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Edible coating of chitosan ionically combined with κ-carrageenan maintains the bract and postharvest attributes of dragon fruit (*Hylocereus undatus*)

[1]Nguyen, T. H., [1,3]Boonyaritthongchai, P., [1,3]Buanong, M.,
[2]Supapvanich, S. and [1,3]*Wongs-Aree, C.

[1]Division of Postharvest Technology, School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok 10150, Thailand
[2]Department of Agricultural Education, School of Industrial Education and Technology, King Mongkut's Institute of Technology Ladkrabang, Bangkok 10520, Thailand
[3]Postharvest Technology Innovation Centre, Ministry of Higher Education, Science, Research and Innovation, Bangkok 10400, Thailand

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Abstract

Dragon fruit (*Hylocereus undatus*) has medicinal properties due to its rich antioxidant profile. Dragon fruit also has an attractive appearance of red peel and green bracts. However, shrivelling and weight loss, bract yellowing, and postharvest diseases are major challenges to the dragon fruit trade. The objective of the present work was, therefore, to formulate a coating composed of chitosan and κ-carrageenan for dragon fruits during storage at 10°C. The composite coating based on 1% chitosan (w/v) and 0.2% (w/v) κ-carrageenan with 0.75% (w/v) glycerol as a plasticiser effectively reduced the physiological weight loss and maintained the freshness of the dragon fruits, while increasing the phenolic content and maintaining the titratable acidity in the pulp. The composite coating delayed chlorophyll degradation by suppressing chlorophyllase and chlorophyll-degrading peroxidase, thereby maintaining the chlorophyll content (45.46 mg/100 g dry weight) and freshness of the bracts. However, the composite coating did not possess a strong effect on enhancing chitinase and β-1-3 glucanase activities of dragon fruits during storage and controlling disease symptoms.

Introduction

Dragon fruit (*Hylocereus undatus*) commercially grown in many countries in the tropics contains an enormous amount of antioxidants which can prevent colon cancer and diabetes, reduce cholesterol and high blood pressure, control high sugar levels, and promote dental health in humans (Dembitsky *et al.,* 2011). However, dragon fruit has some significant postharvest problems such as shrivelling and weight loss, loss of bract greenness, and postharvest diseases which result in rapid deterioration. Dragon fruit pericarp contains active stomata especially in the bracts of the fruit, leading to rapid shrivelling (Mizrahi, 2014). Besides, dragon fruit bracts gradually show discolouration, dehydration, browning, and curling in the postharvest stage (Hoa *et al.,* 2006). Loss of bract greenness due to chlorophyll degradation is one of the major symptoms of senescence in dragon fruit, which substantially reduces the marketability of the fruit. In terms of postharvest disease, notable dragon fruit pathogens are *Alternaria alternata, Bipolaris cactivora, Colletotrichum truncatum, Fusarium dimerum, F. equiseti*, and *Rhizopus stolonifer* (Ngoc *et al.,* 2017).

Fruits and vegetables have natural skins as a protective layer against water loss, pathogenic infections, and other harmful effects (Thakur and Kumar, 2017). However, this protective waxy coating is vulnerable to damage during the postharvest handling processes, which could be reinforced by applying coatings of edible materials to the fruit surface. Therefore, the technology that uses edible coatings has been a growing interest to maintain the quality of fruits and vegetables.

Among edible coatings, chitosan-based coatings have been widely studied for the storage of fruits and vegetables. Chitosan is a linear polyamine copolymer of β-(1-4)-D-glucosamine and acetyl β-(1-4)-D-glucosamine, obtained by alkaline N-deacetylation of chitin, produced mainly from an abundant waste of the shellfish industry (Elsabee and Abdou, 2013). Chitosan maintains the positive charge

*Corresponding author.
Email: chalermchai.won@kmutt.ac.th
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of the molecule, and is considered safe. It is an excellent material for forming coatings and films with selective permeability to gases. Chitosan also exhibits antimicrobial activity against a variety of fungal and bacterial plant pathogens, mainly due to the electrostatic interaction between the positive charge of the protonated amino group of chitosan and the negatively charged molecules on the surface of microbial cells. Therefore, chitosan has been used as an edible coating to extend the shelf life and maintain the nutritional quality of various fruits and vegetables, and inhibit pathogenic growth (Verlee *et al.,* 2017; Chaudhary *et al.,* 2020). However, due to strong hydrophilicity, the moisture barrier property of chitosan coatings is poor (Dutta *et al.,* 2009). Chutichudet and Chutichudet (2011) reported that on dragon fruit, the chitosan coating reduces the stomatal conductance, size, and aperture, thus resulting in maintaining its fresh appearance at room temperature; but, not significantly maintaining its weight. *In vivo*, chitosan treatment could alleviate the symptoms of anthracnose in dragon fruit caused by *C. gloeosporioides* (Ali *et al.,* 2013). However, the effect of chitosan coating on the naturally infected postharvest diseases in dragon fruit and the changes of chlorophylls in the bracts have not been investigated.

To improve chitosan-based composite coatings, κ-carrageenan was introduced as a component. κ-carrageenan is a sulphated anionic polysaccharide extracted from red algae. It is composed of alternating copolymers of α-(1-3)-D-galactose and β-(1-4)-3,6-anhydro-D- or L-galactose. It is reported that κ-carrageenan has excellent film-forming properties (Li *et al.,* 2014). A blend of chitosan and κ-carrageenan has been studied to form a composite film based on the interaction between oppositely charged polysaccharides. After chitosan is mixed with κ-carrageenan, the film made has better water barrier properties, the surface is smooth, uniform, and has greater flexibility (Park *et al.,* 2001; Shahbazi *et al.,* 2016). However, as far as we know, the application of composite coatings based on chitosan and κ-carrageenan in general fruits and vegetables, especially dragon fruit, is limited. Therefore, the present work aimed to produce suitable edible coatings based on the combination of chitosan and κ-carrageenan to reduce the weight loss of fruits, maintain freshness and bract colour, and retain other characteristics of dragon fruits during storage.

