Journal of Food: Microbiology, Safety & Hygiene

OPEN ACCESS Freely available online

Research Article

Alginate Cross-Linking Based Edible Coatings Enriched Hexyl Acetate: Browning Inhibition, Microbial Control and Maintain Quality of Fresh-Cut Rose Apple Cv. 'Tabtimchan'

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ABSTRACT

The edible coating is one of the eco-friendly methods that has been widely applied for extending the shelf life of fresh-cut fruit products including rose apple. However, using only edible coating materials does not fulfill the functions of controlling microbial growth and managing the postharvest quality of the products. This study aimed to develop the coating formulation of biodegradable Sodium Alginate Cross-Linking (SAC) enriched Hexyl Acetate (HA) (HA as natural antimicrobial volatile extracted) for prolong the shelf-life of fresh-cut rose apples. Fresh-cut rose apples were dipped into SAC, HA alone (HA-0.03%), or SAC enriched 0.03% of HA (SAC-HA-0.03%) for 1.5 min, and then stored at 4°C in plastic boxes compared to distilled water dipping served as the control. Changes in postharvest quality, sensory properties as well as microbiological analysis were determined every 2 days for 10 days. The results showed that both of SAC and SAC-HA-0.03% samples had greater acceptance and quality than the control and HA-0.03% alone. SAC-HA-0.03% maintained the freshness appearance and reduced the Browning Index (BI) by decreasing Polyphenol Oxidase (POP) and Peroxidase (POD) activity in comparison with SAC alone. The highest Total Phenolics (TP), antioxidant, and phenylalanine ammonia-lyase activity were found in samples coated with SAC-HA-0.03%. In addition, coated fresh-cut rose apples with SAC-HA-0.03% delayed the loss of Total Soluble Solids (TSS) and Titratable Acidity (TA), maintained firmness, and inhibited the growth of Total Plate Count (TPC), yeasts and moulds and *Escherichia coli* (*E. coli*). Finally, in sensory evaluation, SAC-HA-0.03% coating improved the aroma of fresh-cut rose apples with the highest overall acceptance score. Thus, SAC-HA-0.03% is the best coating formulation to preserve fruit quality characteristics leading to an increase in the shelf-life of fresh-cut rose apples of up to 10 days at 4°C.

Keywords: Antimicrobial, Browning; Coating, Rose apple; Postharvest quality

INTRODUCTION

Fresh-cut fruits can be defined as any fruits physically modified from their original form by peeling, trimming, washing, and cutting to obtain 100% edible product that is bagged or prepackaged and kept in refrigerated storage [1]. In recent years, fresh-cut fruit consumption has currently increased in Asia, Western countries, stimulated by public awareness of health and busy lifestyle of people [1-3]. Fresh-cut has been rapidly growing in response to meet consumer demand for safety, convenient product with freshness quality and nutritional value similar to that associated with intact fruits [4]. In Thailand, fresh-cut fruits products such as durian, mangoes, papayas, guava, pineapple, water melon, cantaloupe, jackfruit, pomelo and rose apple are mainly sold in markets [5,6].

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Received: 05-Dec-2024, Manuscript No. JFMSH-24-35643; **Editor assigned:** 09-Dec-2024, PreQC No. JFMSH-24-35643 (PQ); **Reviewed:** 23-Dec-2024, QC No. JFMSH-24-35439643; **Revised:** 30-Dec-2024, Manuscript No. JFMSH-24-35643 (R); **Published:** 06-Jan-2025, DOI: 10.35841/2476-2059.24.9.319.

Citation: Duong NTC, Uthairanakij A, Laohakunjit N, Jitareerat P (2025). Alginate Cross-Linking Based Edible Coatings Enriched Hexyl Acetate: Browning Inhibition, Microbial Control and Maintain Quality of Fresh-Cut Rose Apple Cv. 'Tabtimchan'. J Food Microbiol Saf Hyg.9:319.

Rose apple fruit cv. Taaptimjaan is one of tropical fruit popular commercial in Thailand because of its ruby-red skin, juicy sweet and pleasant aroma [7]. Recently, not only is intact rose apple fruit economically important but the fresh-cut fruits have also become a popular fresh-cut product and it has been expanded to study for commercial in markets [8-10]. However, the greatest hurdle of fresh-cut products is that they are perishable and difficult to preserve since minimal processing removes fruits natural protection and generates sensory deteriorations such as browning, water loss, off-flavors production, and loss of firmness [11]. Moreover, fresh-cut fruits provide favorable conditions to microorganisms for their growth and proliferation [12]. Browning and microbial growth of fresh-cut products play important roles in their final appearance and quality during marketing. Browning is the main factor that is directly related to the shelf life of fresh-cut products and consumer's purchase decisions. The oxidation of phenolic substrates by PPO or POD is the main cause of enzymatic browning leading to browning pigment [13]. While microbial contamination with food-borne pathogens and spoilage microbes, which leads to a short shelf life, public health risks and economic losses [2,14]. Recently, one approach to reduce the deterioration and preservation of fresh-cut product is to use edible coatings carrying antimicrobials [15]. It can be used as an alternative to improve the aesthetic appeal, quality and increase the shelf stability of the fresh-cut fruits by protecting them from moisture loss, gas exchange or oxidation process and being an effective method to control microbial growth. The addition of Essential Oils (EOs) as functional bio-compounds into the formulation of edible coatings can be used as natural flavorings and antimicrobials in fresh products [16].

