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Effect of Natural Antimicrobial Substances with Packaging System on Improving Quality of 'ETMANI' Guava *(Psidium guajava L.)* Fruit during cold storage

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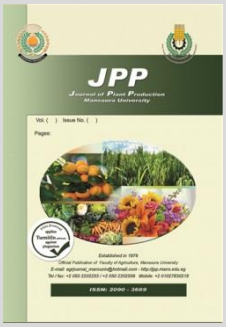

ABSTRACT

The effect of postharvest treatments of thyme oil and chitosan under different concentrations and combined with the packaging on weight loss%, firmness, decay index, malondialdehyde(MDA) content, total chlorophyll, hue angle (h°), ripening index, ascorbic acid and pectin methylesterase enzyme(PME) on 'Etmani' guava (*Psidium guajava* L.) fruit, were investigated during 2017 and 2018 successive seasons. Obtained results indicate that weight loss %was diminished by (T7) thyme oil at 1000 μLL^{-1} + packaging and (T8) chitosan at 1% + packaging, as well as firmness and PME were reduced significantly ($p<0.05$) when treated with chitosan at 1% and 2% (T3 and T 4) and thyme oil at 1000 μLL^{-1} (T2) compared to control treatment (T0). Simultaneously, it indicated that the most effective treatment in decreasing MDA and decay index were the treatment T3 and T4. Therefore, Chitosan and Thyme oils treatments could maintain normal cell membrane structure and function through down-regulating MDA content and pectin methylesterase enzyme activity, which due to reducing softening, weight loss%, and decay index in guava cv. 'Etmani' fruits during storage periods in comparison to other treatments and control

***Keywords**:* Postharvest, Chitosan, Thyme oil, Packaging, Weight loss, Firmness, Decay, Guava fruit.

INTRODUCTION

A global shift towards balanced diets, improvements in customer lives and retail marketing contribute to a substantial rise in the demand for fresh and nutritious fruits and vegetables. (Caleb *et al*., 2013), and its losses are considered the largest proportion of the overall food losses or waste per year (Porat *et al*., 2018). Fungal pathogens are one of the top reasons for post-harvest losses in the global economic sector. (Palou *et al*., 2016).

The storage and transportation of guava as a tropical climate product is restricted by its rapid perishability and high incidence of rot during handling and storage, which consider the principal problem post-harvest, because of extreme metabolism after harvesting (Batista Silva *et al*., 2018).

Modified atmosphere packaging (MAP) technology enables the shelf-life of fresh products to be extended (Kader, 1986), by modifying air inside the packaging (Reche *et al*., 2019), thereby lowering the respiratory rate and decrease water loss (Lufu *et al*., 2020) the tendency of the impact of most packaging materials promotes fruit moisture growth, increase relative moisture and reduce the vapour pressure deficit in the peel during storage conditions after harvest. (Caleb *et al*., 2013 and Lufu *et al*., 2020).

Modified atmosphere packaging technology has already been used successfully in medlar fruit (Ozturk *et al*., 2019), jujube fruit (Reche *et al*., 2019), and loquat (Amorós *et al*., 2008).

Currently, most importantly, there is an enormous concern in the fruit and vegetable sector in an attempt to decrease and avoid the use of disposable plastic packaging or reuse of recyclable materials. An increasing challenge in the fruit and vegetable sector is the emerging shift towards plastic-free packaging (Mukama *et al*., 2020). Furthermore, food-borne disease outbreaks and pathogens that cause food-borne resistance arise in the packaging. (Caleb *et al*., 2013). Therefore, more environmentally-friendly technologies, such as surface waxing and coatings (Motamedi *et al*., 2018), are thus important to mitigate water loss and quality improvement in the supply chain (Lufu *et al*., 2020) and food-borne disease (Sivakumar *et al*., 2016 and Falcó *et al*., 2019).

Furthermore, the application of chemical fungicides as disinfectants gives rise to concerns about contamination, human health, and the production of fungicide tolerant strains. Additionally, countries have led to the strengthening of strict import and export rules on the overall residue limits for the edible part of the fruit (Vilaplana *et al*., 2018), so natural antimicrobials (Danyluk *et al*., 2019, Pisoschi *et al*., 2018 and Federico *et al*., 2015) such as essential oil (Reyes-Jurado *et al*., 2020 and Pinto *et al*., 2021) and chitosan (Zhang *et al*., 2011) and (Miranda-Castro, 2016) should therefore be treated as alternatives.

Interestingly, the edible coating can be an alternative to expensive low-vent packaging to reduce moisture loss and extend the fresh fruit's storage life (Fisk et al., 2008). Additionally, Edible coatings are an ideal replacement for synthetic polymers used for food applications and have gained positive interest in recent years because of their advantage (Saxena *et al*., 2020), Furthermore, the application of various types of coating has shown diverse results because of their various structural and mechanical properties (Saxena *et al*., 2020). Furthermore, coatings can produce a shift in an atmosphere similar to MAP, depending on the coating

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permeability and fruit respiration. Besides, temperature control is an important step because it can affect both the fruit's permeability and the respiration rate (Miranda-Castro, 2016).

Two distinct mechanisms tend to regulate post-harvest diseases by a chitosan or essential oil treatments: a direct effect on pathogenic (Hyldgaard *et al*., 2012 & Palma-Guerrero *et al*., 2009), and indirect effect by inducing pathogenesis-related (PR) expression i.e. β-1,3-glucanase and chitinase (Bill *et al*., 2016). The antimicrobial efficacy of essential oils in direct-contact applications for many microorganisms in food-borne products is known to mainly rely on their chemical composition and the antimicrobial role of essential oils as well as their antioxidant properties (Reyes-Jurado *et al*., 2020 and Hyldgaard *et al*., 2012).

Abdel-Rahim and Abo-Elyousr, (2017) proposed that essential oils, particularly thyme oil, rich in antimicrobial components that can be used as ecological and economic alter to the use of toxic fungicides to manage the post-harvest disease.

Essential oils provided by aromatic and medicinal plants are biodegradable, safety characteristics and considered an environmentally and economically good solution (Sivakumar and Bautista-Baños, 2014) and thyme oil has been found to have antibacterial and antifungal effects on various pathogenic microorganisms. (Cai *et al*., 2019 and Vasile *et al*., 2017). Thyme oil has already been used successfully in guava fruit (Abdel-Rahim and Abo-Elyousr, 2017), avocado (Bill *et al*., 2017 &Sellamuthu *et al*., 2013a) banana (Vilaplana *et al*., 2018), apple (Rabiei *et al*., 2011), sapodilla fruit (Oval, 2015), mango (Cai *et al*., 2020), peach (Cindi *et al*., 2015), and citrus (Pinto *et al*., 2021).

