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FULL PAPER

Maintaining antioxidants in tomato fruit using chitosan and vanillin coating during ambient storage

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Abstract

A high intake of antioxidants in a daily diet could reduce the risk of several diseases, including certain cancers and heart disease. Tomato is one of the rich sources of antioxidant compounds. However, it has a relatively short postharvest life due to several factors such as postharvest diseases, accelerated ripening and senescence that hasten the losses in quantity and quality. Chitosan and vanillin could be an alternative to disease control, maintain the quality and prolong the shelf life of fruit. This research aimed to evaluate the potential of chitosan and vanillin coating on tomato antioxidant properties during storage at 26±2°C and 60±5% relative humidity. Chitosan and vanillin in aqueous solutions of 0.5% chitosan + 10 mM vanillin, 1% chitosan + 10 mM vanillin, 1.5% chitosan + 10 mM vanillin, 0.5% chitosan + 15 mM vanillin 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin, respectively, were used as edible coating on tomato fruit. The analysis was evaluated at a 5-day interval. The results revealed that 1.5% chitosan + 15 mM vanillin have significantly retained tomato's antioxidant properties and prolonged shelf life up to 25 days without any adverse effects on fruit quality. Thus, combining 1.5% chitosan and 15 mM vanillin is highly recommended as a tomato coating to maintain their quality, particularly in the absence of a refrigeration facility during marketing.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill) is among the most popular and consumed vegetables globally after potatoes (Sucharitha *et al*., 2018). Nutritionally, tomato is a rich source of antioxidant compounds such as vitamin C, lycopene and total phenolic contents that support many health benefits. Intake of these exogenous antioxidants through daily diets can reduce the risk of heart disease (Liu *et al*., 2018), cancer (Forni *et al*., 2019), oxidative stress (Jing *et al*., 2019), and cardiovascular diseases (Mehta *et al*., 2018). Vitamin C is a water-soluble vitamin naturally found in fruits and vegetables is vitamin C and it is a powerful antioxidant and acts to prevent or reduce the damage caused by reactive oxygen species in fruit (Valente *et al*., 2011; Khaliq *et al*., 2015). Lycopene is the major carotenoid compound of tomato with bright red carotenoid pigment and phytochemical found in tomato and other red fruit (Abebe *et al*., 2017). In addition to natural antioxidants, oxidation of lipids or other molecules may be postponed or restrained by inhibiting the initiation of oxidative chain reactions (Ali *et al*., 2013). However, tomato is a climacteric fruit has short storage life due to several factors, for instance, disease incidence, high respiration, enhanced ripening. Tomato can be store about 8-12 days after harvest at ambient temperature (Mwende *et al*., 2018). In postharvest handling, maintaining tomato quality is very crucial. In developing countries, the

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quality loss is commonly due to inadequate post-harvest handling, poor transportation systems, fluctuated temperature, low relative humidity (RH), unstable gaseous exchange during storage and invasion of postharvest diseases (Arah *et al*., 2015).

Naturally, the quality of fruit after harvest cannot be improved but it can be preserved by applying appropriate post-harvest management techniques. Recently there are many non-chemical treatments approach has been proposed as an alternative for the control of postharvest diseases prolong storage life and retain antioxidant properties (Ippolito and Sanzani, 2011). However, researchers suggested that edible coatings can be used as one of the alternative treatments in prolonging postharvest life and maintain the quality of fruit as well as keeping low production costs (Mahfoudhi *et al*., 2014). This technique can act as a barrier to water loss, physical, chemical, microbiological activity, anti-browning agents, and exchange of gases and lowering the oxidative reaction rate and maintain the nutritional quality during storage (Kore *et al*., 2017; Bal, 2019). Generally, the materials such as polysaccharides, proteins, lipids, and composites coating were primarily used as an edible coating. (Nor and Ding, 2020). Among them, chitosan is a polysaccharide that has the ability to form semi-permeable films, to retard the fruit deterioration and extend the storage life of fruit by inhibiting the growth of microorganisms and modifying the internal atmosphere to reduce respiration and ethylene production rate that delay changes in antioxidant properties (Safari *et al*., 2020).

Vanillin is an organic phenolic aldehyde and being a powerful antioxidant biophenol is often used as a flavouring and fragrance agent in foods, beverages, and pharmaceuticals (Vijayalakshmi *et al*., 2019). Besides this, it is also used as a food preserving agent, due to its antimicrobial, anti-mould, anti-yeast, and antioxidant activities (Ciriminna *et al*., 2019). The antimicrobial property of vanillin is due to the effect of phenolic compound in its chemical structure, which makes vanillin effective in inhibiting bacteria, yeasts and moulds by direct damage to the fungal cell membrane (Suwarat Rakchoy, 2009). Besides, vanillin has suppressant effects on invasive pathogens including yeasts, moulds, and bacteria, thus control the decay of fruit (Safari *et al*., 2021). Chitosan coating delayed in changes the ascorbic acid content in fruit during storage such as apricot (Ghasemnezhad *et al*., 2010), tomato fruit (Kumari *et al*., 2016) and guava (Thaipong *et al*., 2006). Mandal *et al*. (2018) reported that 2% chitosan coating on tomato fruit caused declining in lycopene content during 22 days of storage in ambient conditions. Up to now, there is limited information on the effect of chitosan combined with vanillin as a coating treatment on antioxidants properties and activities of tomato stored at room temperature 26±2°C/60±5% RH. For this reason, this study was set out to determine the combined effects of chitosan and vanillin as a coating agent on antioxidant properties and activities and storage life of tomato fruit.

