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THE IMPROVEMENT OF TOMATO SHELF LIFE USING CHITOSAN AND STARFRUIT LEAF EXTRACT AS EDIBLE COATINGS

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ABSTRACT

Due to the high degree of perishability and vulnerability to spoilage, tomatoes have limited marketability, which leads to extensive postharvest losses. The edible coatings are generally used to extend the shelf life of fruits and vegetables; therefore, this study investigated the use of chitosan and starfruit leaf extract (SFLE) in the composition of edible coatings for tomato fruit. Firmness, total titratable acidity, reducing sugar content and microbial load were measured every 5 days for 25 days. The results showed that the addition of SLFE to chitosan did not enhance the antimicrobial effect or firmness over the effects made by a separate use of chitosan and SFLE. Both components improved the shelf life of tomato fruits compared to untreated tomatoes.

Key words: firmness, microbial protection, postharvest treatment, storability

INTRODUCTION

Highly nutritious, the tomato (*Solanum lycopersicum*) forms an important horticulture commodity. Raw tomato is a good source of vitamin E, A, and C as well as other micronutrients such as potassium and folate (Canene-Adams et al. 2005). According to the Agricultural Ministry of Indonesia, tomato production and exportation in Indonesia have been increasing every year from 2015 to 2019 (Kementerian Pertanian Republik Indonesia 2020). However, the tomato, like any other fresh produce, is highly perishable, resulting in low marketability. Even after harvest, a continuous metabolic process occurs in tomatoes, which leads to decay (Oms-Oliu et al. 2011).

Based on its ripening mechanism, the tomato is categorized as climacteric fruit. The ripening stage is highly influenced by the ethylene regulation, which triggers ripening-related genes such as texture change, volatile compound production, and ethylene synthesis (Alexander & Grierson 2002). When launched in the market, the tomatoes will have a short shelf life, which is heavily affected by its ripening and senescence rate. This is due to the rapid increase of respiration rate in climacteric fruits (Vaishali et al. 2019).

Edible coating is a preservation method aimed to extend the shelf life of a food product by applying a layer that can act as a barrier for gas exchanges to slowing down the respiration rate (Raghav et al. 2016). The edible coating can enhance the food's safety, nutritional and sensory attributes, consists of a polymer base with various additional active ingredients. Chitosan, a hydrophilic biopolymer extracted from the chitin of crustaceans skeletal, is a common example of the polymer used as the base of edible coating (Zargar et al. 2015).

Another factor affecting the shelf life of tomatoes is the microbiological factor, especially in those tomatoes with damaged skin, where spoilage microorganism can easily penetrate the skin. Various bacterial species found in tomato fruit mostly are *Bacillus subtilis*, *Bacillus coagulans*, *Bacillus*

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stearothermophilus, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Wogu & Ofuase 2014; Bello et al. 2016). The pathogenic *P. aeruginosa*, a frequent etiological factor of nosocomial infections, is especially harmful for patients with cystic fibrosis, a chronic lung condition where thick mucus can form in lungs, pancreas, and other organs (Diggle & Whiteley 2020). Some strains of *P. aeruginosa* can acquire antimicrobial resistance characteristic. The strains of *P. aeruginosa* with carbapenemase-resistant are considered as critical pathogen on World Health Organization priority list of pathogen globally (Asokan et al. 2019). Many types of active ingredients – antimicrobial agents, for instance – are typically added to enhance the properties of edible coating. As previous studies have reported, the leaves of starfruit, also known as carambola (*Averrhoa carambola*), demonstrate high antimicrobial activity, making them a potential antimicrobial agent (Muthu et al. 2016; Phukan & Ahmed 2016; Silva et al. 2020).

This study aimed to explore the enrichment effect of the starfruit leaf extract (SFLE) in chitosan-based edible coating on the shelf life of tomatoes, and especially to the antimicrobial protection.