Alginate is one of the natural polysaccharide edible coatings, extracted from brown seaweed. It is the salts of alginic acid linear of β-D-mannuronic acid (M) and α-L-guluronic acid (G) and is Generally Recognized as Safe (GRAS) by the US Food and Drug Administration (FDA) [17,18]. Alginate has good barrier gas properties and uniform, transparent coatings, which have been successfully applied in minimal products [19]. It could help protect them from moisture loss, gas exchange or oxidation process, browning [20]. However, alginate based coatings provide poor moisture barrier, water-soluble due to their hydrophilic nature [21,22]. Adding Calcium Chloride ($CaCl_2$) cross-linking agents can improve the characteristic, water vapor and oxygen barrier of the alginate based film [23,24]. Previous to our work, alginate-based edible coating cross-linked with $CaCl_2$ in a single step was effective in improving physical-mechanical and barrier gas of alginate [25]. However, alginate based edible coating cannot inhibit microbial growth effectively when used alone [26,27]. Incorporating antimicrobial compounds as plant EOs or volatile compounds into alginate edible coatings as a novel way to ensure safety has been applied for protecting the fresh-cut fruit from microbial spoilage and thus extend their shelf-life [28]. Various studies have demonstrated that the EOs (e.g. lemongrass or thyme, eugenol, citral) incorporated into alginate edible coatings are effective in preventing the growth of pathogen and spoilage microorganisms and delaying browning on fresh-cut fruits such as pineapple, apple and kiwi [29]. However, EOs have limited use for the preservation of fresh products due to EOs components may impact the sensory attributes such as the taste and odor of coated fruit [30].

HA is one of plant volatile compound with fruity odor and an extremely aromatic compound with a green note flavor, and has been widely used as a food flavoring agent and it is generally recognized as safe [31,32]. Generally, the activity of antimicrobial agent of volatile compounds including HA is dependent on its vapor pressure and affected by temperature [33]. As the temperature rise increases, HA tends to pass in the vapor phase and, consequently, the antimicrobial effects of alcohol molecules such as hexanol. Hexanol is six-carbon alcohol with amphipathic, and the hydrophobic region is dissolved in the cell membrane and increases membrane fluidity, damaging the cell membrane [34,35].

Recently, HA has been used as an antimicrobial agent for improving the safety and prolonging the shelf life of minimally processed fruits. Antimicrobial activity of HA at the levels used 150 ppm affected against pathogenic microorganisms (*L. monocytogenes*, *E. coli*, and *S. enteritidis*) was already tested in both model systems and fresh-sliced apples [32]. Additionally, it also found an effect in reducing the growth of Colletotrichum acutatum in strawberry [36]. However, the application of HA volatile compounds has been limited due to their easy evaporation, quick release when exposed to heat, pressure and affected by system composition [37,33]. The incorporation of HA into alginate could prevent volatile compound losses and improve the function of alginate as antimicrobial edible coating. Considering limited studies of the incorporation of HA oil into Sodium Alginate (SA) to inhibit the activity of enzymatic browning and reduce microbial growth on fresh-cut rose apple storability, SA enriched HA was applied. The current study aimed to investigate the possible potentials of coating formulations of SAC incorporated with HA oil for suppressing the browning, inhibiting microbial, and extending the shelf life of fresh-cut rose apples.

Our preliminary work found that HA concentrations of >0.05% (v/v) cause flesh browning as well as a negative effect aroma (strong smell) of fresh-cut rose apples. Therefore, HA at 0.03% (v/v) was selected for this study.

MATERIALS AND METHODS

Materials

Rose apple (*Syzygium samarangense*) fruits cv. 'Tabtimchan' at 45 days after full bloom were harvested from an orchard in Ratchaburi province, Thailand. They were immediately transported to the King Mongkut's University of Technology Thonburi, and selected uniform in size and color with no defect for experiment.

Food-grade SA (Japan), glycerol, and Tween 80 were supplied by Krungthepchemi Co., Ltd (Thailand). Purity HA oil was supplied by Perfumer's World, Thailand.

Preparation of fresh-cut rose apples and edible coating solution

Rose apples fruits were washed with tap water and immersed in 50 ppm sodium hypochlorite solution for 3 min. Fruits were cut into four pieces using a sterile knife. The core and top of each fruit were removed.

SAC was prepared according to the method reported by Song et al., [38]. To prepare 1000 mL of SA solution, 12.50 g of SA powder was dispersed in 1000 mL of distilled water by stirring on a hot plate at 70°C until the solution was completely clear. Then, 1.25% (w/v) glycerol was added followed by 0.3% (w/v) Tween 80. After that 40 mL $CaCl_2$ (1% w/v) aqueous cross linker was added into SA to create SAC. Finally, 0.03% (v/v) of HA was added and homogenized for 3 min.

Fresh-cut rose apple coatings and storage

Rose apple pieces were dipped in the SAC enriched HA solution (SAC-HA-0.03%) for 1.5 min, dripped off the excess coating solution for 2 min in compared with samples were dipped in SAC based edible coating alone and HA alone (HA-0.03%). Uncoated samples were used as the control treatment. After air-drying at room temperature (26°C), the samples were kept in polypropylene boxes (10 cm × 15 cm × 8 cm), each box contained four pieces, stored at 4°C for 10 days. Samples were taken to determine every 2 days. The experiments were carried out in four replications.

Fresh-cut rose apple evaluation

BI: BI was determined based on measurement at a bottom of the cut-side of flesh with three points by using a colorimeter (Model CR-400, Konica Minolta, Japan). CIE L*, a* and b* values of the flesh were recorded. The BI was calculated as described by Palou, et al., [39].

$$BI = \frac{100(x-0.31)}{0.172}$$

Where,

$$x = \frac{(a^*+1.75L^*)}{(5.645L^*+a^*-0.3012b^*)}$$

Determination of PPO, POD and Phenylalanine Ammonia Lyase (PAL) activities: A 2 g of samples were homogenized with 10 mL of extraction buffer containing 0.2 g of polyvinylpolypyrrolidone. The mixture was centrifuged at 4°C and 15,000 × g for 20 min, and the supernatant was analyzed the enzyme activities immediately. Cold sodium phosphate buffer (50 mM, pH 7.0) was used for the extraction of PPO. The PPO assay was performed according to Teisson (1979) by adding 0.5 mL of enzyme extract to the reaction with 1.8 mL phosphate buffer (0.05 M, pH 7.0) and 0.05 mL catechol (10 mM) and incubated in a water bath at 30 °C for 30 min. A 0.8 mL aliquot of perchloric acid (2N) was added to stop the enzymatic reaction, and then readings the change of absorbance at 395 nm. The activity of enzyme increased in absorbance per minute under the conditions tested defines one unit of PPO activity.

