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Fabrication of *Cleistocalyx operculatus* extracts/chitosan/gum arabic composite as an edible coating for preservation of banana

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ABSTRACT

The phenolic compounds from natural plant extracts, which have strong antioxidant properties as inhibitors for browning retardants of fruits, have been extensively investigate in the last few decades. In this study, the polyphenols in *Cleistocalyx operculatus* (Roxb.), a distinct plant in tropical region, was successfully extracted and determined. The obtained Roxb. extract (CE) as an antioxidant additive was employed in Chitosan/Gum Arabic (CH/GA) edible coating for the fruit preservation. The resultant CE/CH/GA coating revealed the high effectiveness in improving the freshness of banana at ambient storage conditions. The properties of bananas before and after coated by CE/CH/GA were investigated and assessed. The surface structure of banana was examined by using scanning electron microscopy, showed wrinkle and crack structure for uncoated banana and smooth surface for banana coated with CE/CH/GA coating. The freshness of banana treated with CH/GA/CE edible coating could last for 21 days of storage at room conditions. Thus, it is promising for good potential practical application to preserve fruits.

1. Introduction

It has been well-known that banana is one of the most widely growth and consumed fruit in the world, especially in the tropical/subtropical regions [1]. Bananas contain all the essential nutrients with valuable nutrition and calories such as vitamins and minerals [2]. However, banana is a climacteric fruit, which is highly biodegradable and facilely infected by diseases leading to shorten the shelf life of fruit [3]. Therefore, the control of postharvest losses of bananas fruit will reduce the cost of cultivation and distribution, as a result, lowering price for consumers and increasing income for the farmers. Thus, prolonging the shelf life of postharvest bananas are received extensive attention [45]. Many techniques have been employed to effectively minimize the postharvest losses including low-temperature storage [42,43] and controlled atmosphere and hypobaric storage [5,6]. However, using these approaches damage physical properties of fruits causing chilling injury, being expensive to operate. Thus, it is necessary to find alternative cost-effective methods to extent the shelf life of postharvest bananas [7]. One of the cost-effective methods for improving the bananas' shelf-life is to apply the safe coatings on the surface of banana [8]. The mechanism of protecting perishable fruits from deterioration by edible coatings is to suppress respiration, enhance textural quality, maintain volatile of the flavour, minimize the microbial growth and retard dehydration [9]. Many edible coating systems have been developed to remain the quality as well as prolong the freshness of fruits including, but not limited to, lipid-based coatings (oils, waxes, fatty acid and

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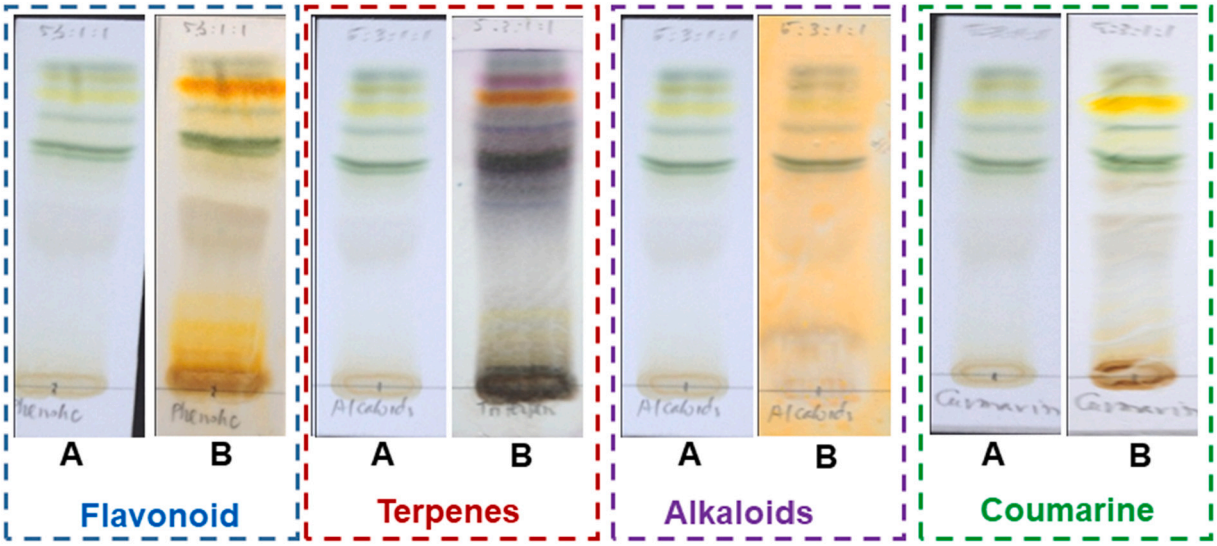

Fig. 1. Phytochemical screening of CE by TLC. The sample was run on TLC using a suitable solvent system (TEAF - toluene:ethyl acetate:acetone:formic acid 5:3:1:1) (A) and then stained with a specific reagent to detect the presence of the compounds of interest (B).

monoglycerides and resins), protein-based coatings (casein, whey protein, wheat gluten, corn zein, collagen and gelatin, and surimi), carbonhydrate-based coatings (cellulose, pectin, sucrose ester, chitin/CH, starch, *aloe vera*, alginate and carrageenan) [10–12]. The edible coatings could be formulated with singe or combination of two or three coating materials along with addition of antioxidant and antimicrobial additives [13–15, 44].