2. Materials and methods

2.1 Fruit materials

Pink colour tomato (10 to 30% of the surface is yellow to pink according to USDA class 3 colour) from Syngenta 1039 variety was obtained from Weng Seng Vegetable Products Sdn. Bhd., Cameron Highlands, Pahang, Malaysia. On the same day of harvesting, the tomato was transported to Postharvest Laboratory Science, Faculty of Agriculture, Universiti Putra Malaysia. The fruit was sorted in terms of uniformity, maturity, weight (ranged between 90-110 g) and free from any blemishes and damages.

2.2 Preparation of coating solutions

Commercial chitosan originated from shrimp-shell crustaceans with 85% deacetylation was purchased from Enviro Clean Energy Sdn. Bhd., Perintis Teknologi Pertanian, Malaysia (ECO. www.kitosan.my). Meanwhile, vanillin (99%) was purchased from Evergreen Engineering and Resources Sdn. Bhd., 43500 Semenyih, Selangor, Malaysia. Chitosan solution at 0.5, 1 and 1.5% v/v were prepared and adjusted to pH 5.6 with 1 M NaOH. Tween 20 at 0.1% was supplemented to improve the solution wettability. Distilled water without chitosan containing 0.1% Tween 20 was served as control. Vanillin powder was dissolved in distilled water in concentrations of 10 and 15 mM and heat at 83°C for 5 mins using a hot plate magnetic stirrer until vanillin powder was completely dissolved. Then, each vanillin solution was mixed with three concentrations solution of chitosan to form 0.5% chitosan + 10 mM vanillin, 1% chitosan + 10 mM vanillin, 1.5% chitosan + 10 mM vanillin, 0.5% chitosan + 15 mM vanillin 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin, respectively.

2.3 Postharvest coating treatments

Tomato was dipped in chlorinated water that was prepared from 0.05% sodium hypochlorite for 3 mins prior to coating treatments (Ali *et al*., 2010). Next, the fruit was rinsed three times using running water and air-dried for 1 hr. The fruits were distributed into seven lots and dipped for 1 min according to their coating solutions. The fruit was then dried for 2 hrs at 26±2°C/60±5% relative humidity (RH). Each coating treatment carried six fruits per replicate. The treated fruits were packed in

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18-holes 0.5 cm diameter perforated plastic bag 18 cm × 26 cm of 0.05 mm thickness. Six plastic bags were placed in a commercial corrugated fibreboard carton of 30 cm × 25 cm × 15 cm and the fruit were stored at 26±2°C/60±5% RH for 25 days. Each treatment was repeated four times and analysis was carried out at every 5-day interval. In each replication, six fruit was analysed.

2.4 Determination of disease incidence

The incidence of the disease was measured as a percentage of fruit exhibiting symptoms of fruit rot according to the method of Khaliq *et al*. (2015). The incidence of the disease was determined as the number of infested fruits showing symptoms of the disease such as dots and rots of the total number of tomato fruit at each batch of storage interval. The fungal growth symptoms on the fruit surface were observed visually by using a scale where 0 = no symptoms of decay, 1 = 1-10% decay, 2 = 11-25% decay, 3 = 26-50% decay, 4 = 50-75% decay, 5 = >75% decay. The percentage of disease was determined using diseases incidence scale by the following formula as reported by Abebe *et al*. (2017).

$$\text{DI (\%)} = \frac{\sum(\text{DI scale}) \times (\text{Number of tomato fruit at the DI level})}{\text{Total number of tomato fruit in the treatment} \times \text{The highest score(5)}} \times 100\%$$

2.5 Disease severity

Tomato fruit disease severity (DS) was evaluated as described by Mohamed *et al*. (2017) with slight modification. Fruit disease severity was evaluated based on visible symptoms, spots, rot and decayed area on each fruit skin in each storage interval. For disease severity assessment, five points of disease severity score were used as shown in Table 1. Fruit with disease severity index scores of two, three and four were considered to have no commercial and marketing value anymore.

$$\text{DS (\%)} = \frac{\sum(\text{Severity rating} \times \text{Number of tomato fruit clusters in the rating})}{\text{Total number of tomato fruit clusters assessed} \times \text{Highest DS scale}} \times 100\%$$

2.6 Determination of antioxidant properties

2.6.1 Vitamin C determination

Vitamin C content was determined according to the method described by Ding and Mashah (2016) using the direct colourimetric method. Fruit with peel (5 g) were homogenized with 45 mL of 2% cold metaphosphoric acid (HPO_3). The juice was filtered and added with 2% HPO_3 to make up 100 mL. Then, 1 mL of extract was diluted with 2% cold HPO_3 to make up 5 mL. Ten mL of dye solution was added and measured at 518 nm wavelength using a spectrophotometer (S1200, Spectrowave spectrophotometer, Cambridge, England) immediately. The concentration of tomato vitamin C was noted from the standard curve using vitamin C (R^2 = 0.96).

2.6.2 Lycopene determination

The lycopene content of the tomato fruit was evaluated according to Nagat and Yamashita (1992) protocol with minor modifications. A total of 800 mg of tomato pulp without seed was weighed and crushed using pestle and mortar. The sample pigments were extracted in 10 mL acetone and hexane (4:6). After the homogenization process, the extraction was transferred to a 50 mL separating funnel. It was allowed to stand for approximately 15 mins to allow phase separation. Finally, the top pigment layer was collected carefully in a quartz cuvette (10 mm path length) and measured by using a spectrophotometer at several wavelengths (663, 645, 505 and 453 nm). The measured wavelengths were used to estimate total lycopene content using the following equation as described by Nagata and Yamashita (1992):

Lycopene mg/kg fresh weight = $-0.0458A_{663} + 0.204A_{645} + 0.372A_{505} - 0.0806A_{453}$

Where A_{663}, A_{505}, and A_{453}, are absorbance at 663, 505 and 453 nm, respectively.

