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Formulation of Edible Coatings from Alfalfa Saponins to Enhance the Postharvest Quality of Tomatoes

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ABSTRACT: Tomatoes are a major crop for global exports and have significant nutritional benefits. However, their lifespan is limited due to various biotic and abiotic factors. This study aimed to formulate an edible coating using crude alfalfa saponins coupled with decaglycerol monolaurate (ML-750) and polyoxyethylene (20) sorbitan monolaurate (Tween 20), to enhance the postharvest quality and shelf life of tomatoes by preventing spoilage. The effectiveness of alfalfa saponins coatings, both alone, and with ML-750 and Tween 20, was evaluated by comparing their impact on color, texture, overall acceptability, and % weight loss at 4°C and 25°C for 7 days. Significant improvements were observed in the quality attributes of tomatoes, including firmness, aroma, color, texture, and overall acceptability. Crude alfalfa saponins in emulsified form with Tween 20 increased the shelf stability of tomatoes more effectively than uncoated and ML-750 combined coatings. The total soluble solids (TSS) and pH also play a crucial role in determining the quality of the fruits. The results indicated no significant changes in the TSS of tomatoes coated with encapsulated saponins. Subsequently, a gradual increase in the pH of the coated tomatoes was observed on days 5 and 7, respectively. The findings of this study revealed that alfalfa saponins coupled with synthetic emulsifiers may be a beneficial strategy for prolonging the shelf life and improving the postharvest quality of tomatoes.

Keywords: alfalfa, emulsions, polysorbates, saponins, tomatoes

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most cultivated, world's largest processed, fresh, and extensively consumed fruits (Feng et al., 2019). Tomato is ranked the second most widely distributed agricultural crop in the world and its annual production rate is about 182 million tons (Kamali et al., 2019). Tomato is a significant cash crop due to its relatively short ripening period, and Asia accounts for 61.1% of the total tomato yield globally (Ahmad et al., 2019). Tomatoes are consumed as fresh vegetables throughout the world. The nutritional components of tomatoes include 95% moisture, 1.2% protein, 3% carbohydrates, and 1% lipids. They are also rich in both water-soluble and fat-soluble vitamins and minerals (Salehi et al., 2019). Due to improper pre- and postharvest practices, 25～42% of tomatoes are lost globally (Arah et al., 2015). Modern and effective preservation techniques, such as modified atmospheric pressure, use of edible coatings, proper transportation, appropriate packaging, and fungicide treatment, can be adopted to minimize pre and post-harvesting losses.

To prevent postharvest losses, aloe vera has been utilized to formulate coatings due to its biodegradability, antibacterial, and film-forming properties. Consequently, the shelf life of the tomato was increased up to 35 days with an 80% coating of aloe vera gel (Firdous et al., 2020). In another study, electron beam (e-beam) treatment was applied to assess the microbiological inactivation and bioactive profile of cherry tomatoes. e-Beam treatment of 3.6 kGy at 4°C significantly reduced mesophilic bacteria, foodborne pathogens, and filamentous fungi for 14 days. Furthermore, the application of 3 kGy e-beam on tomatoes preserved their antioxidant activity and total lycopene content. An *in vivo* study demonstrated an anti-proliferative effect on human lung cancers cells and no cytotoxic effect on healthy cells (Madureira et al., 2019).

Roma tomatoes were coated with a mixture of 1.5% guar gum and 30% glycerol (plasticizer) and subsequent-

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ly stored at ambient temperature for 20 days in another study. The findings of the study demonstrated that guar gum reduced the respiration rate and also delayed the ripening process. The application of the coating improved the firmness, reduced the respiration rate and weight loss, and also slowed down the changes in total solid soluble content. In addition, it also enhanced the sensorial quality and physicochemical properties of the Roma tomatoes, while reducing their microbial load.

Cellulosic packets coupled with silver nanoparticles were used as a packaging material for tomatoes. The presence of silver particles in the packaging material enabled efficient control of microbial activity. The utilization of cellulose packaging material impregnated with silver nanoparticles to cover tomatoes and cabbage improved their nutritional value and shelf life (Singh and Sahareen, 2017). Among the above-mentioned techniques, emulsions are one of the most widely used formulations in pharmaceutical, agrochemical, and food industries to protect, deliver and encapsulate bioactive material (vitamins, antioxidants, nutraceuticals, antimicrobials, and drugs) (Mcclements, 2012). To prevent coalescence and decrease surface tension, an emulsifying agent should adsorb in the dispersed phase (Gurpreet and Singh, 2018). While both natural and synthetic emulsifiers can stabilize emulsions, consumers tend to prefer eco-friendly, safe, and natural emulsifiers due to potential health-related issues associated with synthetic stabilizers (Zhu et al., 2019).

Alfalfa (*Medicago sativa*) is a significant forage legume, and is sometimes referred to as the "father of all foods". Alfalfa is a rich source of saponins, and due to their structural formula, they exhibit various biological activities, like anti-parasitic, antimicrobial, antifungal, anti-inflammatory, anti-tumor, and hypocholesterolemic, and immune stimulatory activity. Saponins act as natural emulsifiers to stabilize nanoemulsions (Oakenfull, 1981). Edible coatings based on nanoemulsions proved to be effective in enhancing the shelf stability of tomatoes by controlling the gaseous exchange, preventing moisture loss, retarding pathogen growth, and reducing oxidative reactions. Moreover, saponins themselves possess antimicrobial properties and free radical scavenging properties that can reduce oxidative stress. Due to their biodegradable nature and easy availability almost all year long, saponin coatings can be considered a suitable substitute for commercial hydrocolloid-based coatings. Given the importance of saponin, this research was conducted to formulate and characterize alfalfa saponins using different extraction techniques and to formulate oil in water (O/W) emulsions using alfalfa saponins or in combination with synthetic emulsifiers. Lastly, the shelf stability of the tomatoes was studied using crude and emulsified forms of alfalfa saponins.

