

Quality changes of Hoa Loc mangoes (*Mangifera indica* L.) during storage: Effect of chitosan-based nano-silver film coating

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ABSTRACT

The aim of this study was to evaluate the possibility of storage of mangoes (*Mangifera indica* L.) coated with chitosan-based nano-silver films. Chitosan-based nano-silver films were first made using chitosan with three degrees of deacetylation of 70, 80, and 90%. The films were then used for coating the mango fruits. All coated mangoes and the control samples were stored at 12 °C in a cooling room. The respiration rate, ethylene production rate, weight loss, firmness, total soluble solids, total acid, vitamin C, total sugar, and change of peel colour were evaluated once every 5 days during the storage period. As observed, the coating using the chitosan-based nano-silver films did not induce significant negative alterations on most of the physical characteristics and chemical constituents of the fruits. In addition, the coating using those materials helped reduce the respiration and the ethylene gas production and retard the ripening process of mango fruits. The chitosan-based films with higher deacetylation degrees (CN80 and CN90) better moderated the respiration of mangoes. The coating using chitosan-based nano-silver films prolonged the shelf-life of mangoes (up to 35 days) compared to the control (less than 10 days).

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KEYWORDS

chitosan-based nano-silver film, coating, deacetylation degree, *Mangifera indica*, mango

1. INTRODUCTION

Chitosan-based film can protect food products, including fruits, efficiently. The film makes a protective layer around food products with good selective permeability to CO₂, O₂, and ethylene, while controls respiration process of fruits and reduces condensation of water vapour. Chitosan is a positively charged bio-polymer with antimicrobial activity depending on pH value, molecular weight, and cross-linkage (Elsabee and Abdou, 2013). Chitosan solution possesses a high stability for a long period, however, if the pH value is not maintained, the antibacterial activity of chitosan will decrease (Alishahi and Aïder, 2012).

Commercial chitosan exists in a flake form with diversified deacetylation degrees, average of 80%. Chitosan is a biopolymer with large molecular weights of about 10–1,200 kDa depending on production conditions. Chitosan is biodegradable, non-toxic to the environment and humans, so it has potential of being used in many applications (Ong et al., 2008; Aranaz et al., 2009).

Currently, thanks to the advanced development of technology, the production of antibacterial substances using silver as a main constituent has advanced to develop new types of products like nanoscale particles (Metak, 2015; Huang et al., 2018; Hoffmann et al., 2019). Silver nanoparticles are reported as non-toxic and non-tolerant disinfectant (Dastjerdi and Montazer, 2010). The nano-silver crystals easily penetrate the cell to inhibit the growth of bacteria. Silver's antiseptic activity is dependent on nanoparticle size. As reported, the 10 nm-sized particles show strong interactions with bacteria (Morones et al., 2005). Apart from possessing the antimicrobial activity, silver nanoparticles can catalyse the absorption and decomposition of ethylene produced from fruit metabolism, it has been postulated as an ethylene blocker. Fruit ripening and rotting caused by ethylene gas can, therefore, be retarded with the extension of product shelf-life (Huang et al., 2015). Rhim et al. (2006) and Zhao et al. (2011) mentioned potential applications of nanotechnology in the development of natural biopolymer-based biodegradable packaging materials with additional bioactive function. According to Almeida et al. (2015), packaging materials developed using nanotechnology will possess stronger mechanical properties and reduced hydrophilic properties. Silver and nano-silver show a better capacity over other substances for binding to biological materials (starch, chitosan, cellulose) to produce packaging materials. A study comparing the shelf-life of silver-with zinc nanoparticles in orange juice stored at 4 °C concluded that zinc nanoparticles had a much shorter shelf-life compared to silver nanoparticles (112 days) (Beigmohammadi et al., 2016).

