fruits. This indicates that, besides the potential of the CMC and MLE coatings to extend shelf life, they can also

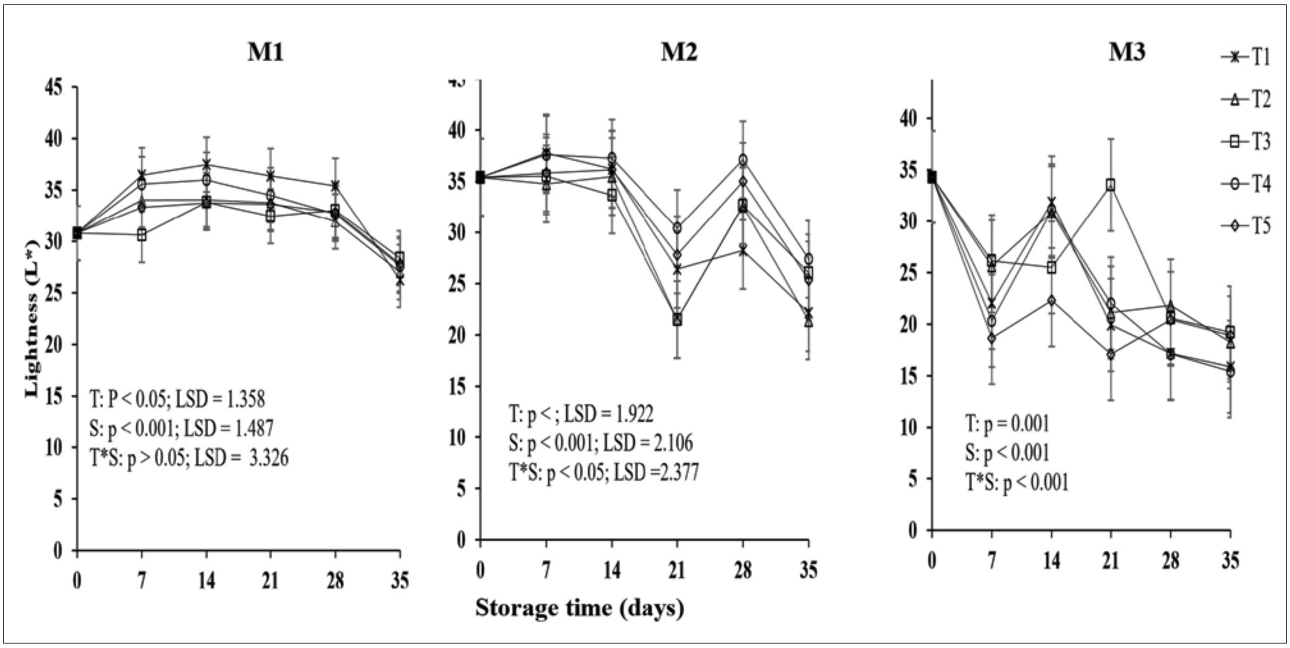

Figure 4: The effect of CMC and MLE composite coating on the exocarp colour (L*) of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

