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***Abstract*—** The effect of calcium carbonate ($CaCO_3$)-nanoparticles-longkong peel extracts (LPE) coating on quality and browning of longkong after harvesting were evaluated. Edible coating (carrageenan) was used as a component of 2 mM $CaCO_3$ and 2.00 mg LPE. Longkongs were coated with 0 (control), 1 and 2% carrageenan and then stored at 13˚C and 90-95% relative humidity for 14 days. Longkong were analyzed for changes in browning pigment, L* value, pH, total phenolic content, activities of polyphenol oxidase (PPO) and total sugar. The results showed Longkong coating with 1 and 2 % carrageenan (CGN) lower browning pigment, which correlated with a decrease in PPO and total phenolic content than control. However, longkong coating with 1% CGN delayed browning more than longkong coating 2% CGN during 10 days of storage. While the sugar was significantly higher in control compared to 1 and 2% CGN. Longkong treated with 1 and 2% CGN showed no significantly a decrease in pH when compared to the control fruit.

***Keywords*—** Calcium carbonate, Longkong peel extract, coating, browning, polyphenol oxidase

I. INTRODUCTION

The major problems of longkong are rapid pericarp browning and desiccation a few days after harvesting. Because of its susceptibility to browning and desiccation, it is difficult to keep longkong in good appearance at room temperature without proper postharvest conditions. Among noble-metal nanomaterials, silver nanoparticles have received positive attention because their desirable physicochemical properties. It is well known that nanomaterials have attracted increasing interest because of their potential impact on an incredibly wide range of food industry [1]. Nano-$CaCO_3$ polymer reduced oxygen permeability an d improved packaging operation [2][3]. Longkong peels that are naturally rich in polymers such as phenolic, lignin and antioxidants could be used in the synthesis of nanoparticles. Even though the coating has been studied extensively to increase the shelf life of many fresh fruits, this research provides the only information regarding the application of nano-CaCO3 longkong peel extracts coating for longkong fruits. The objective of this research was to evaluate the effect of calcium carbonate (CaCO3)-nanoparticles-longkong peel extracts (LPE) coating on quality and browning of longkong after harvesting stored at 13ºC.

II. EXPERIMENTAL METHODS

A. Longkong peels extract (LPE) powder preparation

Longkong peel were prepared longkong peels (*A. dookkoo* Griff.) (120 fruit) were obtained, washed and boiled in distilled water for 30 min at 90ºC. Such peels (100 g) were crushed in 100 ml of distilled water and the extract formed was filtered through a cheese cloth. This filtrate was treated with equal volumes of chilled acetone and the resulting precipitate was centrifuged at 1000 rpm for 5 min. This was air-dried into a powder and used for further experiments [4].

B. Prepare $CaCO_3$ and coating

$CaCO_3$ was prepared by dissolving 1 mM $CaCO_3$ in 100 ml (w/v) distilled water and heating at 70 ºC while stirring until the solution became clear.

Carrageenan coating were dissolving at the following concentrations: 0% (control), 1 or 2 % (w/v) in 100 ml in distilled water, respectively.

Calcium carbonate-nanoparticles longkong peel extract were then incorporated into $CaCO_3$ content at a level of 1.0 mM (1.0 mM $CaCO_3$ at pH 3.0) This reaction condition included incubation at 80 ℃ in a water bath for 3 min.

Carrageenan coating solutions at the following concentrations: 0% (control), 1.0 or 2.0% of carrageenan coating solutions were added to a calcium carbonate-nanoparticles longkong peel extract. The longkong was coated by immersing for 1 min. They were dried at room temperature for 1 h and then immediately transferred to refrigeration storage at 13 °C. Data was recorded every 2 days for 14 days. Each treatment contained 120 fruits/3 replicates.

C. Analysis

1). Determination of fruit browning

Pericarp browning was estimated by measuring the extent of total browned area on each fruit surface with the following scores: 1= no browning, 2= <20% of peel surface, 3= 20-40% of peel surface, 4= 40-60% of peel surface and 5= >60% of peel surface.

