Efficiency of edible coating chitosan and cinnamic acid to prolong the shelf life of tomatoes

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Received 8 February 2018
Accepted 26 May 2018
Online 27 May 2019

Keywords:
Chitosan, cinnamic acid, edible coating, tomato

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Abstract
Tomato (Solanum lycopersicum L.) is a large target commodity for Malaysia's export trade. However, tomato is a perishable climacteric fruit that requires favourable conditions to maintain its freshness from farm to the table. Its fast ageing process tends to attract pathogens that reduce the shelf life of a detached tomato, hence affecting its quality causing severe losses to the agropreneurs (Anderson et al., 2014). Therefore, there is a dire need for a mechanism to maintain the freshness of tomatoes. This study aims to examine the effects of chitosan and cinnamic acid as edible coatings to prolong the shelf life of tomatoes. Chitosan plays a role as an antifungal agent whereas cinnamic acid possesses antimicrobial properties that help to improve the shelf life of tomatoes (Bautista-Baños et al. (2006). In the study, Chitosan and cinnamic acid coatings were applied on fresh graded tomatoes at two maturity stages; breakers and turning. The samples were observed every three days for a total period of 12 days at ambient temperature. The results showed that a single coating of chitosan (0.5 %) had a positive impact on the total soluble solids (TSS), firmness, hue angle and weight loss of the samples. On the other hand, cinnamic acid (2mM) influenced the firmness, weight loss and TSS value of the tomatoes. The coatings inhibit respiration and minimise starch conversion into sugar that could lead to lowered sugar (TSS) content. The restriction on respiration ensures firmness and delay the colour change of fruits at a particular period. The slowing of the ripening process that causes ageing and weight loss in fruits is reduced. Both coatings were proven to be highly feasible for application in various industries due to their edible, non-toxic and biodegradable nature.

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1. INTRODUCTION
Tomato or Solanum lycopersicum L. was previously known as a dangerous and poisonous wild red plant (Thomas, 1996). Since then, it has evolved in terms of colour, flavour, and shape that allow them to be eaten raw or cooked. The varieties of tomatoes differ from one region to another (Guerra et al., 2015) and they include Jaune Flamme, Red Zebra, Silitz, Orange Cherry, Candy’s Old Yellow and Cavern to name a few.

Tomato contains an abundance of vital phytochemical elements such as lycopene and β-carotene, which are higher in processed tomato compared to fresh ones (Friedman, 2013), providing them with a competitive advantage for export purposes (Islam et al., 2012).

However, a detached tomato is an “easy-to-damage” kind of fruit due to its climacteric characteristic where ripening continues well after it has been harvested (Cara and Giovanni, 2008). The presence of ethylene causes the rapid deterioration of the tomato. The ripening process will alter the properties and desirable qualities of the fruit leading to a reduction in its market value (Diaz et al., 2002). Thus, it is crucial that the aesthetical values of the tomato are preserved to prolong its shelf life and maintain its market value.

An edible coating is a safe, biodegradable and non-toxic substance derived from either plants or animals as a substitute for harmful chemicals (Moreira et al., 2011; Sabaghi et al., 2015). Chitosan, for example, is an edible coating that is developed from chitin shells of crustaceans. It is one of the most abundant biopolymers after cellulose (Cheha, 2011). Chitosan is an antifungal agent that can control postharvest diseases (Terry and Joyce, 2004; Bautista-Banos et al., 2006). Additionally, cinnamic acid is a plant-derived soluble compound that exhibits antimicrobial potential (Narasimban et al., 2004; Adisakwattana et al., 2008). Meanwhile, cinnamic aldehydes play a role in the disruption of biological processes that impede microbial growth (Mucho et al., 2011).

The combination action of chitosan and cinnamic acid provides a semi-permeable barrier for oxidative gas that helps to delay the ripening process (Baldwin et al., 1999; Yang et al., 2015). The barrier inhibits respiration and transpiration to preserve the antioxidant capacity of a fruit (Ali et al., 2010). The application of chitosan and
cinnamic acid at a commercial scale aims to replace the usage of synthetic fungicides to control spoilage by the pathogen. Chitosan and cinnamic acid are considered as safer alternatives to the potentially toxic chemicals to treat early deterioration and extend the shelf life of a food product (Win et al., 2007).

Chitosan showed promising effectiveness when used on papaya (Ali et al., 2011), guava (Hong et al., 2012) and strawberry (Hernández-Muñoz et al., 2006). The edible coating is a significant replacement for chemicals in maintaining the freshness and safety of the fruits while in storage (El Ghaouth et al., 1992) as well as preserving their desirable qualities (Garcia et al., 2014a). Postharvest handling is one of the main factors that affect the quality of tomatoes. Therefore, a coating can delay, if not prevent the deterioration of the fruits by minimising the effects of unfavourable conditions and accidental contamination during postharvest handling (Moreira et al., 2011). Understanding the reaction of the fruits to their coating is essential in developing a cost-effective and efficient postharvest handling.