Mangoes originated from India, Myanmar, and other Southeast Asian countries. It is grown widely in Vietnam. According to the Statistical Yearbook of Vietnam (2019) (G.S.O, 2020), the total production area of mango of Vietnam in 2019 was 104,700 hectares (ha) with a production volume of about 839,000 tons. Among mango varieties in Vietnam, Hoa Loc mango is ranked the first for its quality. Hoa Loc is large in size with fruit weight around 350–400 g. When ripe, the fruits are characterised by their thin peel with lemon yellow colour, bright yellow flesh, and they are thick, sweet, and fragrant. Mango is a climacteric fruit, with 20–30% postharvest loss



each year due to short shelf-life and fast ripening (Moss, 2002). Fungal spoilage is a main cause of postharvest loss of mangoes during storage and transport.

In this study, chitosan-based nano-silver films were developed using chitosan with three degrees of deacetylation 70, 80, and 90%. The films were used for coating Hoa Loc mangoes. At the final step, the effects of the coating using chitosan-based nano-silver films on mango quality during storage were evaluated.

2. MATERIALS AND METHODS

2.1. Materials

Chitosans with the degree of deacetylation (DD) of 70, 80, and 90% derived from shrimp peel were donated by a laboratory of Nha Trang University (Nha Trang city, Vietnam). Silver nitrate (AgNO_3 99.9%), sodium citrate dihydrate ($\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ 99%) were supplied by Merck (Missouri, USA). Acetic and citric acids and other chemicals were of analytical grade. Hoa Loc mango fruits (*Mangifera indica* L.), 400–450 g each, VietGAP certified, a very delicious and famous mango variety in Vietnam, were purchased from a mango farm in Tien Giang province, Vietnam. The fruits were collected, washed, packed, and stored at 12 °C for all experiments. Three fruits were selected and a homogenised mixture was taken for each parameter measurement.

2.2. Nano-silver solution preparation

Silver nitrate (0.0425 g) and deionised water (~45 mL) were added to a cleaned and dried 50 mL volumetric flask with continuously stirring to make up a volume of 50 mL of 0.005 M solution. The solution was then thoroughly mixed and boiled, during which 5 mL of sodium nitrate 1% stock solution was added drop by drop until a pale yellow colour appeared.

2.3. Chitosan solution preparation

Samples of two grams of chitosan powder (DD of 70, 80, or 90%) were soaked for 48 h in flasks containing 200 mL of 1% acetic acid/sodium acetate buffers (pH 3.6 for chitosan of 70% DD and 3.8 for chitosan of 80 or 90% DD). The mixtures were then stirred at 50 °C for 30 min using hot plate magnetic stirrer.

2.4. Chitosan-based nano-silver film preparation

Nano-silver solution 0.005 M (75 μL) was well mixed with chitosan 1% solutions (100 mL) of three DD of 70, 80, or 90%. The mixtures were kept stable for 10 min and cast onto glass plates (25 × 25 cm) to form 0.02 mm thick films. The films were dried at 40 °C for ~36 h, stabilised at 12 °C for 24 h and stored for further use.

2.5. Mango storage

The mango storage were conducted at 12 °C for the control sample (uncoated, M0) and the samples coated using the chitosan-based nano-silver films with three different DDs of 70, 80, and 90% (CN70, CN80, and CN90). During the course of storage, physico-chemical attributes



and sensorial quality (firmness, soluble solid-, total acid-, vitamin C-, total sugar contents) of mango fruits were determined once every five days.

2.6. Statistical analysis

The experiments were arranged randomly, repeated three times. Data were collected and processed using Statgraphics Centurion 15.2 software. Analysis of variance (ANOVA) was performed according to LSD test to conclude the difference between the experimental mean at a significance level of 5%, with the assumptions of the model as normal distribution of the errors or residuals, positive variances, and independency. Principal component analysis (PCA) approach using XLSTAT software was performed for multivariate data analysis.