2). Determination of peel color changes

Peel color changes in the middle part of longkong was determined by measuring the L value with a Minolta colorimeter (Model RC-300, Minolta Co. Ltd., Osaka, Japan) and expressed as a lightness value (L* value).

3). pH

The pH of the pericarp tissue was determined by the method according to Underhill and Critchley [5].

4). Total phenolic content

Quantification of the total phenolic content was carried out using the method proposed by Singleton et al [6]. The extraction was separately prepared from the top (around calyx), middle and bottom part of longkong peel. Two grams of each peel section were homogenized with 20 mL of 80% ethanol for 1 min. The extract was filtered and centrifuged at 10,000 × g for 15 min. One mL of the supernatant was mixed with 1 mL of Folin Ciocalteu reagent (SigmaAldrich, Buchs, Switzerland) and 10 mL of 7% sodium carbonate. This was increased to 25 mL with distilled water and left to settle for 1 h. The absorbance was then read at 760 nm by a spectrophotometer (UV- 1601; Shimadsu Co., Kyoto,. Japan). A standard curve of gallic acid was used for quantification of total phenolics.

5). Extraction and assay of PPO activities

Pericarp tissues (2 g) from 20 fruit were homogenized in 20 mL of 0.05 M phosphate buffer (pH 7) and 0.2 g of polyvinylpyrrolidone (insoluble) at 4°C. After filtration of the homogenate through a cheese cloth, the filtrate was centrifuged for 20 min at 19,000 × g and 4°C. The supernatant was then collected for PPO activity assays as the crude enzyme extract. PPO activity was assayed by measuring the oxidation of 4-methylcatechol as the substrate, according to the method of Jiang [7]. Change in absorbance at 410 nm was measured by a spectrophotometer (UV-1601; Shimadsu Co., Kyoto,. Japan). One unit of PPO activity was defined as a change of 0.001 in absorbance per minute.

6). Total sugar content

The quantification of the total sugar was carried out using the method proposed by Phenol-sulfuric method [8]. One gram of tissue longkong was mixed with 10 ml 80% ethanol and incubated in water bath at 60℃ for 1 hour. After that the solution was filtered with Whatman No. 4. One ml of the reagent solution was added with 1 ml of 5% phenol and 1 ml of 100% sulfuric acid and left to settle for until cool. The total sugar content was then read at 490 nm by a spectrophotometer (Shimadzu UV-1750). A standard curve of D-glucose was used to quantify the total sugar content.

D. Statistical Analysis

The experiments were repeated three times, and the results were analyzed using SPSS program at the significant different at 95% confident level.

III. RESULTS AND DISCUSSION

The browning of the longkong peel from all treatments increased gradually over 10 d of storage and then rapidly decreased until the end of storage (Fig. 1).

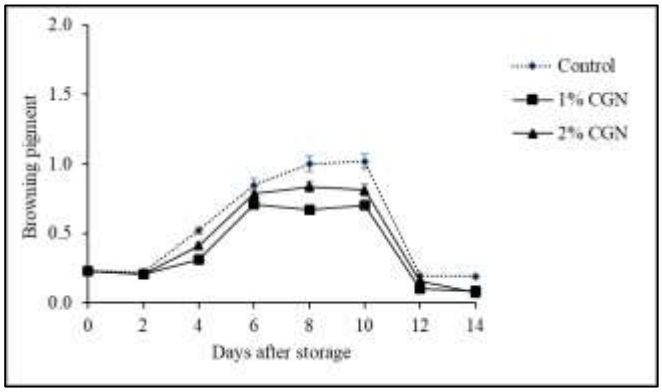

Fig. 1 Browning pigment of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM CaCO3 and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

A browning of control fruit was higher than 1.0 and 2.0 % CGN of fruit although the storage. Browning of the longkong coated with carrageenan at 1.0 and 2.0 % delayed the browning of longkong after 2 d of storage. The browning pigment of longkong coated with carrageenan at 1.0 % was 0.67, significantly lower than the control on 8 d of storage. Edible film made carrageenan possess good mechanical properties, enable stabilization of emulsions and reduction of oxygen transfers, but their highly hydrophilic nature limits the ability to provide a significant moisture barrier [9].

