Maintenance of defense enzymes activities in tomato fruit during storage by chitosan and vanillin coating

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IJASR 2021
VOLUME 4
ISSUE 2 MARCH – APRIL **ISSN: 2581-7876**

Abstract: Tomato is rich sources of minerals, vitamins, polyphenols, and carotenoids that are beneficial for human health. Chitosan and vanillin could be an elicitor to induced defense enzyme activities in host against pathogen causing disease. This study aimed to evaluate the effect of chitosan and vanillin coating on defense-related enzymes (PAL, PPO and POD) activites on tomato fruits during ambient storage Chitosan and vanillin in aqueous solutions i.e. 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 fruits. The results revealed 1.5% chitosan + 15 mM vanillin have significantly lower the activities of defense enzyme i.e. peroxidase (POD) and polyphenoloxidase (PPO), and phenylalanine ammonia-lyase (PAL) while shelf life was prolonged to 25 days at 26 ± 2ºC and 60 ± 5% relative humidity without any negative effects on fruit postharvest quality.

Keywords: Defense enzyme; peroxidase; polyphenoloxidase; phenylalanine ammonia-lyase; vanillin; chitosan

Introduction

Tomato (Lycopersicon esculentum Mill) is one of the most popular and highly consumed vegetables globally (Sucharitha et al., 2018). Tomatoes are rich in antioxidants such as lycopene, carotenoid, total phenolic content, minerals and vitamins (Safari et al., 2020). These valuable antioxidants could reduce the risk of heart disease, cancer, oxidative stress and cardiovascular diseases (Forni et al., 2019; Jing et al., 2019; Liu et al., 2018).

However, being a climacteric fruit, it associated with short postharvest life due to postharvest diseases, accelerated ripening and senescence that resulted losses in quantity and quality. Tomato sustained their shelf life in ambient storage around 8-12 days (Mwende et al., 2018). The quality maintenance of tomato is very crucial in postharvest handling. Commonly, in developing countries, the quality loss was due to insufficient postharvest handling, poor transport systems, fluctuating temperatures, relative humidity (RH), gaseous storage and postharvest diseases (Arah et al., 2015).

Generally, the fresh fruit could not improve their quality after harvest. Nevertheless, itcan be retained by applying effective postharvest management techniques. Researchers indicated that edible coating is one of the alternative treatments to prolong postharvest life by preserving fruit quality, yet, it was low in cost (Mahfoudhi et al., 2014). Coating treatment acted as a barrier to water loss, physical, chemical, microbiological activity, anti-browning agents, and exchange of gases (Safari et al., 2020), lowering the oxidative reaction rate and maintain nutritional quality during storage (Kore et al., 2017; Dhumal and Sarkar, 2018; Bal, 2019). Among structural materials of edible coatings, chitosan from polysaccharides-based has the ability to form semi-permeable films on fresh fruits (Nor and Ding, 2020). It retard the fruit deterioration and extend the storage life by inhibiting the growth of microorganism and modifying the internal atmosphere to reduce respiration and ethylene production rate, thus

delay activities in defense enzymes (Jiao et al., 2019; Rahimi et al., 2019). Phenylalanine ammonia-lyase (PAL), peroxidase (POD), and polyphenoloxidase (PPO), are among the most important enzymes with defensive responses in plants against insects and pathogens (Han et al., 2009).

Vanillin is an organic phenolic aldehyde that has antimicrobial effects against yeasts, molds, and bacteria (Quyen and Rachtanapun, 2016; Rakchoy et al., 2009; Safari et al., 2021). These antimicrobial effects control the decay of fruit (Takma and Korel, 2017). In in vitro study, researchers found out vanillin inhibits mycelium growth of Escherichia coli in food (Stroescu et al., 2015), Anthracnose in mango fruit (Jaimun et al., 2019) and Botrytis cinerea in grapes (Sangsuwan, 2019).

In term of defense-enzyme activities, 1% of chitosan significantly suppressed the activities of POD and PAL compared to 0.5% of chitosan and control in Sponge gourd stored in darkness at 25±1◦C and 90–95% relative humidity (RH) (Han et al., 2014). Wang and Gao (2013) reported that 1% chitosan coating decline POD activity in strawberry fruit during 9 days of storage at 10ºC. In other studies, 1% chitosan reduced PPO enzyme activities in pomegranate fruit during 12 days storage at 4ºC. To date, the combination effects of chitosan with vanillin on defense enzymes activity of tomato stored at room temperature 26 ± 2ºC/60 ± 5% RH is not well-explored. Therefore, this study aimed to determine the combined effects of chitosan and vanillin as a coating agent on defense enzymes activities during storage at 26 ± 2ºC/60 ± 5% RH of tomato fruit.