3. RESULTS AND DISCUSSION

3.1. Respiratory intensity and ethylene production of Hoa Loc mango during storage

Mango fruits were first washed for one minute using hot water at 50 °C. After draining water off their surfaces, the fruits were coated with chitosan-based nano-silver films of different DDs of 70, 80, and 90%. The coated mangoes of all experimental set-ups and the control mangoes were stored in a cooling room at 12 °C for storage investigation. During the storage, the respiratory intensity of mango fruits was determined using the gas analyser ICA-250 (UK). As observed in Fig. 1A, the control mangoes (M0) showed a peak of respiration on the 15th day of storage, while the coated mangoes using chitosan-based nano-silver films of different DDs of 70, 80, and 90% (CN70, CN80, and CN90) shifted the respiratory peaks forward a period of 10 days, to the 25th day. The results of this study are in line with the findings of Zhu et al. (2008), when they investigated the effect of chitosan on the post-harvest quality of mangoes.

The experimental results showed that the respiratory intensity of the control mango (M0) increased rapidly and reached the earliest respiratory peak on the 15th storage day with the highest peak value 56.35 mL CO₂ kg⁻¹ h⁻¹. Meanwhile, the respiratory peaks of the coated mangoes CN70, CN80, and CN09 showed up on the 25th storage day with the peak values of 53.87, 51.21, and 51.12 mL CO₂ kg⁻¹ h⁻¹, respectively. It revealed that the fruit coating using chitosan-based nano-silver films retarded ripening and reduced respiratory rate of mango fruits during storage compared to the control.

As another important indicator, the amount of ethylene gas produced during the storage of mangoes was determined using the ethylene tester ICA-56 (Japan). The experimental results showed that the ethylene amount produced by the control mango (M0) reached the highest level of 45.63 mL C₂H₄ kg⁻¹ h⁻¹ on the 15th day, while those produced by the coated mangoes CN70, CN80, and CN90 achieved their lower peaks at values of 43.10, 42.04, and 41.27 mL C₂H₄ kg⁻¹ h⁻¹ on the 25th day, respectively (Fig. 1B).

Comparing the graphs in Fig. 1A and those in Fig. 1B reveals that the storage time (days) at which the rate of ethylene production reached the peak value coincided with the storage time when the respiratory intensity reached the highest value for all four samples (M0, CN70, CN80, and CN90). In the experimental set-ups, the films as coating materials created a closed environment, reduced water loss from fruits. Besides, according to Huang et al. (2015), silver nanoparticles catalysed the absorption and decomposition of ethylene produced from fruit