2. MATERIALS AND METHODS

2.1. Fruit selection

Fresh Solanum lycopersicum L. were purchased from tomato farms at Lojing, Kelantan and Blue Valley, Cameron Highland, Pahang, Malaysia. The selected fruits are uniform in colour (breakers and turning), size, weight (60 – 90g) with no obvious physical damage or bruising. The tomatoes used are in their breakers stage (<10 % other than green/yellow colour) and turning (<30 % other than green/yellow colour) as stated by the Federal Agriculture Marketing Authority (FAMA) and US Department of Agriculture (USDA).

2.2. Preparation of chitosan

The acetic acid and commercial chitosan were purchased from Sigma-Aldrich, Kuala Lumpur. Chitosan 0.1 % (w/v) was prepared by dissolving it in 1.0 % (v/v) acetic acid. The solution was stirred overnight in ambient temperature. Then, the solution was filtered using a muslin cloth, and its volume was adjusted to 1000ml by adding distilled water. The coating formulation resulted in several sets of treatments: a) control (uncoated tomatoes), b) 0.5 % chitosan acetate solution c) 0.75 % chitosan acetate solution and d) 1.0 % chitosan acetate solution.

2.3. Preparation of cinnamic acid

The cinnamic acid was purchased from Sigma-Aldrich, Kuala Lumpur. Cinnamic acid was added to distilled water and stirred until it was fully dissolved. The coating formulation resulted in several sets of treatments: a) control (uncoated tomatoes), b) 2mM cinnamic acid solution, c) 3mM cinnamic acid solution and d) 4mM cinnamic acid solution.

2.4. Coating application

Tomato samples were divided into the breakers and turning batches. Each batch was separated into three different coating treatments. Then, each batch of the coating treatments was divided into five different groups for 0, 3rd, 6th, 9th and 12th day of observation. The tomatoes were rinsed using distilled water and air-dried prior to the treatments. They were then dipped into chitosan for 30 seconds and 3 minutes in cinnamic acid and allowed to dry in ambient temperature for two hours on permeable tissue paper or simply in a tray to remove any excess solution. Observations were recorded at a three days interval for a period of 12 days.

2.5. Colour assessment

Colour changes of the tomatoes were documented using Minolta chromameter (model CR-400X Minolta Camera Co. Ltd., Japan) every three days. The chromameter was calibrated (L*=98.15, a*=0.13, b*=1.92). The colour was determined from six light pulse points at the equatorial without spots and tissue discoloration from 45 tomatoes per treatment. The values of the hue angle were calculated using (h = tan-1[b*/a*]). Chromometer read L as lightness (black [L* = 0]); (white [L* = 100]), a* indicated redness to greenness (red [a* = 100]); (green [a* = -100]), b* indicated yellowness to blueness (yellow [b* = 100]); (blue [b* = -100]).

2.6. Firmness

Firmness was determined using Brooklyn Texture Analyser using force in gram (g) before being converted to Newton (N). The firmness was determined using the puncture method of TA 39/100 (probe TA39 of TA-MTP). The process was automatically accomplished by using remote control where the readings were transmitted from the machine to the computer to calculate graft firmness. Readings were taken twice at every opposite point (4 or 5cm apart). Texture pressure analyser (TPA) was set at a speed of 10mm/s. Firmness at the first peak using surface penetration was recorded, and the average reading was calculated.

2.7. Total soluble solid

The tomatoes were vertically cut to obtain their filtered residue to measure the suspended solids (Brix, 20 % sucrose) using hand refractometer (Atago) indicated in percentage. TSS was observed on the 0, 3rd, 6th, 9th and 12th day.

2.8. Weight Loss

The weight of the tomatoes was measured using an analytical balance (Kern EMB 2200-00) after they were coated and air-dried. The results were reported as weight loss percentage.
2.9. **Statistical Analysis**

The data obtained were analysed using SPSS by comparing the means using Tukey’s multiple range test at p<0.05.

3. **RESULTS AND DISCUSSION**

3.1. **Colour Attributes**

Fig. 1 shows the influence of edible coatings on the colour changes of tomatoes at different maturity stages after 12 days in storage at ambient temperature. There is a significant difference between the edible coating and colour change with storage time. Hue angle values decreases with time for chitosan coated tomatoes, but increases for cinnamic acid coated samples. The best hue values are recorded by breakers tomatoes coated with 4mM CA and 0.75 % Ch and turning samples coated with 3mM CA and 0.75 % Ch compared to the control. The lowest hue values are charted by tomatoes covered with chitosan at the breakers stage. Cinnamic acid treatment at the turning stage shows a decreasing trend with respect to the control until the 6th day where the values of 2mM and 3mM increased. The results suggest that chitosan as an edible coating delays the ripening process for tomatoes at ambient temperature due to the low hue angle at approximately 9.9 %.