Chitosan is a natural biopolymer produced by the deacetylation of chitin, performing as a potential bio stimulator and producer in agriculture and is biodegradable and not toxic (Hidangmayum *et al*., 2019). With regards to horticultural commodities, chitosan is proven to suppress numerous postharvest diseases, and its mechanisms of action have been well-documented (Bautista-Baños *et al*., 2017). Additionally, chitosan's antifungal properties are primarily related to fungal plasma membrane damage by the unit–NH_2 of chitosan could be tasked with preventing the proliferation of food pathogens causing decay as well as Induce host tissue resistance response (Palma-Guerrero *et al*., 2009, Devlieghere *et al*., 2004 and Romanazzi *et al*., 2012), chitosan has become a promising alternative treatment for fruits and vegetables (De Aquino *et al*., 2015). Chitosan has already been used successfully in guava (De Aquino *et al*., 2015, Batista Silva *et al*., 2018 and Nair *et al*., 2018) and in other fruits such as loquat fruit (Song *et al*., 2016) Pear (Yu *et al*., 2008) kiwi fruit (Fisk *et al*., 2008) citrus (Cháfer *et al*., 2012) apple (Shao *et al*., 2012) mango (Awad *et al*., 2017), fig fruit (Saki *et al*., 2019) blueberry (Vieira *et al*., 2016).

Based on the above information, the objective of the study was to evaluate the assumption that the fundamental quality and sensory parameters of guava fruit were consistent with cold storage conditions with thyme oil (*Thymus vulgaris* L.) and chitosan, as well as evaluate the inhibitory and their effect on decay index.

MATERIALS AND METHODS

This investigation was carried out during the two seasons 2017 and 2018, on guava (*Psidium guajava* L.) CV. 'Etmani' obtained from a commercial orchard located in El Klubia Governorate, Egypt. Fruits were harvested in the morning and transported in an air-conditioned vehicle to the postharvest handling lab. at Horticulture Research Institute, Giza Governorate, to study the effect of different postharvest treatments on fruit quality and storability of guava fruits.

Preparation of guava fruit:

The fruit was selected to be consistent in size and colour, and deteriorating fruits were discarded,600 fruits were allocated into five major groups. The first samples distributed on two treatments (dipping in thyme oil at 500 and 1000 μLL^{-1} for five minutes). The second samples were two treatments (dipping in chitosan at 1% and 2% for one minute) the third batch was placed into unsealed PE, polyethylene film bag (50 µm) thickness, and the fourth was a combination among thyme oil and chitosan with packaging. Every treatment contains 60 fruits which are distributed on three replicates (20 fruits), and the fifth group controlled. The fruits were dry at room temperature for 30 min and were packed in boxes of 3 kg per replicated, then were stored at 8±1º C and 90% relative humidity for 21 days for an estimate of the physical and chemical properties of guava fruit every seven days plus one day at room condition.

Preparation of thyme oils and treatment application:

Thyme oils were purchased from the commercial market at Kafr Elsheikh Governorate, Egypt, oils were dissolved in 0.5% Tween 80 for easy diffusion.

Preparation of chitosan coating and treatment application:

For Chitosan coating, the solution was prepared by dissolving 1% and 2% Chitosan (it was purchased from Cornell Lap Company. Chitosan ($C_6H_{11}NO_4$) n molecular weight:100.000 - 300.000.) in a 0.5% glacial acetic acid and distilled water and left under magnetic stirring for 30 minutes. The pH value of the Chitosan solution was then adjusted to 6.0 using 0.1M NaOH (Tween-80 was added as an emulsifier (Chien and Yang 2007). For one minute, the fruits were immersed in the solution mentioned above and kept on a bench to be dry air for 30 minutes.

Performed assessments:

Fruit weight loss %: Changes in fruit weight were recorded at each sampling date and fruit weight loss was calculated as a percentage from the initial weigh

Fruit firmness: It was measured in three guava fruits per replicate at two equatorial sites to determine the penetration force by using a hand-held fruit firmness tester (FT-327, Italy) equipped with an 8 mm cylindrical stainless-steel plunger tip (Watkins and Harman, 1981). and data was calculated as Newton/ cm^2 (N/cm^2)

Total Chlorophyll

Total Chlorophyll contents in the peel of guavas (three replicates) were spectrophotometrically determined according to the method of (Wellburn, 1994). The absorbance of the extract was measured at a spectrum of 663 nm for chlorophyll a, and 646 nm for chlorophyll b by using a spectrophotometer (UV/Visible spectrophotometer Libra SS0PC). Pigment contents were calculated by the following equations:

Chlorophyll a (µg/ml) = 12.21 E663 - 2.81 E646 & Chlorophyll b (µg/ml) = 20.13 E646 - 5.03 E663

and

Total chlorophyll (µg/ml) = chlorophyll a + chlorophyll b

Sensory evaluation: During the period of study, observations on sensory properties were estimated by using9- point Hedonic scale for their sensory characteristics like appearance, texture and overall acceptability. The scores were assigned from extremely liked (9) to disliked extremely (1) (Kaur and Aggarwal, 2015)

Decay index%

The severity of the disease (fruit rot) was determined visually using the scale of Zahid *et al*. (2015). Dark brown or blackish irregular spots were observed on the surface of diseased fruits in general. Decay index was graded on a six-point scale: 0 = no disease (0 %), 1=1–20 %, 2=21–40 %, 3=41–60 %, 4 61–80 %, and 5=81–100 %. The disease's severity was expressed as a frequency (%) value.

Color evaluation:

The color of the exocarp was calculated using a Minolta colorimeter (Minolta Co. Ltd., Osaka, Japan), as defined by (Mc Gire, 1992). After one day from all storage periods, the L*, a*, and b* parameters were registered, and three replicates in three trays were evaluated per treatment, and the hue angle (HUE) was recorded as H0= tan-1(b*/a*).

MDA content:

Malondialdehyde (MDA) content determination: It was calculated using the thiobarbituric acid (TBA) reaction method. Fresh tissue samples (5 g) were homogenized in 20 mL of 10% trichloroacetic acid (TCA) before being centrifuged at 10,000 g for 10 minutes. 2 ml of supernatant (2 ml of 10% TCA used as a control) was combined with 2 ml of 0.5 % 2-thiobarbituric acid (TBA), heated at 95 °C for 15 minutes, then centrifuged at 1800 g for 10 minutes after cooling to room temperature in an ice-water bath. At 450, 532, and 600 nm, the absorbance was measured by using a spectrophotometer (UV/Visible spectrophotometer Libra SS0PC). The amount of MDA was calculated as follows:(µmol g−1 FW) = [6.452 (OD532−OD600) – 0.559 OD450] * 10 ml / FW, FW was the fresh weight of sample fruit (g) (Zhao *et al*., 2007).

Soluble Solids Content (SSC%), Total acidity (TA%), and Ripening index:

Fruit samples were chosen at random from each treatment and divided into three replicates to determine the soluble solids content (SSC %) using a hand refractometer. Total acidity (TA %) was determined as a citric acid by titration with 0.1 N NaOH (A.O.A.C., 2000), and the Ripening index was calculated as a ratio of soluble solids content (SSC) to titratable acidity (TA).

Ascorbic acid:

Immediately after taking the juice, vitamin C was measured by titrating 3 mL of juice in 3 ml trichloroacetic acid (TCA, (5% w/v)) with 2, 6-dichlorophenolindophenol (DCPIP, (0.03% w/v)) up to colour change to sustainable pink. Results were expressed as mg 100 g−1 of ascorbic acid on a fresh weight (A.O.A.C, 2000) using a standard curve that was made by different concentrations of ascorbic acid.