MATERIALS AND METHODS

Study design

The scheme of study design for one day of measurement can be seen in Figure 1. This study was conducted using a completely randomized design with four edible coating composition. Three boxes of tomatoes with 21 tomatoes per box within the same treatment represent biological triplicates. A tomato was randomly taken from each box for one analysis. The measurements were conducted every five days within 25 days.

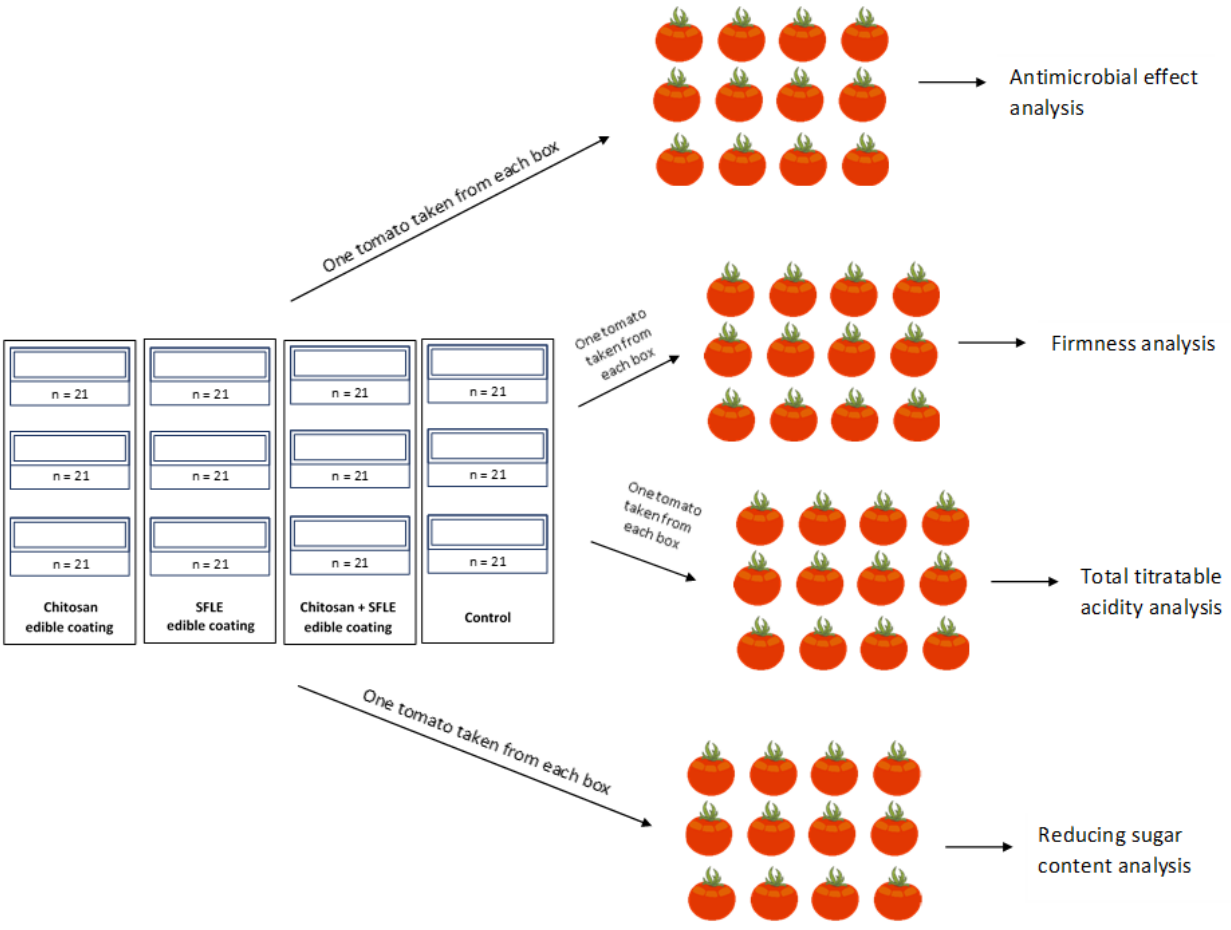

Figure 1. Scheme of study design for one-day measurement

The production of edible coating

In this study, to produce edible coating, SFLE solution was prepared in the beginning. The starfruit leaves were harvested according to the same color and size. The leaves were then washed and cut, followed by sun drying and oven drying at 55 °C for 2 hours. The dried leaves were ground, and 2 g of the ground leaves was dissolved in 5 mL of 96% ethanol. The solution was filtered three times using a medium sieve (mesh size = 0.16 cm) and another three times using filter paper. To obtain chitosan coating, 0.25 g chitosan (Chem-Mix Pratama Laboratory, Yogyakarta, Indonesia) was dissolved in 100 mL of distilled water with 1 mL acetic acid atop a Bunsen burner. To obtain SFLE edible coating, 5 mL of the SFLE extract was dissolved in 100 mL of distilled water. To obtain mixed coating, 5 mL of the SFLE extract was added to 100 mL of chitosan.

Sample preparation

The tomatoes (*Solanum lycopersicum* 'Ruby') were harvested from the plantings in Keteb, East Java, Indonesia. For the experiment purposes, fruits of the same age, size, and maturity were selected (10–30% of the surface with a pinkish or tannish yellow color, stage III – Turning). They were transported to the Postharvest Laboratory in Universitas Muhammadiyah Yogyakarta. Tomatoes were washed, air-dried for 5 min and dipped in the edible coating solutions for 30 sec. There were four treatments: chitosan, SFLE, combination of chitosan and SFLE, and control (no edible coating). The tomatoes were then air-dried for another 5 min before stored. The coated fruits were placed in an open plastic storage box with small holes and set into one layer (Figure 2).

Figure 2. Image of the stored samples