![Figure 1: Hue angle of tomato treated with chitosan (Ch) and cinnamic acid (CA) at the breakers and turning stage after 12 days storage](image)

3.2. **Weight Loss**

The weight loss (%) of fresh tomatoes during storage at ambient temperature is shown in Fig. 2. The fresh tomatoes experience the highest weight loss at the turning stage compared to the breakers stage. Tomatoes covered with chitosan 0.5 % (breakers) and 0.75 % (turning) record significantly lower weight loss at 5.43 % and 6.60 % respectively. Tomatoes coated with 3mM CA (breakers), and 2mM (turning) demonstrate the lowest weight loss at 4.61 % and 3.79 % after 12 days in storage at ambient temperature. The control samples for the breakers stage lost approximately 8.12 % of their weight, notably higher than other treatments.

![Figure 2: Weight loss of tomato treated with chitosan (Ch) and cinnamic acid (CA) at the breakers and turning stage after 12 days storage](image)

3.3. **Firmness**

The firmness of the tomatoes covered with edible coating is significantly reduced at the turning stage compared to the breakers stage (Fig. 3). Tomatoes at the turning stage coated with 0.5 % Ch lose their firmness rapidly, not much different than the control. The firmness of tomatoes at the turning stage that are coated with chitosan fluctuates throughout the experiment in relation to the control, except for those coated with 1.0 % Ch that steadily lose their firmness. Generally, the tomatoes continuously lose their firmness during the 12 days storage with an exception for the control tomatoes at the breakers stage.

![Figure 3: Firmness of tomato treated with chitosan (Ch) and cinnamic acid (CA) at the breakers and turning stage after 12 days storage](image)

3.4. **Total soluble solid (TSS)**

There is no significant difference between TSS values with maturity stages during the storage period. Most of the starter tomatoes record values between 2.5 to 4.5. All the treated tomatoes at the breakers stage exhibit a decreasing trend in TSS value. However, 2mM CA coated tomatoes shows an increase in sweetness or TSS value up to 73 % from day 6 to day 9. A similar situation was observed for 3mM CA at the turning stage (2.97 to 3.27) from day 3 to day 6. However, 0.5 % Ch coated samples demonstrate an abnormal increase in the TSS values from day 9 to the end of the experiment. All treatments including the control at both stages show a certain peak in TSS value during the storage period.
Tomatoes tend to degrade faster than non-climacteric fruits due to the ethylene produced by the fruit (Diaz et al., 2002). The stimulation of phytoalexin by ethylene as a defence mechanism against the pathogen cannot stop the ripening of tomatoes caused by the conversion of starch into sugar (El Oirdi et al., 2011). A study by Vunnam et al. (2014) observed that the respiration rates are lower when the fruits are treated with radiation in a modified atmosphere hence proving that respiration rate influences the shelf life of fruits. Edible coatings serve as a semi-permeable barrier for gases such as oxygen and carbon dioxide thus inhibiting respiration and ethylene production (Rojas-Graü et al., 2005; Yang et al., 2015). The decline in the ethylene level will delay the spoilage of the fruits.

In the present study, the rate of decay of the tomatoes that were treated with 0.50 % chitosan and 2mM cinnamic acid was significantly minimised. Previous studies have shown the efficiency of chitosan and cinnamic acid in delaying the spoilage of fresh fruits such as tomatoes, melons and bananas (Liu et al., 2007; Win et al., 2007; Silveria et al., 2015). The coating of fresh fruits serves as a substance to maintain the freshness of fresh commodities from the farm to the table (Romanazzi et al., 2017).

The weight loss in the treated breakers tomatoes was lower than the untreated tomatoes. Similar results were obtained in a study conducted by Kaya et al. (2016) on the weight loss of uncoated fruits (25.0 %) and commercial chitosan-coated fruits (20.0 %) after 26 days in storage at ambient temperature. Studies conducted by Ali et al. (2011), Benhabiles et al. (2013) and Qiu et al. (2014) also supported the claim that the protective action of chitosan reduces the weight loss in fruits and vegetables. The water vapour pressure between the fruits and the environment has an impact on the transpiration rate which in turn affects the moisture loss in the fruits (Bautista-Banos et al., 2006). The edible coating provides a barrier that helps to regulate the moisture level in the fruits by limiting water loss (Cissé et al., 2015). In contrast, the untreated tomatoes at the turning stage experienced lower weight loss compared to the coated samples. This result showed that adhesion of the coating also affects the coating potential of a substance, even though chitosan is known to enhance water vapour resistance (WVR) (Poverenov et al., 2014). Also, the appearance of wrinkles on the tomatoes was observed at the end of the current study. It contributed to an increased weight loss compared to fruits that are stored in the refrigerator prior to being stored at ambient temperature. Petriccione et al. (2015) had proven that the storage of tomatoes at low temperature lowers the respiration rate to below 2.5 % after 14 days.