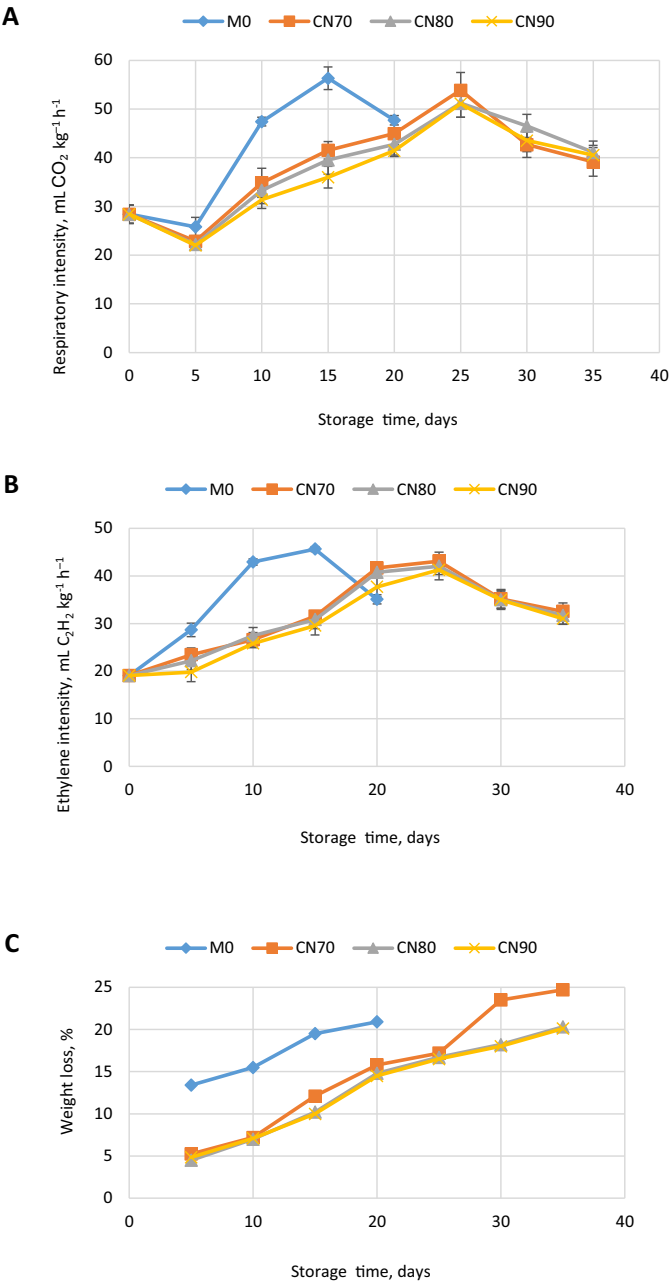


Fig. 1. A: Respiratory intensity; B: Ethylene production; and C: Weight loss of Hoa Loc mangoes coated by chitosan-based nano-silver films of three degrees of deacetylation of 70, 80, and 90% (CN70, CN80, and CN90) and the control (M0) stored at 12 °C



metabolism, therefore, it retarded the ripening of fruits. These findings are in line with those reported by Jongsri et al. (2016), Cosme Silva et al. (2017), and Parvin et al. (2023) on the effect of chitosan film coating on storage quality and physicochemical properties of mango.

3.2. Effect of chitosan-based nano-silver film coating on the quality of Hoa Loc mango during storage

3.2.1. Weight loss. As plotted in Fig. 1C, the weight loss of all samples increased during storage. In more detail, the weight loss of the control sample was much higher than those of the three coated samples. Thanks to the chitosan-based nano-silver film coating, the coated mangoes CN70, CN80, and CN90 could be stored up to the 35th day, at that moment their weight loss increased up to values of 24.7, 20.3, and 20.1%, respectively. As previously mentioned, the chitosan-based nano-silver films can help reduce water loss from fruits by controlling water vapour passing through the films. Also from Fig. 1C, the chitosan-based nano-silver films with the DD of 80 and 90% showed a better control of fruit weight loss compared to that with the DD of 70%. Shao et al. (2012) reported that the chitosan coating combined with heat treatment minimised weight loss and respiration rate of apple.

The data analysis showed significant differences (P -value < 0.05) in the mango weight loss of all four mango samples by the end of storage. On another way of ANOVA, the mango weight loss of the control and those of the coated samples were also significantly different as compared to each other.

3.2.2. Fruit firmness. Firmness is an indicator for investigation of the maturity level of mangoes during storage period. The decrease in fruit firmness during storage is caused by the converting of starch to sugars, the breaking of cell wall, and the changing of insoluble protopectin to soluble pectin. In this study, the firmness of mangoes was determined using a firmness tester (TR Turoni, Italy) with a stainless steel spherical probe, at a speed of 5 mm s⁻¹. Firmness was reported as the force in kilogram (kg) needed for a penetration of 10 mm through the flesh of mango (Robles-Sanchez et al., 2009). The graphs in Fig. 2A show that the firmness of the control and the coated mangoes abated in the progress of storage period. In more detail, the firmness of the control mango (M0) abated hastily compared to those of the coated samples (CN70, CN80, and CN90).

During the course of storage, the firmness of the control mango (M0) abated hastily from the initial to the 5th day (52.4% reduction), thereafter it reduced more slowly, while those of the coated mangoes (CN70, CN80, and CN90) abated 23.1–27.8% in the same period. Concerning the coated mangoes, their firmness abated more slowly, while their shelf-life lasted for 35 days. This result is in line the findings of Cissé et al. (2015), who investigated the preservation of mango quality using functional chitosan-lactoperoxidase systems coatings.