Pectin methylesterase (PME):

PME was calculated by using 50 g of fruit in an equal amount of the solution (50% 2 M NaCl and 50% 10 mM phosphate buffer with pH(7.5) as defined by Anthon and Barrett (2006)). Samples were filtrated then added to 2.5ml of 0.5%pectin solutions. When the sample solution drops down to pH 7, the amount of 0.1M NaOH was added until pH reached 7.5. A time for the solution to drop down to pH 7 again was recorded. PME activity is expressed in µmol of hydrophilic ester conformed as an indicator for PME activity per 50 g of fruit fresh weight during one minute (µmol.g-1 FW.min-1).

Statistical analysis

The data were represented as the mean ± standard deviation (S.D.). Statistical variations were measured using a one-way analysis of variance and a post hoc. Duncan test at a confidence level of 95% (ANOVA) using the CoStat software package, Version 6.303 (789 lighthouse Ave PMB 320, Monterey, CA, 93940, USA).

RESULTS AND DISCUSSION

Fruit firmness and Weight loss%:

The firmness loss of the guava fruit during storage was confirmed by microscopic analysis in (Botelho *et al*., 2016), which was mainly due to the hydrolysis of pectic polymers in cell walls and middle lamella of fruit tissue (Khaliq *et al*., 2015 and Murmu & Mishra, 2018a)

As predicted, the decrease in fruit firmness during storage was observed as shown in Table (1), and the greatest firmness reduction was observed in control fruit (T0) at the end of storage (22.33±0.65 N/cm2 and 12.40±0.50 N/cm2) in both seasons 2017 and 2018 respectively. These findings revealed that the firmness of guava fruit could significantly be enhanced with thyme oil and chitosan alone (T2, T3 and T4) or combined with polyethylene film bag (T7 and T8). The highest concentration of thyme oil or chitosan (T2 and T4) delayed the fruit softening during the storage period compared to other treatments in both seasons (Table 1). Furthermore, high firmness values were found with the chitosan-coated fruit, which may be due to the elastic behaviour (Benítez *et al*., 2013). Moreover, due to the bioactive compounds in the coating, the cell wall of the coated fruit may also be maintained, as well as reducing the respiration and other phases of ripening (Ali *et al*., 2011), and in the meantime, increased firmness of the coated fruit may be attributed to a reduction in PME enzyme activity (Khaliq *et al*., 2017; Wang *et al*., 2020). Thymol can boost the enzymatic and non-enzymatic antioxidants level that causes a decrease in the degradation of the fruit tissue (Sivakumar and Bautista-Baños, 2014).

The fruit weight loss percentage increasing is related to the extending storage period, as a result of vapour pressure between the fruit peel tissue and the surrounding atmosphere (Ojagh *et al*., 2010). Data summarized in Fig. (1) indicated that the weight loss percentage was affected significantly due to the application treatment in comparison to control during storage time. After 7 days of storage, weight loss not occurred, using polyethylene film bag treatment combined with chitosan and thyme oil under the greatest concentration (T7 and T8), which still maintain the minimum weight loss until 14 days in both seasons. At the end of the storage period (21 days), the highest value was measured in control (12.54 and 12.65%) in both seasons respectively, and the least value was found between the highest concentration of chitosan (T4) (2.53 and 2.67%) and thyme oil T2(1.90 and 2.79%) in both seasons respectively. In addition, there were no significant differences between T6, T7 and T8 in both seasons. The obtained results are in agreement with those obtained by Batista-Silva *et al*., (2018); De Oliveira *et al*., (2014); Freitas *et al*., (2015) and Vilaplana *et al*., (2020).

Table 1. Effect of postharvest treatments on firmness (N/cm^2) of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Firmness (N/cm^2)											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	61.40±	0.80g	41.20±	1.10f	22.33±	0.65i	52.30±	0.80g	33.20±	0.70h	12.40±	0.50h
T1	71.20±	0.90ef	67.20±	0.50d	36.20±	0.50f	65.30±	0.90f	46.30±	0.40f	31.30±	0.30e
T2	79.40±	0.50ab	76.20±	0.50b	61.30±	0.90b	72.40±	0.60dc	75.20±	0.60a	61.80±	0.50b
T3	78.33±	0.75bc	75.67±	0.55d	65.50±	0.60a	73.20±	0.60b	66.30±	0.60d	54.43±	0.78c
T4	79.60±	0.50a	78.50±	0.60a	54.40±	1.10c	75.57±	0.55a	71.27±	0.15b	63.80±	0.80a
T5	70.13±	0.95f	65.40±	1.10e	34.20±	0.30g	65.33±	0.25f	44.20±	0.40g	29.50±	0.20f
T6	72.80±	0.80d	67.40±	0.90d	41.20±	0.80e	69.80±	0.40e	46.30±	0.20f	29.90±	0.30f
T7	73.20±	0.40d	75.20±	0.60b	44.20±	0.70d	71.40±	0.50cd	68.80±	0.50c	44.50±	0.70d
T8	77.60±	0.60c	72.20±	0.10c	42.30±	0.30e	70.10±	1.10e	62.10±	0.70e	32.10±	0.30e
T9	72.10±	0.20de	71.30±	0.70c	32.30±	0.60h	70.30±	0.40de	61.30±	1.00e	22.53±	0.45g

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$).

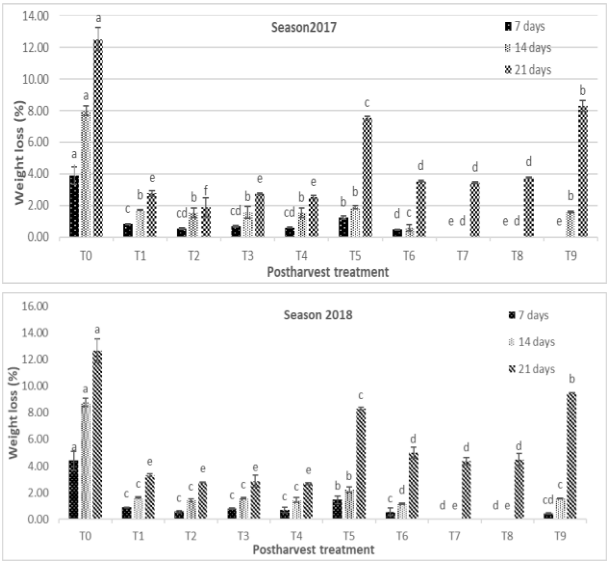

Fig. 1. Effect of postharvest treatments on weight loss % of "Etmani" guava fruits during storage at 8 ºC and 90% RH during 2017 and 2018 seasons.

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$).

In compliance with previous studies of Khaliq *et al*., (2017) chitosan maintained higher firmness and reduce weight loss % of mango fruit, and papaya (Ali *et al*., 2011). Edible coating's hygroscopic properties serve as a semi-permeable barrier to preserve firmness as well as fresh weight in fruit (Khaliq *et al*., 2019 andNourozi & Sayyari, 2020).

Total chlorophyll content:

During the storage time, guava fruits changed colour from green to yellow of the fruit peel (Rehman *et al*., 2020), due to chlorophyll degradation or qualitative and quantitative alterations of green pigment into another pigment as resulting of enzyme activities such as chlorophyll oxidase and peroxidase (Valiathan and Athmaselvi, 2018).