Materials and methods

Fruit materials

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

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, an organic compound of 99% pure vanillin with the molecular formula $C_8H_8O_3$ was bought from Evergreen Engineering & Resources Sdn. Bhd., 43500 Semenyih, Selangor, Malaysia. Chitosan solution with concentration of 0.5, 1 and 1.5% v/v was prepared and the solution pH was adjusted to 5.6 with 1 M NaOH, and 0.1% Tween 20 was added to improve the solution wettability. Distilled water without chitosan containing 0.1% Tween 20 was served as control. Vanillin powder with concentration 10 and 15 mM was dissolved in distilled water. A hot plate magnetic stirrer was used to heat the solution at 83ºC for 5 min until vanillin powder has melted and 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.

Postharvest coating treatments

Tomato was dipped in chlorinated water that prepared from 0.05% sodium hypochlorite for 3 min prior to coating treatments (Ali et al., 2010). The fruit was rinsed and air-dried for 1 h and randomly divided into seven lots. All fruit were dipped for 1 min in coating solution. For control, the fruit was dipped in distilled water containing 0.1% Tween 20. The fruit was then dried for 2 h at 26 ± 2°C/60 ± 5% relative humidity (RH). For each coating, six fruit per replicate was used. The fruit was then packed in 18-holes 0.5 cm diameter perforated plastic bag 18 cm x 26 cm of 0.05 mm thickness. These bags were placed in commercial corrugated fibreboard cartons of 30 cm x 25 cm x 15 cm. The fruit was stored at 26 ± 2°C/60 ± 5% RH for 25 days. Each treatment repeated four times and analysis was carried out at every 5-day interval. In each replication, six fruit was analysed.

Determination defense enzymes activities

Protein content

The extraction and analysis of protein are carried out by using the combined techniques of Safari et al. (2021); Jumnongpon et al. (2012); Raseetha et al. (2011) and Bonjoch and Tamayo (2001) with minor modifications. The chemicals used to extract and evaluate enzymes were analytical grade. Frozen tomato fruit pulp tissue of 0.5 g was immediately ground by using a small ceramic kitchen pestle and mortar (UNITED SCIENTIFIC PPM075 Mortar and Pestle, 125 mL, USA) for 30 s on ice and homogenized with 1 mL ice-cold 50 mM phosphate buffer containing 1 M NaCl (pH7.1). The mixture was centrifuged (Scan Speed 1730R, Scala Scientific, Netherlands) at 16000 x g at 4°C for 20 min. The supernatant was then kept in an ice-water bath prior to the analysis.

The protein content of protein solutions derived from tomato fruit was measured using the Bradford procedure (Bradford, 1976). Bradford reagent was obtained from Bio-Rad Laboratories, Inc., USA. The Bradford reagent was prepared by using distilled water in a 1:4 ratio, 40 mL Bradford reagent was mixed in 160 mL distilled water, then 1.2 mL of Bio-Rad Bradford reagent was added with 120 µL protein supernatant and the mixture was briefly vortexed. The mixture was left to incubate for 30 min at room temperature, and the absorbance was read at 595 nm. The concentration of the extracted protein solutions from the bovine serum albumin standard curve (R^2=97) has been quantified. The measurement was repeated three times. A standard curve plotting absorbance with various concentrations was obtained using bovine serum albumin (Sigma Chemicals Co., St. Louis, USA) in the concentration range 25- 400 µg/mL. The protein content in mg/mL was read against the standard curve and calculated by following formula according to Wang et al. (2020):
Protein content (mg/mL) = protein quality × VT/VS × W
Where:

Protein quality results are collected in agreement with the standard curve; VT is the total volume of extraction and VS is the volume of solution for evaluation while W is the weight of the sample.

Determination of phenylalanine ammonia-lyase (PAL) enzyme activities

The extraction for enzyme phenylalanine ammonia-lyase (PAL) was carried out according to Tamimi et al. (2017) and Han et al. (2014) methods with some modifications. 50 mg frozen tissue was ground in 2 mL cold 25 mM sodium borate buffer (pH 8.8), containing 2 mM β-mercaptoethanol and 0.5 g polyvinylpyrrolidone. The homogenate was centrifuged (Scan Speed 1730R, Scala Scientific, Netherlands) for 20 min at 16000 x g at 4ºC, and the supernatant was used as an enzyme source to determine the PAL activity.