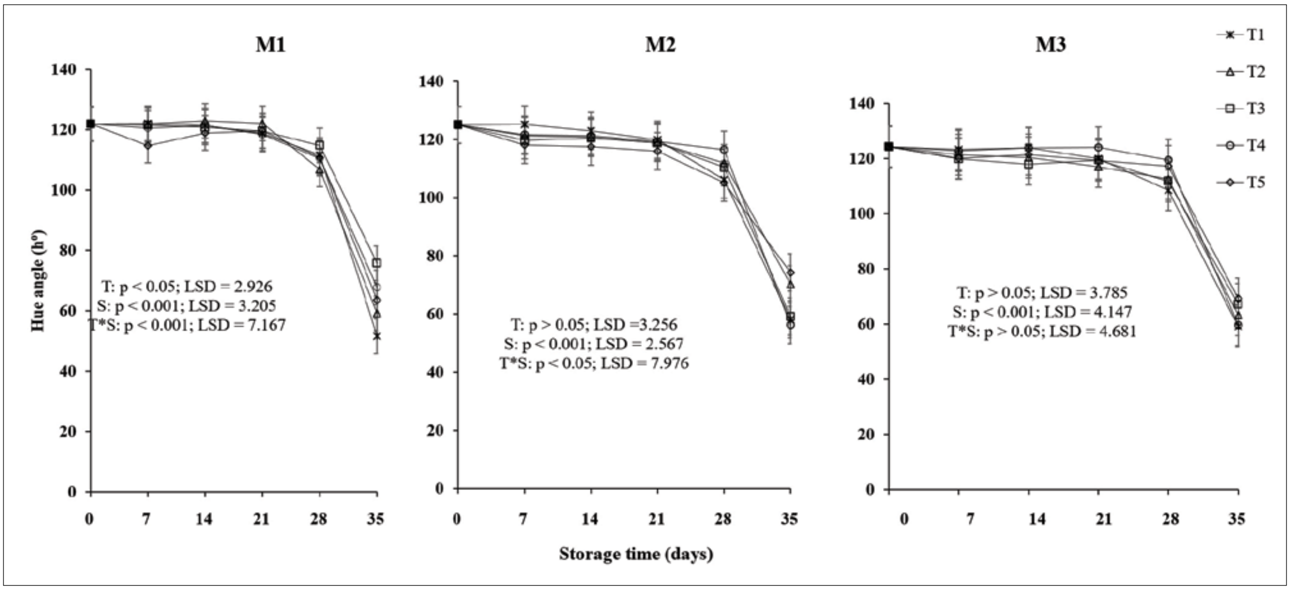

Figure 5: The effect of CMC and MLE composite coating on the exocarp colour (h°) of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 5; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

maintain fruit phenolic concentration. This corroborates with Maringgal *et al*. (2020), who demonstrated that edible coatings help preserve phytonutrients in fruit. Moreover, these results validate those that edible coatings modify the internal atmosphere by serving as selective barriers to O_2 and CO_2, reducing respiration rate and delaying phenolic changes (Awad *et al*., 2017). The findings from this study are consistent with those presented by Chiabrando and Giacalone (2015) where the decrease in phenolic content and antioxidant capacity in blueberries was delayed by applying polysaccharide (chitosan) coatings. However, it is important to mention that CMC, in combination with 16% non-chilled MLE, resulted in a slightly increased phenolic content than all the other treatments at the end of the storage period, particularly in fruit at maturity stages 1 and 3. At the same time, the combination of CMC and 8% chilled MLE produced a slight increase in phenolics in fruit at maturity stage 2. This indicates that the coatings were able to delay fruit senescence which results in disrupted cell structure, thereby leading to reduced phenolics (Riaz *et al*., 2021). Consequently, the observed differences may have resulted from a higher respiration rate in untreated fruit associated with the breakdown of total phenols (Nair *et al*., 2018).

Total Flavonoids content

Flavonoids are secondary metabolites that resemble variable phenolic structures and are involved in colouring of many fruits, vegetables, and flowers. These phytochemicals also have health benefits such as anti-cancer, anti-inflammatory, and antioxidant properties (Zahedi *et al*., 2019). In the present study, total flavonoids were significantly ($p < 0.001$) affected by the storage period and harvest time (Fig. 7). However, the coating treatments did not affect flavonoid concentrations ($p > 0.05$). Cordenunsi *et al*. (2005) reported that storage conditions influence the concentration of flavonoids. The total flavonoid concentration was similar for all treatments and harvesting times; however, the untreated fruit harvested at maturity stage 1 showed an increased flavonoid content after 35 days of storage. This was in contrast to the fruit treated with CMC combined with 16% non-chilled MLE which resulted in fruit with lower flavonoid content than all other treatments, regardless of harvest time. These results are comparable to those presented by Panahirad *et al*. (2019) wherein plums treated with 0.5% CMC-based edible coating resulted in higher flavonoid content. In contrast, those treated with concentrations above 0.5% (1% and 1.5% CMC-based edible coating) had less flavonoid content than the untreated fruit. Langa (2018) also reported a rapid increase of flavonoids in untreated papaya fruit compared to those treated with CMC + moringa leaf or seed extract. Although the results presented in this study are inconsistent, a progressive decline in total flavonoids during the 28 days of cold storage was, however, observed. This was followed by a slight increase during the seven days of shelf life, especially in fruit harvested at maturity stages 1 and 2. Similarly, Ballesteros *et al*. (2022) reported an increase in flavonoid content in goldenberries stored at 20 ºC and 65% relative humidity for 12 days, irrespective of CMC-based coatings; this was, however,