MATERIALS AND METHODS

Materials

Alfalfa seeds were purchased from the local market and sorted by hand to remove all dirt and stones. Oleanolic acid standard (97% pure) was procured from Carbo Synth for the estimation of total alfalfa saponins. For protein quantification, Bradford reagent and bovine serum albumin (BSA) were purchased from Sigma Aldrich Co.. Vanillin was purchased from Daejung. Decaglycerol monolaurate (ML-750, HLB 14.8) was kindly provided by Sakamoto Yakuhin Kogyo Co., Ltd. as a sample. Polyoxyethylene (20) sorbitan monolaurate was procured from Uni-chem chemical reagents. All other chemicals were of analytical grade and used as such to perform different experiments. To formulate and study the saponin coatings, freshly harvested tomatoes that were bright red and near their fully ripened stage were purchased from a commercial market. The selected tomatoes were sorted based on uniformity in size, color, and weight. They were clean and free from defects and any kind of microbial infection. After purchase, the tomatoes were transported to the laboratory within 1 h for further experiments.

Preparation and analysis of alfalfa saponin extracts

The pre-dried seeds of alfalfa were finely ground (<0.5 mm) with multiple food processors (AG-3051, Anex) and added to freshly prepared mixtures of ethanol and distilled water 0~100% (v/v), at a solvent : powder concentration ratio of 10:1. The suspensions were continuously shaken (Lab-Line Environ Orbital Shaker Model 3528, Lab-Line Instruments Inc.) at room temperature for 24 h until the powder was completely whitened and then centrifuged at 4,000 rpm. After centrifugation and filtration, the solid particles were removed and the supernatant was evaporated using a rotary evaporator (model WEV-1001L, Daihan Scientific Co., Ltd.) at 16 hPa at 4°C. To obtain the appropriate concentration of the stock solution, the residues were redissolved in distilled water and stored at 4°C for further use.

Quantification of alfalfa saponins

The method of Taarji et al. (2018) was used to evaluate total saponin content spectrophotometrically. Standard solution of oleanolic acid (2.4 mg/mL) was prepared using ethanol and different dilutions were prepared in distilled water. For the quantification of saponins, the ethanolic mixtures with 8% (v/w) vanillin and 72% (v/v) sulphuric acid were added to a glass test tube and incubated at 60°C for 15 min with continuous shaking. After incubation, the prepared solution was cooled at room temperature for 5 min. The absorbance was measured using a spectrophotometer at 560 nm (Taarji et al., 2018).

Quantification of extracted alfalfa saponin proteins

Bradford's method was used to determine the total protein content of extracted alfalfa saponins. Briefly, 3 mL of Bradford reagent were added to 0.1 mL of extract solution and incubated for 5 min at room temperature. BSA was used as a standard and the total proteins present in the extract were expressed as BSA equivalent using a BSA standard curve. The absorbance was measured at 595 nm using a ultraviolet-visible spectrophotometer (Bradford, 1976).

Preparation of alfalfa saponins-based O/W emulsions

For the preparation of O/W emulsions, the aqueous phase containing 0.1∼2.0% (w/w) alfalfa saponins in distilled water, and soybean oil was used as the dispersed phase. Both phases were homogenized using a rotor-stator homogenizer (Daihan Scientific Co., Ltd.) at 10,000 rpm for 5 min and the stability of emulsions was evaluated by observing the change in droplet size at different temperatures (4°C and 25°C) for 7 days (Taarji et al., 2018).

Effect of saponins-based coatings on postharvest quality of tomatoes

***Selection and preparation of sample*:** The coatings were prepared using the method reported by Norcino et al. (2020), with minor modifications. Pectin solution (1% w/w) was prepared by dissolving pectin powder in distilled water on a hot plate with continuous stirring at 40°C for 10 min and then 1% (w/w) glycerol and a total 0.1∼2.0% (w/w) of saponin extract (0.1, 0.5, 1, and 2%) were added in different concentrations. The tomatoes of equal size with no physical damage were washed properly with distilled water and dried at room temperature.

All the collected samples of tomatoes were divided into five groups. Table 1 shows the distribution of samples and a detailed treatment plan. Each group comprised 32 samples of tomatoes; whereas four different treatments of saponins [T_1 (0.1%), T_2 (0.5%), T_3 (1%), and T_4 (2%)] were applied on tomato samples of each group. For each treatment, four samples of tomatoes were used and all these samples were stored at two different temperatures (16 samples were stored at 4°C and the rest 16 at 25°C to determine the storage stability). The first group of tomatoes was considered as the control group and washed with distilled water only. The second group of tomatoes was dipped in the pectin-glycerol solution, while the third group of tomatoes was coated with sole alfalfa saponin-based emulsions. In group four, tomatoes were coated with a mixture of alfalfa saponins and Tween 20, while the fifth group contained tomatoes coated with a mixture of alfalfa saponins and ML-750.

***Determination of physicochemical properties of sample treatments*:** The pH of all the samples was measured using a pH meter (Hanna, Combo Grocchek). The weight of the samples was measured using a lab scale digital weighing balance. The total soluble solids (TSS) were measured using a handheld refractometer and the results were expressed in °Brix. The color change was observed using a fully automatic colorimeter (Konica Minolta Inc.) on days 1, 3, 5, and 7 respectively (Buendía-Moreno et al., 2019). The color of the tomatoes was determined using a colorimetric assay with L^*, a^*, and b^* values where L^* denoted lightness of the sample (from black to white), a^* represents the green and red color, and b^* represents blue and yellow color (Firdous et al., 2020).