PAL activity was determined by the production of cinnamate at 37ºC for 1 h, the absorbance was measured at 290 nm (Habibi et al., 2019). The assay mixture comprised 1 mL of enzyme extract and 2 mL of 50 mM sodium borate buffer (pH 8.8). The reaction started with 1 mL of 20 mM L-phenylalanine added and incubated at 37ºC for 1 h. Then, the reaction was stopped by adding 1 mL of 1 M HCl. The blank assay was performed with a mixture containing L-phenylalanine at zero incubation times. One unit of PAL activity has been defined as the amount of enzyme that produced an absorbance increase of 0.01 at 290 nm per h (Habibi et al., 2019). The specific activity of the PAL enzyme was expressed as U/mg protein, where one unit of enzyme activity was defined as the production of cinnamic acid and the increase of one unit in absorbance per h. The activity of the enzymes was determined using the analytical approximation as defined in the following equation:

Unit enzyme activity (U/mL) = Δ A 270 nm/min Test- Δ A 270 nm/min Blank *3* df /19.73*0.1
The specific activity of the enzymes was expressed in (U/mg protein) as followed:

Specific activity (U/mg protein) = Unit activity (U/mL) / Protein content (mg/mL) (Sigma Prod. No. P-2126)

Determination of peroxidase activity

Extraction and assay of peroxidase activity were carried out based on the combined procedure of Zhang et al. (2018) and Raseetha et al. (2011) with minor modifications. 0.5 g frozen tomato fruit pulp tissue was immediately ground by using a small ceramic kitchen pestle and mortar (UNITED SCIENTIFIC PPM075 Mortar and Pestle, 125 mL, USA) for 30 s on ice and homogenized with 1 mL ice-cold 50 mM phosphate buffer containing 1 M NaCl (pH7.1). The mixture was centrifuged (Scan Speed 1730R, Scala Scientific, Netherlands) for 20 min at 16000 x g at 4°C. The supernatant was then kept in an ice-water bath prior to the analysis.

The POD activity was determined based on the development of brown coloration in the presence of hydrogen peroxide H_2O_2 arising from the oxidation of guaiacol. A 20 µL sample extract supernatant was well mixed in a clean cuvette with 1.7 mL 0.1 M sodium phosphate buffer pH 7.0, and 200 µL of 1 mM guaiacol. Then the POD reaction was started by adding 100 µL of 1.5% H_2O_2 v/v. The rate of absorbance rise at 485 nm was monitored for 3 min at 20°C. The POD activity was expressed as U/mg protein by Kokkinakis and Brooks (1979) and Ogola et al. (2009) as follows:

Unit activity (U/mL) = (Δ OD /min * V * D) / (26.6 * d* v)
The specific activity of the enzymes was expressed in (U/mg protein) as followed:
Specific activity (U/mg protein) = Unit activity (U/mL) / Protein content (mg/mL)

Polyphenol oxidase activity

Polyphenol oxidases (PPO) activity was determined based on changes in the color intensity of catechol oxidation products as described in combination methods of Indunil Kumari et al. (2017) and Mishra et al. (2012). The extracted POD supernatant was used as the source of the enzyme, which was held at -20°C. Briefly, 200 µL of 0.01 M catechol was supplemented to start the reaction. The absorbance changes were recorded at 495 nm for 1 min. The PPO specific activity was determined by expressing PPO enzyme specific activity (U/mg protein) by the following equation:

Unit activity (U/mL) = (Δ OD/min * V * D) / (11.3 * d* v)
The specific activity of the enzymes was expressed in (U/mg protein) as followed:
Specific activity (U/mg protein) = Unit activity (U/mL) / Protein content (mg/mL)

Experimental design and statistical analysis

The experiments were carried out in a completely randomized design (CRD) with seven coating treatments and four replications (Figure 1). 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 $P \leq 0.05$. All the analyses were conducted using a statistical analysis software (SAS) version 9.4 (SAS Institute Inc., Cary, North Carolina, USA). Pearson's correlation analyses were used to correlate defense enzymes among each other. The entire experiments were repeated three times and the data were pooled before analysis. However, control fruit was discarded for analysis after day 20 due to high disease severity and decay.