Cold extraction buffer (sodium phosphate 50 mM, pH 6.0) was used for POD. The POD activity was determined according to Jiang et al., A 0.55 mL enzyme extract was mixed with 1.8 mL sodium phosphate buffer (50 mM, pH 6.0), 0.1 mL hydrogen peroxide (0.5 mM) and 0.5 mL guaiacol (25 mM) [40]. The mixture was incubated at 30 °C for 5 min. POD activity was measured by the absorbance of the mixture at 470 nm. One unit of POD is defined as the amount of enzymes causing an increase in absorbance per minute under the conditions tested. Cold sodium borate buffer (50 mM, pH 8.8) was used for PAL extraction. A 0.5 mL supernatant was added to a mixture containing 2.5 mL sodium borate buffer (50 mM, pH 8.8) and 1 mL L-phenylalanine (1% w/v). The mixture was incubated at 37°C for 60 min, the reaction was stopped by adding 0.5 mL of 5 N HCl. PAL activity was measured by the absorbance of the mixture at 290 nm [41]. One unit of PAL is defined as µmol cinnamic acid per minute. One unit of enzyme activity (Unit) was defined as the absorbance increase of 0.001 units per minute reaction at 395, 470 and 290 nm for PPO, POD and PAL, respectively, under the conditions tested. All enzyme activities were expressed as units per mg protein (U/mg protein). The protein content was determined as the method of Bradford et al.; by using bovine serum albumin as a standard [42].

TP contents and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity: TP contents was determined according to [43]. A 2.0 g samples were homogenized with 10 ml methanol (80%) and then centrifuged at 15,000 × g for 20 min at 4°C. The supernatant was used to determine TP contents and antioxidant capacity. The reaction was started when 0.2 mL of extract solution was mixed with 0.2 mL of 50% Folin–Ciocalteu reagent and 2 mL of 7% sodium carbonate solution. The mixture was left for 90 min in darkness at room temperature. Absorbance was read at 750 nm by a spectrophotometer (UV 1800, Shimadzu). TP contents was expressed as mg gallic acid equivalent per kg fresh weight (mg GAE/kg FW).

Antioxidant capacity was determined following the method described by Cuvelier et al., using DPPH [44]. A 0.1 mL of the extract was mixed with 2.9 mL of 1 mM DPPH in methanol, and the absorbance at 517 nm was then immediately recorded (A0). The mixture was then incubated in the dark room for 30 min, and the absorbance at 517 nm was again recorded (A2). The percentage of DPPH was calculated using the following equation:

DPPH scavenging

$$(\%) = \frac{A_0 - A_2}{A_0} \times 100$$

Quality attributes: Firmness of slices rose apples was determined by using a texture analyzer (model TA–XT Plus; Stable Micro System Co. Ltd., England) equipped with a 7 cm × 12 cm knife blade at a distance of 25 mm and a speed rate of 5 mm per second. The data was recorded and expressed in Newtons (N).

TSS were measured using a digital refractometer (PAL-1 ATAGO, Japan). Slices of fresh-cut rose apples were minced, squeezed, and then filtered using a cloth sheet. A few drops of the extract were placed on the refractometer prism glass and data was noted from direct reading the result was expressed as a percentage Brix. TA was analyzed by titration of the juice with 0.1 N NaOH (AOAC, 1990). A 2 mL of extract juice was titrated with 0.1 N NaOH using 2–3 drops of 1% (w/v) phenophthalene as indicator. The TA content of the fruit juice was expressed as a percentage of citric acid.

Sensory evaluation: Sensory properties of fresh-cut rose apples were evaluated during days 6 and 10 of storage at 4°C by semi-trained panelists. Seven panelists aged between 28 and 32 years old from faculty students performed the evaluation independently with sensory parameters (aroma, appearance, taste, and overall acceptability) based on a 9-point hedonic scale (1-definitely dislike; 9-definitely like) [45]. Scores higher or equal to 5 were considered acceptable in supporting microbiological shelf-life and retaining quality parameters.

Microbiological analysis: A 25 g sample of fresh-cut rose apples from each treatment was put in a stomacher bag with 225 mL of 1% sterile peptone water, and pummeled in a stomacher (IUL Instruments Masticator; Barcelona, Spain) for 1 min. 10X serial dilutions with sterile peptone water were made in sterile peptone water as required for plating, then 0.1 mL was spread on Plate Count Agar (PCA) for TPCs, Potato Dextrose Agar (PDA, HiMedia, India) for yeast and mould counts, and Eosin Methylene Blue agar media (EMB, HiMedia, India) for *E.coli* counts., PDA plates were incubated at 26 ± 2°C for 5 days, while PCA and EMB plates were incubated at 37°C for 2 days. The results were expressed as log colony forming units per gram fresh weight (log CFU/g FW).

Statistical analysis

All experiments were performed using a Completely Randomized Experimental Design (CRED) with four replicates and statistical analysis was carried out using Analysis of Variance (ANOVA) using SAS version 9.0 (SAS Institute, Cary, NC, USA). Significant differences between means were compared using the Least Significant Difference (LSD) at $p \leq 0.05$. The data was expressed as the mean of four replications (n=4) ± Standard Error (S.E).