As shown in Table (2). At 7 days, the total chlorophyll value was increased only in fruit-coated with thyme oil at 1000 µLL^{-1} (T2) (13.56±0.51 µg/ml) in the first season, but in the second, chitosan at 2% was accompanied with it (T2 and T4) (11.11±0.39 and 11.02± 0.51 µg/ml), respectively. After 14 and 21 days, the same trend was observed. At the end of storage, in the first season (T4, T2 and T3) recorded the highest values (2.84±0.20, 2.76±0.20 and 2.68±0.42 µg/ml) respectively, followed by T7 and T8 (2.55±0.24 and 2.12±0.40 µg/ml), respectively. In the second season (T4 and T2) recorded the highest values (2.64±0.22 and 2.41±0.07 µg/ml), followed by T3, T7 and T8 (2.24±0.07, 2.19±0.24 and 2.01±0.15 µg/ml), respectively. It was also reported that guava fruits coated with chitosan exhibited chlorophyll reduction during storage (Hong *et al*., 2012). The retardation of colour development in papaya fruits treated with 2.0% of chitosan was attributed to a lower rate of ethylene production and slow respiration, which led to a modified atmosphere for the fruit (Ali *et al*., 2011).

Table 2. Effect of postharvest treatments on total chlorophyll (µg/ml) of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Total Chlorophyll(µg/ml)											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	7.05±	0.26**f**	4.27±	0.38d	0.78±	0.30e	7.14±	0.16d	5.22±	0.11c	0.89±	0.08g
T1	8.97±	0.62**d**	6.83±	0.43c	1.58±	0.30b	8.63±	0.27c	6.52±	0.19b	1.72±	0.13e
T2	13.56±	0.51a	9.57±	0.46a	2.76±	0.20a	11.11±	0.39a	8.45±	0.10a	2.41±	0.07ab
T3	11.63±	0.68b	7.82±	0.62b	2.68±	0.42a	10.55±	0.12b	8.43±	1.02a	2.24±	0.07bc
T4	12.24±	0.31b	9.64±	0.50a	2.84±	0.20a	11.02±	0.51a	8.55±	1.02a	2.64±	0.22a
T5	8.07±	0.66**e**	6.49±	0.80c	1.86±	0.20cd	7.42±	0.30d	6.34±	0.70bc	1.75±	0.09de
T6	9.34±	0.59cd	6.82±	0.60c	1.94±	0.30cd	8.53±	0.10c	6.73±	0.58b	1.85±	0.11de
T7	11.53±	0.28b	7.23±	0.30bc	2.55±	0.24ab	10.33±	0.10b	7.25±	0.30b	2.19±	0.24bc
T8	10.08±	0.20c	6.92±	0.47bc	2.12±	0.40bc	8.74±	0.21c	6.85±	0.77b	2.01±	0.15cd
T9	9.91±	0.18c	4.47±	0.17d	1.45±	0.17d	8.86±	0.33c	6.23±	0.59bc	1.31±	0.17f

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

Hue angle

The hue angle (H°) attributes were evident in Table (3). At harvest, hue angle was (97.45 and 96.85°) in both seasons respectively, which decreased with storage time mainly for control fruits, while slightly with treatments, that the highest concentration of thyme oil and chitosan treated fruits recorded the least reduction compared by the other used treatments. At 7 days hue angle values, thyme oil at 1000 µLL^{-1} (T2) and chitosan at 2% (T4) were recorded the highest values (97.32±0.79 and 97.22±0.81°) and (95.74±0.71 and 95.61±0.40°) in both seasons respectively. However, control and packed fruits showed the least hue angle with non-significant differences among them (94.55±0.30 and 95.19±1.00°) and (93.85±0.20 and 94.23±0.22°) in both seasons respectively. After 14 days stored fruit (T2, T3, T4, T7 and T8) recorded the highest hue angle values in both seasons, but at the end of the experiment, chitosan at 2% recorded the highest value (91.54±0.98°) followed by chitosan at 1% with value (90.44±0.68°) in the first season. Furthermore, the control treatment (T0) recorded the least value (70.23±0.80 °), and chitosan at 2% packed in polyethylene film bag (70.65±1.06 °). At the end of the second season, control (T0) recorded the least value (65.84±0.40 °) and there were no significant differences between treatments.

Table 3. Effect of postharvest treatments on Hue Angle (H°)of "Etmani" guava fruits during storage at 8 °C and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Hue Angel (H°)											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	94.55±	0.50d	92.86±	0.27c	70.23±	0.80g	93.85±	0.20d	87.23±	0.21e	65.84±	0.40c
T1	95.86±	0.31bc	93.54±	0.57c	73.83±	0.29f	94.55±	0.68bcd	92.17±	0.50c	85.15±	0.60a
T2	97.32±	0.79a	95.33±	0.46a	90.23±	1.06b	95.74±	0.71a	93.45±	0.41ab	86.44±	0.39a
T3	96.57±	0.30ab	94.96±	0.15a	90.44±	0.68ab	95.31±	0.23abc	93.28±	0.69abc	86.25±	0.28a
T4	97.22±	0.81a	95.10±	0.22a	91.54±	0.98a	95.61±	0.40ab	93.51±	0.59a	86.54±	0.50a
T5	95.19±	1.00cd	93.24±	0.37c	73.87±	0.30f	94.23±	0.22cd	92.22±	0.37bc	84.23±	0.21a
T6	96.07±	0.28abc	93.77±	0.67bc	75.36±	0.20e	94.86±	0.50abcd	92.76±	0.99abc	85.35±	0.32a
T7	96.41±	0.68abc	94.81±	0.21a	88.53±	0.49c	95.22±	0.40abc	93.12±	0.90abc	86.11±	0.89a
T8	96.35±	1.00abc	94.46±	0.66ab	78.84±	0.41d	95.11±	0.60abc	92.98±	0.70abc	85.78±	0.21a
T9	96.30±	0.50abc	93.13±	0.89c	70.65±	1.06g	94.97±	0.90abc	89.54±	0.41d	73.44±	12.16b

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

The change in peel colour is a normal sign of ripening, as such changes are closely related to ethylene biosynthesis (Forato et al., 2015), thus reducing respiratory rates and preventing ethylene biosynthesis could be due to the effects of coating treated fruit, which delay changes of fruit colour (Hong et al., 2012). These findings are in agreements with (Arroyo et al., 2020; De Aquino et al., 2015a; García-Betanzos et al., 2017 and De Oliveira et al., 2020).

Sensory evaluation

The overall edible acceptance of guava fruit was found to be diminished, as storage time pass (Fig. 2). Chitosan at 1% (T3) targeted maximum sensory score at 7 and 14 days of storage in both seasons (8.87 and 8.35) and (8.66 and 8.43) respectively, followed by both concentrations of thyme oils 500 µLL^{-1} and 1000 µLL^{-1}. At the end of the experiment, the highest score recorded with thyme oil at 1000 µLL^{-1} (7.39 and 7.85) in both seasons respectively, followed by thyme oil at 500 µLL^{-1} (7.24 and 7.12) and chitosan at 1% (7.14 and 7.42) in both seasons respectively. On the other hand, the low score of overall acceptability was observed in control (T0), polyethylene film bags (T5) and chitosan at 2% packed with polyethylene film (T9) during all the storage period. These results are in agreement with those obtained by De Oliveiraa et al., (2018) who stated that, in contrast with non-coated guava fruit, sensory tests have better acceptability of the coated fruit.