Gum Arabic, a complex polysaccharide, commonly consists of valuable mineral for human health such as magnesium, calcium, and potassium, which is extracted from part of Acacia plants [16]. The gum arabic (GA) is a safe compound and commonly employed as a food additive in many industrial sectors as well as is utilized as an emulsifier thank to the better solubility in comparison to other hydrocolloids [17,18]. Chitosan (CH) is one of polysaccharides obtained from the shells of crabs, shrimps with chemical structure similar to the cellulose. With good inhibiting properties of reducing dehydration and respiration, the CH could be extensively used as a coating to maintain the food quality by preventing the deterioration of many types of foods [19]. Additionally, the CH has remarkable biochemical and film forming properties, which is commonly employed as an additive for food industry as well as in the coating composition to prolong the shelf life of fruits and vegetables. It has been reported in the literature that, chitosan-based coatings has been extensively studied to prevent the deterioration of postharvest fruits and vegetables including, but not limited to banana, mango, avocado, strawberry, papaya, and tomato [20–22]. Maqbool, Ali, Alderson, Zahid and Siddiqui [1] successfully fabricated the GA and CH edible coating and studied the biochemical and physiological properties of banana fruits after covered with the resultant coating. The banana protected by GA/CH composite coating lasted for 28 days at at 13 °C and 80% relative humidity, and 5 days at 25 °C, 60% RH. The results showed that the composite with 10% GA and 1.0% CH could prolong the storage life of bananas up to 33 days at the temperature of around 5 °C.

Recently, many works have been devoted to find alternative phenolic compounds from natural plant extracts, which have strong antioxidant properties as inhibitors for browning retardants of fruits and food [23,24]. These extracted with high phenolic contents play a significant role as inhibitors for enzyme polyphenol oxidase [25]. *Cleistocalyx operculatus* (roxb.) is widely distributed tropical countries (India, Vietnam, China) which has been well-known as a good source of polyphenolic compounds with strong antioxidant properties [26]. In these countries, *C. operculatus* has been commonly used as drinking tea in daily life. Unlike green tea extracts, roxb. extracts (CE) haven't yet been studied for such applications because of local characteristics, especially for enzymatic browning inhibiting application in food and fruits.

Herein, we report the fabrication of the edible coatings containing chitosan, gum arabic, and CE extract for postharvest banana fruits. The effect of CE contents on the physiological and biochemical properties of banana is studied in detail. The protective efficiency of the prepared CH/GA/CE composite coating is compared with control and the optimized CH/GA composite coating.

2. Experimental section

2.1. Materials collection and extraction

Leaves of *C. operculatus* were collected in Ha Tay province, Vietnam and identified. The CE leaves was dried and ground in powder form before extracted with water for 2 days. The extraction was repeated twice and dried using rotary vacuum.

2.2. Phytochemical screening of CE

A solvent mixture of formic acid, acetone, ethyl acetate, and toluene with volume ratio of 5/3/1/1, respectively was used to separate the extract using thin-layer chromatography (TLC) plates. The specific reagents (polyethylene glycol, anisaldehyde–sulfuric acid, Dragendorff's reagent, 5% KOH, and vanillin-sulfuric acid) were then sprayed on the TLC plates to determine the organic compounds such as flavonoids, alkaloids, terpenes/steroids, and others in the CE extract [27].

2.3. Total polyphenol and flavonoid determination of CE

The reagent of Folin Ciocalteu reported by Singleton and Rossi was utilized to determine the total phenolic contents in the ethanolic CE extracts [28]. Gallic acid was used as standard. Typically, 5 ml of Folin Ciocalteu reagent was mixed with 4 ml sodium carbonate solution (75 g/l) with addition of 1 ml CE extract solution, the mixed solution was kept stirring for 30 min. The mixed solution was then measured the absorbance at the wavelength of 765 nm using garlic acid as standard to determine the total phenolic content. The polyphenol content was calculated as mg phenolic/g of gallic acid.