RESULTS AND DISCUSSION

BI and visual appearance

BI of fresh-cut rose apple gradually increased in all treatments throughout the entire 10 days storage period and there was significantly different among treatments (Figure 1). It is known that browning occurs cause of enzymatic browning reaction and the moisture loss from the cut surface [7,9]. Herein, SAC coating delayed the increase in BI of fresh-cut wax apple fruits during storage. Amongst treatments, those samples coated SAC-HA-0.03% expressed the lowest BI on the cut surface while the contrast results were observed in the HA-0.03% and uncoated sample. After 10 days of storage at 4°C, the BI of SAC-HA-0.03% treatment had the lowest BI (31.97), followed by SAC (39.7) and HA-0.03% (45.75), while the control one had the highest BI value (47.6), indicating that SAC incorporated with HA effectively prevented the development of BI and delay flesh browning of fresh-cut rose apple compared to the other treatments and control one. A previous study reported that the addition of EOs (cinnamon or rosemary) and antioxidants was more effective than alginate alone in reducing browning, preserving the original color and lightness, and extending the shelf-life of fresh-cut apple by suppressing the activity of enzymes PPO and POD [46]. Nonetheless, another study by Raybaudi-Massilia et al., suggested that browning of cut apple surface was notably affected by the incorporation of EOs (cinnamon, lemongrass) into the alginate coating due to phenolic compounds from EOs might be substrate themselves for PPO activity and an increase in the permeability of plant cell membrane cause of volatile compounds might cause a higher leakage of PPO and polyphenols from the cell cytoplasm [26,47]. Interestingly in this study, fresh-cut rose apples coated with SAC-HA-0.03% showed lower BI than those of SAC and HA-0.03% alone coatings and uncoated samples. These results are consistent with our previous works. We reported that SAC-HA-0.03% formulation coating had a lower oxygen permeability than SAC alone due to the addition of HA as a plasticizer agent improving the gas barrier properties of SAC. Moreover, homogenization of thickness coating under scanning electron microscope was found in SAC-HA-0.03% which could help prevent the oxygen supply for enzymatic browning (PPO and POD) reaction better than SAC alone. These later changes could explain the observation of lower PPO and POD in the sample coated with SAC-HA-0.03%.

The visual appearance changes of fresh-cut rose apples are presented in Figure 2. It was found that slight browning at the bottom of the cut fruit surface was observed on the control and HA-0.03% samples on the first 6 days of storage period, however, it was not found on the SAC and SAC-HA-0.03% samples. Dark brown, water soaking, and wilting on the top of the cut surface were found on the control and HA-0.03% samples at the end of the storage period. SAC incorporated with 0.03% HA was effective in not only inhibiting the browning on the cut surface but also preserving the freshness and glossy of the peel of fresh-cut rose apples.

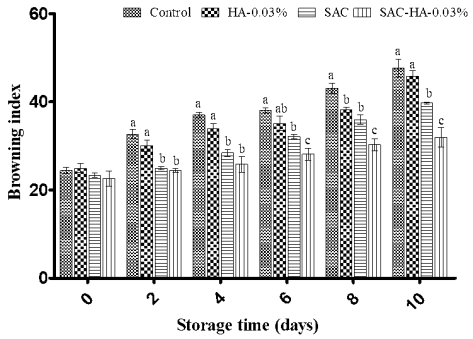

Figure 1: Browning index of fresh-cut rose apple coated with Sodium Alginate Crosslinking with or without Hexyl Acetate (SAC-HA-0.03%, SAC), Hexyl Acetate (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C for 10 days. Mean (n=4). Bars with the same letters are not significantly different between treatments using Lysergic Acid Diethylamide (LSD).

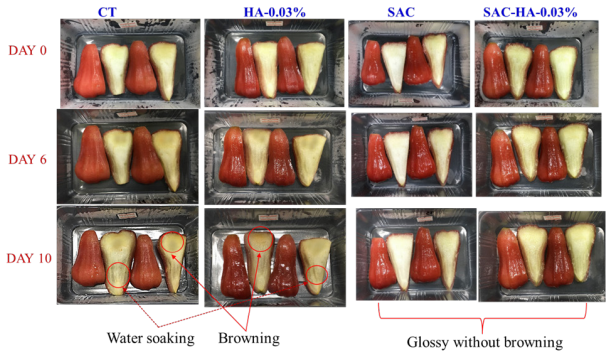

Figure 2: Visual appearance of fresh-cut rose apple coated with SAC with or without HA (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) during storage at 4 ± 1 °C for 10 days.

PPO, POD and PAL activities

The activity of enzymes associated with the browning of the fresh-cut rose apple such as PPO, POD and PAL enzymes were significantly affected by edible coating formulations during cold storage (Figure 3). As shown in Figure 3A, the activity of the PPO enzyme in fresh-cut rose apple was increased during storage in all treated and control samples. However, the activity of PPO in the control and HA-0.03% samples were 1.61 and 1.31 folds higher than those coated with SAC and SAC-HA-0.03% after 10 days of storage. The PPO activity was inhibited by alginate crosslinking coating (SAC) or alginate crosslinking combined with HA (SAC-HA-0.03%). The increased POD activity was observed in all treatments during 6 days of storage, except on day 2 (Figure 3B). However, the control samples increased and reached maximum values on day 6, showing POD activity higher than those at day 0, approximately 79%, followed by a slow decrease, while samples coated with SAC and SAC-HA-0.03% caused a significant decrease reduced activities of POD at beginning of storage, it slightly increased at the middle stage of storage and then decreased until the end of storage period. From the Figure 3C, shows that, the PAL activity of the control and HA-0.03% samples decreased throughout the entire storage periods, except on day 2, while samples coated with formulation SAC-HA-0.03% sharply increased level of PAL activity until peaking on days 6 of storage and decreasing gradually afterward, however, it had no significant differences with SAC coated samples.

It is well known, minimal processing (peeling, cutting and slicing) tends to destroy tissues and cells. Loss of membrane integrity is condition for phenolic compounds are exposed to oxygen and

oxidized by the catalytic activity of PPO and/or POD leading to browning reactions [48]. In general, browning reactions are assumed as a consequence of PPO and POD acceleration of the monophenols hydroxylation and the oxidation of o-diphenols into o-quinones which polymerize and produce color changes in fresh-cut fruit products [49]. It also found that the activity of POD enzyme is one of the reasons that affect undesirable quality such as discoloration, off flavor, and nutritional destruction through storage of fruit and vegetables [50]. This enzymatic browning can be reduced at low levels of oxygen [51]. In this study, increase of PPO and POD activity was observed in all treatments of fresh-cut rose apple during storage due to the oxidization of phenolic compounds occurring during this process led to lower amounts of polyphenol compounds, especially in uncoated samples and HA-0.03% samples (Figure 4A). However, lower PPO and POD activities of samples coated with SAC and SAC-HA-0.03% may be attribute to higher phenolic compounds by accumulating PAL enzyme. PAL is the first key enzyme involved in the biosynthesis of phenols in fruits and vegetables [52]. It found that increase of the activity of PAL enzyme correlated with increase of phenolic compounds when application of edible coating [53]. In addition, it is known that edible coatings act as a gas barrier on the surface of fresh products, reduce oxygen supply, delay ripening and senescence, as well as inhibit browning of fresh commodities by restricting activities of enzymes PPO and POD, for example carrageenan coating on fresh-cut banana and different edible coatings on fresh-cut "Royal Gala" apple [54,55]. Alginate is a polysaccharide edible coating with good barrier gas, alginate coating forms a protective barrier on the surface of samples, reducing the supply of O_2 which could help reduce PPO and POD activity [56]. Moreover, our previous work found that the incorporation of HA (0.03%) with SAC reduced oxygen permeability, thus samples coated with SAC-HA-0.03% inhibited PPO and POD activities and maintained the phenolic compound higher than SAC alone. Finding similar results to those reported by Chiabrando et al., who reported that application of alginate enriched with cinnamon or rosemary EOs coatings on fresh-cut apple was an effect in reducing PPO and POD activities, resulting in retarding browning on the cut surface [57]. In addition, using alginate coating enriched with thyme oil could reduce the browning of fresh pistachios during storage by suppressing PPO activity [58].