Skin browning is carried out due to phenolic oxidation, subsequently leads to the production of o-quinones, which by the polymerization led to brown pigments, which an undesirable change in colour that negatively affects the visual quality of fruits and vegetables (Altunkaya and Gökmen, 2009). Additionally, the coating inhibits the oxidation of phenols and delays the fruit and vegetable browning (Ali et al., 2019). Conversely, Yahyazadeh et al., (2009) pointed that an important multiparty interaction in fruit-sweetness and the overall acceptance attributes between the polyethylene type, essential oils and oil concentration. Furthermore, fruit treated with essential oil without packaging was found with multiple sensory defects. De Aquino et al., (2015) also found that chitosan-coated guava fruit was better preserved when compared to uncoated ones due to colour enhancement.

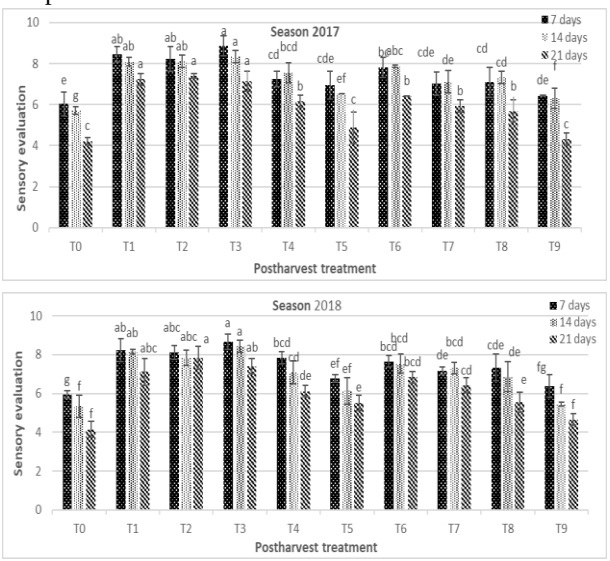

Fig. 2. Effect of postharvest treatments on sensory evaluation of "Etmani" guava fruits during storage at 8 °C and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

Determination of Lipid Peroxidation (Malondialdehyde, MDA) Content:

Increased MDA content which is an indicator of lipids oxidized in the epidermis, that associated with membrane damage in fruit(Rehman *et al*., 2020)and(Sun *et al*., 2011). As shown in (Fig 3), the high concentration of thyme oil at 1000 μLL^{-1} and chitosan at 2.0% coating retarded increments MDA content of guava fruit compared to the other applications. This impact continues to 21 days, with the lowest values observed in the treatment 2.0% of chitosan (5.9and6.59) in both seasons respectively, and thyme oils at 1000 μLL^{-1}(6.02 and6.55) in both seasons respectively, and the maximum value was recorded in control (T0)(11.29 and13.24) in both seasons, respectively. Our findings also correspond to (Hong *et al*., 2012) in guava fruit, as well as Khaliq *et al*., (2017)indicated that mango quality is maintained by improving the antioxidant protection mechanism by reducing MDA throughout storage.

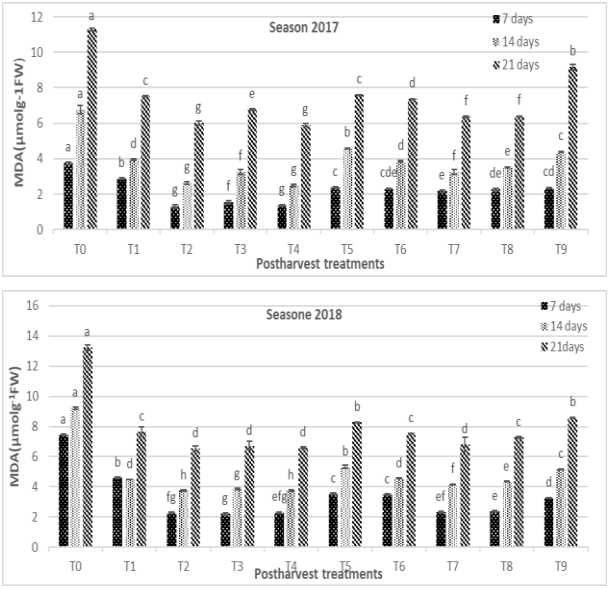

Fig. 3. Effect of postharvest treatments on MDA(µmolg^{-1}FW) content of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatments: (T0) control,(T1) thyme oil at 500 μLL^{-1}, (T2) thyme oil at 1000 μLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 μLL^{-1}+ packaging, (T7) thyme oil at 1000 μLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

Pectin methylesterase (PME) enzyme (µmol.g^{-1}FW.min^{-1}):

The rapid performance of the PME enzyme breaks down pectin in the middle of the chain in the cell wall and seems important in terms of the shelf life and consistency of the postharvest guava fruit (Botelho *et al*., 2016 and Hailu *et al*., 2014).PME is substantially increasing as fruit ripening before decreasing in the overripening period, according to (Goulao & Oliveira, 2008). The activity for pectin methylesterase (PME) in all treatments was increased, which was expected during storage. In Fig.(4) The control showed higher values in the activity of PME in all storage period in both seasons, for instance in 21 days recorded (3.51 and 3.61 µmolg^{-1}FWmin^{-1}) respectively. At seven days of storage (T3, T4, T7 andT8) showed a lower activity of PME in the first season, but in the second (T3 and T4) recorded the lowest values with (0.45 and 0.24 µmolg^{-1}FWmin^{-1}). On the other hand, at the end of storage, chitosan at 1% and 2% (T3 and T4) showed a lower activity of the enzyme PME, with a value of (1.93 and 1.95 µmolg^{-1}FWmin^{-1}) in the first season and (T4) with value (1.74 µmolg^{-1}FWmin^{-1}) in the second followed by (T3) with value (1.94 µmolg^{-1}FWmin^{-1}). The treatments had a lower PME activity was related to higher firmness in these fruits.

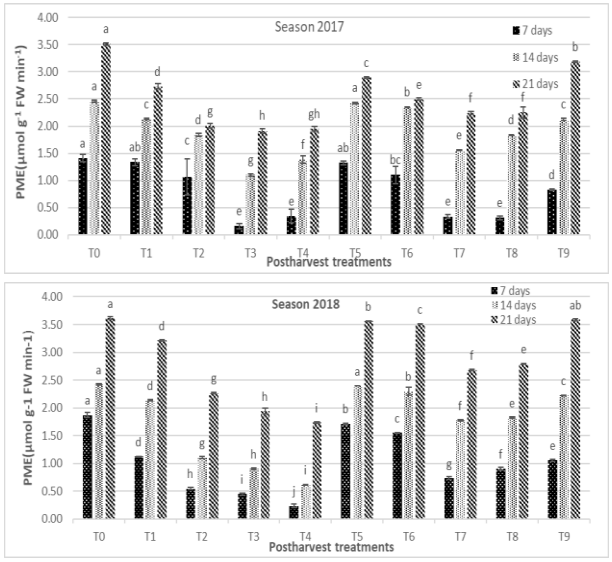

Fig. 4. Effect of postharvest treatments on Pectin methylesterase(µmolg^{-1}FWmin^{-1}) of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatments: (T0) control,(T1) thyme oil at 500 μLL^{-1}, (T2) thyme oil at 1000 μLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 μLL^{-1}+ packaging, (T7) thyme oil at 1000 μLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

After treatments, a decrease in the activity of PMEs may result in decreased pectin breakdown and enhanced firmness during storage(Botelho *et al*., 2016 and García-Betanzos *et al*., 2017). Chitosan coating treated -fruit showed significant inhibition in metabolic enzymes of pectin, especially pectin methylesterase (PME), and expression *FaPME1* (Wang *et al*., 2020), as well as increased overall activity of the fruit phenolic and antioxidants in mango fruit (Khaliq *et al*., 2017).