The bottom part was added with tissues to avoid direct contact with the plastic. The samples were stored at room temperature (25–27 °C), 90% RH, and away from direct sunlight for 25 days. No other fruits or other ethylene source aside from the samples were present in the room. Randomly selected fruits were further analyzed for total microbial load, firmness, total titratable acid and reducing sugar content in experimental triplicates.

Antimicrobial effect analysis

To investigate the antimicrobial properties of the edible coatings, the total microbial load was scored using the total plate count (TPC) method. The sample was mashed and weighed for 1 g to be transferred to a volumetric flask. Distilled water was added until the mark in the volumetric flask to dilute the samples. A dilution series until 10^{-5} were made from the first dilution. The dilution series of 10^{-3} to 10^{-5} were plated out using the spread plate method to a Petri dish containing nutrient agar. The plates were incubated for 48 hours before colony counting. The results were expressed in log CFU per mL.

Firmness

Sample firmness was measured using the digital PCE-PTR 200 penetrometer (PCE Instruments, Southampton, UK) with 8 mm diameter probe. The result was recorded and expressed in N per mm.

Total titratable acidity

In this study, the total titratable acidity (TTA) analysis was conducted according to the AOAC (Horwitz 2000). The samples were mashed, then 5-gram samples were inserted into a volumetric flask, which was filled with water to the mark and mixed until homogenous. This solution was filtered, and 10 mL of the filtrate was transferred into an Erlenmeyer. Two to three drops of phenolphthalein were added, and the solution was titrated with NaOH 0.1 N. The TTA was reported in percentage (%).

Reducing sugar content

The analysis for reducing sugar content was conducted by referring to the Nelson–Somogyi method for sugar determination (Somogyi 1952). The 1 g of mashed samples were transferred into a test tube to be diluted with 9 mL of distilled water. Afterwards, 1 mL alkaline copper tartrate was added. The test tubes were placed in a water bath for 30 min. Then, the samples were held under tap water to be cooled.

After cooling, 1 mL of arsenomolybdic acid reagent was added. The mixture was diluted to reach a volume of 10 mL with distilled water before it was measured for its absorbance in 620 nm using a spectrophotometer. The value of reducing sugar was expressed in percentage (%).