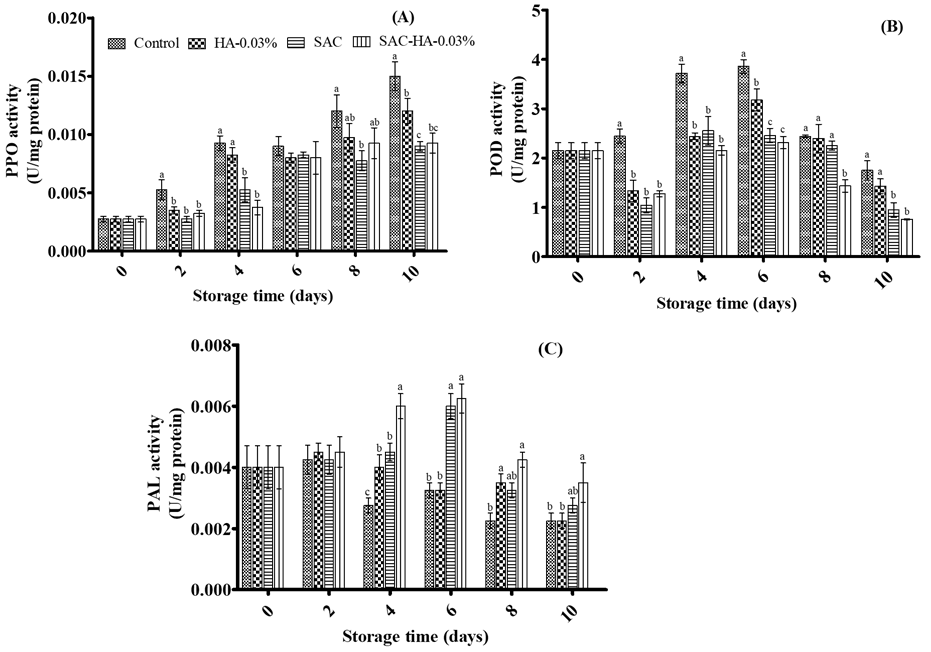

Figure 3: PPO (A), POD (B) and PAL (C) activities of fresh-cut rose apple coated with Sodium Alginate Crosslinking with or without Hexyl Acetate (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C for 10 days. Mean (n=4). Bars with the same letters are not significantly different between treatments using LSD.

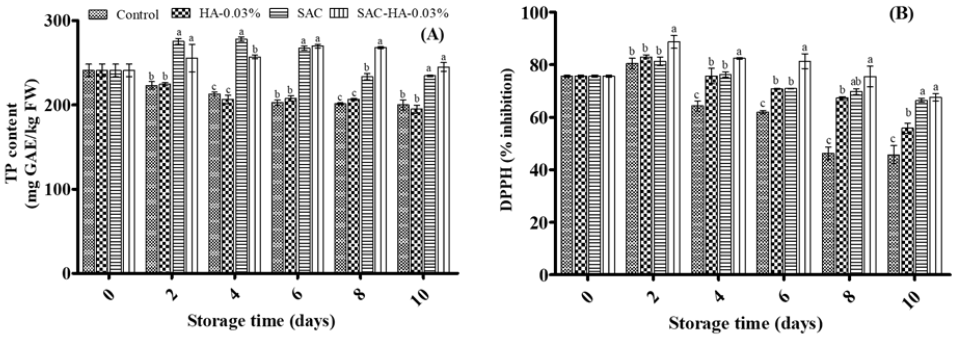

Figure 4: Total phenolic content. **Note:** A) DPPH; B) of fresh-cut rose apple coated with SAC with or without HA (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C for 10 days. Mean (n=4). Bars with the same letters are not significantly different between treatments using LSD.

TP contents, DPPH radical scavenging activity

Effects of different formulation edible coatings on changes in TP contents and DPPH• radical scavenging activity of fresh-cut rose apple are presented in Figure 4. TP contents showed significant differences among treatments (Figure 4A). TP contents of control and HA-0.03% samples exhibited the decline of TP contents throughout the storage, while the increased TP contents in SAC and SAC-HA-0.03% samples were observed during the first 4 days of storage and maintained a relatively stable of TP contents until 10 days. Among different treatments, fruits coated with formulation SAC-HA-0.03% showed the highest TP contents (244.94 mg/kg FW) after 10 days of storage. However, fruits treated with HA-0.03% showed the lowest TP contents (194.97 mg/kg FW). According to Figure 4B, the DPPH• radical scavenging activity of the fresh-cut rose apple at the beginning of storage was 75.66%. The raised in DPPH radical scavenging activity of all treated and the control samples was observed during the first 2 days of storage and then decreased throughout the storage period. However, the control and HA-0.03% samples dramatically declined in DPPH level and reached the lowest level (45.69% and 55.83%, respectively) after 10 days of storage, while SAC and SAC-HA-0.03% samples preserved a relatively steady DPPH level (66.44%, 67.63%, respectively) and higher than the control samples in all storage period.