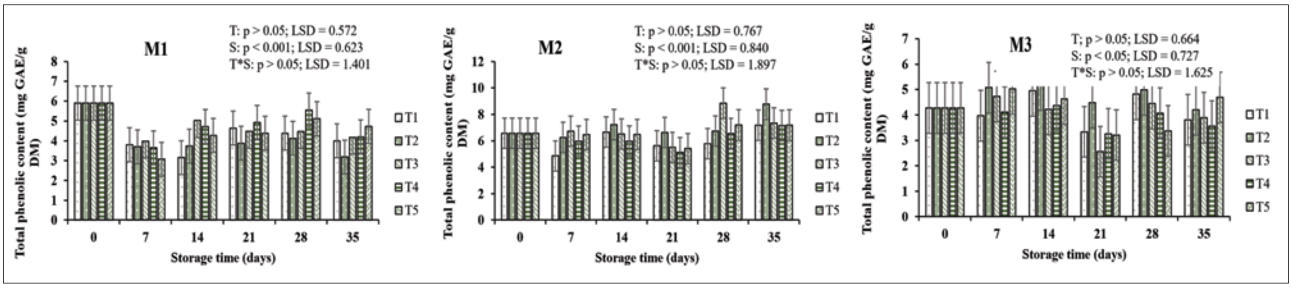

Figure 6: The effect of CMC and Moringa-based edible coatings on the changes in phenolic content of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

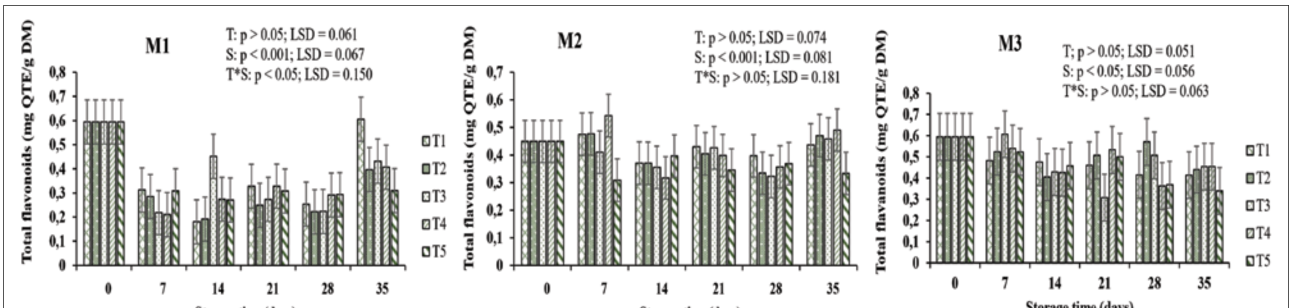

Figure 7: The effect of CMC and Moringa-based edible coatings on the changes in flavonoid content of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

inverse for fruit stored for 28 days at 4 ºC and 95% RH. These results show that storage conditions influence flavonoids. In addition, cold storage tends to decrease flavonoid content, while high temperatures increase flavonoid content.