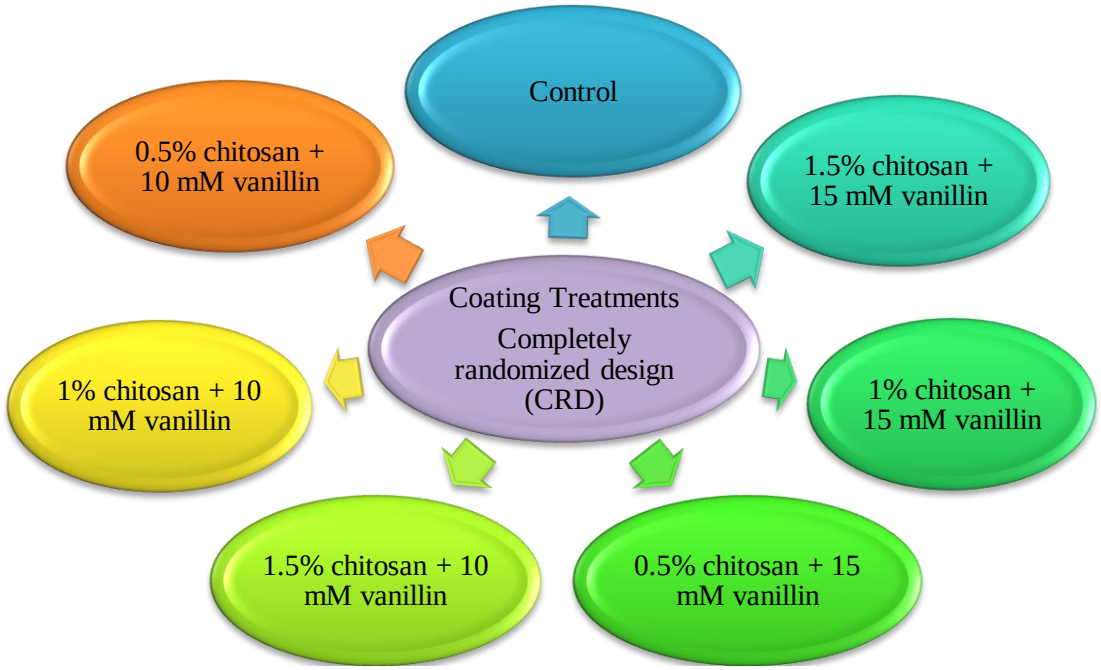

Figure 1. Schematic diagram showing coating treatments.

Result

Defense-related enzymes (PAL, PPO, and POD) activity

In the present study, there were significant interaction effects between coating treatments and storage days in PAL, PPO, and POD activities of tomato fruit (Table 1).

Table 1: Main and interaction effects of different coating treatments and storage days on defense-related enzyme activity of tomato fruit stored at 26 ± 2°C 60 ± 5% relative humidity for 25 days

Factor	PAL specific activity (U/mg protein)	PPO specific activity (U/mg protein)	POD specific activity (U/mg protein)
Treatment			
Control	0.95 a[z]	1.25 a[z]	1.55 a[z]
0.5% chitosan +10 mM vanillin	0.92 a	1.17 a	1.51 a
1% chitosan +10 mM vanillin	0.88 ab	1.10 b	1.48 a
1.5% chitosan +10 mM vanillin	0.81 b	1.27 a	1.36 b
0.5% chitosan +15 mM vanillin	0.91 a	1.08 ab	1.44 ab
1.0% chitosan +15 mM vanillin	0.82 c	0.88 c	1.09 c
1.5% chitosan +15 mM vanillin	0.74 c	0.77 c	1.03 c
Storage day			
0	0.59 d	0.69 c	1.16 c
3	0.69 c	0.72 c	1.10 c
6	0.76 b	0.87 b	1.29 b
9	0.85 ab	0.94 ab	1.39 b
12	0.94 a	1.15 a	1.54 a
15	1.09 a	1.17 a	1.45 a
Interaction			
Treatment* Storage day	**	**	**

[z]Means values in a column followed by different letters indicate significantly different according to Duncan's multiple range test at P < 0.05. [**]Highly significant at P ≤0.01. (n=24)

Figure 2 displays that there were no significant changes in PAL enzymes activity among treatments at day 0 and day 5. However, at storage day 10, fruit treated with 0.5% chitosan + 10 mM vanillin 1% chitosan + 10 mM vanillin, 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin shows lower PAL activity but not different with fruit control and those coated with 0.5% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin. By storage day 15 fruit coated with 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin had lower PAL activity than than control fruit and those coated with 0.5% chitosan + 10 mM vanillin, 1% chitosan + 10 mM vanillin, 1.5% chitosan + 10 mM vanillin and 0.5% chitosan + 15 mM vanillin. This trend continued-up until the end of storage day 25 where starting from storage day 10, the PAL activity has increased gradually to the maximum level.