Decay index

Two different mechanisms tend to regulate post-harvest diseases through chitosan or essential oil therapies: the direct germicidal effect on pathogens; and the indirect effect through induction of defence mechanisms in tissue fruit (Murmu and Mishra, 2018b). Chitosan coating is known to enhance resistance to pathogens. (Romanazzi *et al*., 2017), and this is the same trend with Sivakumar *et al*., (2016)who stated that the direct fungal behaviour of Penicillium spp depends on the concentration of chitosan. Hosseinnejad and Jafari, (2016); Kumar *et al*.,(2020) reported that based on the chelating properties of chitosan, amine groups, and molecular weight the chitosan may be responsible for the prevention of food pathogens that cause decay. Furthermore, chitosan induces related defence genes and protects fruit quality by jasmonic acid signalling against Botrytis infection(Peian *et al*., 2021). It

also can increase the production of defence-related enzymes (e.g. chitinase and β-1,3-glucanase), and antioxidant activity(De Oliveira *et al*., 2014 and Ma *et al*., 2013). As well Essential oils obtained from medicinal and aromatic plants produce antimicrobial and antifungal substances especially Thymus species (Diniz-Silva *et al*., 2019 and Khalili *et al*., 2015). Furthermore, Thyme oil has proven to inhibit the fungal growth of C. *gloeosporioides* in vitro and in vivo experiment with avocado cultivars Has and Fuerte, as well as induce anthracnose disease resistance (Bill *et al*., 2017 and Sellamuthu, *et al*., 2013b). Also, using the essential oil has prevented the growth of microorganism, due to the presence of volatile compounds and thereby extending the shelf-life of guava fruit (Aquino *et al*., 2015) (Botelho *et al*., 2016) the same trend was observed in orange fruit (Pinto *et al*., 2021), may dissolve the phospholipid biomass in its microorganism cell membrane and aligned with fatty acid chains leading to death (Ultee *et al*., 2000). Furthermore, in vivo and in vitro the experiment with avocado cultivars has demonstrated that thyme oil inhibits fungal growth (Sellamuthu *et al*., 2013b) Thymol is shown to improve the mechanisms of antioxidants, contributing to improved tolerance to pathogens for fruit tissues (Sivakumar and Bautista-Baños, 2014). Conversely, thyme applied on orange fruit in polyethylene bags made successful reductions in diseases(Yahyazadeh *et al*., 2009).

In decay index values, has been found through the storage period the control had higher index decay values (Fig.5). There has been no decay index in both chitosan and thyme oil levels or combined with polyethylene compared to the control after 7 days of storage in both seasons. On the 14 days of storage, chitosan-treated fruit at 2% and oil at 1000 µLL−1 had lower decay index values (3.55 and 3.80) and (2.97 and 3.48) in both seasons respectively compared with the other treatments. At the end of storage, the highest decay index (13.73 and 14.22) was observed in control in both seasons respectively, and the lowest (7.63) was observed in chitosan at 1%in the first season, beside chitosan at 1%, 2% and oil 1000 µLL−1combined polyethylene recorded (8.29,8.44and 8.47) without significant in the second season respectively. It is observed that chitosan 2% of treatments still maintain the lowest decay index until the end of storage. Previous studies showed that chitosan coating prevented symptoms of decay in different fruit, against *Botrytis cinerea* and *Penicillium expansem*, such as table grapes (De Oliveira *et al*., 2014 and Freitas *et al*., 2015), as well as in blackberries fruit (Vilaplana *et al*., 2020), guava (Arroyo *et al*., 2020) (Hong *et al*., 2012), mango (Cai *et al*., 2020), furthermore essential oils can effectively be used to manage fungal pathogens after harvest (Seshadri *et al*., 2020)

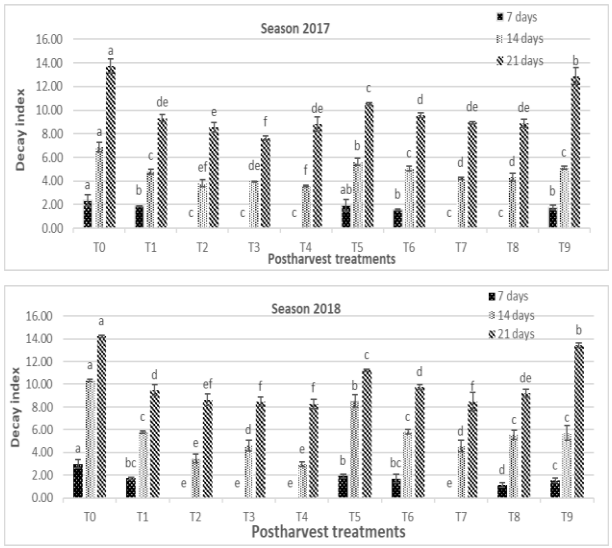

Fig. 5. Effect of postharvest treatments on decay index of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

Soluble solids content (SSC%) Titratable acidity%

The SSC increases and acidity decreases in most stored fruits(Khaliq *et al*., 2015). Organic acids are the energy source of the fruits that are consumed during the ripening of the fruit by increasing metabolism during the oxidation of acids in the tricarboxylic acid cycle. (Batista-Silva *et al*., 2018), as well as the gradual rise in free sugars of fruit during storage and the oxidation of organic acids by the respiration process (Khaliq *et al*., 2015 and Parven *et al*., 2020). In the guava fruit during storage the SSC% increased, similar to several other results (Etemadipoor *et al*., 2019; Khaliq *et al*., 2015 and Vilaplana *et al*., 2020) Before storage, soluble solids content (SSC%) were (7.00 and7.80) in both seasons respectively, and increased during storage for all treatments (Table 4).