2,2' Diphenyl-1-picrylhydrazyl (DPPH) Antioxidant Assay

Antioxidant activity is a very important parameter that determines health-related benefits and is usually determined using different methods, including the DPPH radical scavenging assay. This technique is one of the most popular methods to measure antioxidant activity due to its accuracy and convenience. The fruit's antioxidant properties are greatly influenced by the presence of various secondary metabolites, including flavonoids and phenolics (Maringgal *et al.*, 2020). This study showed a significant effect of the storage period ($p < 0.001$) on antioxidant activity of avocados. Figure 8 shows an increasing trend in the DPPH radical scavenging activity over time during the cold storage period and a decrease at ambient temperature, regardless of harvest time. Although there was no significant difference between treatments ($p > 0.05$), the reduction in antioxidants was more pronounced in uncoated fruit than in MLE and CMC-coated avocados, which may indicate a positive effect of these composite coatings. The high decline in anti-

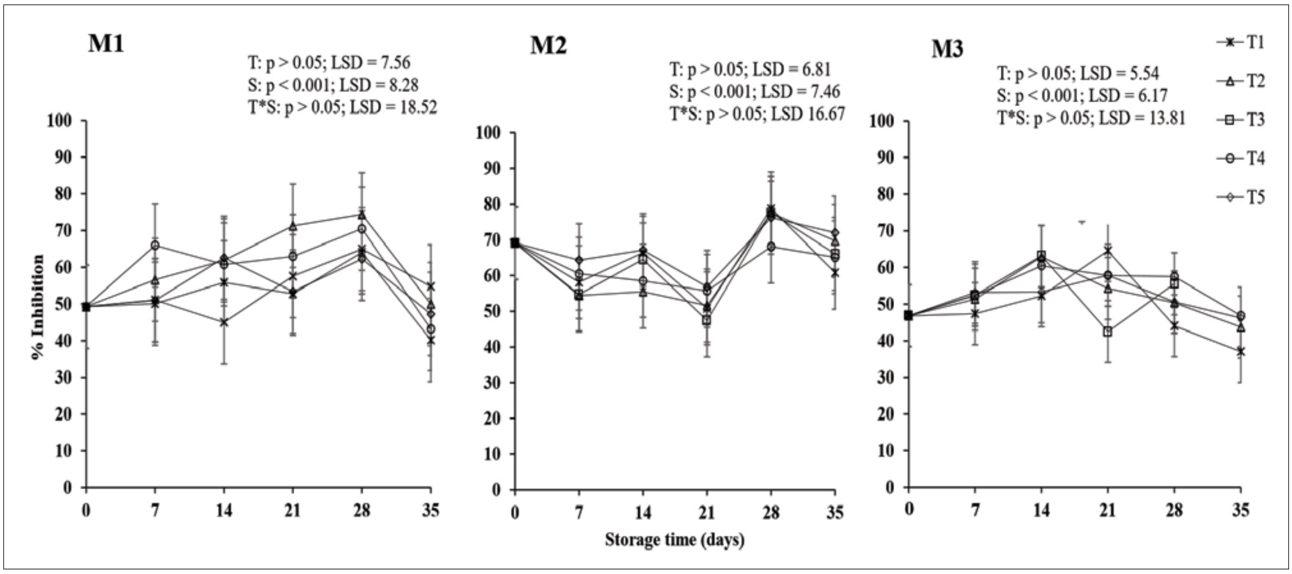

Figure 8: The effect of CMC and Moringa-based edible coatings on antioxidant activity of 'GEM' avocado fruit harvested at maturity stages M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 3; H; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