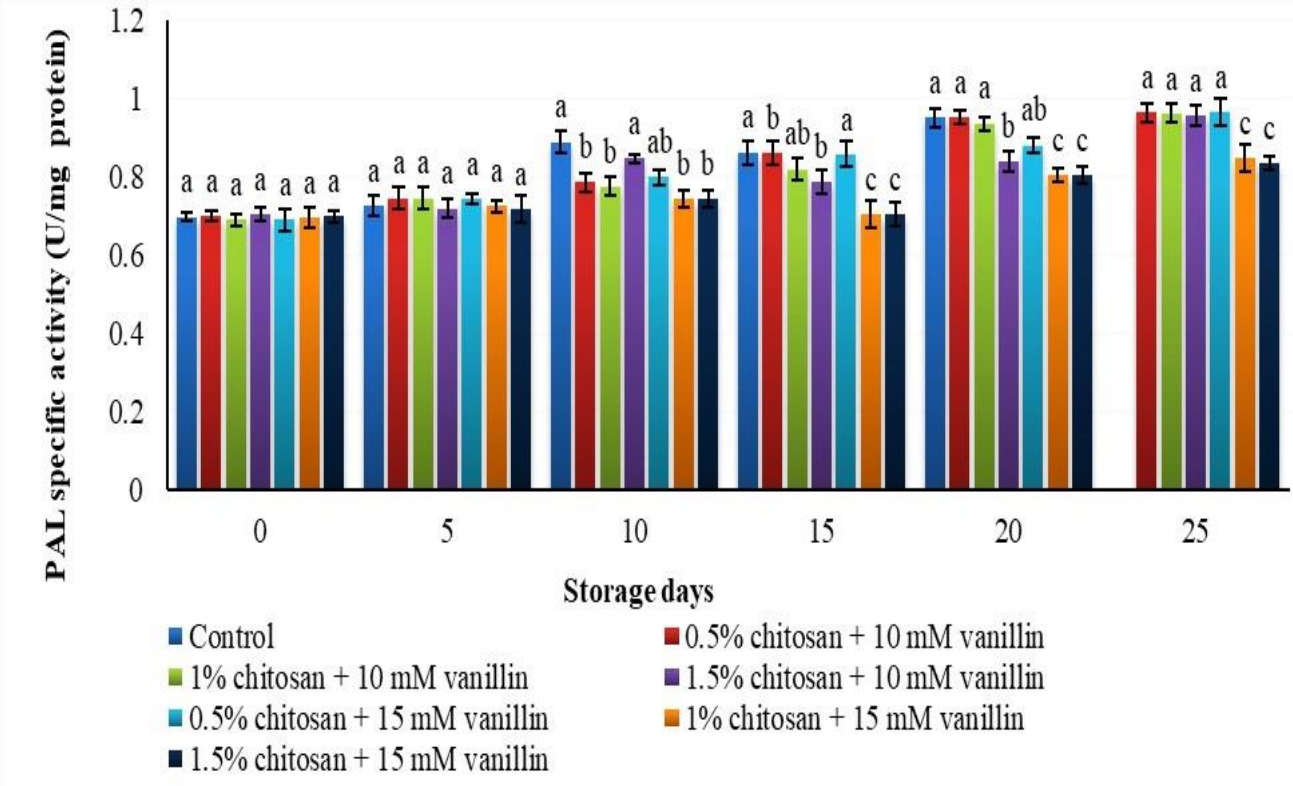

Figure 2: Effects of coating treatment on PAL specific activity in tomato fruit stored for 25 days at 26 ± 2°C and 60 ± 5% relative humidity. Means value in a column followed by different letters in each storage days differed significantly by DMRT at P ≤ 0.05. Vertical bars indicate standard error of means for four replicates. (n=24)

Figure 3 displays no significant PPO enzyme activity changes among all treated fruit at day 0 up to day 15. However, at storage day 20, the activity of the enzyme was lower in fruit treated with 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin than control fruit and those coated with 0.5% chitosan + 10 mM vanillin, 1% chitosan + 10 mM vanillin, 1.5% chitosan + 10 mM vanillin and 0.5% chitosan + 15 mM vanillin. This trend continued until the end of storage day 25.