Materials and methods

Raw materials

Dragon fruits (maturity stage: > 80%) with a bright red surface of fruit pericarp were harvested from an orchard (latitude: 14.135052; longitude: 100.824257) in Pathum Thani province, Thailand. After harvest, the fruits were transported to the School of Bioresources and Technology, King Mongkut's University of Technology Thonburi, Bangkok within 2 h. Uniform-sized (average weight 300 g) and defect-free fruits were selected and cleaned with tap water, and sanitised with 150 mg/L sodium hypochlorite.

Preparation of coating solutions

Chitosan coating

Low-molecular-weight chitosan (50,000 - 190,000 Da) was purchased from Sigma Chemical Co., USA. One litre of 0.5, 1, or 1.5% (w/v) chitosan solution was prepared by dissolving 5, 10, or 15 g of chitosan in 950 mL of acetic acid solution at 1.0% (v/v), and stirred for 5 h at room temperature. The pH was adjusted to 5.6 using 1 M NaOH, and then the solution was made up to 1 L.

κ-carrageenan coating

In the present work, 0.2% (w/v) κ-carrageenan solution was selected because it could thoroughly mix with chitosan to prepare the highest level of the composite coating as previously observed during concentration screening. The κ-carrageenan solution was prepared by dissolving 0.2% (w/v) κ-carrageenan (sulphated plant polysaccharide; Sigma Chemical Co., USA) in distilled water, and stirred for 1 h at 60°C. The solution was kept for 1 h to cool to room temperature.

Chitosan- and κ-carrageenan-based composite coating

The composite coating solution was prepared according to the method previously described by Olaimat *et al.* (2014) with slight modifications (Figure 1). Firstly, acetic acid (1.0%, v/v) was added to the 0.2% (w/v) κ-carrageenan solution. Subsequently, chitosan (1%, w/v) was added and mixed with magnetic stirring until completely dissolved. Plasticiser (if any) was then added to the mixture, and stirred thoroughly for 1 h. The coating solution was then homogenised at 10,000 rpm for 2 min. Finally, 1 M NaOH was used to adjust the pH to 5.6.

Experimental designs

Preliminary experiment on bilayer coating and composite coating

Dragon fruits were divided into four groups:

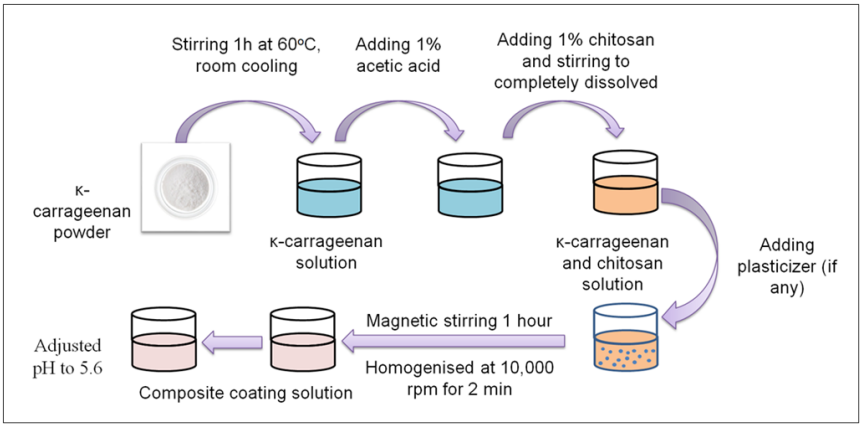

Figure 1. Flow diagram of composite coating preparation.

(1) 1% chitosan and 0.2% κ-carrageenan based composite coating; (2) double-layer coatings: the first layer was 1% chitosan, and the second layer was 0.2% κ-carrageenan; (3) 1% chitosan alone; and (4) uncoated (control). In this experiment, plasticisers were not used in the coating solution. The dragon fruits were evaluated in terms of physiological loss in weight, appearance of bracts, and body the appearance following the scale of 0 (without shrivelling on the peel) to 3 (shrivelling in > 50% of the area of the fruit surface) as described by Woolf *et al.* (2006). Results indicated that although the weight loss was not significantly different between treatments and as compared to control, the composite coating based on 1% chitosan and 0.2% κ-carrageenan maintained a better appearance of fruit body and bracts. Therefore, the composite coating combining chitosan and κ-carrageenan was selected for subsequent experiments.

Preliminary experiment on chitosan concentration to combine with 0.2% κ-carrageenan in the composite coating

Five treatments were prepared: (1) composite of 0.5% chitosan and 0.2% κ-carrageenan, (2) composite of 1% chitosan and 0.2% κ-carrageenan, (3) composite of 1.5% chitosan and 0.2% κ-carrageenan, (4) 1.0% chitosan alone, and (5) uncoated (control). Glycerol 0.75% (w/v) was added as a plasticiser to all coating solutions. Physiological weight loss and disease severity were evaluated in dragon fruit samples. Results indicated that although all-composite coatings significantly reduced dragon fruit weight loss, the 1.0% chitosan and 0.2% κ-carrageenan composite showed the lowest severity of disease in the dragon fruits stored. Therefore, a composite coating of 1% chitosan and 0.2% κ-carrageenan was selected for the subsequent experiment.

Preliminary experiment on plasticiser used for the composite of 1% chitosan and 0.2% κ-carrageenan

Glycerol or sorbitol 0.75% (w/v) was added and mixed thoroughly with the composite of 1% chitosan and 0.2% κ-carrageenan, and compared to the composite without plasticiser. The physiological weight loss of the dragon fruits was evaluated to determine the appropriate plasticiser to improve the water vapour barrier for the composite coating. Results indicated that 0.75% glycerol reduced weight loss more effectively, and was selected as an appropriate plasticiser for a composite coating based on 1% chitosan and 0.2% of κ-carrageenan. The summary of the preliminary experiments is shown in Figure 2.