Statistical analyses

Statistical analysis was performed using the R program (version 1.3.1093). To analyze the antimicrobial effect, multiple testing of the one-way ANOVA was conducted on each observation day. This analysis was to investigate whether there is a difference of TPC, which indicates the level of microbial growth inhibition in the treated and control samples in a single measurement. This analysis was continued with the post hoc analysis of Tukey HSD with Bonferroni adjustment for the days observed with a significantly different result between treatments.

For the shelf-life parameter analysis, the trend of 25 days of observation was analyzed for its regression model using different regression types: firmness analysis used the spline function, TTA used simple linear regression model, and reducing sugar content used polynomial regression. After the regression of firmness was obtained, the days to reach 1.45 $N \cdot mm^{-1}$ firmness that is the acceptable limit to be marketed (Batu 1998) was calculated. Then, the significant difference between the days and its 95% confidence interval of four different treatments were analyzed using the Kruskal–Wallis nonparametric test and Nemenyi post hoc.

RESULTS AND DISCUSSION

Antimicrobial effect analysis

According to Table 1, a significant difference in the total microbial load on different treatments was found on observation day 10 and 15. On day 10 and 15 a total microbial load was significantly lower in the samples coated with SFLE addition. Microbial counts were highest in the samples not protected with coatings, although the differences between the control and treated samples were not always significant. After 15 days of storage, microbial growth was not significantly hindered by chitosan or SFLE.

There was no difference between the SFLE treatment and the SFLE combined with chitosan at each analyzed time period. According to Silva et al. (2020), SFLE contains secondary metabolites of tannins, steroids, and saponins that are theoretically responsible for the antimicrobial properties of SFLE.

Firmness

A decreasing, linear trend was found in the linear regression model of all the treatments tested, a trend that was also observed in the previous study where firmness between two different tomato cultivars was investigated (Sinha et al. 2019). The loss of firmness results from the plant's cell wall or pectin degradation, which impacted texture integrity loss. Firmness, since it is the limiting quality factor for tomatoes stored above 13 °C, is an important factor of determining shelf life (Tadesse et al. 2015). According to Batu (1998), the acceptable firmness value limit for marketable tomatoes is above 1.45 $N \cdot mm^{-1}$. As this value was inside the data for linear regression, an interpolation using the fitted model equation was used to investigate how many days it took for the tomatoes to reach the firmness value limit in each treatment.

Table 1. Total plate count (log CFU per mL ± SD)

Day	Chitosan	SFLE	Chitosan + SFLE	Control	p-value
5	5.70 ± 0.35^{a}	6.09 ± 0.44^{a}	5.67 ± 0.31^{a}	6.50 ± 0.35^{a}	0.330
10	6.12 ± 0.34^{ab}	5.73 ± 0.40^{a}	5.45 ± 0.45^{a}	7.12 ± 0.56^{b}	0.043
15	6.76 ± 0.41^{b}	5.90 ± 0.09^{a}	5.81 ± 0.26^{a}	7.01 ± 0.3^{b}	0.008
20	6.66 ± 0.70^{b}	6.28 ± 0.27^{b}	6.17 ± 0.38^{b}	7.02 ± 0.81^{b}	≈1.000
25	7.07 ± 0.71^{b}	7.11 ± 0.73^{b}	6.88 ± 0.66^{b}	7.81 ± 0.58^{b}	≈1.000

The p-value indicates to F-statistical probability of each day separately as a result of one-way ANOVA test. The results with the same superscript letter(s) and order indicate that no significant difference was found between the results ($p > 0.05$) according to the Tukey HSD test with Bonferroni correction