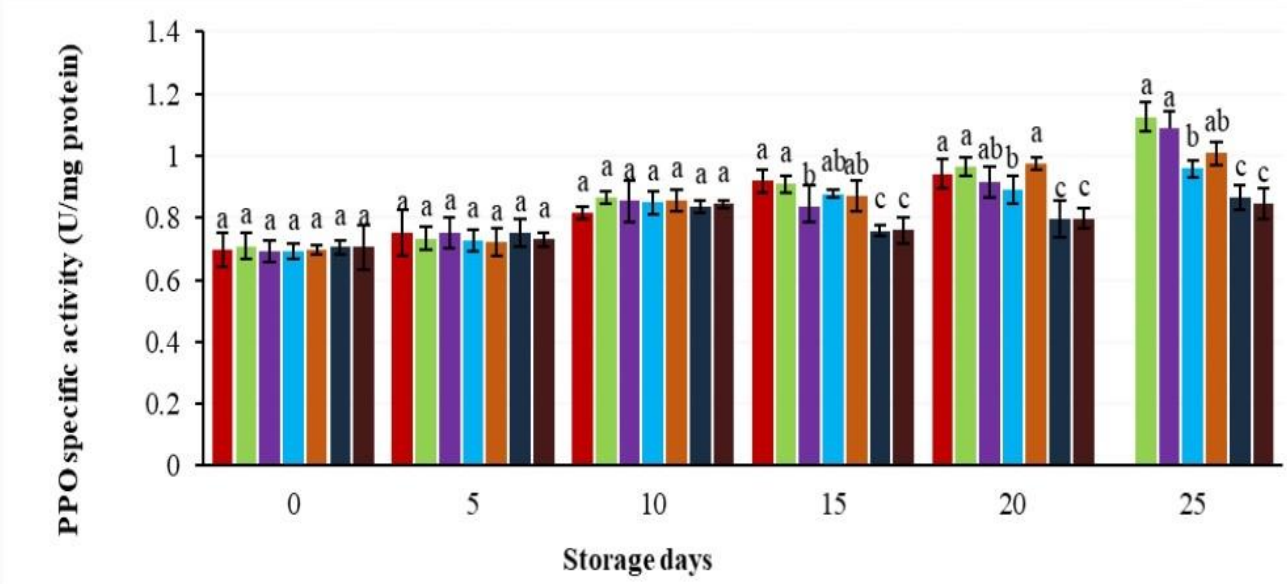

Figure 3: Effects of coating treatment on PPO specific activity in tomato fruit stored for 15 days at 26 ± 2°C and 60 ± 5% relative humidity. Means value in a column followed by different letters in each storage days differed significantly by DMRT at P ≤ 0.05. Vertical bars indicate standard error of means for four replicates. (n=24)

Figure 4 exhibits no significant changes in POD enzyme activity among treatments at day 0 and day 5. However, at storage day 10, the activity of the enzyme was lower in fruit treated with 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin than control fruit and those coated with 0.5% chitosan + 10 mM vanillin, 1% chitosan + 10 mM vanillin, 1.5% chitosan + 10 mM vanillin and 0.5% chitosan + 15 mM vanillin. This trend continued until the end of storage day 25.