The spectrophotometry was employed to determine the total flavonoid content in the extract [29]. The quercetin solution was used as

standard. Typically, 1 ml CE extracts was dissolved in 1 ml aluminum chloride methanolic solution 2%, followed by incubating for 15 min at room temperature. The mixed solution was then measured the absorbance at the wavelength of 430 nm using Milton Roy 601 UV–Vis spectrophotometer. The flavonoid content was calculated by mg flavonoid/g of quercetin.

2.4. Phytochemical screening and flavonoid content of CE

The phytochemical screening on the TLC plates revealed the presence of terpenes and flavonoids in the extract with the flavonoids content of up to 6.8 mg/g dried CE leaves. The antibacterial properties of the polar compounds in the CE extracts against *S. aureus* and *S. mutans* pathogenic bacteria was observed by bioautography, which is indicated by the low R_f value in TLC plates (Fig. 1).

2.5. Antioxidant activity of CE

The capability of removing the free 1, 1- diphenyl-2-picryl hydrazyl (DPPH) radicals was employed to determine the antioxidant property of the extract. When the extract was added to the DPPH solution, the radicals was scavenged and the color of the DPPH solution was changed This from purple to yellow. The color changing degree determines the strength of the antioxidant property of the extract. In the typical experiment, 1 ml DPPH 0.1 mM solution in ethanol was added to 3 ml of the extracts with various concentration ranging from 5 to 30 μg/ml. The mixture was vigorously shaken and measured the absorbance at the wavelength of 517 nm after 30 min of stand at room temperature. The experiment was repeated for three times with ascorbic acid as standard. The log dose inhibition curve was utilized to determine the IC_{50} value of the mixture. The following equation: DPPH scavenging effect (%) = $(A_0 - A_0)/A_0 \times 100$ (where, A_0 was the absorption intensity of initial DPPH solution and A_1 was the absorption intensity of the DPPH solution in presence of the extract), was used to determine the DPPH radicals scavenging capability of the extract [30].

2.6. Fabrication of CH/GA/CE edible coatings

The optimized CH and GA contents of 1% and 10% *w*/w, respectively, were adopted from previous study [1]. In this work, edible coatings containing CH 1% and GA 10% were prepared upon addition of various CE contents. Typically, the solution A was prepared by dissolving 20 g gum arabic in 100 ml with vigorous stirring for 2 h at 40 °C. In order to prepare solution B, 2 g chitosan was dissolved in 100 ml water with addition of 0.5 ml acetic acid for 2 h at room temperature. The diluted NaOH or H_2SO_4 solution was utilized to adjust the pH solution to around 5.5. The solution A was then mixed with solution B with addition of various CE extract concentrations to obtain the edible coatings for the preservation of the bananas.

2.7. Covering bananas with edible coatings

The 0.01% NaClO solution was employed to clean the post-harvest bananas for 5 min. The treated bananas were immersed in the various prepared edible coatings solution of 1%CH + 10%GA, 1%CH + 10%GA with addition of various CE extract concentration ranging from 0.05% to 0.5% CE, until form the uniform film on the surface of bananas. The bananas with coating were used as controlled samples. 90 bananas were divided into 6 groups (15 bananas/group) for different treatments and each experiment was repeated for 3 cycles. The edible coating-treated bananas were natural dried at 25 °C and 70% for 21 days. After interval of 4 days, the one banana of each treatment was taken out to determine the physicochemical properties of the bananas.

Table 1
Polyphenolic and flavonoid contents of the extract.

Plant extract	Content (%)
Polyphenol	12.4 ± 1.48
Flavonoid	0.62 ± 0.15

2.8. Colors

7-Stage point ladder was employed to measure the color of bananas' peel, which determine the ripening degree of the bananas. The ripening of bananas are recognized as following: point 1 when 0–10% of peel is yellow; point 2 is from 10 to 30% yellow; point 3 is 30 to 50% yellow; point 4 is from 50 to 70% yellow; point 5 is from 70 to 90% yellow; point 6 is from 90 to 100% yellow; and point 7 meant the banana is rotten.

2.9. Weight loss

The weight loss of each tested banana was calculated by mass differences of banana weighted on the basic of 1 day during the storage period.

2.10. Banana firmness

The banana firmness was estimated by the force amount (N) applied to leave a hole on the banana's surface. The Instron Universal Testing Machine with a 8 mm-diameter tip connected to a desktop and the compression speed of 20 mm/min. Each banana was measured the firmness for three times to obtain the average firmness data.

2.11. Acidity of bananas

Titratable acidity of bananas was determined using the titration approach. In a typical experiment, certain tested bananas were removed the peel and blended with 40 ml water. The resultant mixture was filtered by centrifugation at 7000 rpm for 15 min followed by filter paper. 03 drops of 0.1% phenolphthalein indicator were added to the 5 ml filtered sample. 0.1 M NaOH solution was then drop-wised to the solution until the color of the solution changed to pink, which indicated the reaction between base and acids in the bananas. The malic acid percentage in the banana pulp is the acidity of bananas.