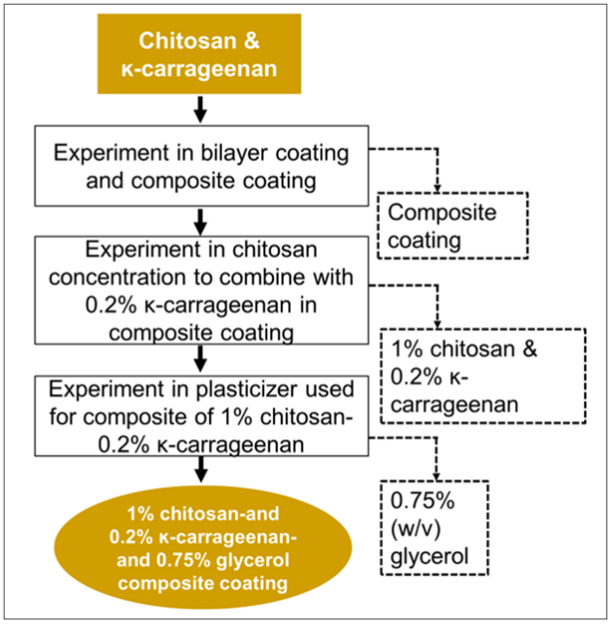

Figure 2. Flow diagram of preliminary experimental design.

Effect of composite coating on quality of dragon fruits during storage

Two groups of 60 clean dragon fruits were

separated for the treatments. The dragon fruits in the first group received no treatment, and served as control. The dragon fruits in the second group were completely immersed in the composite of 1% chitosan and 0.2% κ-carrageenan with added 0.75% glycerol. All samples were air-dried for 1 h, and then stored in a plastic basket at 10°C with 85 - 90% relative humidity. Fruit sampling for quality evaluation was carried out at 6-d intervals. Chitinase and β-1-3 glucanase activities were measured in the first three days. Fifteen fruits of each treatment were used for non-destructive measurements. For the biochemical analyses, four fruits were removed at scheduled intervals for each analysis.

Physicochemical analyses

Bracts and peel colour

Bracts and peel colour were measured based on the Commission International del' Eclairage (CIE) colour system (L*, a*, b*) using a colourimeter (model CR-400, Minolta, Japan). For bract colour, measurements were done on the largest area of three random bracts on top of the fruits. The hue angle (h°) used to express the colour of the bracts was calculated using Eq. 1. The colour of the dragon fruit peel was evaluated at three points of the pericarp of each fruit. The ΔE value, which indicates the total colour difference, was calculated using Eq. 2.

$$h° = \tan^{-1}\left(\frac{b^*}{a^*}\right) \quad \text{(Eq. 1)}$$

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad \text{(Eq. 2)}$$

Physiological loss in weight

The weight loss of the dragon fruits was determined as a percentage using Eq. 3:

$$\text{Weight loss (\%)} = [(m_0 - m_i) / m_0] \times 100 \quad \text{(Eq. 3)}$$

where, m_0 = weight of dragon fruit on day 0, and m_i = weight of dragon fruit on the day of the evaluation.

Ethanol and acetaldehyde in dragon fruit tissues

Ethanol and acetaldehyde concentrations in dragon fruit tissues were determined as described by Choosung *et al.* (2019). Briefly, 5 g of meat tissues was incubated in a polytetrafluoroethylene septum-sealed bottle at 50°C in a water bath for 15 min. Then, 1 mL of headspace gaseous mixture was taken and measured using a gas chromatograph (GC-2014; Shimadzu, Kyoto, Japan) equipped with an FID detector and a Porapak Q column (2.0 m long, 3.00 mm of I.D.). The injector, detector, and column temperatures were set at 120, 23, and 200°C, respectively.

Firmness

Dragon fruit flesh firmness (N) was measured in the middle of a cut surface of each fruit using a texture analyser (TA-XT2, Stable Micro Systems Ltd., UK) with a plunger (8 mm diameter) and a moving speed of 5 mm/sec for a 10 mm depth.

Total soluble solids (TSS)

The TSS of the fruit juice was determined using a digital refractometer (PAL 1, Atago Co Ltd.), and expressed as °Bx.

Titratable acidity (TA)

The TA of the fruit juice was determined by the titration method using a 0.1 N NaOH solution, and expressed as a percentage (w/w) of citric acid.

Total phenolic content (TPC)

The TPC of the fruit pulp was determined using the Folin-Ciocalteu reagent (FCR) (Singleton *et al.,* 1999), and expressed as g/kg of gallic acid equivalent (GAE) based on fresh weight.

Vitamin C content

The total vitamin C was determined by the 2,4 dinitrophenylhydrazine (DNP) method (Roe *et al.,* 1948), and expressed as mg/100 g of fresh weight.

2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity

The DPPH assay was performed according to Li *et al.* (2017). The supernatant extracted with 80% methanol was added with 110 μM DPPH (working DPPH). The reaction mixture was incubated at 25°C for 30 min. The absorbance was measured at 517 nm. The working DPPH solution without the addition of the supernatant served as control. The DPPH removal activity was calculated using Eq. 4:

$$\text{\% Inhibition} = [(A0 - A1) / A0] \times 100 \quad \text{(Eq. 4)}$$

where, A0 = absorbance of the control, and A1 = absorbance of test samples.

Ferric-reducing antioxidant power assay (FRAP)

The FRAP was determined following the method of Benzie and Strain (1996), and expressed as g/kg of ascorbic acid equivalent (AsA) based on

fresh weight.

Chlorophyll content of bracts

The chlorophyll content of the bracts was determined according to Moran (1982), and expressed as mg per 100 g of dry weight.

Disease incidence and severity

Disease incidence

The disease incidence was expressed as the percentage of fruits that showed disease symptoms of the total number of fruits observed in each treatment.

Disease severity

The disease severity of the fruit disease was scored based on a rating scale (0 = 0% of the fruit body surface rotten; 1 = 0 < rotten area ≤ 5% of the body surface; 2 = 5% < rotten area < 10% of the body surface; 3 = 10% < rotten area < 25% of the body surface; 4 = 25% < rotten area < 50% of the body surface; and 5 = > 50% rotten area).

Chlorophyll-degrading enzymes and pathogen-related proteins activities assay

Preparation of chlorophyll a, chlorophyllin a, and pheophytin a

Chlorophyll a

Chlorophyll *a* was prepared according to Aiamla-or *et al.* (2010) from spinach leaves. Chlorophyll *a* was separated from petroleum ether soluble chlorophylls using powdered sugar column chromatography (Perkins and Roberts, 1962). Finally, chlorophyll *a* was prepared at 500 µg/mL in acetone.