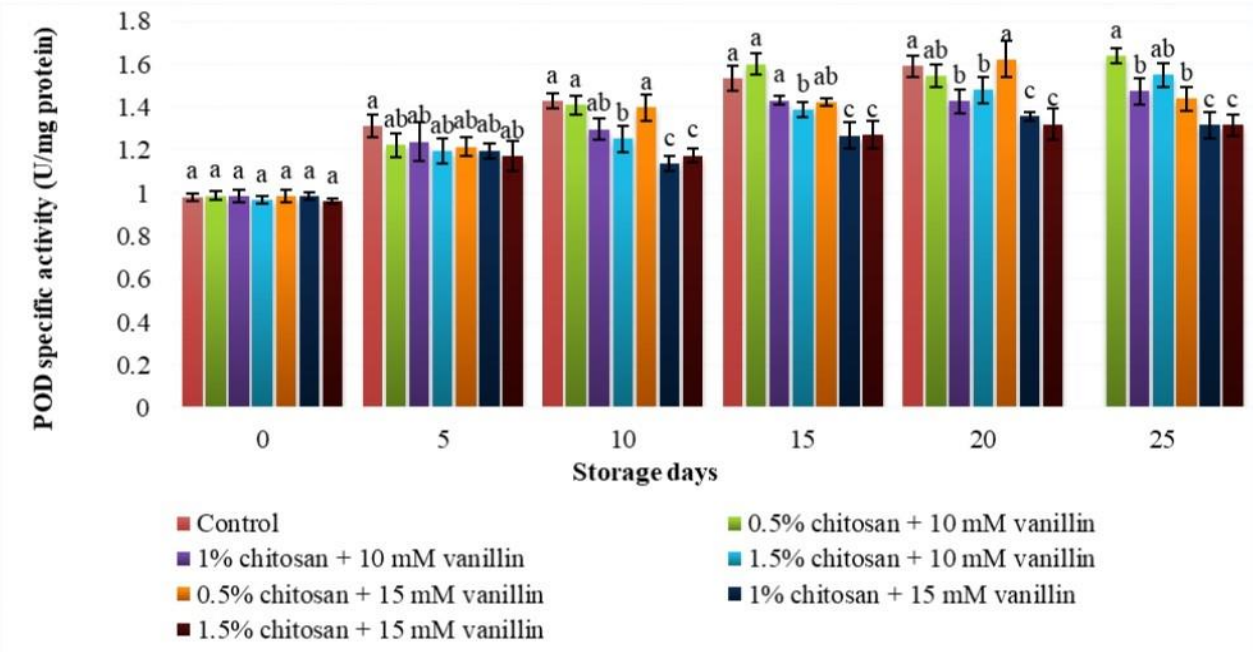

Figure 4: Effects of coating treatment on POD specific activity in tomato fruit stored for 15 days at 26 ± 2°C and 60 ± 5% relative humidity. Means value in a column followed by different letters in each storage day differed significantly by DMRT at P≤0.05. Vertical bars indicate standard error of means for four replicates. (n=24)

There was a significant correlation among defense-related enzymes. Pearson's correlation analysis shows that there was a highly significant positive correlation between PAL and PPO (r = 0.76), an intermediate positive correlation between PAL and POD (r=0.71), also a positive correlation between POD and PPO (r = 0.69) (Table 2).

Table 2: Pearson's correlation coefficients for diseases incidence and severity of Fusarium oxysporum inoculated tomato fruit stored at 26± 2 °C 60 ± 5% relative humidity for 15 days

	PAL	PPO	POD
PAL	-		
PPO	0.76 **	-	
POD	0.71**	0.69**	-

PAL = Phenylalanine ammonia-lyase, POD = Peroxidase and PPO = Polyphenoloxidase. ** Significant correlation at P ≤0.05 and P ≤0.01. (n=24)

Discussion

Effects of coating on the activity of defense-related enzymes (PAL, PPO, and POD)

There are many defense enzymes involved in defense reactions against plant pathogens including oxidative enzymes such as POD, PPO, and PAL (Lavania et al., 2006). Defense-related enzymes such as PAL, PPO, and POD are important biochemical indicators for pathogen resistance in host plants (Han et al., 2009). PAL is a leading enzyme in the metabolism of phenols that protect plants against stress conditions (Soleimani et al., 2012). Interestingly, there was a significant interaction effect between treatment and storage day on tomato fruit defensive enzyme PAL activity (Figure 2). As storage day advanced, PAL activity increased, contrary as the concentration of chitosan and vanillin increased, the PAL activity decreased (Table 1). However, at the end of storage day, PAL activity in fruit coated with 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin had 28.82 and 31.85%, respectively. It was lower than the fruit coated with 0.5% chitosan + 10 mM vanillin. Overexpression of PAL activity might be due to injury that caused by disease attack, fruit senescence, and ethylene production in control fruit and those coated with 0.5% chitosan + 10 mM vanillin. Zhan and Zhu (2011) found a declining trend of PAL activity of water caltrop fresh fruit (Trapa natans L.) coated with 1 and 2% chitosan compared to 0.5% chitosan during 15 days of storage at 4 ± 1ºC and 80-85% RH. Previous research also reported tomato fruit that coated with 1.5% chitosan has lower PAL activity than those coated with 0.5% stored at 25ºC (Lu et al., 2019). In the present study, the layer created by the higher concentration of coating such 1.5% chitosan + 15 mM vanillin was probably reduced ethylene production rate and slowed down the ripening process of tomato fruit that may lead to low PAL activity in this fruit.