Table 4. Effect of postharvest treatments on Soluble solids content (SSC%)of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Soluble solids content (SSC%)											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	9.30±	0.50a	11.23±	0.80a	12.30±	0.60a	10.50±	0.60a	11.55±	0.50a	12.62±	0.72a
T1	8.87±	0.75ab	10.52±	0.45ab	10.80±	0.50bc	10.30±	0.70a	10.83±	0.73ab	12.20±	0.70ab
T2	7.20±	0.90d	8.68±	0.60cd	9.85±	0.40c	8.30±	0.60b	9.45±	0.60c	10.30±	0.60de
T3	7.40±	0.70d	8.90±	0.90cd	9.80±	0.70c	8.63±	0.63b	9.52±	0.48c	9.75±	0.50e
T4	7.50±	0.30cd	8.45±	0.50d	9.65±	0.60c	8.10±	0.50b	9.35±	0.40c	9.70±	0.70e
T5	8.77±	0.51ab	10.00±	0.90abc	10.40±	0.92bc	8.95±	0.90b	10.75±	0.70ab	11.90±	0.90abc
T6	8.30±	0.80abcd	9.90±	0.80bc	10.30±	0.90bc	8.75±	0.70b	10.44±	0.31abc	11.47±	0.45bc
T7	7.80±	0.80bcd	9.35±	0.60bcd	9.95±	0.50c	8.63±	0.35b	10.23±	0.75bc	10.95±	0.30cd
T8	8.10±	0.60bcd	9.35±	0.98bcd	10.20±	0.70c	8.75±	0.20b	10.30±	0.60bc	11.30±	0.60bcd
T9	8.60±	0.60abc	9.70±	0.40bcd	11.43±	0.16ab	8.65±	0.70b	10.86±	0.79ab	12.58±	0.17a

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

As shown in Table (5). At the seven-day, SSC % was significantly higher among the different treatments in control fruits (9.30±0.50 and 10.50±0.60) in both seasons respectively, compared with fruits of other treatments. After 14 days The lowest value of SSC was found in the coated fruits without packed (T2, T3 and T4) followed by fruit treated with thyme oil 500µLL^{-1} and chitosan at 2% were packed with polyethylene bag (T7 and T8) in both seasons respectively. Where, at the end of storage there are no recorded significant SSC values between treatments compared with control in the first season, but the lowest values were (9.70±0.70,9.75±0.50 and10.30±0.60)by (T4, T3 and T2) respectively in the second season. In contrast SSC, total acidity was (1.31&1.11g 100 g^{-1}) in the first experiment in both seasons respectively, and decreased during storage. These results showed that acidity levels during the storage period were reduced. The edible fruit coating, including, guava, lower respiration rate by limits the usability of O2 and retains organic acid activity, resulting in a slower ripening process (Batista Silva *et al*., 2018 and Santos *et al*., 2018).

Table 5. Effect of postharvest treatments on Total acidity(TA%) of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Total acidity(TA%)											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	0.75±	0.06c	0.61±	0.11c	0.48±	0.05f	0.72±	0.05c	0.40±	0.04e	0.28±	0.03e
T1	0.84±	0.09bc	0.70±	0.05bc	0.53±	0.05ef	0.79±	0.09bc	0.50±	0.05d	0.33±	0.02e
T2	0.85±	0.05bc	0.71±	0.04bc	0.63±	0.08cd	0.86±	0.12abc	0.60±	0.07bc	0.47±	0.06bcd
T3	1.19±	0.11a	0.93±	0.06a	0.74±	0.06ab	0.90±	0.09ab	0.67±	0.07ab	0.55±	0.04a
T4	0.97±	0.10b	0.82±	0.11ab	0.76±	0.09a	0.97±	0.05a	0.73±	0.02a	0.56±	0.02a
T5	0.77±	0.10c	0.69±	0.05bc	0.52±	0.03ef	0.83±	0.10abc	0.53±	0.06cd	0.41±	0.08d
T6	0.85±	0.08bc	0.78±	0.10b	0.65±	0.03bcd	0.85±	0.11abc	0.56±	0.02cd	0.43±	0.01cd
T7	0.95±	0.06b	0.82±	0.06ab	0.76±	0.03a	0.94±	0.04a	0.66±	0.05ab	0.51±	0.04ab
T8	0.98±	0.02b	0.78±	0.10b	0.73±	0.04abc	0.90±	0.04ab	0.66±	0.05ab	0.50±	0.04abc
T9	0.93±	0.05b	0.71±	0.04bc	0.58±	0.04de	0.85±	0.04abc	0.62±	0.04bc	0.41±	0.03d

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

At seven days, there are no significant variances between treatments in both seasons. At the end of storage the control fruit showed the lowest acidity (0.48±0.05 and 0.28±0.03) respectively in both seasons, during the same storage period, (T3, T4, T7 and T8) showed higher acidity values of (0.74±0.06, 0.76±0.09, 0.76 ±0.03and0.73±0.04) respectively in the first season (Table). But in the second season chitosan at 1%and2% (T3 andT4) recorded the lowest with values (0.55±0.04 and 0.56±0.02) followed by (T7, T8 and T2)with values (0.51±0.04, 0.50±0.04 and 0.47±0.06)respectively. Obtained similar results in blackberries (Vilaplana *et al*., 2020), papaya fruit, (Ali *et al*. 2011) , guava fruit (Hong *et al*. 2012) , and grapes(Sánchez-González *et al*. 2011a). Due to the coatings, the degradation of organic acids will decrease, resulting in a regulated environment with little oxygen (Siddiqui and Goyal, 2015). Khaliq *et al*.(2015) stated that there is a rise in SSC% and a decrease in acidity until the fruit reaches senescence, and by coating occur reduction in fruit respiration, resulting in a lower concentration of soluble solids. Edible coatings are avoided significant changes in SSC% and fruit acidity by restricting gas exchange, diminished respiration and the metabolism in the fruit (Naeem *et al*., 2018).

Ripening Index

Table (6): shows the changes in ripening index (SSC/acid ratio) during storage. The ripening index of treated -fruit during storage periods showed a substantial increase, and the delay in ripening index increase is accompanied within chitosan and thyme oil alone or was packed with polyethylene bags treatments compared with control.

Table 6. Effect of postharvest treatments on Ripening Index of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons.

Treatment	Ripening index											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	12.47±	0.34a	18.53±	1.86a	25.73±	1.28a	14.53±	0.28a	28.81±	2.77a	43.42±	1.22a
T1	10.65±	1.60abc	15.16±	1.53b	20.44±	1.39b	13.11±	1.25ab	21.57±	0.99b	37.38±	1.77b
T2	8.62±	1.64cde	12.32±	1.25cdef	15.70±	1.92cd	9.87±	1.98c	15.75±	1.15def	22.24±	2.50d
T3	7.09±	1.75e	9.79±	1.58f	13.41±	1.91d	9.60±	0.97c	14.31±	1.85ef	17.96±	3.24ef
T4	7.75±	0.75de	10.50±	1.77ef	12.80±	0.66d	8.24±	1.54c	12.75±	0.38f	17.47±	2.44f
T5	11.55±	1.96ab	14.49±	1.98bc	20.11±	0.83b	10.76±	0.24bc	20.52±	3.41bc	29.30±	4.29c
T6	9.74±	0.72bcd	12.76±	1.00bcde	15.87±	1.60cd	10.38±	1.32c	18.77±	0.64bcd	26.68±	1.46c
T7	8.21±	1.67cde	11.42±	1.38def	13.42±	1.91d	9.51±	1.99c	15.52±	0.92def	21.41±	1.82de
T8	8.51±	1.53cde	11.73±	0.87def	14.04±	1.73d	9.89±	1.32c	15.78±	1.98def	22.79±	0.66d
T9	9.24	0.35bcde	13.88±	0.16bcd	18.26±	2.26bc	10.07±	1.44c	17.51±	0.14cde	30.53±	2.24c

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

There was a delay in the increasing of ripening index in the fruit coated with chitosan at 1%and 2% were recorded, that chitosan at1%(T3) (13.41±1.91and 17.96±3.24) and 2%(T4) (12.80±.66 and 17.47±2.44) could significantly ($P < 0.05$) restrict the increase in ripening index at 21 days of storage. it is observed that there is not significant between (T3&T4&T7&T8) with values (13.41±1.91&12.80± 0.66&13.42±1.91&14.04±1.73) respectively at the end of the first season, and between (T2&T8) with values (22.24±2.50&22.79±0.66)respectively in the second one. SSC/acid ratio was the highest values with control which ranged from the initial value of (12.47–25.73) and (14.53-

43.42) by the end of the storage period in both seasons respectively.