2.12. Reducing sugar in bananas

Tested bananas were blended with the distilled water using a blender to obtained a homogeneous solution. The obtained solution was centrifugated 7000 rpm for 15 min and filtered through a filter paper. 1 ml of the filtrate solution was introduced to the glass tube with addition of 2 ml dinitrosalicylic acid (DNS) as a standard agent to determine the reducing sugar. The mixed solution was then heated at temperature of 100 °C for 5 min and naturally cooled down to room temperature. The absorbance of the resultant solution was studied at wavelength of 540 nm using a UV-Spectrophotometer.

3. Results and discussion

3.1. Phytochemical screening on the TLC plates of the extract

Phytochemical screening was carried out to investigate the presence of the main group of secondary metabolites in the CE. Our data indicated the presence of the flavonoid, triterpenes and coumarin groups in this extract, while alkaloid was not found.

3.2. Polyphenol and flavonoid contents

It was known that CE has high polyphenol content and flavonoids

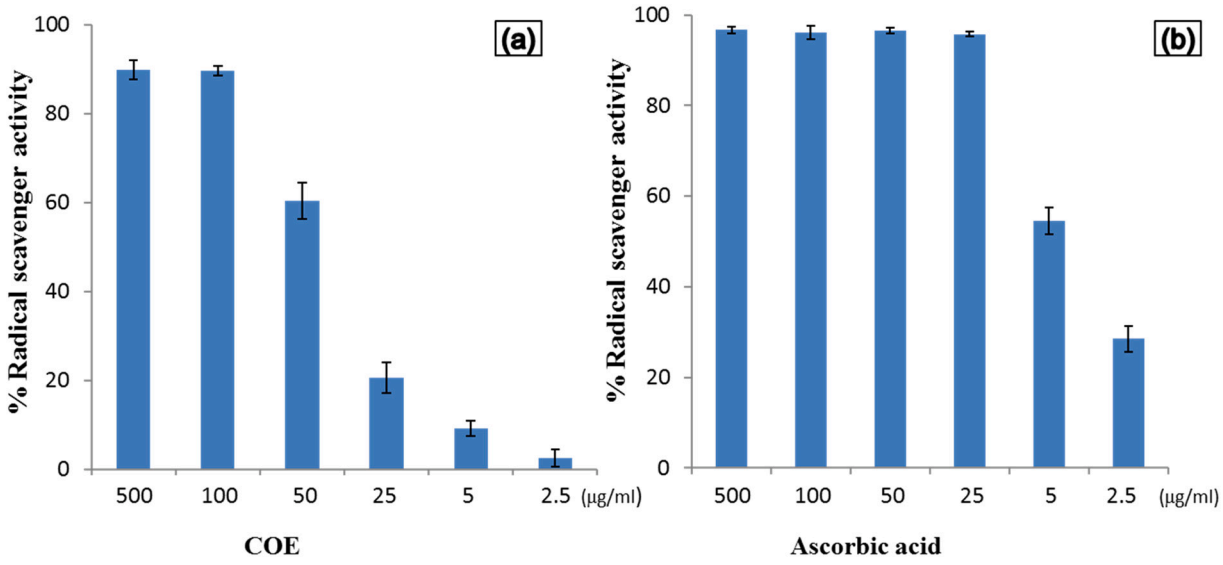

Fig. 2. Free radical scavenging activity of CE.