***Sensory evaluation of saponin-coated tomatoes*:** The sensory evaluation of all the tomatoes treated with extracted alfalfa saponin extract was conducted using a 5-point hedonic scale. The sensory evaluation of the intact toma-

Table 1. Description of groups and treatment plan used in the study to increase the shelf life of tomatoes

Group	Treatment used	Composition of treatment	Sample size
1	Tap water	Tomatoes washed with tap water	2
2	Crude alfalfa saponins [0.1~2.0% (w/w)]	T_1 = 0.1% T_2 = 0.5% T_3 = 1.0% T_4 = 2.0%	32
3	Emulsified alfalfa saponins [0.1~2.0% (w/w)]	T_1 = 0.1% T_2 = 0.5% T_3 = 1.0% T_4 = 2.0%	32
4	0.25% (w/w) Tween 20 + alfalfa saponins extract [0.1~2.0% (w/w)]	T_1 = 0.25% + 0.1% T_2 = 0.25% + 0.5% T_3 = 0.25% + 1.0% T_4 = 0.25% + 2.0%	32
5	0.5% (w/w) decaglycerol monolaurate + alfalfa saponins extract [0.1~2.0% (w/w)]	T_1 = 0.5% + 0.1% T_2 = 0.5% + 0.5% T_3 = 0.5% + 1.0% T_4 = 0.5% + 2.0%	32

All treatments contain 1% (w/w) pectin and 1% (w/w) glycerol mixture.

toes was initially performed. The panelists for the study were selected based on their knowledge and sensory skills, which included expertise in evaluating characteristics such as aroma, appearance, color, and texture. The aroma of both intact tomatoes and of those that had been coated with saponin extract was determined before and after cutting. The scale for the sensory evaluation was chosen from 1 to 5, where 1 represented extremely poor and 5 indicated excellent quality. The sample that scored less than 3 was considered unacceptable for the commercial market.

Statistical analysis

In this study, the data were analyzed using the SPSS Statistics 16.0 software (SPSS Inc.). One-way analysis of variance (ANOVA) was used to determine significant differences in the emulsifying properties of alfalfa-rich saponins and other synthetic emulsifiers. All the performed experiments were conducted in triplicate, and mean values were calculated. The ANOVA was applied at a 95% confidence level and multiple mean comparisons were performed using Tukey's test.

Institutional Review Board statement

The sensory evaluation of treated tomatoes was approved by the Ethical Review Committee of the University of Management and Technology (approval no. UMT/IRB/PostGrad/Res/2020-09-R001) and written consents were recorded before sensory evaluation. Moreover, the study was conducted in accordance with the principles of the Declaration of Helsinki Protocol.

RESULTS AND DISCUSSION

Effect of extraction methods on the quality and quantity of extracted saponins

Alfalfa seeds contain a significant amount of saponins and might possess emulsifying properties that reduce surface tension (Eslahati et al., 2020). The saponins were quantified using the oleanolic acid standard curve at 560 nm. The absorption values showed perfect linearity with a coefficient of determination (R^2=0.9974). Due to the presence of hydrophobic and lipophilic ends, the proteins and saponins both demonstrated emulsifying properties (Öztürk, 2017).

Different concentrations of aqueous-ethanolic extract 0, 20, 40, 60, 80, and 100% (v/v) (with powder solvent ratio of 1:10) were used to evaluate saponin content from alfalfa seeds. The results revealed that 40% (v/v) ethanolic extract contains the maximum concentration of saponins (2,300 μg/mL). Fig. 1A showed that alfalfa powder contains saponins that significantly increased from 0 to 40% (v/v) ethanolic concentration. The results were similar to those reported by Xu et al. (2018) who showed that ethanol and water increase the extraction of saponins by enhancing the interaction between the surface contact area of solvent and plant material. Moreover, saponins required a selective ethanol concentration for extraction. Various studies have shown that by increasing the ethanol concentration from 50% to 90% the saponin contents keep on decreasing (Akbari et al., 2019; Dahlawi et al., 2020). In another study, saponins were extracted from fenugreek seeds using different concentrations of ethanolic extract. The results of the study demonstrated that concentrated ethanolic extract of 40% (v/v) was highly effective in saponins extraction (Akbari et al., 2019). Dahlawi et al. (2020) extracted saponins from onion skin waste using a powder solvent ratio of 1:5 with 60% (w/w) ethanolic extraction solution.

Effects of ethanol concentration on total protein content

Proteins undergo conformational and structural changes due to the presence of ethanol; different concentrations of ethanol were used to study the protein precipitation (Yoshikawa et al., 2012). Initially, by increasing the ethanol content [20∼40% (v/v)] the protein content gradually decreased (3.5 μg/mL) until it became constant at higher concentrations of ethanol [80∼100% (v/v)] (Fig. 1B). Proteins and saponins both possess hydrophobic hydrophilic moieties and show emulsifying properties (Taarji et al., 2018).

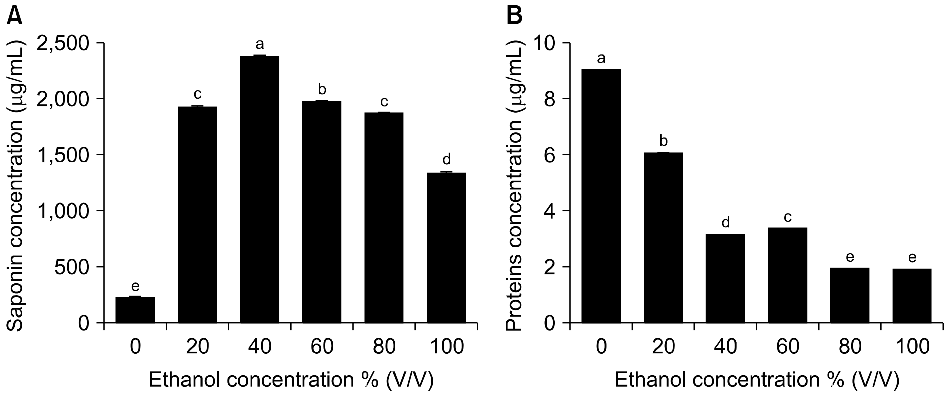

Fig. 1. The effect of ethanol concentration on (A) total saponin content and (B) total protein content from alfalfa seed powder. The letters on each bar (a–e) indicate significant difference between different treatments at P<0.05.