In this study, it was predicted that the addition of SFLE as an antimicrobial agent to a coating containing chitosan would extend the time to reach a boarder firmness value of 1.45 N·mm^{-1} compared to control. However, only chitosan and SFLE given as a single addition increased significantly number of days needed to reach a critical firmness point (Figure 3). A possible reason of this result is that the concentration of SFLE was too low to give significant maintenance of firmness. A previous study compared the effect of chitosan-based edible coating incorporated with two different concentrations of *Mentha* × *villosa* Huds. and *M. piperita* essential oils to enhance the shelf life of papaya (dos Passos Braga et al. 2020). After 20 days of storage, papaya fruit that was coated with higher concentrations of oils resulted in reduced firmness loss. The correlation between concentration and antimicrobial activity was also seen in the edible film of chitosan and bergamot oil (Sánchez-González et al. 2010). When chitosan-based edible film combined with bergamot oil applied directly or in contact with oil vapor, a significant inhibition on the growth of *Penicillium italicum* was reported. This inhibitory effect was dependent on the concentrations of bergamot oil. The study also reported that by using bergamot oil to chitosan ratio of 3 : 1, the water vapor permeability was decreased by 50%.

In Figure 4, the significant difference of days needed to reach 1.45 N·mm^{-1} from all treatments, including the 95% confidence interval, was analyzed. The samples treated with chitosan had a longer shelf life compared to the control. There was no significant difference in between samples treated with chitosan, SFLE, and chitosan added with SFLE. Firmness loss can not only be caused by pectin degradation but also by fungal colonization and Peralta-Ruiz et al. (2020) reported less firmness loss in tomatoes not colonized with fungi.

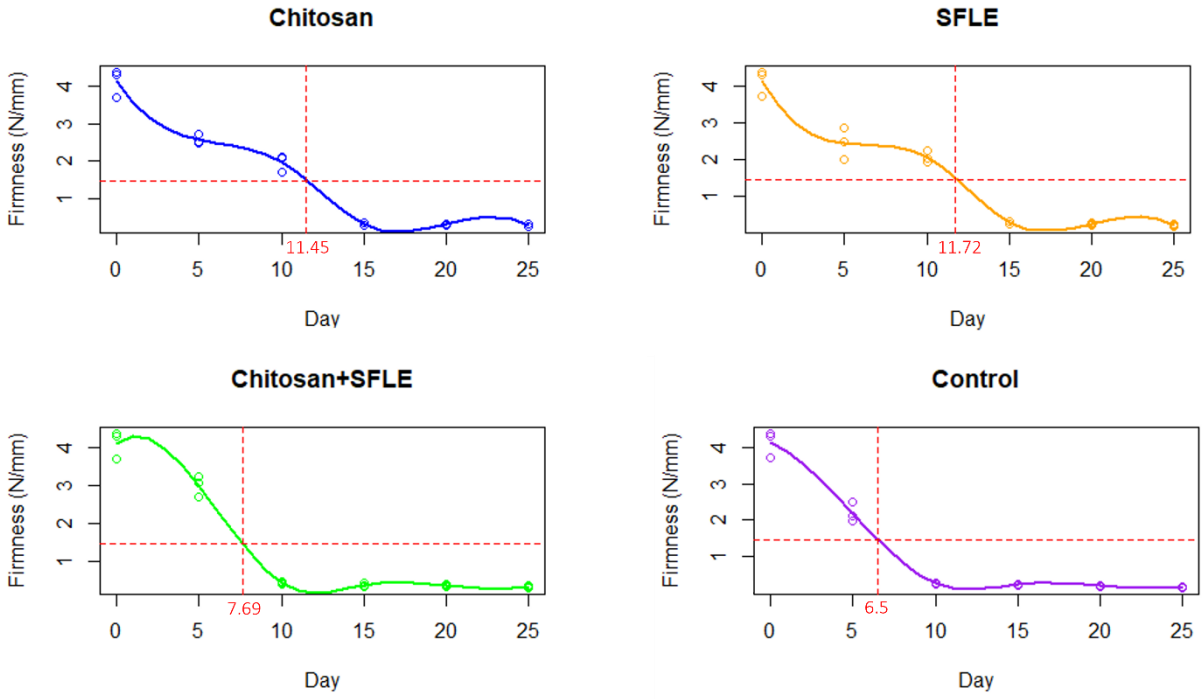

Figure 3. Firmness as a function of observation days in tomato coated with different composition of chitosan-based edible coating enriched with SFLE