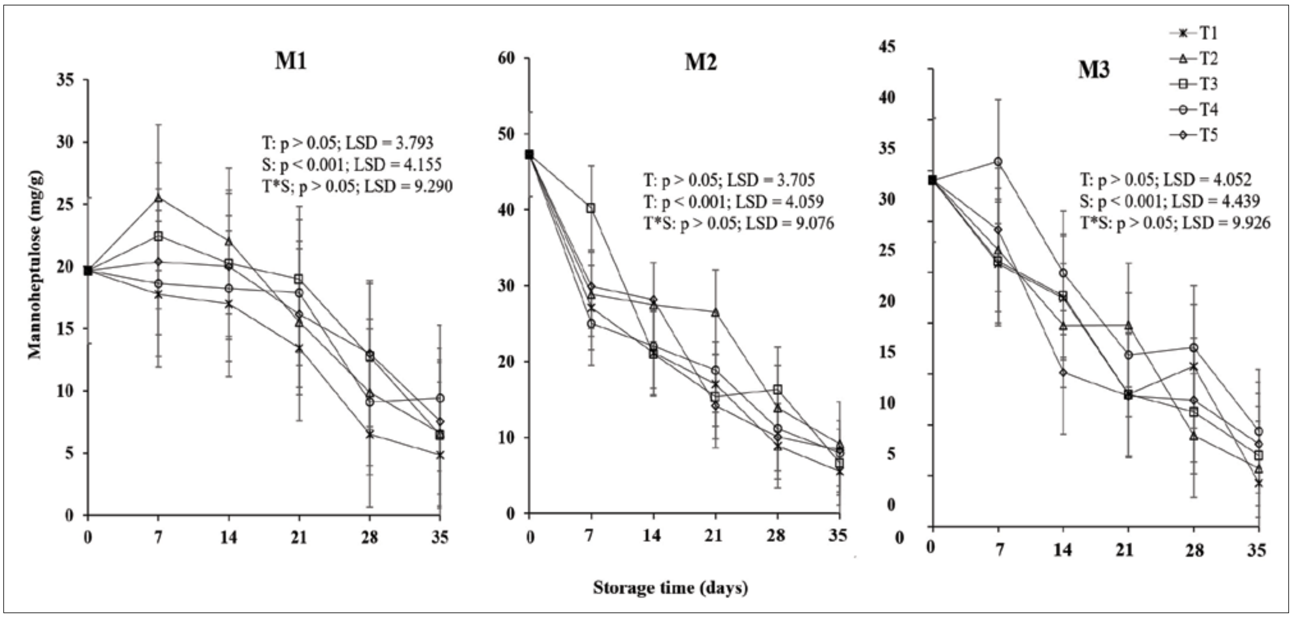

Figure 9: The effect of CMC and MLE-based edible coatings on mannoheptulose of 'GEM' avocado harvested at different maturities: maturity M1, M2, M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