Effect of extracted saponins on the stability of O/W emulsion

The O/W emulsions were prepared using [0.5∼2% (w/w)] extracted alfalfa saponins alone and coupled with Tween 20 [0.25% (w/w)] and ML-750 [0.5∼1.5% (w/w)]. The emulsions formulated with sole saponins were highly unstable and exhibited foaming at a rapid rate. The optical microscopic graphs (Fig. 2) showed a larger droplet size of >100 μm that indicates high polydispersity in formulated emulsions. The emulsions formulated with alfalfa saponins (Fig. 2A and 2B), synthetic emulsifiers (Fig. 2C and 2D) Tween 20 [0.25% (w/w)] and ML-750 [0.5∼1.5% (w/w)] were highly stable (Fig. 2E and 2F) with no significant change in creaming index over 7 days. Furthermore, optical microscopic graphs (Fig. 2A and 2B) confirmed the smaller droplet size of 2∼10 μm in alfalfa saponins-Tween 20 stabilized emulsions and size of 5∼10 μm (Fig. 2E and 2F) in alfalfa saponins-ML-750 emulsions.

The stability of different emulsions containing Tween 20, ML-750, and alfalfa saponins was observed at temperatures of 4°C and 25°C for seven days. The results revealed a significant change in particle size in emulsions with sole alfalfa saponins compared to those coupled with Tween 20 [0.25% (w/w)] emulsion and those with ML-750 [0.5∼1.5% (w/w)] emulsions. The change in particle size insole saponin-based emulsion was mainly due to the poor surface activity of alfalfa saponins. However, the quantification of the surface activity of alfalfa saponins is beyond the scope of the present study.

Effectiveness of saponins coatings on tomatoes

The application of various coatings increased the shelf life of tomatoes, and the most effective coating was evaluated based on the tomato's % weight loss, acidity, TSS, and color at 4°C and 25°C. The pictures of coated tomatoes at 4°C and 25°C are presented in Fig. 3. At 4°C and 25°C, % weight loss of tomatoes coated with alfalfa saponins was recorded and the results revealed the lowest level of weight loss with treatment on day 3 as presented in Fig. 4A. At 4°C, a significant (P=0.00) weight loss was observed at day 7 with all treatments except T_2 that showed minimum weight loss (2%). On the 7th day of storage, T_3 and T_4 exhibited maximum weight loss (14∼16%) while T_1 and T_2 showed minimum weight loss (5%) compared to other treatments at 25°C. These results revealed that saponins with pectin-glycerol solution inhibit respiration and moisture loss and reduce % weight loss. This is probably because pectin is used as an active packaging material in food-grade emulsions

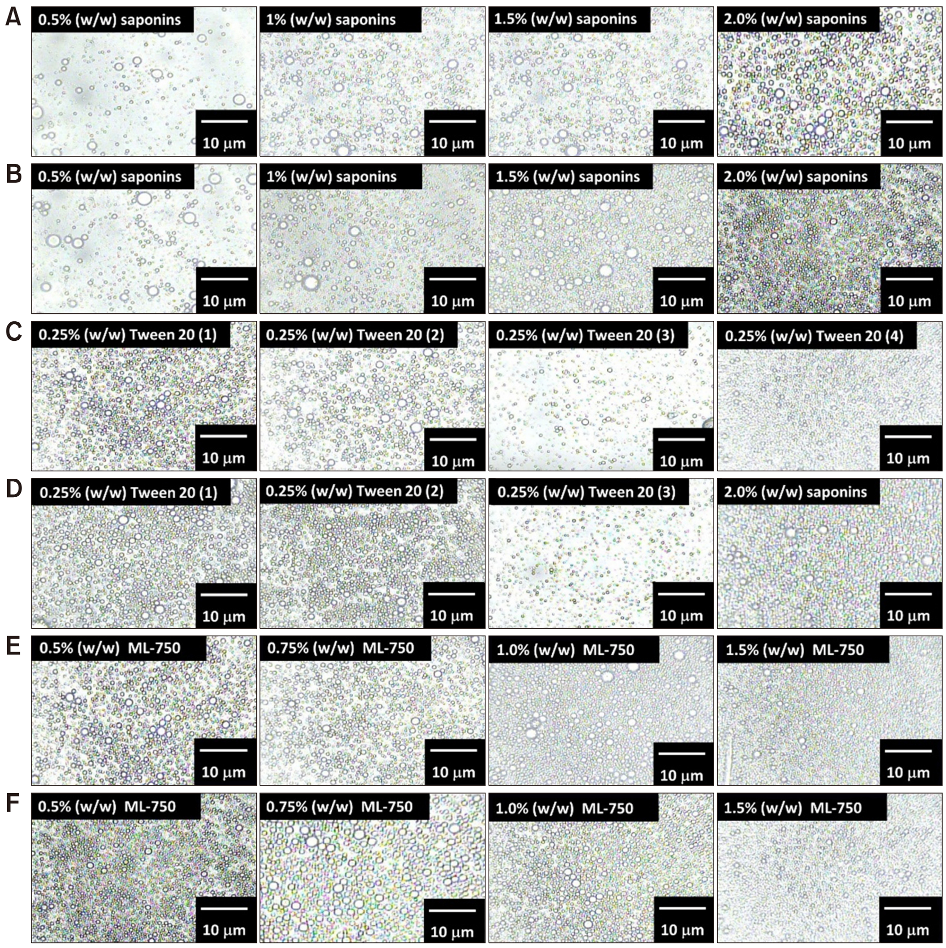

Fig. 2. Optical microstructure representation of different oil in water emulsions. Microstructure of emulsions stabilized with saponin on (A) day 3 and (B) day 7. Microstructure of emulsions stabilized with Tween 20 on (C) day 3 and (D) day 7. Microstructure of emulsions stabilized with ML-750 on (E) day 3 (F) day 7.