2.7 Determination of total phenolic content

2.7.1 Sample extraction

The extraction of tomato pulp tissue for total phenolic content (TPC) and antioxidant activities (DPPH, ABTS, and FRAP) assays followed the method described by Chiabrando and Giacalone (2019) and Petriccione *et al*. (2015) with slight modification. Concisely, 4 g of tomato tissue from each replicate were frozen in liquid N_2 and ground by using a small ceramic kitchen pestle and mortar for 30 s. The grounded tissue was dissolved in 10 mL 80% (v/v) methanol. Subsequently, the homogenate was extracted under reduced light conditions by spinning using an orbital shaker at 180 rpm for 1 hr. After shaking, the homogenate was filtered by Whatman No.1 filter paper and transferred to a vial, which was covered by aluminium foil, and then the supernatants were kept at -

Table 1. Disease severity score of disease assessment for tomato fruit

Diseases score	Description	Inference
0	No visible symptoms on fruit	No infection
1	1-25% of the area covered by slight necrotic inoculations	Mild infection
2	26-50% of the inoculated area covered by necrotic and white fungal mycelia	Moderate infection
3	51-75% of the sample is necrotic with the presence of spore mass	Severe infection
4	> 76% Necrotic tissue with fungal mass appears soft and decay	Very severe/Devastating

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20ºC for antioxidant activity and capacity analyses.

2.7.2 Total phenolic content

The total phenolic was estimated following the method described by Kaewseejan and Siriamornpun (2015) with some modifications. Briefly, 150 µL aliquot of supernatant extract and 750 µL 10% (v/v) Folin-Ciocalteu reagent were mixed in test tubes that were covered by aluminium foil and incubated for 5 min in the dark. This followed by addition of 600 µL 7.5% (w/v) Na_2CO_3. The mixture was then incubated in dark for 30 mins at 26±2°C/60±5% RH before measuring the absorbance at 765 nm by using a spectrophotometer. The TPC was expressed as milligrams of gallic acid equivalents (GAE) per 100 g fresh weight (FW) using gallic acid as the standard with R^2 = 0.97, and calculated using the following equation:

$$\text{TPC mg GAE/100 g FW} = \frac{\text{TPC per mL sample} \times \text{dilution factor (2.5)} \times \text{total sample volume used(1.5)}}{\text{Sample weight (4 g)}}$$

2.8 Antioxidant activity and capacity

2.8.1 Radical scavenging activity by using 2, 2-diphenyl-1-picrylhydrazyl free radical (DPPH)

The TPC of the antioxidant activity of tomato extracts was determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) according to Briones *et al*. (2019) method with some modification where 1 mM of DPPH was prepared by dissolving 0.197 g of DPPH powder in 1 L of methanol before the analysis. Then, 1 mL of sample extracts was applied to 1 mL 1 mM DPPH solution; the mixture was vortexed and then held at room temperature in the dark for 30 min. After incubation, the absorbance of the mixture was measured at 517 nm using a spectrophotometer. The assay was performed in triplicate per sample. Meanwhile, 1 mL 80% methanol was applied as the control solution to 1 mL DPPH reagent. The results were expressed as percentage inhibition of DPPH, and calculated using the following equation:

$$\text{DPPH inhibition (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where A_0 = Absorbance of the control and A_1 = Absorbance of sample

2.8.2 ABTS (2, 2-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)

The antioxidant activity of tomato was measured using 2, 2-azino-bis, 3-ethylbenzthiazoline-6-sulfonic acid according to the method of Briones *et al*. (2019) with slight modification. ABTS was formed by reacting 7 mM ABTS aqueous solution with 2.45 mM of potassium per sulphate at 26±2°C/60±5% RH for 16 h in the dark. This solution was diluted in ethanol (around 1:89 v/v) before the test and equilibrated at 30°C to provide an absorbance of 0.700±0.02 at 734 nm. The addition of 1 mL diluted ABTS solution in ethanol to 10 µL sample extract was incubated at 30°C for 6 min before absorbance. The inhibition percentage for the blank absorbance was then calculated at 734 nm. The percentage of ABTS free radical inhibition was determined using the equation below:

$$\text{ABTS inhibition (\%)} = (A_0 - A_1)/A_0 \times 100$$

Where A_0 = Absorbance of the control and A_1 = Absorbance of sample

2.8.3 Ferric reducing antioxidant power

The tissue of tomato antioxidant capacity was assayed using ferric reducing antioxidant power (FRAP). The assay was prepared following Thaipong *et al*. (2006) method with slight modification. In FRAP assay, the FRAP reagent was freshly prepared by mixing 10 mM 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) in 40 mM HCl solution, 300 mM acetate buffer ($C_2H_3NaO_2.3H_2O$, pH 3.6), and 20 mM ferric chloride in the ratio of 1:10:1 (v/v/v). An aliquot of 50 µL sample extracts was added to 950 µL of FRAP reagent and incubated in a water bath at 37°C for 30 min. Absorbance was measured at 593 nm against a control that was prepared by adding 50 µL 80% methanol to a 950 µL FRAP reagent. The standard curve was a linear line between 0 and 800 mM Trolox. The achieved results were expressed as µM Trolox equivalent (TE) of tomato fresh weight using a standard with R^2 = 0.98. The obtained FRAP results were expressed in µM TE/g fresh weight and then calculated using the formula below:

$$\text{FRAP µM TE/g FW} = \frac{\text{TE µM per mL} \times \text{dilution factor (2.5)} \times \text{total sample volume used (10)}}{\text{Sample weight (4 g)}}$$

2.9 Experimental design and statistical analysis

The experiments were carried out in a completely randomized design (CRD) with seven coating treatments and four replications i.e. Control (T0), 0.5% chitosan +10 mM vanillin (T1), 1% chitosan +10 mM vanillin (T2), 1.5% chitosan +10 mM vanillin (T3), 0.5% chitosan +15 mM vanillin (T4), 1% chitosan +15 mM vanillin (T5) and 1.5% chitosan +15 mM vanillin (T6). Totally 168 fruits were used in this experiment. The obtained data were analysed using analysis of variance and mean comparisons were performed using Duncan's multiple range test (DMRT) in the significance level of P≤0.01. All the analyses were conducted using statistical analysis software (SAS) version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). The data in percentage was transformed using square root transformation before determining the significance level using DMRT. Pearson's correlation analyses were used to correlate antioxidants and antioxidants capacity among each other.