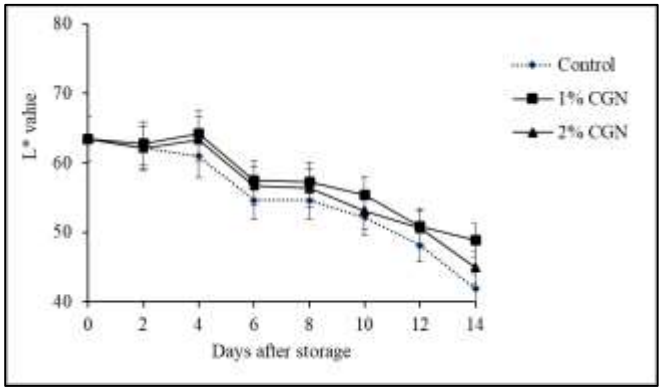

Fig. 2 L* value of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM

$CaCO_3$ and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

The longkong peel of control fruit had lower L* value than 1.0 and 2.0% CGN of fruit during storage (Fig. 2). L* value of all treatments were decreased with relation to browning pigment were increased until the end of storage. Longkong fruit of control featured an increase in browning as evidenced by a decreased in L* value [10]. The increase in longkong browning was might be the oxidation of phenolics by PPO enzyme with can be active by air induced fruit surface damage [11].

The pH of the longkong fruits decreased in all treatments during the 8 days and then their maintained until the end of storage (Fig. 3).

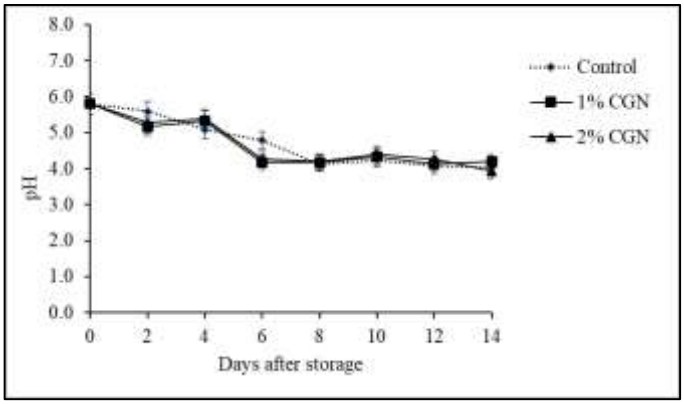

Fig. 3 pH of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM $CaCO_3$ and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

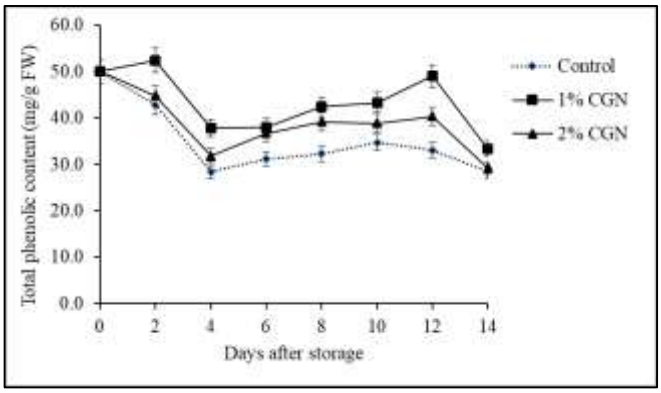

Fig. 4 Total phenolic content of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM $CaCO_3$ and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

The total phenol content from all treatments decreased in the first 4 d, followed by a rapid increase until to 12 day and decreased in the end of storage (Fig. 4). The total phenolic content of the longkong fruits coated with 1% CGN was significantly higher than that of fruits coated with at the onset of the experiment. The postharvest browning of fruit and vegetables is also related to the synthesis of phenolic compounds, which are oxidized into quinines and polymerized into brown polymers [12]. As show in Fig. 4, the initial total phenolic content of the longkong fruits coated with 1% CGN was considerably higher than that in the control (P<0.05); after 2 d of storage, the fruits coated with 1% CGN had the highest total phenolic content, at 52.40 mg/g FW.