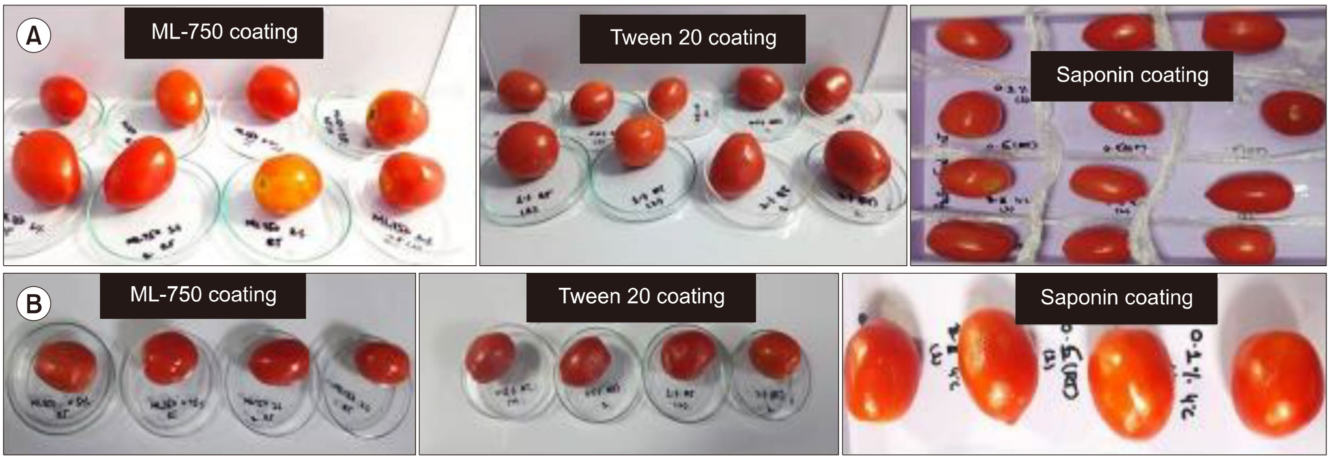

Fig. 3. Effect of different coatings on weight, pH, and the visual appearance of samples after various coating treatments. The efficiency of ML-750, Tween 20, and saponins coating on tomatoes at (A) day 3 and (B) day 7.

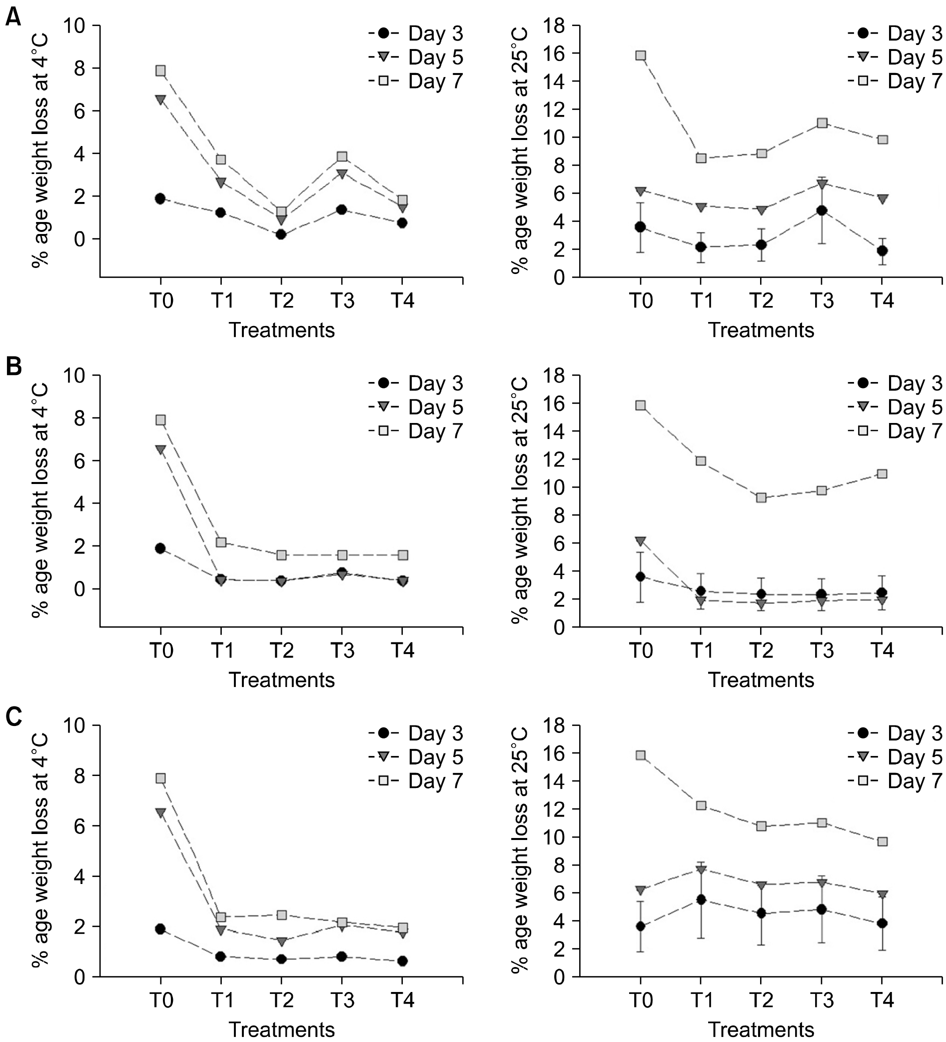

Fig. 4. Efficiency of different coatings on weight loss of tomatoes at days 3, 5, and 7. Weight loss percentage of tomatoes coated with (A) alfalfa saponins, (B) saponins-ML-750, and (C) saponins-Tween 20.