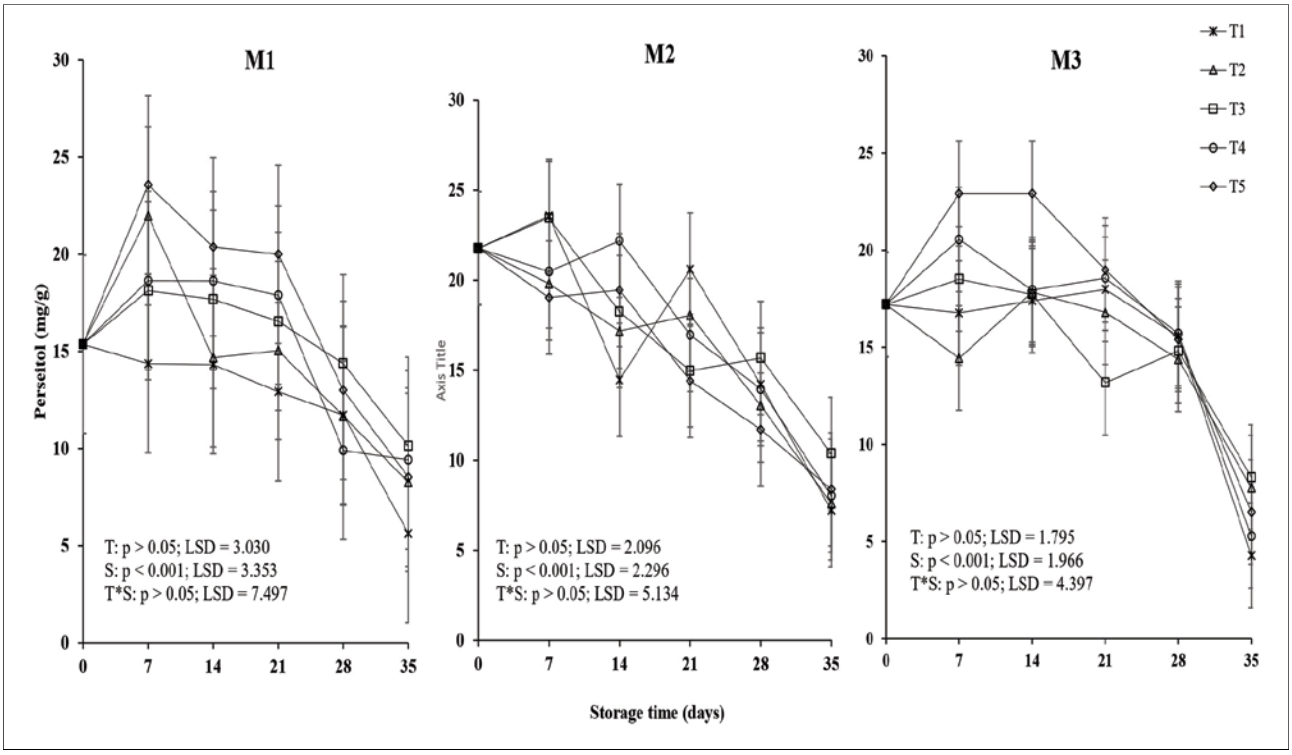

Figure 10: The effect of CMC and MLE-based edible coatings on perseitol of 'GEM' avocado harvested at maturity M1, M2, and M3 during 28 days of cold storage and seven days of shelf life. *The vertical bars represent standard error (SE) at n = 3; T, treatment; S, storage period; T1, control; T2, CMC + 8% chilled MLE; T3, CMC + 16% chilled MLE; T4, CMC + 8% non-chilled MLE; T5, CMC + 16% non-chilled MLE.

oxidant activity in untreated fruit could be attributed to the fast rate of ripening which is associated with fruit senescence and decay (Wang and Gao, 2013). The trend displayed by the antioxidant activity in this study contradicts the results by Kumar *et al*. (2021) on bell peppers treated with a chitosan-pullulan composite coating and stored for 18 days at 4 ºC. These authors reported a decreasing trend in antioxidant activity. However, the present study aligns with Fernando *et al*. (2014) who reported an increase in antioxidant activities as bananas ripen and decline with senescence. Another study by Thakur *et al*. (2018) revealed the same trend: the free radical scavenging activity in uncoated plums declined with ripening. Zahedi *et al*. (2019) also reported that chitosan coated 'Langra' mango fruit had higher antioxidant activities than controls after 24 days, at 15 ± 2 °C and 85-90% RH storage conditions. The authors further stated that this may result from edible coatings forming a protective barrier on the fruit surface, which reduces the decline in antioxidant activity, nutrient loss, and water evaporation. This could show the potential of the MLE and CMC used in this study in retaining the scavenging activity of antioxidants in avocado fruit at 5 ºC and 23 ºC. Usually, the bioactive compounds in a fruit have an impact on its antioxidant activity (Maftoonazad and Ramaswamy, 2005). This was supported by the results of this study, where the trend between phenolics and flavonoids showed an inverse relationship with antioxidant capacity, which could be associated with changes in these compounds (Awad *et al*., 2017).