Ripening of fruits can be delayed by the use of edible coating as the fruit's interior environment will then be metabolized selectively (Naeem *et al*., 2018). Furthermore, Sánchez-González *et al*., (2011) have also stated that adding EOs to coatings will lead to lower consumption of oxygen and the production of carbon dioxide and this is due to the lipophilic character of the essential oils as a coating resists the spread of gas.

Ascorbic acid content:

On average, the content of ascorbic acid was steadily reduced as storage increased (Table 7). At harvest, ascorbic acid content was (125.44 and 118.35(mg 100 g^{-1})) in both seasons respectively, which decreased with the storage time. In our findings, the ascorbic acid levels were higher in fruit coated with chitosan and thyme oil alone or were packed with polyethylene bag than in control. At the end of 21 days, the lowest level of ascorbic acid (81.18 ±6.40 and 82.26± 5.99 (mg 100 g^{-1})) was observed in the control fruits in both seasons respectively, whereas the levels in the fruit treated with chitosan and thyme oil alone or were packed with polyethylene bag coated fruits were almost similar (Table 7). Oxidation during post-harvest storage, however, is one of the key triggers for the ascorbic acid decrease. Ascorbic acid is usually subject to the availability of O_2 for storage and oxidation reactions (Ali *et al*., 2019). Coatings like chitosan and oils minimize O_2 availability and oxidation, It implies that vitamin C loss is prevented in the changed environment provided by coating (Batista-Silva *et al*., 2018; Etemadipoor *et al*., 2019). Furthermore, the phenolic antioxidant in essential oils, which can boost antioxidant function, can also contribute to increased concentrations of ascorbic acid in fruit (Naeem *et al*., 2018; Seshadri *et al*., 2020), as well as the efficacy of essential oils can be ascribed to a reduction of ROS accumulation in guava that can, in turn, prevent vitamin C loss to scavenge ROS and enable the fruit protection mechanism, as shown by an increased antioxidant activity(Murmu and Mishra, 2018a). Similar results were reported using coating in stored guava (Batista Silva *et al*., 2018; Botelho *et al*., 2016; De Aquino *et al*., 2015b; Etemadipoor *et al*., 2019; Hong *et al*., 2012; Murmu and Mishra, 2018b and Seshadri *et al*., 2020).

Table 7. Effect of postharvest treatments on Ascorbic acid content (mg 100 g^{-1}) of "Etmani" guava fruits during storage at 8 ºC and 90% RH plus 1 day at ambient temperature during 2017 and 2018 seasons

Treatment	Ascorbic acid (mg 100 g^{-1})											
	Season 2017						Season 2018					
Day	7		14		21		7		14		21	
T0	110.48±	1.90b	95.65±	2.00b	81.18±	6.40c	95.82±	3.01a	91.88±	3.51b	82.26±	5.99b
T1	110.69±	0.49b	98.87±	6.00b	91.49±	1.50b	97.45±	2.01a	94.43±	3.99ab	90.82±	3.00a
T2	113.78±	0.30a	107.44±	0.40a	94.98±	3.03ab	103.78±	2.00a	98.85±	1.01a	96.62±	6.00a
T3	113.43±	0.59a	107.32±	0.31a	101.93±	7.42a	103.70±	2.99a	98.52±	2.95a	96.72±	2.94a
T4	113.65±	0.51a	107.82±	0.31a	97.48±	2.43ab	104.86±	3.99a	98.88±	5.01a	95.37±	3.94a
T5	111.08±	0.01b	104.47±	0.62a	94.50±	2.49ab	102.78±	8.50a	96.91±	3.02ab	94.16±	0.96a
T6	111.23±	0.71b	104.66±	0.50a	94.79±	3.12ab	102.85±	8.54a	96.98±	3.00ab	94.03±	4.01a
T7	112.16±	0.96ab	106.22±	2.00a	96.30±	1.88ab	103.13±	3.99a	97.44±	2.00ab	94.78±	2.01a
T8	112.22±	2.00ab	106.11±	0.90a	94.99±	2.54ab	103.46±	6.96a	98.15±	1.03ab	94.97±	4.01a
T9	111.87±	0.20ab	105.76±	0.68a	89.83±	4.07b	103.29±	7.56a	97.06±	2.99ab	88.97±	6.99ab

Treatments: (T0) control,(T1) thyme oil at 500 µLL^{-1}, (T2) thyme oil at 1000 µLL^{-1} (T3) chitosan at 1%, (T4)chitosan at 2%, (T5) packaging, (T6) thyme oil at 500 µLL^{-1}+ packaging, (T7) thyme oil at 1000 µLL^{-1} + packaging, (T8) chitosan at 1% + packaging and (T9) chitosan at 2%+packaging. Values represent the mean ± SD for three replications. Different letters indicate significant differences among different treatments ($p < 0.05$)

CONCLUSION

Overall, the above-mentioned findings suggest that postharvest applications of chitosan and thyme oil as coating treatments are a promising strategy for the management of fruit quality of 'Etmani' guava (Psidium guajava L.) fruits during cold storage and increasing storage life by retaining fruit quality, by diminishing the weight loss, as well as increment reduction in softening.

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تأثير المواد الطبيعية المضادة للميكروبات مع نظام التعبئة والتغليف في تحسين الجودة لثمار الجوافة أثناء التخزين المبرد

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قسم بحوث تداول الفاكهه-معهد بحوث البساتين-مركزالبحوث الزراعيه-الجيزه

تأثير معاملات ما بعد الحصاد الشيتوزان و زيت الزعتر بتركيزات مختلفة مع التعبئة على نسبة فقدان الوزن ، والصلابة ، ومحتوى malondialdehyde(MDA)، والكلوروفيل وحمض الأسكوربيك ، ومؤشر النضج ، ونشاط إنزيم البكتين ميثيل استيريز PME على ثمار الجوافه صنف العتماني خلال موسمي 2017 و 2018 المتتاليين. تشير النتائج التي تم الحصول عليها إلى أن نسبة فقدان الوزن للثمار قد انخفضت بزيت الزعتر عند 1000 ميكرولتر + العبوة والشيتوزان عند 1٪ + العبوة ، وكذلك تم تقليل الصلابة و PME بشكل ملحوظ عند معالجتها باستخدام الشيتوزان بنسبة 1٪ و 2% وزيت الزعتر عند 1000 ميكرولتر مقارنة بالكنترول ،بالاضافه الي ذلك تحافظ معاملات الشيتوزان و زيت الزعتر على هيكل ووظيفة غشاء الخلية الطبيعي من خلال خفض محتوى MDA و نشاط إنزيم البكتين ميثيل استيريز PME للتخفيف من ليونه الثمار وتقليل الفقد في الوزن وتلف ثمار الجوافة خلال فترات التخزين