(Nisar et al., 2018).

To further enhance the effect of the saponin coating, the tomatoes were additionally coated with the saponins and ML-750. As shown in Fig. 4B, minimum changes in weight loss were observed at 4°C and 25°C on days 3 and 5 after applying this combined coating. The minimal weight loss could be attributed to the use of saponins combined with ML-750, but significant (P=0.00) weight loss was observed on the 7th day at 25°C and 4°C, probably due to the weakening of emulsified layers. These results indicated that T_2 was effective in reducing the % weight of tomatoes. The tomatoes were also coated with the alfalfa saponins and Tween 20, and weight loss was minimal on day 3 (Fig. 4C). Samples stored at ambient temperature showed a significant (P=0.00) weight loss of 10∼12% on days 5 and 7 respectively, with T_1 exhibiting the maximum weight loss of 14%. At 4°C the weight loss with all the treatments was minimal and T_3 and T_4 proved to be most effective. These results showed that between T_3 and T_4, T_4 is effective at both temperatures. Among all the samples, the tomatoes coated with alfalfa saponins and Tween 20 showed significant reduction in the % weight loss of the sample compared with other coatings. At 4°C, all the samples were fresh with minimum weight loss because low temperature reduced the rate of respiration and evaporation (Gurpreet and Singh, 2018).

Effect of saponin coatings on the physicochemical quality of tomatoes

TSS and pH (Fig. 5) are essential to determine the quality of the fruit. The TSS of tomatoes gradually increases as they ripen, and it is consumed in respiration for the hydrolysis of polysaccharides (Tadesse et al., 2015). No significant change was observed in the TSS of all coated samples; coating provided a barrier and less reduction was observed in oxygen from the surface of the fruit. A similar study reported that 1% chitosan and aloe vera coatings applied in combined form tend to significantly improve the shelf life of mangoes. Moreover, this coating also reduced fruit decay, weight loss, respiration rate, and ethylene production. Further, this coating also helped to maintain the fruit quality parameters such as titratable acidity, TSS, fruit firmness, ascorbic acid, and peel color (Shah and Hashmi, 2020).

Fresh tomatoes used in this study were reported to have acidic pH <5 and it gradually increased with ripening of the fruits. A relevant study was also performed in this regard in which the pH of tomato fruits was reported to be 4.38∼4.57 (Altuntas and Ozkurt, 2019). The pH can be determined by the total acid content present in the tomatoes; citric acid is the most important acid present in tomatoes and at maturity, it gradually decreases due to its consumption in respiration (Anthon et al., 2011). Dehydration causes quality deterioration and weight loss, and also affects the flavor and the physical appearance of fresh fruits (Shete et al., 2018). Over time the TSS contents (fructose and glucose) of fruits de-

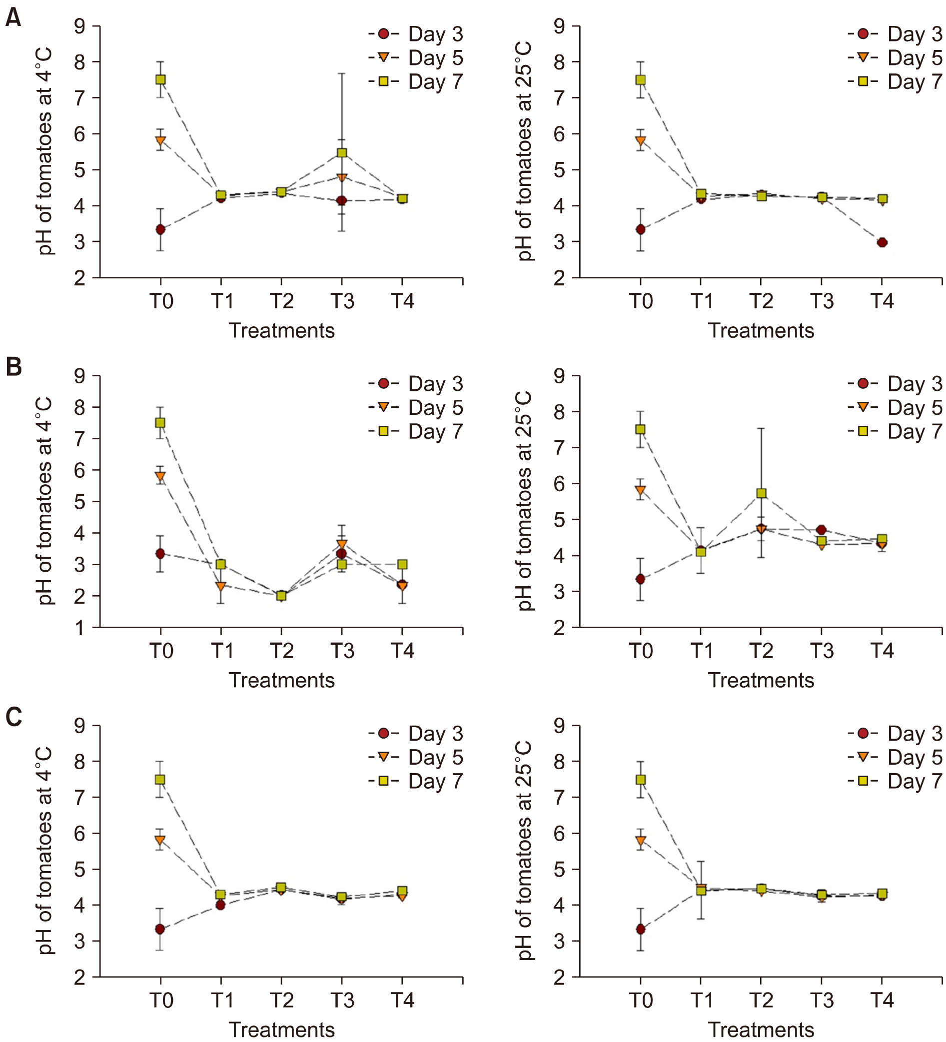

Fig. 5. Effectiveness of different coatings on pH of tomatoes. Change in pH of tomatoes coated with (A) alfalfa saponins, (B) saponins-ML-750, and (C) saponins-Tween 20 at 4°C and 25°C.

creased, ultimately reducing the sweetness of fruits (Gao et al., 2018).