The high colour percentage (Hue angle) of the tomatoes at the end of this study indicated the delay in their colour changes. This was mainly because greener tomatoes (maturity stage) were selected for this experiment to ensure their low lycopene content. The coatings used were believed to be responsible for preventing the lycopene from affecting the shelf life of tomatoes. Colour change is an indicator of the ripening process that occurs due to the ethylene action that stimulates lycopene production of the red pigments in the presence of oxygen (Vunnam et al., 2014). In the case of tomatoes coated with 4mM cinnamic acid at the turning stage, their colour changed gradually with storage time. Since their ageing process began after coating, the fruits’ ability to delay colour change was maximised. The coating had an effect on lycopene levels in tomatoes that were coated at the turning stage (pink stage) whereas lycopene levels in the fruits at breaker stage were not affected (Dávila-Aviña et al., 2014). Lu et al. (2007) had proven that the application of cinnamic acid coating inhibited the browning effect in apple slices [3 % ΔL] compared to sodium chloride [6 % ΔL] after 14 days in storage. Roller (2002) also conducted a similar study using fresh-cut melons and apples by using cinnamic acid as a coating. Other studies focused on the microbial inhibition and the antimicrobial properties of cinnamic acid. There is very few researches regarding the potential of cinnamic acid as a coating for tomatoes.

The coating of 0.75 % chitosan and 2mM cinnamic acid on tomatoes were proven effective due to a high level of firmness after 12 days in storage, indicating a reduction in fruit softening. It was also observed that uncoated tomatoes at breakers stage tended to show a high firmness value compared to the coated tomatoes. The maturation of polysaccharide cell wall and degradation of lamella caused changes in the firmness, but the complex intracellular process that may be a factor that affects the firmness remained unclear (Garcia et al., 2014b). There is a need for further studies to determine the effective levels of coatings on tomatoes at different maturity stages.

Besides that, the physical condition of fruits also influences the application of treatments. For example, a study conducted by Hong et al. (2012) found that 2.0 % of chitosan was proven effective in maintaining the
structural integrity of guava while Ali et al. (2011) discovered that 1.5 % of chitosan is a useful coating in retaining the firmness in papaya. A higher concentration of chitosan decreases water vapour transmission rate due to its hydrophobic nature (Bedane et al., 2012; Fan et al., 2015). In this study, a low chitosan concentration was enough to maintain the firmness in tomatoes because of their thin exocarp. The deformation test had identified that tomatoes lost their firm texture due to the breakdown of their cell wall. Therefore, the function of coatings is mainly to provide a semi-permeable barrier that prevents the respiration process to maintain firmness in the fruits (Yang et al., 2015). The results from a study by Batu (2004) discovered that the firmness of two commercial tomato varieties ranges from 1.45 N mm-1 to 1.46 N mm-1. The firmness of tomatoes commonly used in home dishes such as salads should not be lower than 1.22 N mm-1 or 1.28 N mm-1.

In terms of Total Soluble Solids (TSS), most of the samples recorded a peak sucrose level (20 %) on day 3 of storage before exhibiting a decreasing trend; an indication of fast-ripening process where starch was converted to sugar (Petriccione et al., 2015) as well as the conversion of sugar into carbon dioxide and water (Ghasemnezhad et al., 2011). The TSS values for all the tomato samples ranged from 2.5 to 4.5. The TSS values decreased gradually for tomatoes at the breakers stage than at the turning stage suggesting that they underwent lower metabolic reaction. A fluctuation in TSS values was recorded in a study by Tigist et al. (2013) where different varieties of “green mature” tomatoes were evaluated for quality changes. Meanwhile, Genanew (2013) identified the relationship between TSS values with titrable acidity (TA). The results of the study revealed that as sugar content increased, the level of acidity decreased due to oxidation which eventually decreased the quality of tomatoes. Chitosan as an edible coating reduces respiration besides inhibiting the activities of polyphenol oxidase (PPO) and peroxidase (POD), hence preventing respiration besides inhibiting the activities of polyphenol oxidase (PPO) and peroxidase (POD), hence preventing the respiration process to maintain firmness in the fruits.

**Acknowledgement**

This project was funded by Research Acculturation Grant Scheme (RAGS). This study is part of Master of Science Thesis of Wan Norin Syerina.

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