Chlorophyllin a

Chlorophyllin (Chlin) *a* was prepared from 500 µg/mL chlorophyll *a* according to Vicentini *et al.* (1995).

Pheophytin a

Pheophytin (Phy) *a* was converted from chlorophyll *a* by acidifying with 0.1 N HCl, according to Aiamla-or *et al.* (2012). The Phy *a* concentration was determined by absorbance at 409 nm, and was calculated using the extinction coefficient of 156,000 M/cm.

Chlorophyll-degrading enzyme activities assays

Acetone powder (250 mg) from bracts was added with 7.5 mL of 50 mM phosphate buffer (pH 7.0) containing 50 mM KCl and 0.12% Triton X-100 for Chlase and MgD, 50 mM Tris-HCl buffer (pH 8.0) for pheophytinase (PPH), or 10 mM phosphate buffer (pH 7.0) for Chl-POX. The mixture was stirred for 1 h on ice, and then centrifuged at 16,000 *g* at 4°C for 20 min. The supernatant was used as the crude enzyme extract.

Chlorophyllase activity

Chlorophyllase activity (Chlase) was determined according to Aiamla-or *et al.* (2010), using 500 µg/mL of chlorophyll *a* as a substrate. The reaction mixture contained 0.5 mL of enzyme solution, 0.5 mL of 10 mM phosphate buffer (pH 7.5), 0.1 mL of 1% 3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), and 0.2 mL of 500 µg/mL of chlorophyll *a* acetone solution. The reaction mixture was incubated at 25°C for 40 min, and the reaction was terminated by adding 4 mL of 80% acetone. Chlorophyllide *a* (Chlide *a*) was removed by adding 4 mL of hexane. Chlase activity was determined by the formation of Chlide *a* at 667 nm per unit per mg of protein.

Mg-dechelatase activity

The Mg-dechelatase (MgD) activity was determined as described by Aiamla-or *et al.* (2010). Chlin *a* (OD687 nm = 0.2) was used as substrate. The reaction mixture contained 0.75 mL of 50 mM Tris-HCl buffer (pH 8.0), 0.3 mL of Chlin *a* (OD687 nm = 0.2), and 0.2 enzyme solution. The mixture was incubated at 37°C. MgD activity was calculated based on the formation of pheophorbide (Pheide) *a*, which was determined by the change in absorbance of the reaction mixture at 686 nm after 3 min.

Pheophytinase activity

The activity of phenophytinase (PPH) was determined as described by Aiamla-or *et al.* (2012). The reaction mixture contained 0.5 mL of enzyme solution, 0.5 mL of 50 mM Tris-HCl buffer (pH 8.0), and 0.1 mL of Phy *a* acetone solution. The reaction mixture was incubated in a water bath at 25°C for 40 min, and the reaction was stopped by adding 2 mL of acetone. The Pheide *a* formed was separated from Phy *a* by adding 2 mL of hexane. The activity of PPH was determined by the formation of Pheide *a* at 667 nm per unit per mg of protein.

Chlorophyll-degrading peroxidase activity

Chl-POX was determined following the method of Yamauchi and Watada (1994) with some adjustments. The reaction mixture contained 0.5 mL of crude enzyme extract, 1.5 mL of 0.1 mM phosphate buffer (pH 6.0), 0.1 mL of 1% Triton-X 100, 0.1 mL of 5 mM *p*-coumaric acid, 0.2 mL of 500 µg/mL Chl *a* acetone solution, and 0.1 mL of 0.3%

hydrogen peroxidase. The mixture was incubated for 20 min at 25°C, and then the reaction was stopped by adding 2.0 mL of acetone and 2.0 mL of hexane. The Chl-POX activity was determined by non-degraded Chl *a* in hexane layer measured at 663 nm.

Pathogen-related proteins activities assays

Enzyme crude extraction

Chitinase and β-1-3 glucanase assays were performed on dragon fruit peel. Frozen samples (2 g) were homogenised in 10 mL of 100 mM sodium acetate buffer at pH 5.0. Subsequently, the homogenates were centrifuged at 16,000 *g* for 20 min at 4°C.

Chitinase (CHI) activity

CHI activity was measured as described by Nguyen *et al.* (2012) with some modifications. The reaction mixture contained 500 µL of crude enzyme and 500 µL of 3% colloidal chitin in 0.1 M sodium acetate buffer at pH 5.2. The mixture was incubated for 2 h at 37°C, and the reaction was stopped by centrifugation at 12,000 *g* for 20 min. Later, the reducing sugar produced was determined by 1% 3,5 dinitrosalicylic acid (DNS) using N-acetyl-D-glucosamine as a standard. One unit of CHI activity was defined as µmol of N-acetyl-D-glucosamine produced per minute under the assay conditions.

β-1-3 glucanase activity

GLU activity was determined according to Zheng *et al.* (2011). The crude enzyme (100 µL) was incubated with 50 µL of 0.4% laminarin at 37°C for 1 h. After that, 1 mL of 1% DNS was added and boiled for 5 min. The mixture was then cooled on ice, and the absorbance at 540 nm was measured. One unit of GLU activity was defined as mmol glucose equivalents released per hour from laminarin under the assay conditions (reducing sugar in the enzyme blank was subtracted).

Statistical analysis

An analysis of variance (ANOVA) was performed, and Fisher's least significant difference (LSD) test was used to compare the mean values measured on each sampling day at $p \leq 0.05$ and $p \leq 0.01$ using SPSS software version 19.0 for MS-Windows.

Results and discussion

Bract's colour, chlorophyll content, and chlorophyll-degrading enzymes activities

The colour of the dragon fruit bracts changed from bright green to yellow, as shown by the decreasing hue angle (h°) in Figure 3a. However, dragon fruit bracts coated with 1% chitosan- and 0.2% κ-carrageenan-based composite coating maintained a significantly higher h° value as compared to control after 24 and 30 days of storage, as demonstrated by the fresher and greener colour shown in Figure 4. Also, the chlorophyll content in the dragon fruit bracts decreased during storage as shown in Figure 3b. The initial chlorophyll content in the dragon fruit bracts was 66.32 mg/100 g dry weight, which decreased to 39.09 and 45.46 mg/100 g in control and coated dragon fruits, respectively.