3.2.3. Titratable acidity. As shown in Fig. 2B, the titratable acidity of all mango samples abated during storage period. As the titratable acidity of mangoes decreased during fruit ripening, the fruits became sweeter. At the 35th day of storage, the total acid content of the three coated mango samples CN70, CN80, and CN90 continued decreasing to levels of 0.45, 0.50, and 0.53%, making up final acid reduction of 58.7, 54.1, and 51.4% (P -value < 0.05), respectively. These results are supported by the findings of Khairul Islam et al. (2013), who investigated the storage



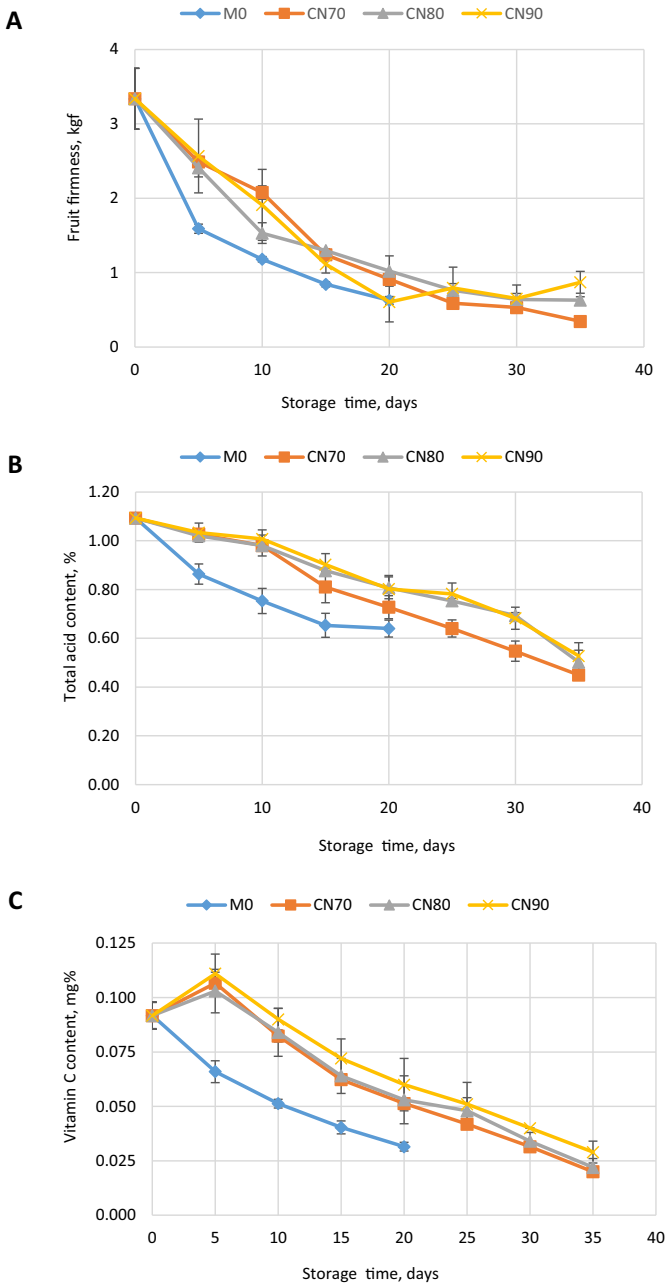


Fig. 2. A: Reduction of firmness; B: total acid content; and C: vitamin C content of Hoa Loc mangoes coated by chitosan-based nano-silver films of three degrees of deacetylation of 70, 80, and 90% (CN70, CN80, and CN90) and the control (M0) stored at 12 °C



of mangoes of two varieties, Khirshapat and Langra. These authors stated that the total acid content of mangoes decreased sharply after three days of storage and stabilised after 12 days of storage.

3.2.4. Vitamin C content. The results showed that the vitamin C content of the control sample (M0) decreased rapidly during storage, while those of the three coated samples (CN70, CN80, and CN90) diminished slightly slower (Fig. 2C). Observing until the 35th day, the vitamin C content of the three coated samples remained 21.7, 23.9, and 31.5% (P -value < 0.05). Statistically, for the whole period of storage, there was a significant reduction in the vitamin C content of the stored mango samples.