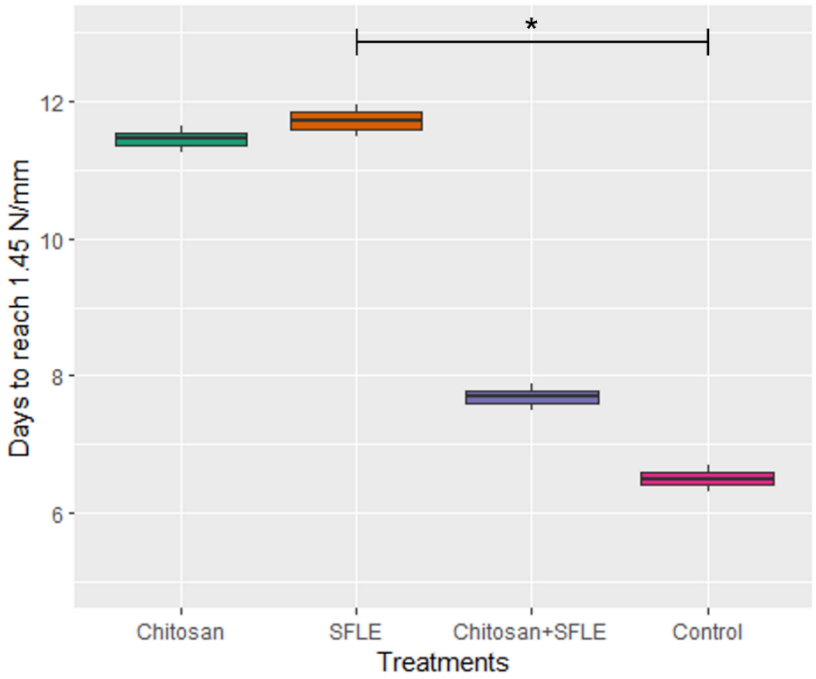

Figure 4. Days needed to reach 1.45 N·mm^{-1} firmness for tomato treated with different composition of chitosan-based edible coating enriched with SFLE