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The entire experiment was repeated three times and the data were pooled before analysis. However, the control fruit was discarded for analysis after day 25 due to high disease severity and decay.

3. Results

3.1 Disease incidence and diseases severity

Disease's incidence and severity are measuring tools that indicate disease development in the fruit. In the present study, Table 2 shows a significant interaction between coating treatment and storage days in the disease incidence and disease severity of tomato fruit.

Figure 1 shows that there was no significant difference in disease incidence among treatments on day 0 as there was no disease incidence occurred during this period of time. However, by day 5, control started to infect by disease but no significant difference was found at this stage. By storage day 10, the disease incidence occurred in most of the treatments except tomato fruit coated with T5 and T6. On storage day 15, although fruit coated with T5 and T6 have infected by a disease, the disease incidence was much lower than control and those coated with T1, T2, T3 and T4. This trend continued until the end of storage day. However, at day 25 control fruit and those coated with T1, T2 and T4 were 100% infected by diseases incidence.

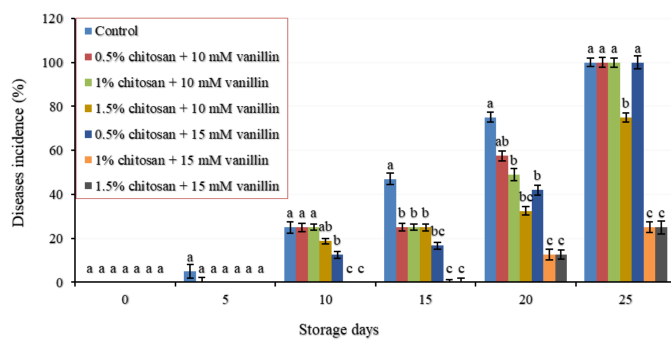

Figure 1. Effects of coating treatment on disease incidence of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars with different letters notation in each storage day differed significantly by DMRT P≤0.01. Vertical bars indicate the standard error of means for four replicates. Prior to analysis, the data were square-root transformed while non-transformed means were shown (n = 24)

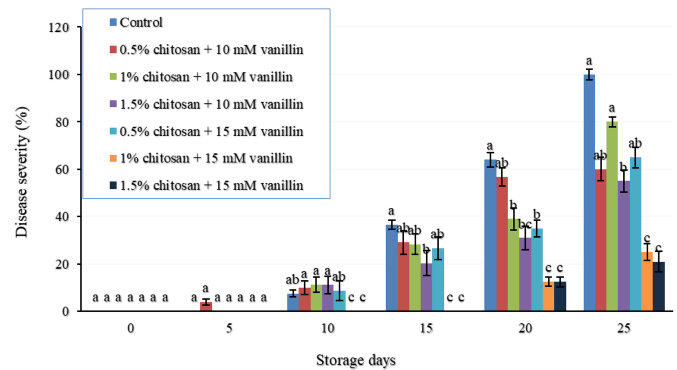

Figure 2. Effects of coating treatment on disease severity of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates. Prior to analysis, the data were square-root transformed while non-transformed means were shown. (n = 24)

3.2 Antioxidants

3.2.1 Vitamin C content

There was a significant interaction between treatments and storage days on vitamin C content during storage (Table 3).

Table 2. Main and interaction effects of different coating treatments and storage days on disease incidence and severity of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days

Factor	Disease incidence (%)	Disease severity (%)
Treatment		
Control	37.36^{a}	29.12^{a}
0.5% chitosan +10 mM vanillin	37.42^{a}	21.49^{ab}
1% chitosan +10 mM vanillin	31.50^{ab}	19.71^{b}
1.5% chitosan +10 mM vanillin	29.34^{ab}	18.46^{b}
0.5% chitosan +15 mM vanillin	28.48^{ab}	20.33^{ab}
1.0% chitosan +15 mM vanillin	6.69^{c}	2.45^{d}
1.5% chitosan +15 mM vanillin	6.69^{c}	1.46^{d}
Storage days		
0	0.00^{d}	0.00^{d}
5	0.81^{d}	0.79^{c}
10	15.27^{c}	6.75^{b}
15	23.45^{bc}	12.78^{b}
20	36.85^{b}	28.84^{ab}
25	75.120^{a}	55.57^{a}
Interaction		
Treatment* Storage days	**	**

Means values in a column followed by different letters indicate significantly different according to Duncan's multiple range test at P < 0.01. **Significant at P ≤0.01. (n=24)

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Table 3. Main and interaction effects of different coating treatments and storage days on vitamin C content, lycopene and Total phenolic content of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days

Factor	Vitamin C content (mg/100 g FW)	Lycopene (mg/kg FW)	Total phenolic content (mg GAE/100 g FW)
Treatment			
Control	37.19[a]	38.77[a]	77.96[a]
0.5% chitosan +10 mM vanillin	37.69[a]	39.16[a]	80.53[a]
1% chitosan +10 mM vanillin	37.61[a]	36.74[ab]	78.06[a]
1.5% chitosan +10 mM vanillin	35.04[b]	34.06[b]	73.05[b]
0.5% chitosan +15 mM vanillin	37.31[a]	37.66[ab]	72.73[b]
1.0% chitosan +15 mM vanillin	31.67[c]	29.93[c]	54.86[c]
1.5% chitosan +15 mM vanillin	30.81[c]	28.68[c]	52.41[c]
Storage days			
0	31.72[bc]	16.71[d]	49.53[d]
5	33.07[b]	19.96[d]	50.40[d]
10	33.06[b]	29.50[c]	58.34[c]
15	33.97[b]	34.60[b]	69.48[b]
20	36.74[b]	43.84[a]	86.14[a]
25	40.47[a]	44.96[a]	89.23[a]
Interaction Treatment* Storage days	**	**	**