3.2.5. Total soluble solids. As plotted in Fig. 3A, the total soluble solid content (TSS) of mangoes tended to increase gradually during storage period. The control sample's TSS increased

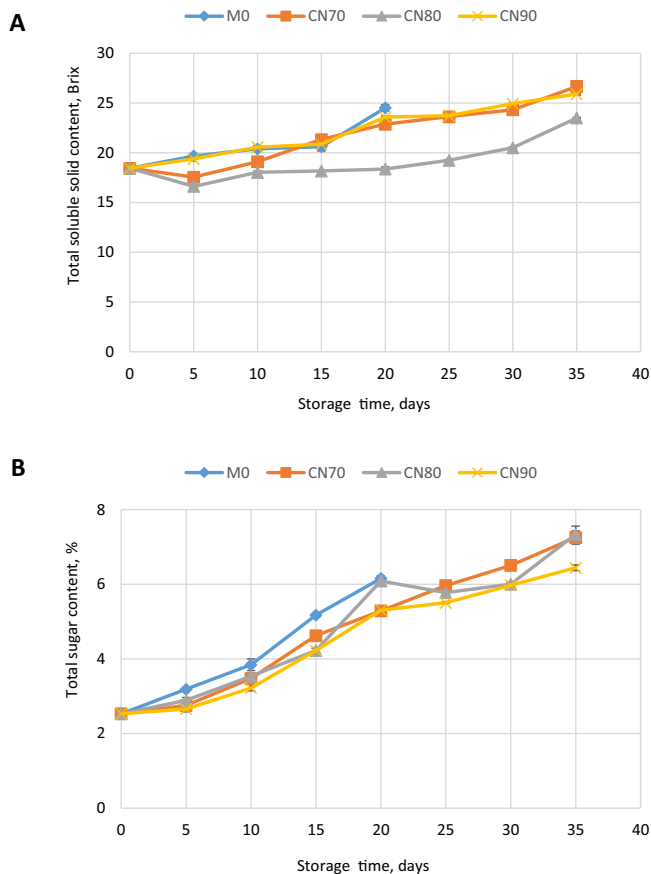


Fig. 3. A: Change of total soluble solid content; B: total sugar content of Hoa Loc mangoes coated by chitosan-based nano-silver films of three degrees of deacetylation of 70, 80, and 90% (CN70, CN80, and CN90) and the control (M0) stored at 12 °C



faster than those of the coated mangoes. These findings are supported by the report of Ueda et al. (2000) that during the postharvest storage of mangoes, the content of dissolved dry matter increased and then remained stable at the end stage.

3.2.6. Total sugar content. As plotted in Fig. 3B, the total sugar content (TSC) of all mango samples increased continuously during storage period. The increase in TSC is due to conversion of complex starch or carbohydrate into simple compound. These results are in line with the findings of Khairul Islam et al. (2013). With regard to the effect of coating, there was a statistically highly significant difference between the coated and uncoated mangoes in terms of the TSC of pulp at different days of storage. The control mangoes generated a higher amount of TSC than the coated mangoes during storage period. TSC in the three coated mango samples increased more slowly and steadily.

Multivariate data analysis was performed using XLSTAT (Academic & basic version, Addinsoft, 75,018 Paris, France) (Figs 4 and 5). In the bi-plot of Fig. 4, fruit attributes grouped close to each other can be considered to have similar trends of change during storage, whereas the increasing distance between them indicates a larger difference in trends of change during storage. It can be observed (Fig. 4) that respiratory intensity, ethylene production, and weight loss of mango fruits during storage are considered being in close relation, while vitamin C, total acid content, and firmness of the fruits are noticed to be in good correlation.