Enzymatic browning in fruits and vegetables is assumed to be a consequence of the reaction between polyphenol substrates and oxygen with PPO and POD. The primary enzyme responsible for the browning reaction is PPO, which is able to catalyse the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to their corresponding o-quinones [13]. The PPO activity in the treatments with 1.0 and 2.0 % was lower than that of the control samples until the end of storage (Fig. 5).

PPO is a key enzyme for enzymatic browning in many fruits. The latent form of PPO is often activated during ripening, senescence or stress conditions when the membrane is damaged, which results in an increase in PPO activity [14]. The PPO activity in longkong fruits increased during the first 4 d of storage. However, at the end of storage, the PPO activity in the treatments with 1.0%CGN was lowest at 0.76 units/mg protein.

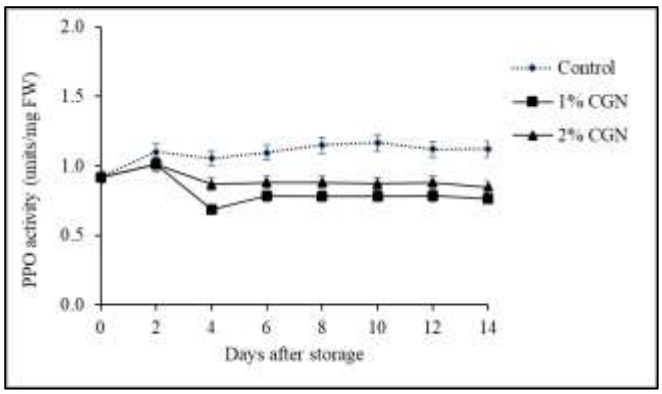

Fig. 5 PPO activity of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM $CaCO_3$ and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

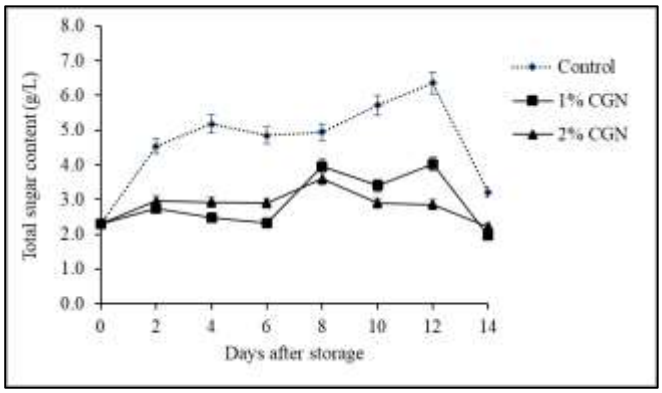

Fig. 6 Total sugar content of longkong coated with 0, 1 and 2% carrageenan LPE of reaction mixtures containing 1.0 mM $CaCO_3$ and 2.0 mg LPE powder, followed by storage at 13 °C and at a relative humidity of 90±5%.

The total sugar of control fruit was significantly increased more than 1 and 2 % CGN of longkong fruit (Fig. 6). The increase in sugar was probably due to the solubilization of neutral sugar from carbohydrate polymer residues [15].

IV. CONCLUSION

Longkong fruits coated with 1.0 and 2.0% CGN delayed browning and decreased the activities of PPO during storage. Longkong fruits coated with 1.0 and 2.0% CGN had a higher total phenolic content than that of the control. Longkong fruits coated with 1.0% CGN delayed browning more than longkong coating 2%CGN during storage.

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