Chlorophyll breakdown is an integral process in leaf senescence and fruit ripening which is accelerated by key enzymes such as Chlase, MgD, and PPH (Hörtensteiner and Kräutler, 2011). Also, Chl-POX induces chlorophyll loss by oxidising phenolic compounds in the presence of hydrogen peroxide to form $C13^2$-hydroxychlorophyll (Yamauchi *et al.,* 2004). As shown in Figure 5a, the Chlase activity in the bracts of control dragon fruits increased significantly after 18 and 24 days of storage, and showed a great difference from that of coated dragon fruits. Chl-POX activity in control dragon fruits increased almost twice after 24 days of storage as compared to day 0, while it decreased in coated dragon fruits during storage, particularly on days 18 and 24 (Figure 5c). Also, the PPH activity in control dragon fruits increased gradually over 18 days of storage before decreasing slightly but was quite stable in coated dragon fruits during storage (Figure 5d). Meanwhile, when dragon fruits were kept at 10°C, MgD activity significantly decreased, and remained stable in both control and coated dragon fruits (Figure 5b). These results indicated that the composite coating based on 1% chitosan and 0.2% κ-carrageenan significantly suppressed Chlase, Chl-POX, and PPH activities in dragon fruit bracts stored at 10°C. However, the coatings did not provide an inhibitory effect on MgD activity.

It is believed that Chitosan-based coatings will produce a modified atmosphere that reduces ethylene production, thereby delaying senescence and retaining the chlorophyll content in sponge gourd (Han *et al.,* 2014). Besides, chitosan coatings were reported to increase internal CO_2 while decreasing O_2 concentration in papaya, thereby delaying changes in physicochemical characteristics including peel colour (Ali *et al.,* 2011). Hamzah *et al.* (2013) also described that the κ-carrageenan-based coating can provide an oxygen barrier, thus reducing respiration rate and ripening, thereby leading to colour retention in papaya. According to Song *et al.* (2015), a high

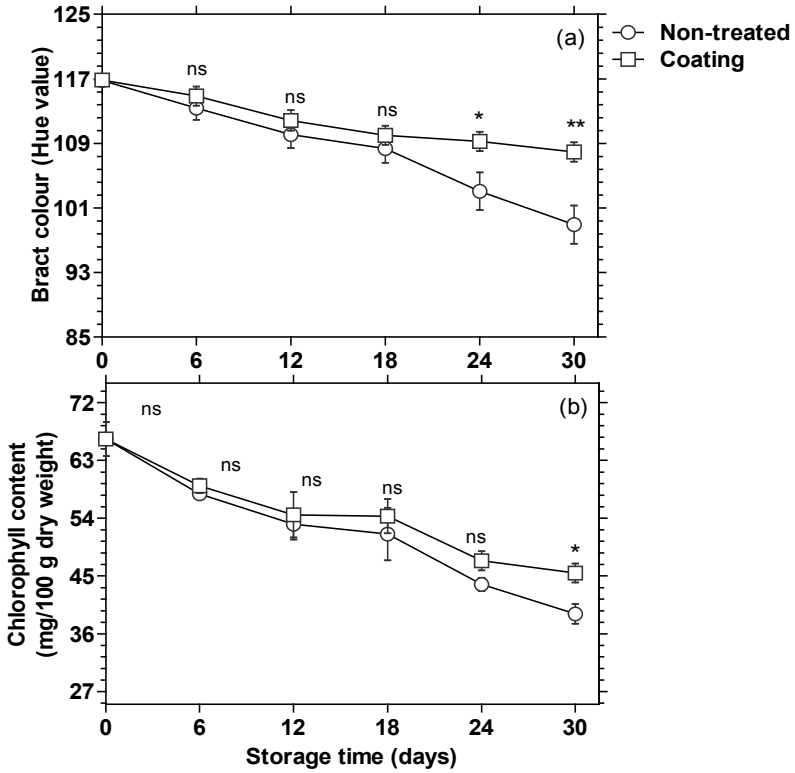

Figure 3. Changes in (a) h° value (n = 5), and (b) chlorophyll content in bracts of dragon fruits coated with chitosan- and κ-carrageenan-based composite coating, as compared to control dragon fruits, during storage at 10°C. Vertical bars represent the standard error (SE) of mean for triplicates. ns = not significant; *significant at $p < 0.05$; and **highly significant at $p < 0.01$ by Fisher'st LSD test.

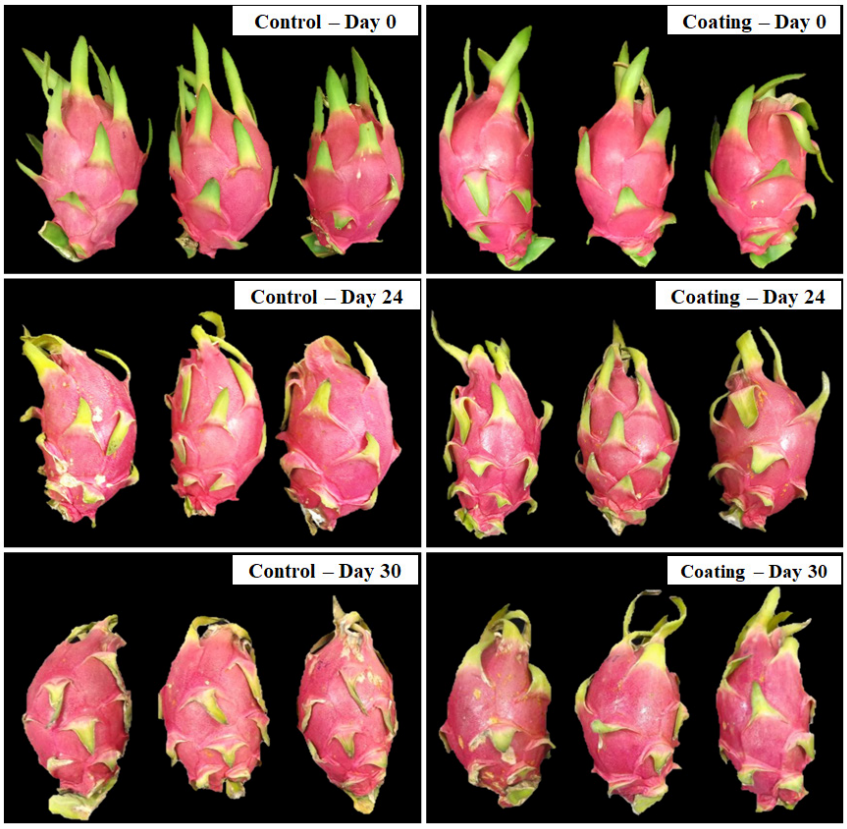

Figure 4. Changes in visual appearance of dragon fruits coated with chitosan- and κ-carrageenan-based composite coating, as compared to control dragon fruits, during storage at 10°C.