Means values in a column followed by different letters indicate significantly different according to Duncan's multiple range test at P < 0.01. **Significant at P ≤0.01. (n = 24)

Figure 3 displays that at storage day 10, fruit treated with T5 and T6 exhibited lower vitamin C as compared to control and those treated with T1, T2, T3, T1. However, by storage day 15, there was a significant difference between treatment and storage day, fruit coated with T5 and T3 has lower vitamin C content than other treatments. This trend sustained until the storage period of days 20 and 25.

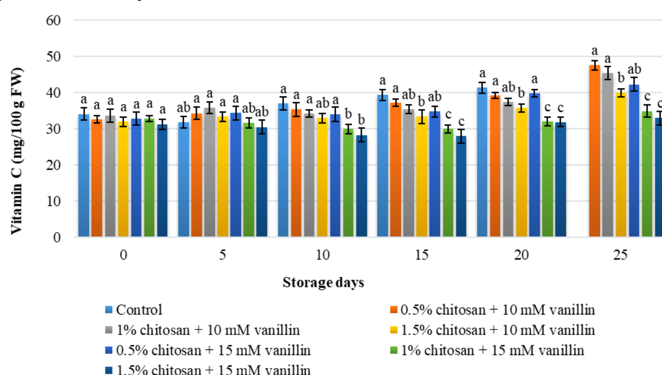

Figure 3. Effects of coating treatment on vitamin C content of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates.

3.2.2 Lycopene

Lycopene pigment is associated with red colour in tomatoes. Table 3 indicates that there was a significant interaction between treatments and storage days on tomato lycopene. Figure 4 exhibits there was no significant difference in tomato lycopene among treatments on days 0, 5 and 10. However, by day 15, the lycopene content of fruit coated with T5 and T6 was lower in lycopene content than control tomato and those coated with T1, T2, T3 and T4. A similar trend was also found in tomatoes of days 20 and 25.

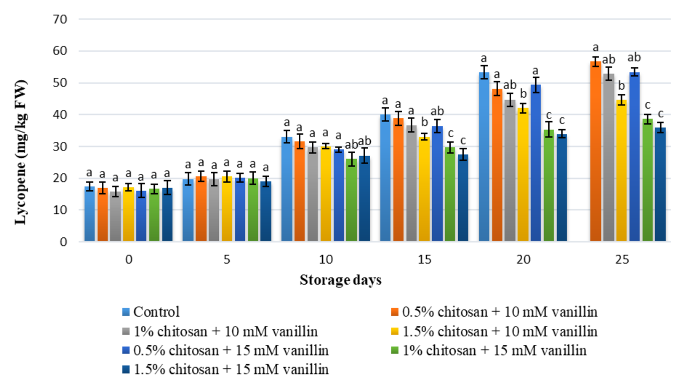

Figure 4. Effects of coating treatment on lycopene content of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates.

3.3 Total phenolic content

There was a significant interaction between coating treatments and storage days on tomato TPC (Table 3). Figure 5 shows there was no significant difference between treatment on day 0 and day 5. By day 10, fruit with T5 and T6 were lower in TPC than control and those coated with T1, T2, T3 and T4. This trend continued to storage days 15, 20 and 25.

3.4 Antioxidant capacity (DPPH, ABTS, FRAP)

Table 4 presents significant interaction effects between treatments and storage days on tomato antioxidant capacity (DPPH) during storage.

As per the mean separation in Figure 6, on days 0

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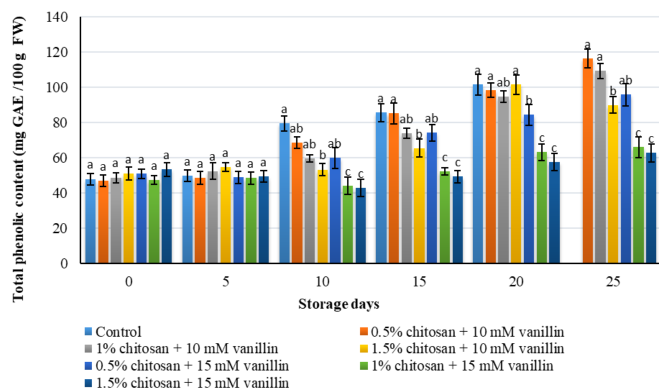

Figure 5. Effects of coating treatment on the total phenolic content of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates.

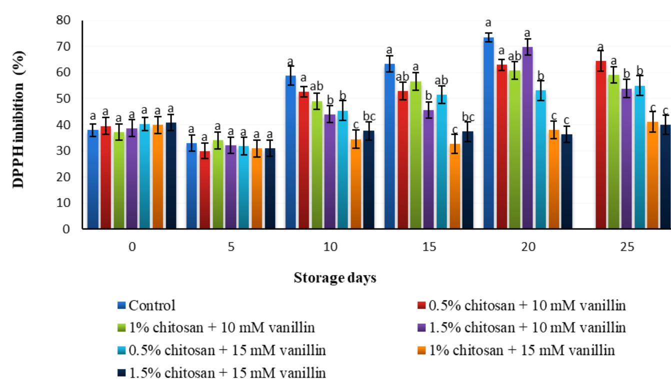

Figure 6. Effects of coating treatment on DPPH of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates.

and 5, there was no significant difference among treatments. However, on day 10, fruit coated with T5 and T6 showed lower DPPH than control and those coated with T1, T2, T3 and T4. This trend continued to the end of storage days 20 and 25.