PPO is a crucial defense enzyme against pathogen reaction by the oxidation of polyphenols into quinines which have antimicrobial activity and also strengthen the resistance of plant cells during the microbial attack (Wang et al., 2019; Prasannath, 2017). The tomatoes' PPO activity was significantly affected by the interaction between treatment and storage day (Figure 3). Table 1 shows that as storage day advanced, PPO activity increased. Conversely, as chitosan and vanillin concentration increased, i.e. 1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin, the PPO activity was decreased. However, PPO activity at the end of storage day, fruit coated with 1% chitosan + 15 mM vanillin, and 1.5% chitosan + 15 mM vanillin had 29.65 and 33.7% lower PPO activity than fruit coated with 0.5% chitosan + 10 mM vanillin, respectively. This reduction and inhibition PPO activity might be due to chitosan coatings, which had reduced the exposure of the fruit to oxygen and suppressed enzyme activity by wrapping the fruit's surface. In agreement to Minh et al. (2019) study, PPO activity in 1.5% chitosan-coated fresh mushroom was lower than those coated with 0.5% chitosan. Ghasemnezhad et al. (2013) demonstrated that PPO activity in pomegranate fruit coated with 1% chitosan was lower than those coated with 0.5% chitosan. A similar finding reported in litchi fruit (Eissa, 2007a) and tomato fruit (Badawy and Rabea, 2009) during storage. In the current study, the film created by the higher concentration of coating 1.5% chitosan + 15 mM vanillin has slowed down respiration, ethylene production rate, and senescence process. Thus PPO activity is lower in this fruit.

POD is one of the enzymes expressed upon different inducements, including pathogenic challenge and have important roles during pathogenesis, oxidative burst, and resistance to infection (Kuvalekar et al., 2011). Interestingly, there was also a significant interaction effect between treatment and storage day on tomato fruit defensive enzyme, i.e. POD activity (Figure 4). As storage day advance, POD activity increased, and contrariwise as the chitosan and vanillin concentration increased, the PAL activity decreased (Figure 4). However, fruit coated with 1% chitosan + 15 mM vanillin and 1.5% chitosan +15 mM vanillin had 33.62 and 37.3%, respectively lower POD activity than fruit coated with 0.5% chitosan + 10 mM vanillin at the end of storage day 25. The inhibitory effects of chitosan and vanillin on POD activities may be because the coatings reduced the respiration that restricts the cell membrane and structure damaged by pathogen attack and delayed ripening and senesces process. Inline with this study, Elsayed et al. (2019) found that fresh green bean coated with 1.5% chitosan had lower POD than those coated with 0.5% chitosan stored at 4ºC and 85-90% RH for 28 days. In agreement with this study, previous researchers reported that 1.5% chitosan had lower POD in the fruit than those coated with 0.5% chitosan as found in tomato fruit (Liu et al., 2007), strawberries (Wang and Gao, 2013) and mushroom (Eissa, 2007). In the current study, the film formed by the higher concentration of coating 1.5% chitosan + 15 mM vanillin has reduced disease attack, cell structure damaged by the pathogen, also has slowed down respiration rate, ripening, and senescence process of tomato fruit, and thus POD activity is lower in this fruit. It is suggested that a higher concentration of chitosan and vanillin coating such as 1.5% chitosan + 15 mM vanillin has reduced diseases attack and ripening and senescence process. Also has slowed down the rate of respiration and ethylene production of tomato fruit; therefore, the activity of the defense-related enzymes i.e. PAL, PPO and POD are lower in this fruit which instigated to prolong the tomato fruit shelf life.

There was a significant positive correlation among defense-related enzymes. From the Pearson's correlation analysis, there was a significant positive correlation between PAL, PPO and POD (Table 2). The result was in agreement with Adiletta et al. (2018), who found a higher correlation between PPO and POD ($r = 0.79$) in loquat fruit coated with 1% chitosan and stored at 7 ºC for 21 days. In line with this study, Pasquariello et al. (2015) also found out a highly positive correlation between PPO and POD ($r = 0.87$), PPO and PAL ($r = 0.71$) in strawberry fruit coated with 1% chitosan stored at 2 ºC and 95% RH for 14 days. This result indicated that defense-related enzymes such as PAL, PPO and POD are the main contributor to oxidation of polyphenols into quinines, which strengthen and resistance the plant cells during the microbial attack.

Conclusion

Chitosan combined with vanillin in different concentrations was used as edible coatings to examine its effect on tomato defense enzymes during storage at tropical conditions. Results exhibit that higher concentration of chitosan and vanillin coatings (1% chitosan + 15 mM vanillin and 1.5% chitosan + 15 mM vanillin) has slowed down the defense enzymes activity of tomato. PAL activity was 31.5, the PPO 33.7 activity was and the activity of POD was 37.3% lower in in tomato by coating1% chitosan + 15 mM vanillin during storage at 26 ± 2°C/60 ± 5% RH for 25 days.

Authorship statement

All persons who meet authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in the work to take public responsibility for the content, including participation in the concept, design, analysis, writing, or revision of the manuscript. Furthermore, each author certifies that this material or similar material has not been and will not be submitted to or published in any other publication before its appearance in International journal of applied science and research

Declaration of Competing Interest

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

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