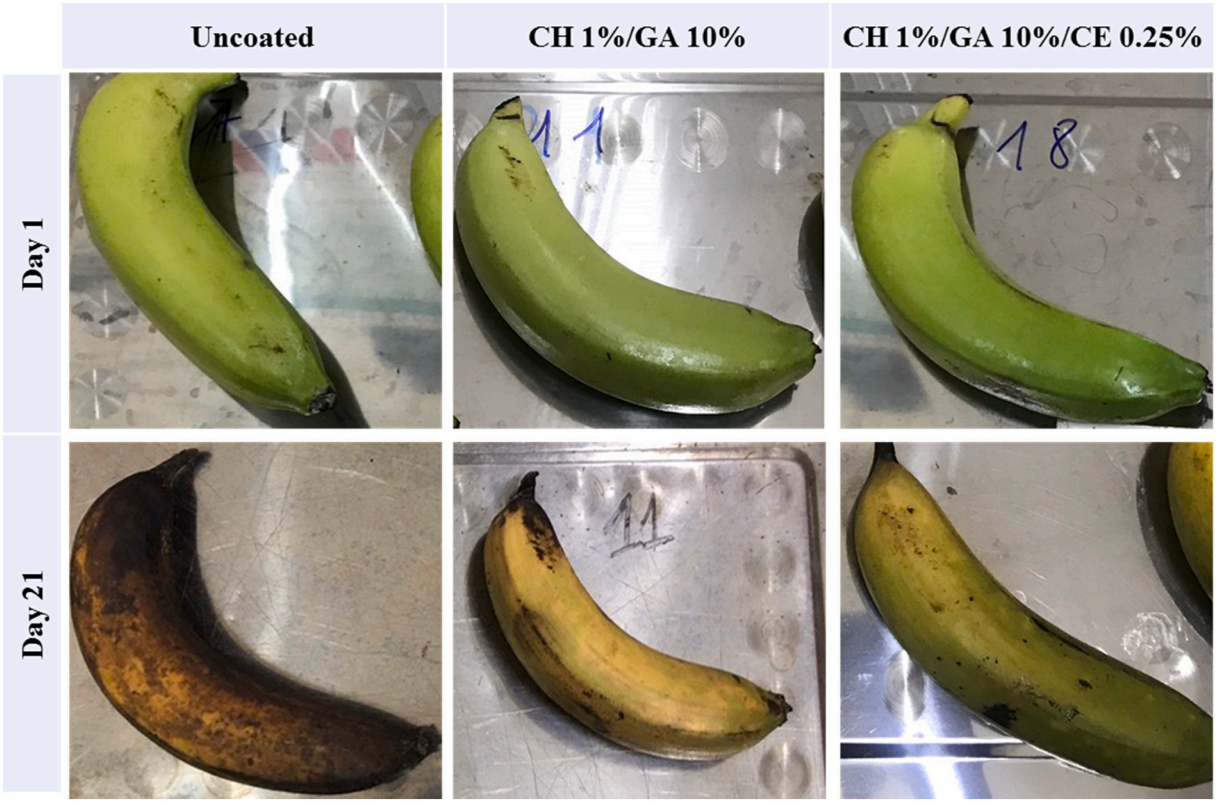

Fig. 3. Physical appearance of bananas with and without coatings (uncoated, coated with CH 1% + GA 10%, and CH 1% + GA 10% + 0.25 CE%) before and after 21 days of storage at 25 °C and 70% relative humidity.

were the major constituents of the extract [31]. The data in Table 1 indicated that the polyphenol content in the test extract here is 12.4% and flavonoid concentration is 0.62% of dry material.

3.3. Anti-oxidant activity of the extract

Illustrated in Fig. 2 is the antioxidant property of the CE extract, which was presented in a dose-dependent manner. It can be clearly seen that CE extract exhibited a high free radical scavenging in the DPPH assay even at low concentrations. Above 60% of activity was found at concentration of 50 μg/ml.

3.4. Color investigation

The bananas aging was primarily studied based on the color changing of bananas appearance from green to yellow, experienced ripening processes before rotten in brown color at the end of the banana's life. The change of bananas colors upon application of various edible coating

Table 2
Colors of uncoated bananas (control) and bananas coated with various coating composites at different days of storage at 25 °C and 70% relative humidity.

Days	Control	CH 1% + GA 10%	CH 1% + GA 10% + CE 0.05%	CH 1% + GA 10% + CE 0.15%	CH 1% + GA 10% + CE 0.25%	CH 1% + GA 10% + CE 0.5%
0	1	1	1	1	1	1
3	1	1	1	1	1	1
6	2	1	1	1	1	1
9	4	2	1	1	1	1
12	5	4	3	3	2	1
15	6	5	5	4	2	3
18	7	6	6	5	3	3
21	7	7	6	5	3	3
1	**2**	**3**	**4**	**5**	**6**	**7**

The coloring point ladder with 7 stages (after Hossain and Iqbal, 2016).