Table 4 indicates that there were no significant interaction effects between treatments and storage days on antioxidant capacity assayed using ABTS. However, there was a main effect among treatments on the ABTS content of tomatoes. Control and those treated with T1, T2, and T4 had higher ABTS than T3, T5 and T6. As storage days advanced, ABTS of tomato increased, with day 25 higher than day 0 by 43.3%.

There were significant interaction effects between treatments and storage days on FRAP of tomato (Table 4). The result of Figure 7 exhibits that by day 10 fruit coated with T5 and T6 had lower FRAP than control and those coated with T1, T2, T3 and T4. This trend continued to the end of storage day 25.

There were positive correlations among TPC, vitamin C, lycopene, DPHH, ABTS and FRAP (Table 5). From Pearson's correlation analysis, there was a significant positive correlation between TPC and vitamin C (r = 0.75), TPC and lycopene (r = 0.72), TPC and DPPH (r = 0.76), TPC and ABTS (r = 0.58) and positive correlation between TPC and FRAP, there was as well a significant positive correlation between vitamin C and lycopene (r = 0.76), vitamin C and DPPH (r = 0.73), vitamin C and ABTS (r = 0.58) and positive correlation between vitamin C and FRAP (r = 0.63). There was also a significant positive correlation between lycopene and DPPH (r = 0.79), lycopene and ABTS (r = 0.47) and

Table 4. Main and interaction effects of different coating treatments and storage days on antioxidants capacity of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days

Factor	DPPH (%Inhibition)	ABTS (%Inhibition)	FRAP (mM TE/g FW)
Treatment			
Control	55.21[a]	50.95[a]	183.37[a]
0.5% chitosan +10 mM vanillin	39.29[b]	53.16[a]	179.49[a]
1% chitosan +10 mM vanillin	38.71[b]	52.07[a]	166.23[b]
1.5% chitosan +10 mM vanillin	40.37[ab]	45.64[b]	167.61[b]
0.5% chitosan +15 mM vanillin	42.63[ab]	52.97[a]	184.21[ab]
1.0% chitosan +15 mM vanillin	34.87[c]	43.36[b]	159.11[c]
1.5% chitosan +15 mM vanillin	33.81[c]	44.49[b]	156.83[c]
Storage days			
0	54.58[a]	37.19[e]	153.67[b]
5	27.68[c]	39.55[e]	133.84[c]
10	35.43[cb]	44.99[d]	145.67[b]
15	37.68[b]	50.13[c]	148.47[b]
20	41.19[ab]	58.26[ab]	161.89[ab]
25	44.85[ab]	65.69[a]	174.81[a]
Interaction Treatment* Storage days	**	ns	**

Means values followed by different letters indicate significantly different according to Duncan's multiple range test P < 0.01. **Significant at P ≤0.01. nsNot significant. (n=24)

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Table 5. Pearson's correlation coefficients for TPC, vitamin C, lycopene, DPPH, ABTS and FRAP of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days.

	TPC	Vitamin C	Lycopene	DPPH	ABTS	FRAP
TPC	-					
Vitamin C	0.75**	-				
Lycopene	0.72**	0.76**	-			
DPPH	0.76**	0.73**	0.79**	-		
ABTS	0.58**	0.56**	0.47**	0.52**	-	
FRAP	0.74 **	0.63**	0.73**	0.76**	0.54**	-

TPC = Total phenolic content, DPPH = 2, 2-diphenyl-1-picrylhydrazyl, ABTS = 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) and FRAP = ferric reducing antioxidant power. ** Significant correlation at P ≤0.01.

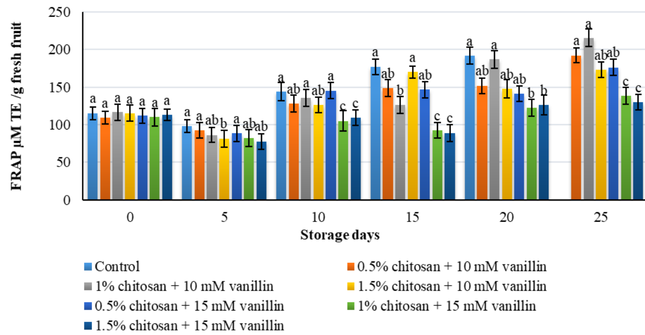

Figure 7. Effects of coating treatment on FRAP of tomato fruit stored at 26±2°C and 60±5% relative humidity for 25 days. Bars different letters notation in each storage day differed significantly by DMRT at P≤0.01. Vertical bars indicate the standard error of means for four replicates.

positive correlation between lycopene and FRAP (r = 0.73). There was well a significant positive correlation DPPH and ABTS (r = 0.52) and positive correlation between DPPH and FRAP (r = 0.76) (Table 5).

4. Discussion

4.1 Effects of edible coating on diseases incidence and diseases severity

Postharvest fungal disease is one of the important factors that restrict the storage period and marketing life of perishable fruit (Hossain *et al*., 2010), and it is a major concern in influencing consumer prices, requirements and mode of transportation, and even result in serious economic losses worldwide (Zhang *et al*., 2019). Severity is the quantity of disease affecting entities within a tomato fruit, where fruit will lose quantity, quality, and marketing value when severity has occurred and progressed (Zhang *et al*., 2017). The capacity of chitosan coating in inhibiting the growth of several fungi has been reported for a wide variety of harvested commodities (Garcí, 2013). This was in agreement with Xing *et al*. (2016) who found that chitosan coating formed a thin film on fruit surface and inhibit the growth of inoculated bacteria, yeast and moulds. This inhibition could due to chitosan activity in the disturbing cell membrane of pathogen that led to intracellular leakage and finally cells death (Matica *et al*., 2017). According to Chang (2019), chitosan could interfere with both cell membranes and cell walls of a pathogen and inactivated the function of the genetic material of the pathogen.