In this study, different coatings were applied to enhance the shelf stability of tomatoes to create a barrier and reduce the rate of respiration. Tomatoes were coated with the alfalfa saponins and no significant change in pH was observed with treatments T_1 and T_2 at both ambient temperature and 4°C (Fig. 5A). The possible reason for the high standard deviation observed was due to the insignificant change in the results reported on coated tomatoes.

At 4°C a significant (P=0.00) change in pH was noticed at days 3, 5, and 7 with T_3. There was a significant increase in pH reported from 4 to 5.5 in uncoated tomatoes as compared to the coated tomatoes. Tomatoes coated with ML-750 and alfalfa saponins were quite stable at both temperatures 4°C and 25°C (Fig. 5B). At 25°C, a significant pH change in T_2 was observed on days 3, 5, and 7 respectively. An increase in pH in uncoated tomatoes was observed on days 3, 5, and 7 respectively. The coated tomatoes were highly resistant to changes in pH as compared to the uncoated tomatoes. In a controlled sample the increase in pH was observed due to the fast respiration rate. The study also reported that the chitosan-based coatings proved effective against changes in weight loss, respiration rate, fruit firmness, pH, and titratable acidity when applied on fruits (Bal, 2018).

Effect of saponin-based coatings on the physicochemical quality of tomatoes

The appearance and color of fruit are crucial to determine the quality and freshness of fruits. Tomatoes with a bright red color and shiny surface have higher overall acceptability among consumers. At the time of maturity, chlorophyll starts to degrade while carotenoid and lycopene start to increase and play a vital role in the red color of tomatoes (Banu et al., 2020). The tomato is a climacteric vegetable and continues to ripen even after harvesting. The respiration process continues during ripening, resulting in an increase in CO_2 levels and decrease in ethylene, which affects the color of tomatoes.

In this study, different coatings were applied to observe the overall color change in tomatoes after coating at 4°C and 25°C (Fig. 6). The tomatoes were dipped in saponins coating and a significant change in color value was observed with T_1 and T_4 while no significant change (Fig. 6A) was observed with T_2 and T_3. These results confirmed that 1% and 1.5% (w/w) alfalfa saponin were effective in maintaining the color of tomatoes. Moreover, tomatoes dipped in the ML-750 and alfalfa saponins coatings showed a significant change (Fig. 6B) in color values in T_2, and L value significantly increased at day 7 while T_1, T_3, and T_4 showed minor color differences. The tomatoes coated with alfalfa saponins coupled with Tween 20 (Fig. 6C), showed no significant change in color with T_2, T_3, and T_4 at 4°C and 25°C. The results indicated that tomatoes coated with combined emulsions, ML-750, and Tween 20; showed slight and no significant change in color, respectively, and were effective against only saponin-based emulsion.

Sensory evaluation of the saponin-coated tomatoes

The quality and freshness of fruits can be evaluated via sensory attributes such as texture, odor, flavor, firmness, and color. Three coatings with different concentrations were applied to tomatoes to observe sensory attributes for 7 days. The sensory quality results of tomatoes are presented in Fig. 7. In tomatoes coated with alfalfa saponins (Fig. 7A), no significant difference was observed in the overall acceptability attributes. T_1 scored better in terms of overall acceptability, color, firmness, and aroma compared to other treatments, however a significant change in texture was observed. Tomatoes coated with ML-750 and alfalfa saponins T_4 showed better firmness, texture, color, aroma, and overall acceptability on a hedonic scale. No significant change in visual appearance was observed with T_1, T_2, and T_3 and only T_2 showed a significant (P=0.01) change in color as presented in Fig. 7B. In tomatoes coated with Tween 20 and alfalfa saponins, no significant change was observed in terms of overall acceptability, texture, firmness, and color with T_1, T_2, and T_4 but a significant change in texture was observed with other treatments. T_3 showed a minimum score as compared to other treatments in terms of firmness as presented in Fig. 7C. The results revealed that the alfalfa saponins when combined with ML-750 and Tween 20 proved more effective as compared to saponins only.

This study was carried out to evaluate the effectiveness of alfalfa saponin as an emulsifying agent in emulsions to enhance the shelf life of tomatoes. The 40% (w/w) ethanolic concentration was selected for the extraction of saponin and used as an emulsifier for the formulation of O/W emulsions. The results demonstrated that alfalfa saponin alone has weak foaming and emulsifying properties that led to instability of O/W emulsions. To enhance its efficiency, 40% (w/w) ethanolic extract was combined with other synthetic emulsifiers such as Tween 20 and ML-750, and the stability was evaluated at 4°C and 25°C. The results showed that such emulsions were stable for more than 7 days. After the evaluation of stability, these emulsions were further used as a coating material to enhance the shelf stability of tomatoes. All the results indicated that tomatoes coated with the alfalfa saponin and Tween 20 exhibited better firmness, texture, aroma, and color than tomatoes coated with ML-750. Our findings proved that the shelf stability of tomatoes could be enhanced by using cost effective alfalfa