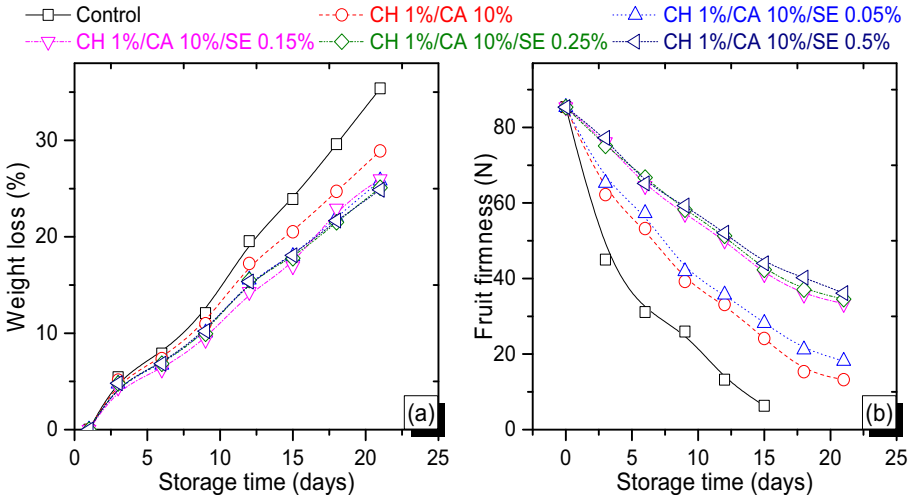

Fig. 4. (a) weight loss and (b) firmness of bananas with and without coatings (uncoated, coated with CH 1% + GA 10%, and CH 1% + GA 10% + CE various concentrations) before and after 21 days of storage at 25 °C and 70% relative humidity.

after preservation for 30 days at 21 days at ambient condition of 25 °C and 70% relative humidity (RH) was investigated. It is well-perceived that the yellowing of the fruits is ascribed to the chlorophyll biodegradation by the chlorophyllase enzyme to form yellow pigments such as xanthophyll and carotene [32]. The brown appearance is due to the formation of the brown pigment by the polymerization of small molecules to macromolecules as well as the reaction between phenol and polyphenol oxidase (PPO) [33]. It can be seen from the Fig. 3 that the color of uncoated banana is extremely changed to brown, even rotten. The banana coated with CH 1% and GA 10% composite shows the changes from green to totally yellow color after 21 days of storage. Interestingly, when coated banana with CH1% + GA10% + 0.25%CE edible composite only partial yellow appearance of banana was observed after 21 days. These results indicate that the used of CH1% + GA10% + 0.25%CE composite coating significantly prolongs the storage life of bananas. This is ascribed to the capability of the edible coating against the moisture escaping and slower the respiration rate of the bananas, as a result, the degradation rate of chlorophyll is decreased, indicating a decrease in the banana aging. These results indicate the CH1% + GA10% + 0.25%CE edible coating can effectively prolong the freshness of bananas by more than 21 days stored at the ambient conditions.

The change in different color stages of bananas during storage was evaluated visually by numerical rating scale of 1 to 7 illustrated in Table 2. The color of uncoated banana stored at 25 °C and 70% RH starts to change after 6 days and totally ripen after 18 days of storage. With CH1% + GA10% coating system, the banana changes color after 9 days of storage and rotten after 21 days. Interestingly, the bananas coated with CH1% + GA10% + CE significantly slower the decay of the banana as bananas only start to change the color after 12 days and not be rotten

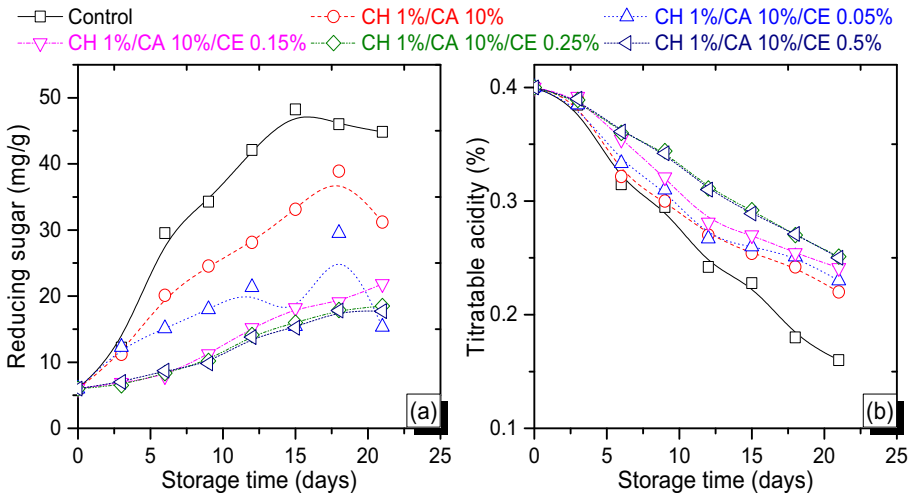

Fig. 5. (a) Reducing sugars and (b) Titratable acid with and without coatings (uncoated, coated with CH 1% + GA 10%, and CH 1% + GA 10% + CE various concentrations) before and after 21 days of storage at 25 °C and 70% relative humidity.

after 21 days. The most effective composite coating was observed with 0.25% *CE* as the coated banana with this coating system after 21 days was only at stage 3 of ripening process.