Based on the results, sodium alginate crosslinking coating in formulation SAC and SAC-HA-0.03% increased and maintained higher TP contents and DPPH radical scavenging activity than the control and HA-0.03% samples over the entire storage time. Suggesting that the loss of TP in the control and HA-0.03% samples was caused by oxidizing the phenolic compounds to the production of o-quinones. In contrast, the increase in TP contents and DPPH radical scavenging activity was observed in SAC and SAC-HA-0.03% samples due to stimulation of PAL activity. Previous studies also found that fresh pistachio coated with 1% alginate+0.3% thyme resulted in higher TP contents, antioxidant capacity, and PAL activity compared to uncoated samples [59]. In this study, the results revealed the increment of DPPH radical scavenging activity of fresh-cut rose apple coated with SAC-HA-0.03%. Therefore, the increase in antioxidant capacity is correlated to the increase in the TP contents and stimulates and enhances the activity of PAL enzyme. It could be explained due to volatile compounds play positive roles in secondary plant metabolites and stimulate the biosynthesis of phenolic compounds by inducing an increase in the activity of PAL enzyme as previously reported [60]. These effects could be the result of the oxygen barrier properties of edible coating and the capacity of essential oil components to retain fruit quality attributes along with the inhibition of PPO and POD activities and increasing activity of PAL which could retard loss of antioxidant compounds [61]. The results obtained are in agreement with previous reports, which indicated that alginate-enriched essential oil coating enhanced phenolic compounds in a range of fresh-cut products such as kiwi, fresh-cut melon [57,62]. Another study found that the phenolic compounds increased with the increasing antioxidant capacity of fresh-cut melon during 14 days of storage at 4°C.

Quality attributes

Different formulation coatings significantly affected the changes in quality attributes such as firmness, TSS and TA of fresh-cut rose apples during cold storage (Figure 5). Initially (day 0), the firmness of fresh-cut rose apples in all treatments was in the range of 36.96-39.24 N, it declined to 26.12 N and 27.02 N in the control and HA-0.03% samples after 10 days of storage, whereas samples coated with SAC and SAC enriched HA (SAC-HA-0.03%) preserved the firmness at 32.03 N and 34.83 N, respectively (Figure 5A). Reducing the firmness is one of the main factors in reducing the freshness and shelf life of fresh-cut products during storage and handling. Brummell et al., confirmed that loss of firmness is correlated with disassembly of the primary cell wall, with solubilization and depolymerization of pectin [63]. The alginate-based edible coating that could maintain firmness has been reported in fresh-cut apples [48]. The reasons for alginate coatings preserving the firmness of apple slices could be explained by the thin layer coating on the cut surface working as a water barrier, preventing loss of turgor in fresh-cut products [48,62]. In addition, alginate coating combined with EOs for delaying loss of firmness in fresh-cut fruits such as alginate enriched eugenol and citral oil or citrus oil on fresh-cut kiwi fruit, alginate incorporated with lemongrass coated on fresh-cut pineapple, fresh-cut Fuji apple [26,27,57,63]. The EOs present in coatings may act over the cell tissue of the fruit, which possibly undergo structural changes that directly affect fruit firmness [28]. In the current work, the addition of HA to SAC (SAC-HA-0.03%) was significantly effective in maintaining firmness higher than SAC and HA-0.03% alone. It could be explained by the hypothesis and previously reported mentioned above.

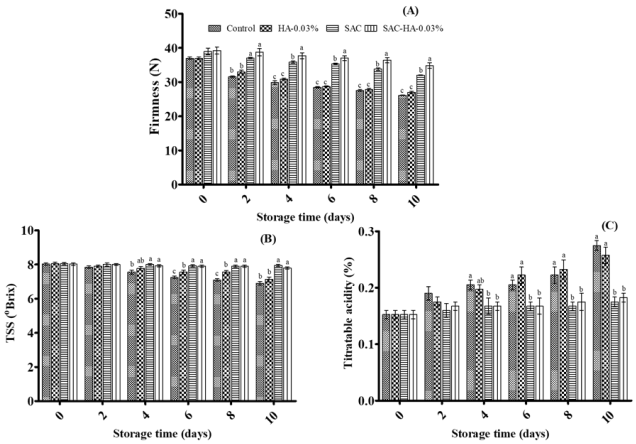

Figure 5: A) Firmness; B) TSS; C) TA of fresh-cut rose apple coated with SAC with or without HA (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C for 10 days. Mean (n=4). Bars with the same letters are not significantly different between treatments using LSD.

At the beginning of the storage period, TSS in all samples was 8.05, it gradually declined during storage time in the control and HA-0.03% samples. After 10 day's storage, TSS value in control samples decreased at a lower level (1.61-fold) than on day 0, while SAC and SAC-HA-0.03% coating seemed stable during the storage (Figure 5B). During storage, the increasing of TA content in fresh-cut rose apples was found in the control and HA-0.03% samples, showing value was 1.8-fold and 1.6-fold higher than on day 0, respectively, at the end of storage period. In contrast, samples coated with SAC and SAC-HA-0.03% slightly increased during the first 6 days and maintained a stable of TA content until 10 days (Figure 5C). This trend may be due to lower respiration rates in samples coated with SAC and SAC combined with HA oil. Previous studies proved that edible coatings are capable modified atmosphere on coated surface by isolating the coated product from the environment, acting as a barrier to oxygen, carbon dioxide, and water vapor, and then decreasing respiration rate [12]. Other researchers have reported alginate alone or enriched essential oil reduced respiration, resulting in retarded loss of TSS and TA in fresh-cut apples [26,48]. Moreover, as in our previous study, when adding HA into SAC edible coatings for fresh-cut rose apples, samples coated with SAC-HA-0.03% lower respiration than in the other treatments, although all formulations of edible coatings were better than in the uncoated. This study confirmed that SAC and SAC-HA-0.03% formulation coatings were effective in reducing loss of TA and TSS by reducing respiration rate as well as reduction of other vital processes. These results indicate that the SAC coatings enriched HA maintained the eating quality of the fresh-cut rose apple by delaying loss of firmness and preventing loss of TSS and TA. A similar effect was found in alginate enriched EOs coated on fresh-cut apple, fresh-cut kiwifruit [26,46,57]. The effect of the SAC-HA-0.03% coatings showed the greatest positive effects on preventing the losses in firmness and TSS and preserving TA. It indicates that the combination of SAC coatings and HA was effective for reducing browning and maintaining the storage quality of fresh-cut rose apples when compared to the SAC and HA alone.