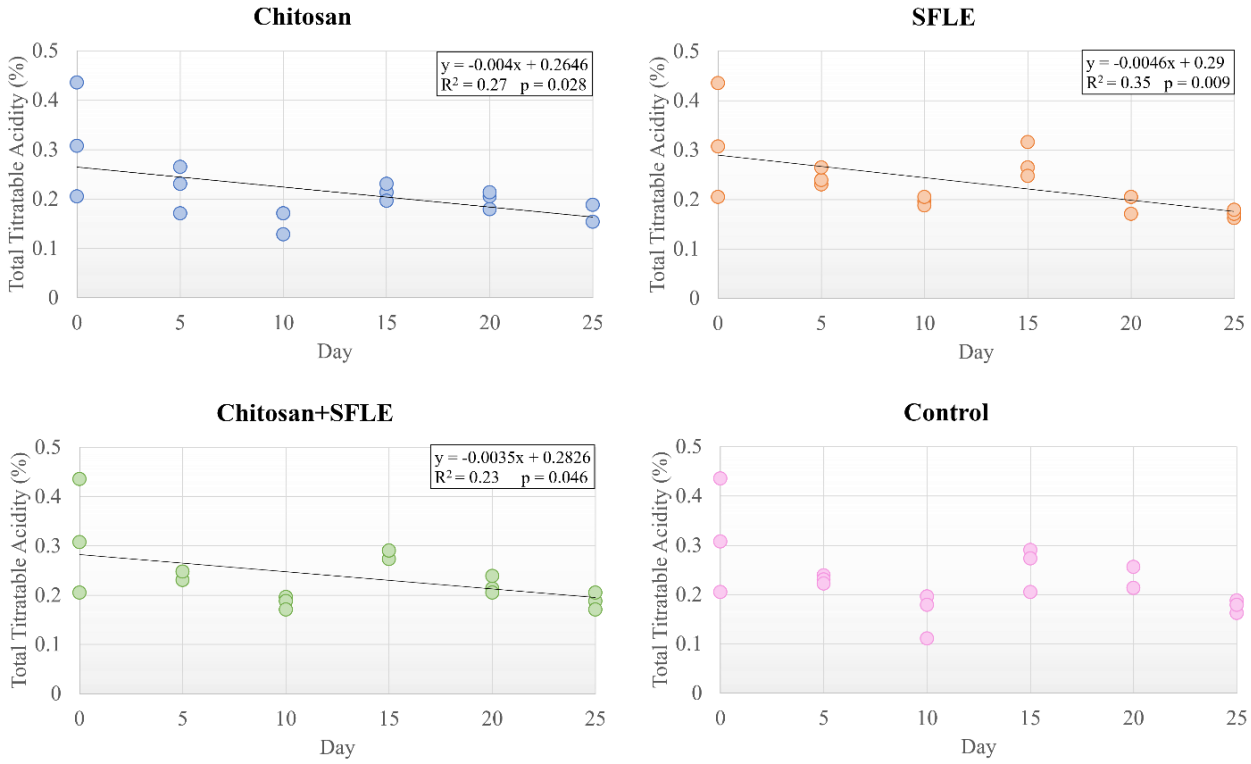

Figure 5. Total titratable acidity as a function of observation days in tomato coated different composition of chitosan-based edible coating enriched with SFLE

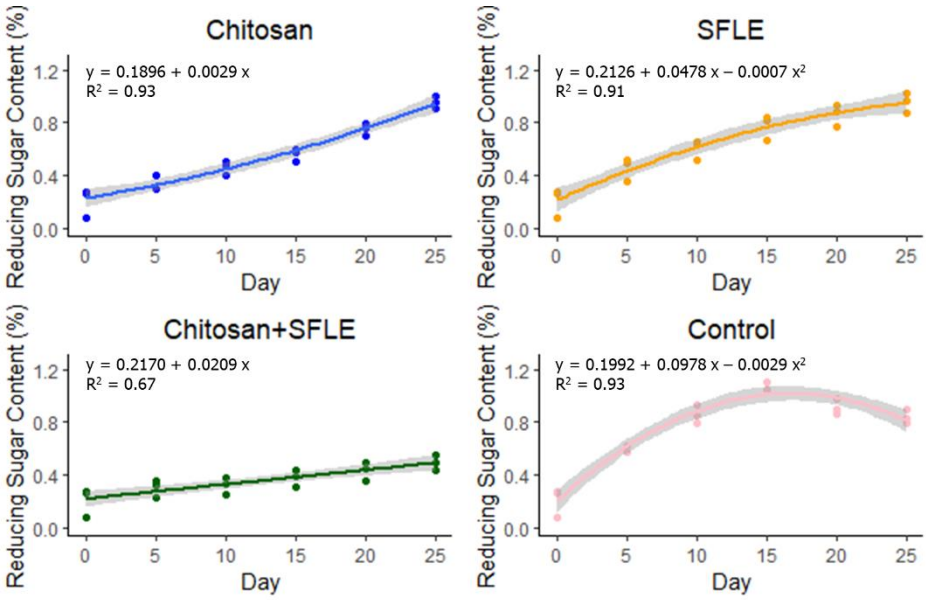

Figure 6. Reducing sugar content as a function of observation days in tomato coated different composition of chitosan-based edible coating enriched with SFLE

Total titratable acidity

The linear regression of TTA and days can be seen in Figure 5. As more days passed, the regression showed a linear and decreasing trend of the TTA, except for the control where only a slight and not significant decrease can be seen. The decreasing trend in treated samples was also reported in a previous study that investigated the TTA of tomatoes when they ripen (Anthon et al. 2011). The study explained that the decrease of TTA during ripening was because organic acids are metabolized even after the tomatoes are harvested. Tomato cultivars contain different titratable acidity level (Tigist et al. 2013), which might be related to the weight of the fruit characteristic to the cultivar. The above study also reported higher TTA found in the larger-sized tomato.