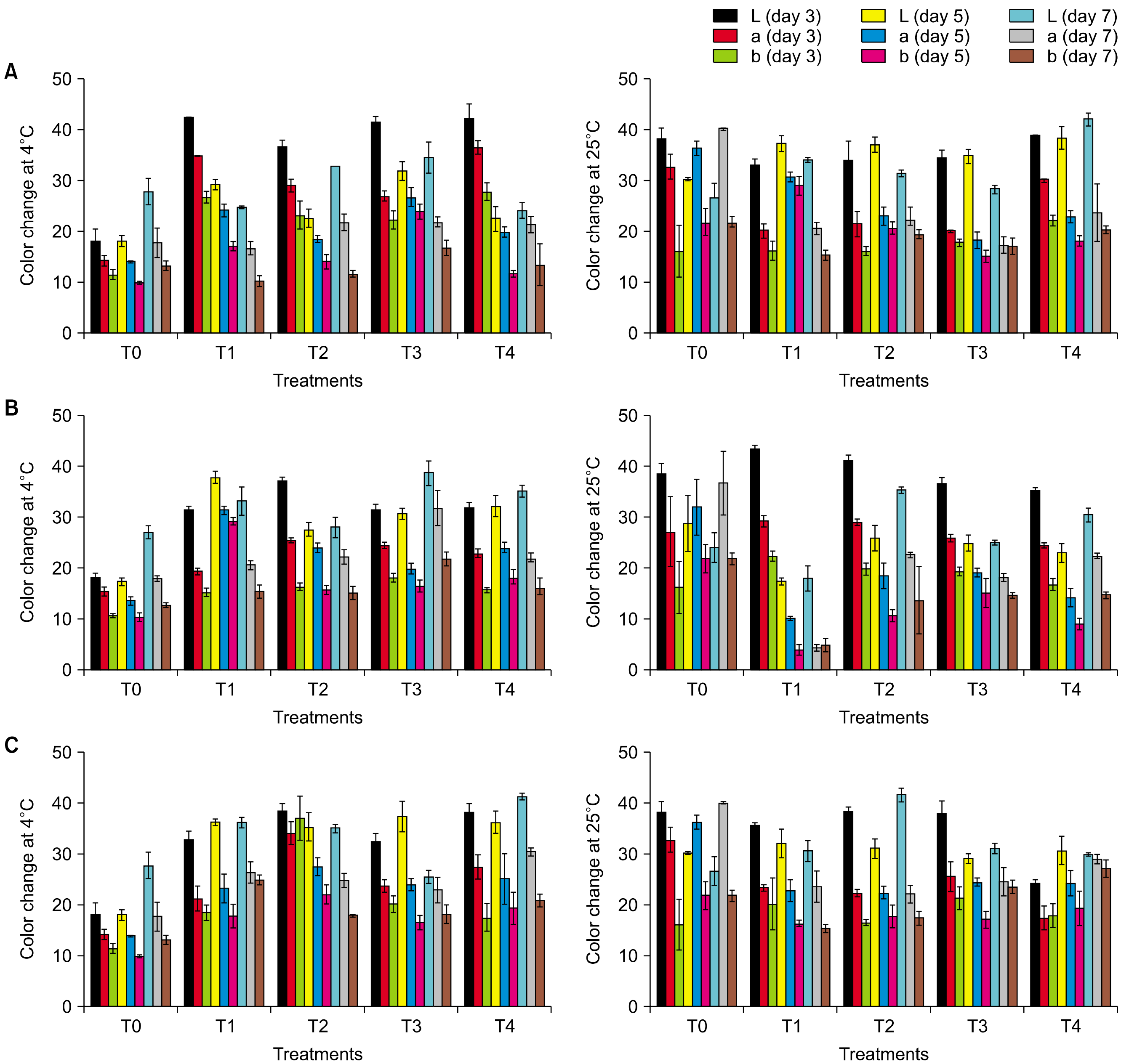

Fig. 6. Efficiency of different coatings on color change of tomatoes. (A) Alfalfa saponins coatings, (B) saponins-ML-750, and (C) saponins-Tween 20 at 4°C and 25°C.

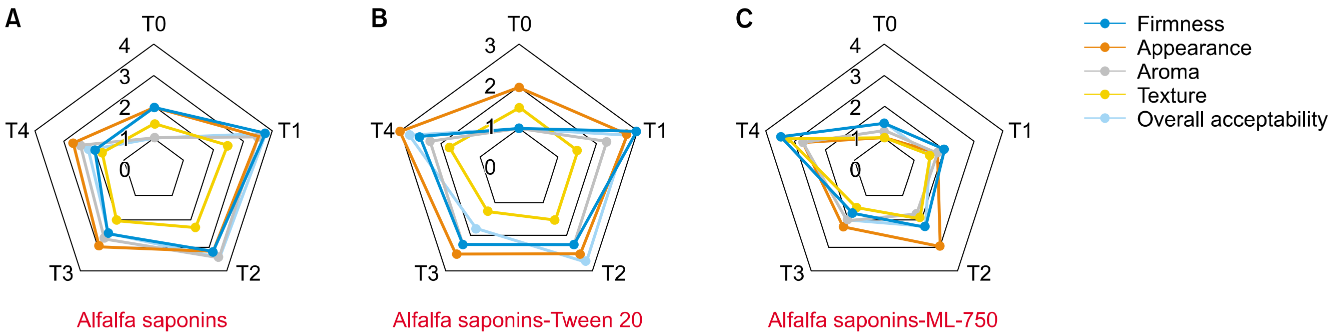

Fig. 7. Sensory evaluation of tomatoes coated with different of (A) saponin coatings, (B) saponins-Tween 20, and (C) saponins-ML-750 stored at 4°C and 25°C for 7 days.

saponins coupled with synthetic emulsifiers.

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AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Concept and design: NK. Analysis and interpretation: MA, AA, WN. Data collection: MA, WN. Writing the article: all authors. Critical revision of the article: NK, AA. Final approval of the article: all authors. Statistical analysis: WN. Overall responsibility: NK.

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