Sensory evaluation

The sensory evaluation (aroma, color, taste, and overall acceptability) based on hedonic scale (1-9) of fresh-cut rose apple coated with or without HA during days 6 and 10 of storage are displayed in Figure 6A. Results show that during 6 days of storage, both of samples coated and uncoated were accepted in range 5.76 -7.47 score. However, the samples coated with SAC and SAC-HA-0.03% had higher acceptance regarding color attributes and taste ($p<0.05$). The analysis of aroma was significant difference higher in SAC-HA-0.03% when compared to the control (uncoated) and SAC alone even samples treated with hexyl HA was a good sensory appreciation for SAC-HA-0.03% and SAC coated samples with 6.87 and 7.00 score, respectively, while control and HA-0.03% treated samples were ranked negatively (approximately 4.8-4.9) accompany with aroma and taste evaluated lower than all coated samples (Figure 6B). Based on the taste panelists, fresh-cut rose apple scored higher in overall acceptability in SAC-HA-0.03% coated samples with glossy appearance attributes. The sample coated with SAC alone was less appreciated in aroma, and taste, although it maintained a good appearance and totally unaccepted with the worst appearance (water soaking) in the control and HA-0.03% treated samples (around 4 score, which corresponded with the limit of edibility) after 10 day's storage. Interestingly, the aroma was positively affected by the coatings enriched HA, with the formulation coating SAC-HA-0.03% maintaining the aroma better appreciated than the SAC and HA-0.03% alone. Physical injury from fresh-cut processing including peeling and cutting increased respiration and ethylene production caused by biochemical changes, microbial growth, and decreasing the organoleptic parameters leading to deterioration [64,65]. The alginate edible coating creates a thin layer on the cut surface, it acts as a barrier which could help slow down respiration rates, prevent oxidation, delay biochemical changes, and maintain sensory parameters of fresh-cut fruits products [26,48]. In addition, the barrier effect also increased in alginate-enriched EOs [25,66]. In this study, SAC enriched HA (SAC-HA-0.03%) was effective in delaying a degradation of fresh-cut rose apple appearance and preserving the taste of samples by reducing the loss of quality attributes (Figure 6). Also, it could inhibit the flesh browning of fresh-cut rose apple by suppressing the activity of PPO and POD enzymes and reducing oxidation of phenolic compounds (Figure 2). In addition, SAC containing HA coatings could lower the population of microbial, such as TPC, yeast and mould and *E. coli*. (Figure 5). These results obtained in this study was also found in previous studies, which reported the highest sensory evaluation score in fresh-cut fruits that were coated with an alginate enriched essential oil based edible coating such as alginate enriched with lemongrass on fresh pear fruit Gago et al., with thyme oil on fresh pistachio with orange essential oil on fresh-cut kiwi fruit with eugenol oil on Arbutus fresh fruit with thyme oil on fresh-cut cantaloupe with lemongrass on fresh-cut pineapple [67]. Moreover, SAC coating enriched HA certainly improves the aroma of fresh-cut rose apples during 10 days of storage at 4°C. HA has a fruity odor and is found in fruit ripening such as apples, bananas, guava and rose apple [68-71]. Therefore, SAC-HA-0.0.3% maintained the natural aroma of fresh-cut rose apple throughout the storage period. This result is in contrast with the findings of Sarengaowa et al., [30]. They reported that adding thyme oil into alginate impacted sensory attributes such as the taste and odor of fresh-cut apples.

Microbiological analysis

Overall, microbial population increased in all treatments throughout the storage period, however, incorporating HA in so SAC as antimicrobial edible coatings significantly reduced TPCs, moulds and yeast and *E. coli* in fresh-cut rose apple during storage at 4 °C (Figure 7). As shown in Figure 7A, TPCs in the control samples showed fast growth with peaking (4.85 log CFU/g FW), followed by HA-0.03% (4.69 log CFU/g FW) and SAC (4.50 log CFU/g FW) samples. However, samples treated with SAC-HA-0.03% inhibited growth of TPCs, showing value 1.57-fold lower than control samples after 10 days storage.

At the beginning (day 0), mould and yeast in all treatments were in the range 1.47-1.62 log CFU/g FW. It sharply increased in control samples from days 4 and reached highest value on day 10 (3.82 CFU/g FW) followed by HA-0.03% (3.71 CFU/g FW). Samples coated with SAC reduced the mould and yeast growth during 6 days, however, they fast growth from day 8 until the end of storage. The inhibition level of mould and yeast during stages of storage was found in sample coated with SAC-HA-0.03%. It showed approximately 42% lower mould and yeast in compared with control on the last day of storage (day 10). As expected, SAC coating enriched HA significantly affected the spoilage of fresh-cut rose apples (Figure 7B).

E. coli counts in control samples gradually increased after 4 days of storage and reached peaking on day 10 (4.8 CFU/g FW) followed

by SAC coatings (4.60 CFU/g FW). SAC coatings enriched HA the *E. coli* population was inactivated immediately after coating and during first 4 days, it slightly increased on day 6 and remained stable level until the end of storage time, whereas fresh-cut apples treated with HA-0.03% presented 4.55 CFU/g FW of *E. coli* after 10 days. Formulation of SAC-HA-0.03% coatings exhibited a faster and enhanced inactivation of *E. coli* on fresh-cut rose apples during storage time in comparison with SAC and HA-0.03% alone (Figure 7C).