The interaction was significant between treatment and storage days on tomato fruit diseases incidence and severity (Figures 1 and 2). Generally, the present study found that the disease incidence and severity increased with the advancement of the storage period. Coating significantly affected the percentage of disease incidence and severity during the storage period. As the storage period progressed, control fruit and those treated with a low concentration of chitosan and vanillin T1 and T4 showed severe infection and reflected as greater disease incidence. At the end of storage day 25, the disease incidence and severity increased by 75 and 55.17%, respectively, as compared to the initial day of storage. While a high concentration of chitosan and vanillin T5 and 1.5% chitosan + 15 mM inhibited the progression of diseases in tomato fruit as found in Figures 1 and 2. By increasing the concentration of chitosan and vanillin, diseases incidence and severity decreased. However, by day 25, coating of T5 and T6 inhibited diseases incidence by 75 and 74.16%, respectively while severity was by 75 and 79%, respectively.

The decrease of disease incidence and severity in a high concentration of coating could due to the formation of semi-permeable film around the fruit surface. This thin layer could prevent the growth of pathogens by disturbing the cell membrane that caused intracellular leakage and finally cell death of pathogens. Besides, chitosan and vanillin coating can enhance the epidermal structure of fruit and limit the spread of pathogens. Abebe and Mohammed (2017) expressed that coating could assist the cell wall to retain its integrity against fungal attack and help in delaying pathogenic infection. This finding was in agreement with Sikder *et al*. (2019) who found mature green banana coated with 1% chitosan and stored at room temperature (28±2ºC) has remarkably inhibited disease incidence and severity than fruit coated with 0.5% chitosan. Hewajulige and Sivakumar (2007)

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also found that anthracnose disease incidence and severity on papaya fruit coated with 1% chitosan was 4.5 times lowered as compared to those coated with 0.5% chitosan. Taghinezhad and Sharabiani (2018) found out similar finding that 1.5% chitosan coating reduced the decay rate in cucumber and bell pepper than those coated with 0.5% chitosan. In the current study, the film created by the higher concentration of T5 and T6 coating has slowed down the ripening and senescence process of tomato fruit and therefore disease incidence and severity were less in this tomato fruit during 25 days of storage at 26±2°C and 60±5% RH.

4.2 Antioxidant properties

4.2.1 Vitamin C content

The result of Figure 3 shows a significant interaction effect between treatments and storage days in vitamin C content of tomato. By increasing chitosan and vanillin concentration, the vitamin C content of tomato decreased (Table 3). Hence, fruit with T5 and T6 had lower vitamin C content than control and those coated with T1, T2, T3 and T4. By storage day 25, the vitamin C content of fruit with T5 and T6 was 34 and 38%, respectively lower than fruit coated with T1.

This might be due to the coating of chitosan that has inhibited vitamin C synthesis and delayed the changes in vitamin C content. In line with this study, Petriccione *et al*. (2015) reported that 1.5% chitosan-coated sweet cherry showed lower vitamin C content than control during storage at 2°C for 14 days. Similarly, Kibar and Sabir (2018) reported that vitamin C content in tomato coated with 1% chitosan decreased during storage at 21° C. The film created by a high concentration of T5 and T6 coating could reduce oxygen diffusion. Thus, resulting in the slow ripening and senescence process of tomato and therefore, vitamin C content is retained at a lower level in this fruit during 25 days of storage at 26±2°C/ 60±5% RH.

4.2.2 Lycopene

Lycopene gives characteristic of red colour and due to its strong colour and non-toxicity, it is a good food colouring (Alda *et al*., 2009). There was a significant interaction between treatments and storage days (Figure 4). From day 15 until the end of storage day 25, fruit coated with T5 and T6 had lower lycopene content than control fruit and those coated with T1, T2, T3 and T4. At the end of storage day 25, fruit coated with T5 and T6 has 39.72 and 44.02% lower lycopene content than fruit coated with T1. This may duet coatings reduce the respiration rate and ethylene production of fruit by forming a barrier around its surface. In line with this study Mandal *et al*. (2018) reported that 2% chitosan coating had lower lycopene in fruit than those coated with 0.5% chitosan during 22 days of storage in ambient conditions (25±2°C). Abebe *et al*. (2017) also found out that 3% chitosan coating reduced lycopene content in fruit than those coated with 0.5% chitosan during storage at 22±1°C/75±1% RH. It has been claimed that the formation of lycopene depends on the rate of respiration and ethylene production during storage. This could explain fruit coated with a higher concentration of coatings such as T5 and T6 have lower lycopene content than other coatings.

4.3 Total phenolic content

Phenolic compounds are one of the major contributors to the antioxidant properties of fresh products (Pinheiro *et al*., 2016). Phenolic compounds are important plant elements with redox properties that are responsible for the antioxidant activity, where the hydroxyl groups in plant extracts are responsible for facilitating free radical scavenging (Aryal *et al*., 2019). Plants need phenolic compounds for pigmentation, reproduction, growth, and resistance to pathogens (Lattanzio *et al*., 2006). Phenolic compound possesses antioxidants and serves as protective mechanisms in fruit (Khaliq *et al*., 2016). As per the result of Figure 5, there was a significant interaction effect between treatments and storage days. The result indicates that fruit coated with T5 and T6 had lower total phenolic content (TPC) than control and fruit that coated with T1, T2, T3 and T4. However, by storage day 25, T5 and T6 had 56.13 and 49.5% lower TPC than fruit coated with T1.