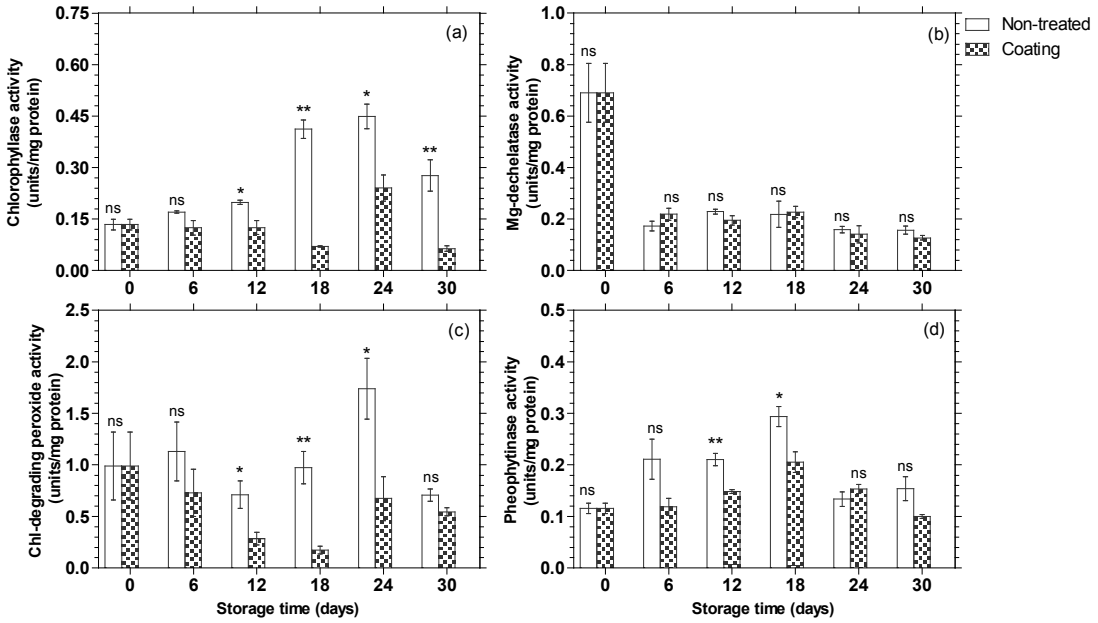

Figure 5. Changes in activities of (a) Chlase, (b) MgD, (c) Chl-POX, and (d) PPH in bracts of dragon fruits coated with chitosan- and κ-carrageenan-based composite coating, as compared to control dragon fruits, during storage at 10°C. Vertical bars represent the standard error (SE) of mean for triplicates. ns = not significant; *significant at $p < 0.05$; and **highly significant at $p < 0.01$ by Fisher's LSD test.

level of CO_2 can delay the degradation of chloroplasts in plant tissues and the reduction in the level of mRNA of the Rubisco small subunit (SSU), which is a down-regulated gene for senescence or fruit ripening. On the other hand, it was reported that chitosan coatings improve the antioxidant system and decrease the accumulation of H_2O_2 during the ripening stage in nectarine fruit, which delays senescence. Low and high molecular weight chitosan treatments delayed pulp colour change in nectarine fruit during storage (Zhang *et al.,* 2019). Jongsri *et al.* (2016) found that the chitosan coating increased CAT and APX activities and reduced the H_2O_2 content in mango, which protected the fruit from oxidative stress during ripening and extended the fruit's shelf life. We assume that the suppressed activity of Chl-POX in the present work could be attributed to the effect of chitosan on H_2O_2 regulation.

Physicochemical quality of dragon fruits

Generally, rapid shrivelling and weight loss of fruits and vegetables after harvest is one of the main problems that reduce visual quality and marketability. Data from our preliminary experiment indicated that chitosan and κ-carrageenan-based composite coatings significantly reduced the weight loss of dragon fruits after the storage period. However, when compared with control, the 1.0% chitosan coating alone even increased the weight loss of dragon fruits. Chitosan coating has a poor moisture barrier due to its strong hydrophilicity (Dutta *et al.,* 2009).

The chitosan film alone was opaque, non-flexible, and brittle (Shahbazi *et al.,* 2016). Salvador-Figueroa *et al.* (2017) reported that chitosan-based coatings could not significantly reduce weight loss in papaya cv. Maradol during storage. Similarly, the carboxymethyl cellulose and chitosan-bilayer coating was not effective in preventing weight loss in citrus (Arnon *et al.,* 2014). According to Poverenov *et al.* (2014), a composite coating of chitosan and gelatine failed to reduce weight loss in bell pepper. Meanwhile, Shahbazi *et al.* (2016) reported that the water vapour permeability of chitosan films decreased after being mixed with κ-carrageenan. This could be due to the formation of the hydrogen-bond network between chitosan and κ-carrageenan, which would increase the barrier property of the film against moisture movement. Pinheiro *et al.* (2012) also found that the κ-carrageenan/chitosan nanolayer coating had a lower permeability to water vapour as compared to conventional chitosan-based films.

Colour, firmness, and off-flavour are the attributes that influence the sensory quality of fruits. However, the composite coating did not affect the colour and firmness of the dragon fruits during storage (Figure 6a and 6b). Also, ethanol and acetaldehyde were not detected in either control or

coated dragon fruits.