It is well known that fresh-cut fruit have big area of cutting surface with high moisture conditions and a rich source of nutrients, which supplies a good environment for growth of microorganisms [12]. Antimicrobial alginate edible coating by adding EOs could improve antimicrobial action and protect fresh fruit products from microbial spoilage [72,73]. In present work, the application of alginate coating enriched HA yeast and mould and *E. coli*. According to Bierhals et al., the limiting criteria for the consumption of ready-to-eat fruit products are 6 Log CFU/g [74]. TPCs, yeasts and mould and *E. coli* found in this study did not reach that limit in all the treatments. However, SAC-enriched HA (SAC-HA-0.03%) had the greatest inhibitory effect on microbial growth during cold storage may be attributed to presence of HA. It is acknowledged that antimicrobial activity of volatile esters is low, however they exhibit strong activity when they are adsorbed to the superficial layer of cells and hydrolyzed by cellular esterase to highly antimicrobial acid and alcohol [75]. Characteristic of alcohol is amphipathic, and the hydrophobic region is dissolved in the cell membrane and increases membrane fluidity, destroying the cell membrane [34,35]. The gradual release of antimicrobial HA from alginate coatings to the fresh-cut rose apple surface could have an advantage over the direct application of single HA in food systems, similar to previous report [76]. It also proved that HA vapor at 150 ppm combined with modified atmosphere packaging could inhibit pathogenic bacteria such as *L. monocytogenes*, *E. coli* and *S. enteritidis* and prolong the shelf life of sliced apples Lanciotti et al., thus explaining the behavior observed. In addition, the highest TP contents (244.94 mg/kg FW) was found in SAC-HA-0.03% treatment (Figure 4A) [32]. According to Dorman et al., the antimicrobial activity of plant extracts may be due to the presence of phenolic compounds [77]. Meaning that, the hydroxyl (–OH) groups in phenolic compounds can interact with the cell membrane of bacteria to disrupt membrane structures and cause the leakage of cellular components [78]. Therefore, the incorporation of HA and SAC (SAC-HA-0.03%) could inhibit the growth of TPCs, yeasts and moulds, and *E. coli* on fresh-cut rose apples better than HA alone. However, there are no previously published about the application of volatile HA incorporated with alginate edible coatings, there is proof that would assist this hypothesis. In addition, Rojas-Graü et al., found that the alginate edible coating cannot reduce microbial growth effectively when used alone, but it creates a barrier and protects cut surface fruit from microbial contamination [26,27]. Several publications on EOs incorporated in alginate coatings strongly demonstrated an inhibitory effect against microbial growth and extended shelf life of fresh fruit products. SA in combination with lemongrass EOs reduced the growth of moulds, yeast, and bacteria in the apple fruit slice and fresh-cut pineapple alginate coating enriched eugenol and citral oil also reduced microbial spoilage on Arbutus fruit [79-83].

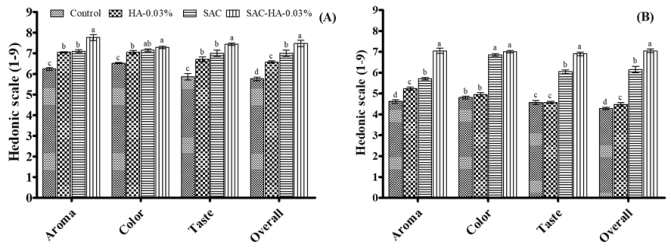

Figure 6: A) Sensory evaluation on day 6; B) day 10 of fresh-cut rose apple coated with SAC with or without HA (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C. Mean (n=4). Bars with the same letters are not significantly different between treatments using LSD.

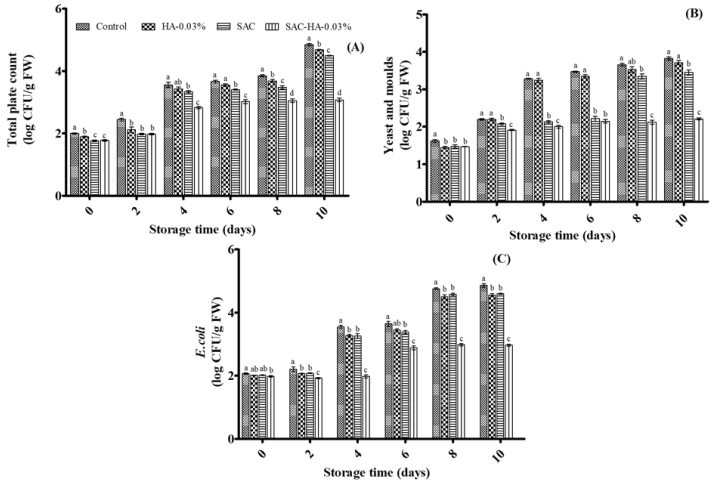

Figure 7: A) Total plate count; B) Yeast and mould; C) *E. coli* population of fresh-cut rose apple coated with SAC with or without HA (SAC-HA-0.03%, SAC), HA (HA-0.03%) solution and the control (uncoated) stored at 4 ± 1°C for 10 days. Mean (n=4). Bars with the same letters are not significantly different between treatments using LSD.

CONCLUSION

Although, SAC coating was effective in reducing browning, however, SAC alone is not enough functional to extend the shelf life of fresh-cut products. EOs can be incorporated into edible coatings to solve this problem, therefore, enhancing the safety of coated fresh-cut products and meeting benefits to the health of consumers. The results of this present study demonstrated that the formulation of SAC-HA-0.03% coating could maintain freshness appearance and reduce the development of flesh browning by suppressing the PPO and POD enzymes activity, stimulating PAL activity, enhancing antioxidant and maintaining high TP contents. Also, it could delay losses in firmness, TSS and TA, especially, it could improve the sensory properties of fresh-cut rose apples with the highest overall score. Moreover, the highest inhibitory effect on the reduction of microorganisms (TPCs, yeast and moulds, and *E. coli*) was also obtained in the sample coated with SAC-HA-0.03% which was selected as the best formulation for prolonging the storage life of fresh-cut rose apple up to 10 days at 4°C. It could suggest that SAC-based coatings incorporated with HA are considered one of the approaches with the greatest interest for the application of fresh-cut products.

ACKNOWLEDGMENTS

The first author would like to thank Petch Pra Jom Klao Ph.D.'s Degree Research Scholarship from King Mongkut's University of Technology Thonburi, Bangkok, Thailand for supporting this study (agreement No. 43/2561). The authors also thank the United Graduate School of Agricultural Science (UGSAS), and Gifu University, Japan for providing the facility to conduct our research.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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