The increase of phenolic levels in low concentration chitosan and vanillin coating (T1 and T4) might be due to the higher rate of respiration that caused degradation of certain phenolic compounds. In agreement with the current study, Petriccione *et al*. (2015) found that TPC was higher in sweet cherry coated with 0.5% chitosan than fruit treated with 1% chitosan during 14 days of storage at 2°C. Yang *et al*. (2014) also reported that TPC content was lower in blueberry coated with 2% chitosan than those coated with 0.5% chitosan during storage at 2±1ºC/95±2% RH for 35 days. In the present study, the barrier created by the higher concentration of coating T5 and T6 has slowed down the ripening and senescence processes and suppressed abiotic stresses on produce which modified the metabolism of tomatoes. Therefore, the TPC was lesser in this fruit.

4.4 Antioxidant capacity (DPPH, ABTS, FRAP)

In the present study, Figure 6 shows significant interaction between treatments and storage days on tomato DPPH. At the end of storage, fruit coated with T5 and T6 had 45.06 and 50.30% lower DPPH than control

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and those coated with T1, T2 and T4.

A study by Kou *et al*. (2014) reported that DPPH in pear fruit coated with 2% chitosan decreased during storage. Zahedi *et al*. (2019) also found out that DPPH in mango fruit coated with 2% chitosan decreased during storage for 24 days at 15±2°C/85-90% RH. In line with this study Zam (2019) found out that DPPH of sweet cherry coated with 1% chitosan decreased during 20 days of storage. In the present study, the film created by the high concentration of coating T5 and T6 has caused modification to internal atmosphere modification of phenolic compounds of tomato and therefore DPPH is lower in this fruit.

Table 4 shows the coating of T1, T2, T3, T4, T5 and T6 affected fruit antioxidant capacity (ABTS). Fruit coated with T3, T5, T6 had lower ABTS than control, and those coated with T1 and T4 during entire storage. The increase in antioxidant capacity might occur due to faster senescence and decay of tomatoes. In line with this study, Martínez *et al*. (2018) found that strawberry coated with 1.5% chitosan had lower ABTS than fruit coated with 0.5% chitosan and 0.5% chitosan + 1% *Thymus capitatus* essential oil during 15 days of storage. Hosseini *et al*. (2018) also reported that ABTS of banana treated with 1% chitosan decreased when stored at 15±2°C/85-90% RH for 20 days. In the current study, the barrier formed by the high concentration of coating T5 and T6 has slowed down the senescence process and reduced decay in tomato and thus ABTS is lesser in this fruit.

Figure 7 shows significant interaction between treatments and storage days on tomato FRAP. However, fruit coated with T5 and T6 had lower FRAP than control and fruit coated with T1, T2, T3 and T4 during entire storage. As storage days advanced, FRAP increased, where FRAP at day 25 was 13% higher than day 0. However, by storage day 25, fruit coated with T5 and T6 has lower FRAP than control and fruit coated with T1, T2, T3 and T4.

This finding might be due to the formation of a protective barrier on the surface of fresh fruit and inhibited the decline of antioxidant activity (Kou *et al*., 2014). In agreement with this study's finding, Ghasemnezhad *et al*. (2013) found out that the antioxidant capacity (FRAP) of pomegranate treated with 1% chitosan decreased during 12 days of storage at 4°C. In line with this study, FRAP in strawberry treated with 1.5% chitosan and stored at 10°C for 12 days has decreased (Wang and Gao, 2013). Tomato coated with 10% gum Arabic had lower FRAP than those treated with 0 and 5% gum Arabic stored at 20°C/80-90% RH for 20 days (Ali *et al*., 2013). Therefore, a barrier formed by the higher concentration of coating has slowed down the senescence process and reduced decay in tomatoes thus led to lower FRAP in the present study.

There was a significant positive correlation between antioxidants i.e. TPC, vitamin C, lycopene and antioxidant capacity (DPPH, ABTS and FRAP) in tomato fruit treated with chitosan and vanillin during entire storage (Table 5). This was in agreement with Lugasi and Helyes (2006), who found a positive correlation TPC and lycopene content (r = 0.72) in tomato fruit during ripening. A similar finding was also found out by Izawa *et al*. (2018) where the correlation was positive between TPC and vitamin C (r = 0.75) in tomato fruit. In line with this study, Alda (2009) also found a positive correlation between TPC and lycopene (r = 0.72) in tomato fruit. In agreement with the study by Palonen and Weber (2019) who reported a strong positive correlation between TPC and vitamin C (r = 0.75). A similar result was also reported by Floegel *et al*. (2011) that there was positive significance in apple fruit that the correlation between TPC and DPPH (r = 0.76), significant correlation TPC and ABTS (r = 0.58) and significant correlation of TPC and FRAP (r = 0.74). In line with this study, Fu *et al*. (2010) also found a highly significant positive correlation between TPC and antioxidant capacity (FRAP) (r = 0.74) in *Ficus benjamina*. The finding of this study indicated that TPC is the major contributor to tomato fruit antioxidants and antioxidant capacity.

5. Conclusion

Chitosan combines with vanillin in different concentrations was used as edible coatings to examine its effect on the antioxidant and antioxidant properties of tomato. Results exhibit that a higher concentration of chitosan and vanillin coatings 1.5% chitosan + 15 mM vanillin has retained low antioxidant content (TPC, vitamin C and lycopene content), antioxidant activity, and capacity of tomato during storage at 26±2°C/60±5% RH for 25 days. At day 25 mentioned coating agent retained low vitamin C up to 30.47%, TPC 50%, DPPH 48.21%, ABTS 9.82% and FRAP up to 33.65% as compared to control and fruits those coated with 0.5% chitosan + 10 mM vanillin. This study indicates that 1.5% chitosan + 15 mM vanillin is recommended to coat tomato when a refrigeration facility is not available during marketing.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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