The TSS and TA contents of dragon fruits in the full maturity stage (75 to 100% of the surface of the red pericarp) varied from 12.4 to 13.6 °Bx and 0.24 to 0.6%, respectively (Ortiz and Takahashi, 2015). Changes in TSS and TA in the storage period ultimately affect the taste of the fruit. Figure 6c shows that the TSS content remained constant in the stored dragon fruits. Furthermore, the TA also did not change considerably in coated dragon fruits, but decreased significantly in control dragon fruits (Figure 6d). The composite coating could cause the modified atmospheric condition, thus slowing down respiratory metabolism, and retaining the main organic acids in dragon fruits. Edible coatings based on chitosan were also found to slow TA loss in freshly cut kiwi and Chinese cherry (Xin *et al.,* 2017; Vivek and Subbarao, 2018).

Figures 6e and 6f show the changes in the TPC and vitamin C content in dragon fruits. Although these did not change significantly during storage, the TPC in coated dragon fruits was significantly higher than in control dragon fruits on day 18 and day 24 (Figure 6e and 6f). Chitosan is an elicitor that induces defence-related secondary metabolites accumulation such as phytoalexins, lignin, and phenolic compounds in plant tissues (Xing *et al.,* 2015). Chitosan up-regulates the gene expression of phenylalanine ammonia-lyase involved in the synthesis of phenolic compounds in avocado

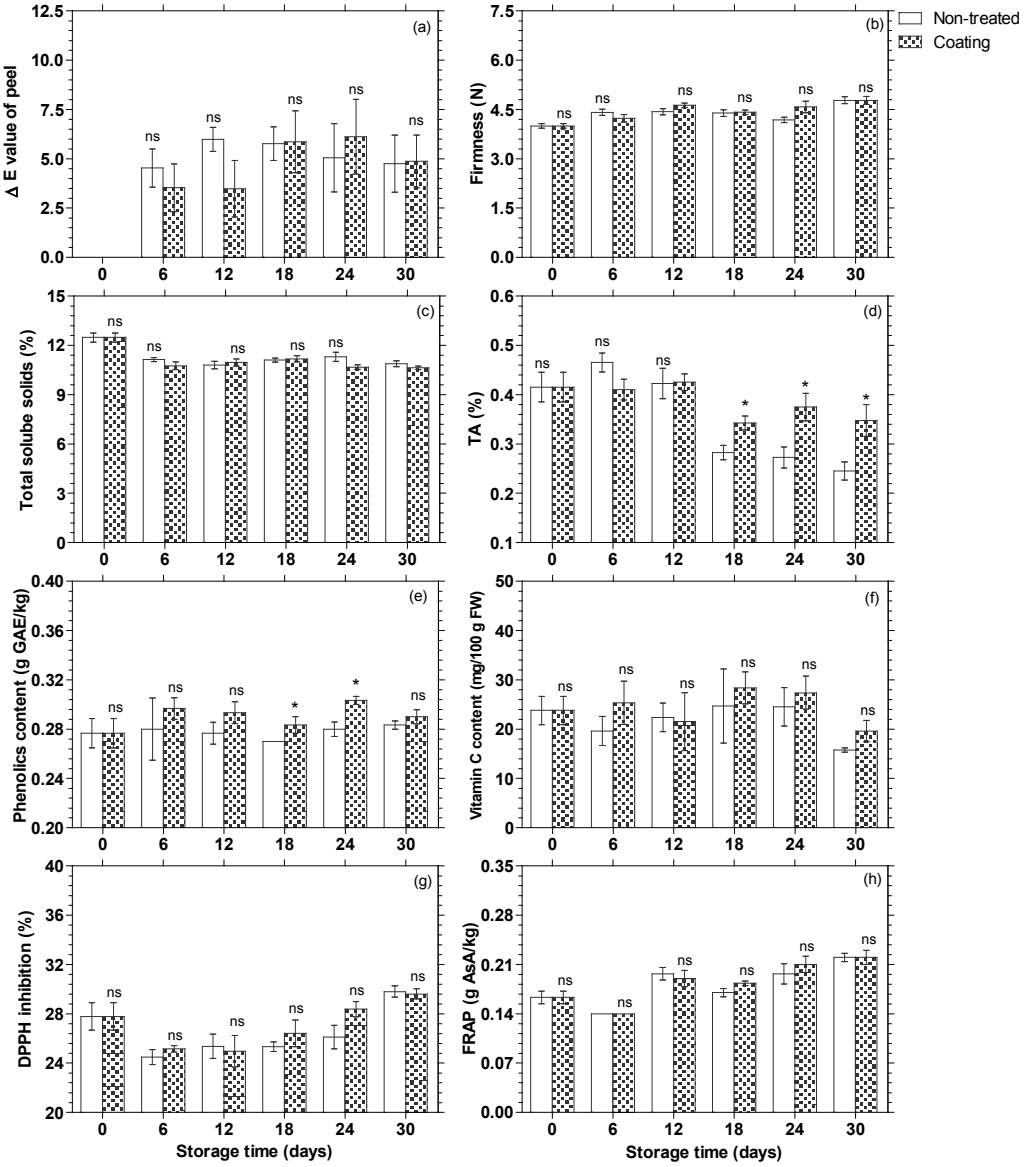

Figure 6. Changes in (a) peel colour, (b) firmness, (c) total soluble solids, (d) titratable acidity, (e) phenolics content, (f) vitamin C content, (g) percentage of DPPH inhibition, and (h) FRAP in dragon fruits coated with chitosan- and κ-carrageenan-based composite coating, as compared to control dragon fruits, during storage at 10°C. Vertical bars represent the standard error (SE) of mean for triplicates. ns = not significant; and *significant at $p < 0.05$ by Fisher's LSD test.

(Obianom *et al.,* 2019). The chitosan coating also increased the TPC in grapefruit (Shi *et al.,* 2018). The antioxidant activities in terms of inhibition of DPPH and FRAP showed an increasing trend during storage; but, there were no significant differences between control and coated dragon fruits (Figures 6g and 6h).