Illustrated in Fig. 4a is the weight loss of banana before and after coated with various edible coating formulas in ambient conditions. One of the most affecting factors for the effectiveness of a coating for storage life and quality of banana is the weight loss. It can be seen from the Fig. 4a that the use of coating significantly reduces the weight loss of the banana. With uncoated banana, the mass loss was calculated to be around 34% after 21 storing days. The calculated weight loss of banana coated with CH/GA coating system to be approximately 28%. Upon addition of CE, the weight loss of banana reduces significantly with a minimal loss of 20% observed at the CE content of 0.25%. This demonstrates that the addition of CE improves the effectiveness of CH/GA coating system for postharvest bananas. It has been demonstrated that the vapor pressure through the peel of fresh fruit and vegetables is responsible for the weight loss and this weight loss caused metabolic reactions in the fruit, as a result, the fruit was soften and ripen [34]. Furthermore, the respiration phenomenon in the fruit involving the losing process of carbon atom in each cycle could also trigger the weight loss. The utilization of coating on the banana fruit could serve as barrier against penetration of moisture, carbon dioxide and oxygen, as a result, decreasing the water loss, metabolic reaction and respiration process [35]. The CE is of high content of polyphenols (~13%) as free radical scavenging agents for reducing the oxidative species in contact with banana, which cause the metabolic reactions in fruits. It was observed that the increase of CE content reduces the weight loss of banana treated with the CH/GA/CE coatings and reach a weight loss minimal at the CE content of 0.25%. Further increase of CE content of higher than 0.25% demonstrated a negligible reduce of weight loss. Thus, 0.25% can be considered as optimized concentration of CE extract in the CE/CH/GA edible coating for minimizing the mass loss of banana.

Another decisive parameter to determine the effectiveness of an edible coating for the freshness and quality of fruit is the firmness. Fig. 4b shows the firmness of banana before and after coated with various edible coatings experiment at 25 °C and relative humidity of approximately 70%, which indicates that firmness of uncoated banana significantly decreases from 86 N to less than 10 N after 15 storing days. The banana firmness is significantly improved when coated with CH/GA coating, which is 22 N after 15 days and still remains about 12 N after 12 days of storage. Especially, when CE as an antioxidant was introduced to the CH/GA system, the firmness of bananas is further increased with a firmness retention of approximately 38 N after 21 days of storage with 0.25% added CE. It has been demonstrated that the deterioration of cell structure, cell wall, and intracellular materials are responsible for the softening of the fruit [36]. The decay of fruit causes the shorten of the length of pectin compounds in banana leading to enhance the activities of two enzymes: pectinesterase and polygalacturonase [37]. The application of coatings on the banana plays a significant role in hindering the penetration of the oxygen and carbon dioxide, which lower the activities of these enzymes, as a result, the firmness of bananas is improved. This is explained why the retention of the fruit firmness when bananas were treated with CH/GA/CE coating systems.

The ripening process of banana fruit is further evaluated by monitoring the change in sugars of fruit. It has been well-known that during the first stage of ripening process the starch content in banana are constantly reduced [38], leading to generation of sugar, and this sugar content in later stage of ripening process will be consumed by the respiration process, which reduce the sugar content [39]. The amounts of RS in uncoated and coated bananas over storage time were determined and the results are showed in Fig. 5a. In the controlled banana, the RS value increased steadily until 15 days of storage before suddenly decreased and continued until the end of tested storage time. Similar trend was observed with the bananas preserved with 0.05% CE/CH/GA and CH/GA coating systems, which the RS reached a maximal after 18 days of storage. On the other hand, the bananas treated with 0.15% CE/CH/GA, 0.25% CE/CH/GA, and 0.5% CE/CH/GA reveal a steady increase in RS until the end of storage period. These results demonstrate that the ripening process of banana are significantly improved by utilization of CH/GA/CE edible coatings.

The major organic acid in the banana is the malic acid, thus the value of malic acid could be used as an indicator for evaluating the effectiveness of coatings for bananas preservation. The change in acidity is also involved to the aging of bananas, which change the color of banana. The Fig. 5b shows the titratable acid (TA) values in bananas with uncoated and coated by CH/GA and CH/GA/CE coating systems. It is obvious that without coatings, the TA values decrease significantly over storage time and the value recorded after 21 days of storage is approximately 0.15% in comparison with initial TA value before storage of 0.4%. The decrease of TA values slows remarkably upon the use of coatings, which is demonstrated that after 21 days the calculated TA values remain around 0.23% and 0.26% for CH/GA and CH/GA/0.25% CE coatings, respectively. This is because the coatings restrict the ripening process of banana fruit by introducing a protective coating around the banana, thus the levels of TA in coated bananas are higher than that of controlled banana over storage time. The primary signs of respiration process in banana is to consume the malic acid in banana, therefore, the more decrease in the values of acidity causes the higher