C7 Sugars (mannoheptulose and perseitol)

Sugar content in avocado fruit is a critical quality indicator (Le *et al*., 2021). When compared to other fruits, avocado consists of unique sugars, such as perseitol, d-mannoheptulose (reducing sugar), and the seven-carbon sugar alcohol. Avocados produce d-mannoheptulose and perseitol in higher concentrations than other sugars, such as hexoses (Tesfay and Magwaza, 2017). Generally, the ripening of this fruit is associated with increased glucose and fructose and decreased concentrations of d-mannoheptulose and perseitol. It is still best to evaluate how long fruit can be stored and still have optimal sugar concentrations without losing its quality. Figures 9 and 10 show the changes in sugar content, mainly mannoheptulose and perseitol respectively, of CMC-moringa coated avocado fruit at 7-day intervals during storage. The initial sugar concentrations before treatment were determined to be 19.67, 34.04, and 47.34 mg/g DW for mannoheptulose and 15.37, 21.77, and 17.2 mg/g DW for perseitol and for fruit at maturity stages M1, M2, and M3, respectively. The results showed a significant effect ($p > 0.001$) of storage period on the concentrations of mannoheptulose and perseitol. The concentrations for these two sugars progressively decline throughout the storage period. In the present study, untreated fruit had less mannoheptulose concentrations of 4.85, 4.28, and 5.54 mg/g DW for fruit at maturity stages M1, M2, and M3, respectively, than fruit coated with different concentrations of moringa and 5% CMC. This indicates a 75.3, 87.4, and 88.3% reduction from the initial concentrations for the fruit at the three maturity stages, respectively. Similar to mannoheptulose, the greatest reduction in perseitol concentrations was observed in untreated fruit at maturity stages 1 and 2. The reductions in these C7 sugars is due to their larger contribution to total carbohydrate concentration than the 6-carbon (C6) sugars (sucrose, starch, and hexose), with perseitol being dominant (Liu *et al*., 2002). This reduction validates the assertion by Wolstenholme (2012) that the concentration of C7 sugars depends on the ripening stage of the avocado, and its reduction can go above 80% and, in some cultivars, can be depleted. In addition, it was previously reported that ripening and its associated physiological processes such as increased ethylene production and respiration do not occur until the C7 sugars drop to below a threshold of 20 mg/g DW (Liu *et al*., 2002). This could indicate that the C7 sugars are metabolised during ripening or are the main contributors that control the ripening process (Landahl *et al*., 2009; Blakey *et al*., 2012). The trend observed in this study is similar to that reported by Shezi *et al*. (2020) for 'GEM' avocado fruit harvested inside and outside the canopy during storage. Moreover, these results are comparable to those reported by Tesfay and Magwaza (2017) who observed a decrease in soluble sugars in 'Fuerte' and 'GEM' avocados treated with CMC and chitosan-based on moringa extracts. Similarly, Kubheka *et al*. (2019) reported a higher d-mannoheptulose in 'Maluma Hass' avocado fruit treated with 1% CMC and MLE. Although there was no significant difference among treatments based on their performance in the retention of sugars, 8% non-chilled MLE was consistent in the fruit at harvested stages. Overall, based on these findings, it was clear that treating fruit with CMC (5%) and moringa leaf extract is beneficial in minimising the reduction in C7 sugars.

CONCLUSION

Based on the results of the current study, it can be concluded that CMC and MLE were effective in preserving the postharvest quality of 'GEM' avocado fruit during a storage period of 35 days. Different moringa-based treatments successfully inhibited firmness and mass loss, consequently extending fruit shelf life. The coated fruit also had a reduction in soluble sugars, especially mannoheptulose. This is the best indication that CMC and moringa-based edible coatings can be the best alternative for substituting risky chemicals and costly avocado preservative techniques to extend the shelf life of this fruit. This research has also shown the ability of the coatings used to extend the shelf life of avocados without compromising the nutritional quality or the compounds of interest of this fruit. This is an effective, environmentally friendly, and affordable technique that can be useful in commercial consignments. The observed fluctuation in the concentrations of most biochemical parameters could be due to the fact that the fruit were not distinguished based on position within the tree canopy. Previous research has reported the effect of canopy position on fruit ripening patterns and biochemical quality. Therefore, this must be considered for further investigation.

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