Diseases and pathogen-related proteins

The disease incidence occurred on dragon fruits since the 24th day of storage. After 30 days of storage, the incidence of the disease reached 93.33% in control dragon fruits, while it was 66.67% in coated dragon fruits. However, the incidence of disease in coated dragon fruits was not significantly lower than in control dragon fruits. Similarly, no significant differences in disease severity were observed between control and coated dragon fruits. These results demonstrated that the composite coating based on 1% chitosan and 0.2% κ-carrageenan showed the potential to control symptoms of disease in dragon fruits, but not with strong effect. Consistently, Shi *et al.* (2018) found that chitosan treatment did not significantly reduce the disease incidence caused by *Penicillium digitatum* in grapefruit during prolonged storage. Arnon *et al.* (2014) also reported that the carboxymethyl cellulose-chitosan bilayer coating did not provide any effect on reducing decay in four citrus fruit cultivars. According to Wang *et al.* (2011), the chitosan solution exhibited good antimicrobial activity on nutrient agar medium, while the chitosan film did not have a remarkable effect. When chitosan binds within the film matrix, no diffuse antimicrobial agent is produced. However, these are in contrast to reports that the chitosan coating reduced postharvest decay in avocados and Chinese cherries (Xin *et al.,* 2017; Obianom *et al.,* 2019).

CHI and GLU are two pathogenesis-related proteins (PR proteins) that accumulate in plant tissues after the invasion of pathogens (Ferreira *et al.,* 2007). Figures 7a and 7b show that CHI activity was not found in control dragon fruits during storage, but was detected at a low level in dragon fruits coated with chitosan and κ-carrageenan composite coating. However, the significant difference in CHI activity between control and coated dragon fruits was observed only on day 18. As shown in Figures 7c and 7d, the highest GLU activity was observed in coated dragon fruits after 24 h of treatment, but it was not significantly different from that in control dragon fruits. In the last stage of storage, the GLU activity in dragon fruits was quite low, although a significant difference was observed between the two samples on day 6 and 12. It is known that CHI and GLU show an inhibitory action against fungal pathogens. Obianom *et al.* (2019) reported that chitosan treatment-induced CHI activity in avocado, which contributed to the

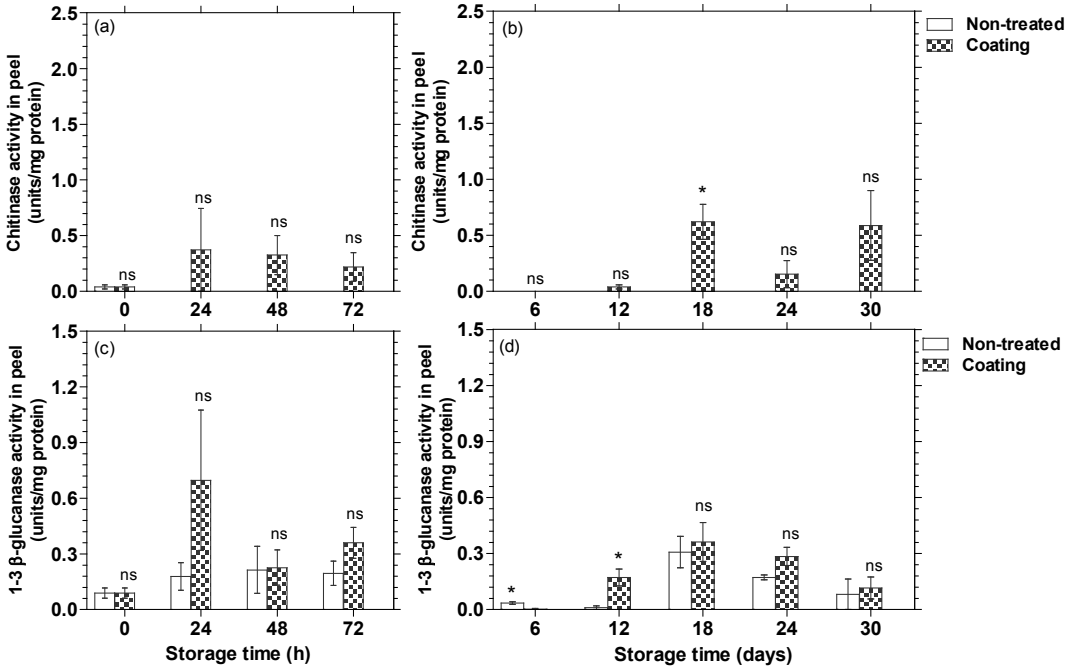

Figure 7. Changes in activities of (a, b) CHI and (c, d) GLU (n = 15) in dragon fruits coated with chitosan- and κ-carrageenan-based composite coating, as compared to control dragon fruits, during storage at 10°C. Vertical bars represent the standard error (SE) of mean for triplicates. ns = not significant; and *significant at $p < 0.05$ by Fisher's LSD test.

control of stem-end rot. However, although CHI and GLU show antifungal activity, some forms of these PR proteins do not have or appear to lack in this activity. Some PR proteins can release elicitor molecules from plant cells, and stimulate the biosynthesis of secondary metabolites such as phenolics (Vidhyasekaran, 2008). In the present work, as compared to control dragon fruits, the activities of CHI and GLU in coated dragon fruits were slightly increased, but did not have a significant effect on alleviating the disease symptoms of dragon fruits.

With an effectiveness on the freshness of the dragon fruits and their bracts, the coating composed of chitosan and κ-carrageenan could be a crucial step in the post-harvest handling of dragon fruits due to its safe, practical, and economical procedure. However, it may be necessary to further investigate to improve post-harvest disease control during long-term low-temperature storage.

Conclusions

The present work demonstrated that the composite coating based on chitosan and κ-carrageenan could reduce the physiological weight loss, improve the accumulation of phenolics, and maintain the titratable acidity without affecting other physicochemical attributes in the dragon fruits stored at 10°C. Also, the formulated coating could suppress the activities of the major chlorophyll-degrading enzymes including Chlase and Chl-POX, resulting in the retention of the bracts green colour. However, the coating did not effectively induce CHI and GLU activities or control postharvest disease in the dragon fruits. An alternative technology to combine with the composite coating should be investigated to maintain the overall quality of the dragon fruits.

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