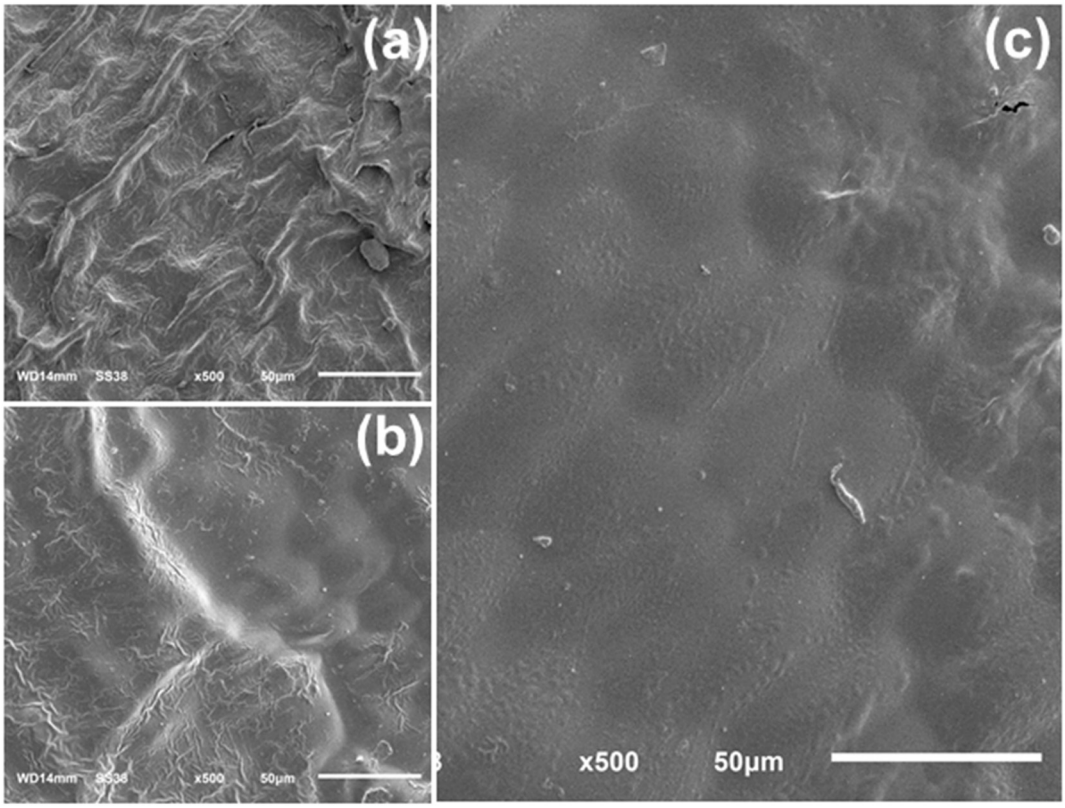

Fig. 6. SEM images of banana peels of (a) uncoated, (b) coated with CH/GA, and (c) coated with CH/GA/CE edible coatings.

respiring fruit [40].

It has demonstrated that the cuticle surface of banana fruit accelerated the loss of water leading the high speed of respiration, which eventually caused the invasion of fungus. Thus, the application of coatings on the banana's surface would block the pores of the cuticle, which minimize the water loss of banana [41]. Furthermore, the coating also prevents the contact between external and internal environment of banana, which reduce the effect of outside atmospheres on the ripening process of banana.

The surface structure of the banana's skin with uncoated and coated with edible coatings were observed using scanning electron microscopy as shown in Fig. 6. The surface structure of uncoated banana shows the wrinkle and cracks on the epidermal cells of the skin (Fig. 6a). When banana was coated with CH/GA coatings, the skin's surface is much smoother than controlled banana with significantly reduce of cracks on the pericarp surface (Fig. 6b). The smooth surface with no cracks on the banana's skin is observed when banana was treated with CH/GA/CE coating system (Fig. 6c). This enhanced smoothness of banana surface by these edible coatings could significantly slow down the respiration rate, improving internal environment, and suppressing transpiration losses, as a result, maintaining the freshness of bananas.

4. Conclusion

The natural polyphenols were successfully extracted from *C. operculatus* plants in water. The total polyphenol in CE was 12.4 ± 1.48 mg/ml. This extract was employed as antioxidant additive in fabrication of newly edible coatings of CH/GA/CE. The resultant edible coatings with CE exhibited a significant improvement of banana shelf-life in comparison with control and banana treated with CH/GA edible coating. The CH1% + GA10% + 0.25%CE composite coating could effectively prolong the freshness of bananas up to 21 storing days at the ambient conditions. After coated with CH/GA/CE systems, the banana showed smooth surface and maintained the necessary nutrients such as RS and fruit firmness. This CH/GA/CE edible coating could be employed as an effective pathway to prolong the shelf-life of banana.

where the control is uncoated, and treated bananas are coated with CH 1% + GA 10%, and CH 1% + GA 10% + CE at various concentrations).

Declaration of competing interest

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

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