According to a study of Kayode and Afolayan (2014), the TTA in a spoiled tomato fruit was 0.03%. The limit value falls out of the data used to make the linear regression in this study. As it is not allowed to do an extrapolation in statistical analysis, which will lead to a confusion in interpreting the result, the shelf-life determination of tomatoes cannot be done using the TTA linear regression in this study.

Reducing sugar content

Figure 6 exhibits the result of the linear regression between reducing sugar content with days on different treatments. The regression model showed an increasing and linear trend in all treatments. However, in the regression model of control, it was found that the increasing trend occurred from day 0 until day 15, then followed with the decrease of reducing sugar content. A previous study reported a similar trend of an increase but which continued to decrease after 16 days of storage (Tadesse et al. 2015). The study explained that the increase of reducing sugar content was because of the breakdown of tomato's polysaccharide content into sugars. In contrast, the following decrease trend is due to the sugar content being used by tomato for respiration.

As previously reported by Jamir and Khawlhring (2017), overripe tomatoes after storage in ambient temperature for 25 days had reducing sugar content of 1.44%. This limit value is not in the data used to make the polynomial regression in this study; thus, the shelf-life determination of tomatoes cannot be done by the reducing sugar content regression. This is due to the limitation of extrapolation in statistical analysis that can lead to confusion in interpreting the result.

The major saccharides content in tomato consists of fructose and glucose, while sucrose, arabinose, xylose, and galactose were found to have lower levels (Mendelová et al. 2021). The reducing sugar level of tomatoes differ with ripening stage (Dalal et al. 1965; Jamir & Khawlhring 2017). According to the color change, tomato undergoes six stages of ripening: I – Green (completely green surface), II – Breakers (indicated by a definite break in color on not more than 10% of the surface), III – Turning (around 10–30% of the surface shows definite change of color in the aggregate), IV – Pink (color change to pink or red in the aggregate between 30–60% surface change), V – Light Red (more than 60% of the surface shows pinkish-red or red in the aggregate), and VI – Red (more than 90% of the surface in the aggregate is red (Garcia et al. 2019). The reducing sugar increased during storage, regardless of which ripening stage the tomato was at harvest (Moneruzzaman et al. 2008).

Another factor affecting the reducing sugars of tomato is the genotype. Several studies have reported that different cultivars lead to varying reducing sugar levels (Beckles et al. 2012; Ibrahim et al. 2017; Tadesse et al. 2012), which means that sugar metabolism depends on genotype (Beckles et al. 2012).

LIMITATIONS

This study has some limitations that need attention. For the trend of the firmness regression model, a cut-off point at observation day 15 can be seen in samples treated with chitosan and SFLE, whereas the cut-off points for samples treated with chitosan enriched with SFLE and control samples were at day 10 of observation. This indicated a limited decrease in firmness after the cut-off point, illustrated by the flat data at the end of observation period. This trend of regression can be analyzed using a more complex statistical method such as the piecewise or segmental regression, hyperbolic curve fitting, ANOVA with different ordinal data days, or polynomial contrast for regression. However, for some of the complex statistical methods, it may be unsuitable. Further comparison between these statistical methods might be of interest to find the best regression model for the firmness value and observation day.

CONCLUSION

The present study showed that the addition of starfruit leaf extract cannot improve the antimicrobial properties of chitosan-based edible coating in tomatoes but it can be an alternative in use for antimicrobial protection. Similarly, estimation of firmness value, indicated longer shelf-life of tomato fruit coated with chitosan or SFLE compared to untreated tomatoes and those coated with a mixture of chitosan and SFLE.

Conflict of interest

The authors declared no conflict of interest in this study.

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