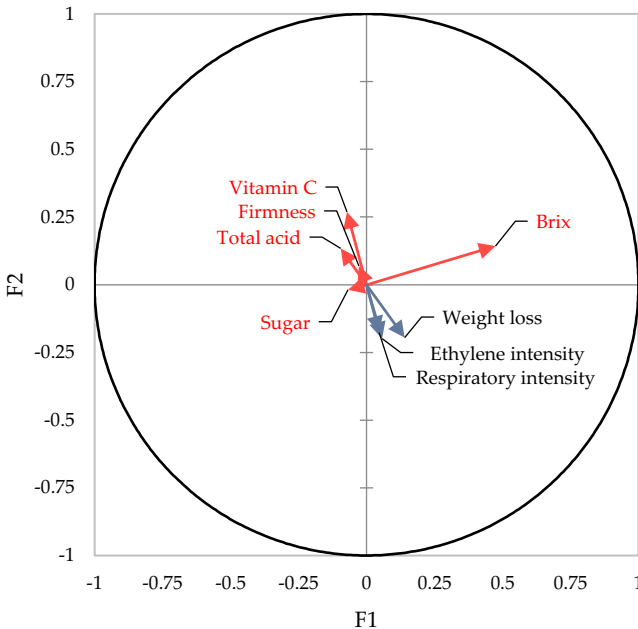


Fig. 4. A bi-plot based on PLS-DA model selected to describe the relationship of the fruit attributes of Hoa Loc mangoes during storage



In order to compare the impact of coating treatments on the quality characteristics of mangoes, Partial Least Square Discriminant Analysis (PLS-DA) was applied, where the mango fruit attributes were considered as X-variables and the coating treatments (CN70, CN80, CN90, and M0) as categorical Y-variables (i.e. classes). Figure 5 shows the corresponding PLS-DA bi-plot for the coating treatments CN70, CN80, and CN90 versus the control (M0). The separation between the treatments in the bi-plot signifies the relative differences between their fruit attributes, which clearly shows the impact of the coating compared to the control (without coating).

3.3. Spoilage rate of Hoa Loc mango during storage

During storage, black spots appearing on the peel of mangoes were considered as an indicator of fruit spoilage. Table 1 shows the spoilage level of the control and the coated mango samples

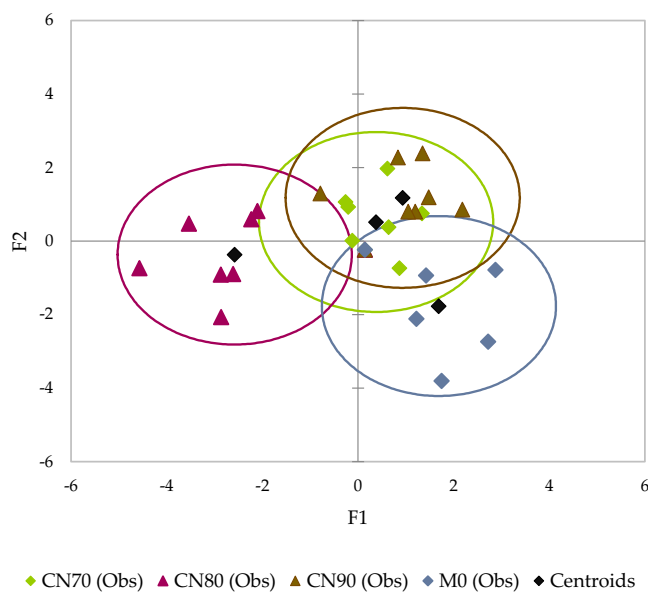


Fig. 5. A bi-plot describing the effect of coating using chitosan-based nano-silver films of three degrees of deacetylation of 70, 80, 90% (CN70, CN80, CN90) and the control (M0) on mango fruits during storage

Table 1. Spoilage levels of different mango samples during storage period, %

Coating treatment	Time of storage, days							
	5	10	15	20	25	30	35	40
M0	0	7.8	19.2	61.6	100	–	–	–
CN70	0	0	0	0	0	0	0	38.2
CN80	0	0	0	0	0	0	0	25.6
CN90	0	0	0	0	0	0	0	19.1



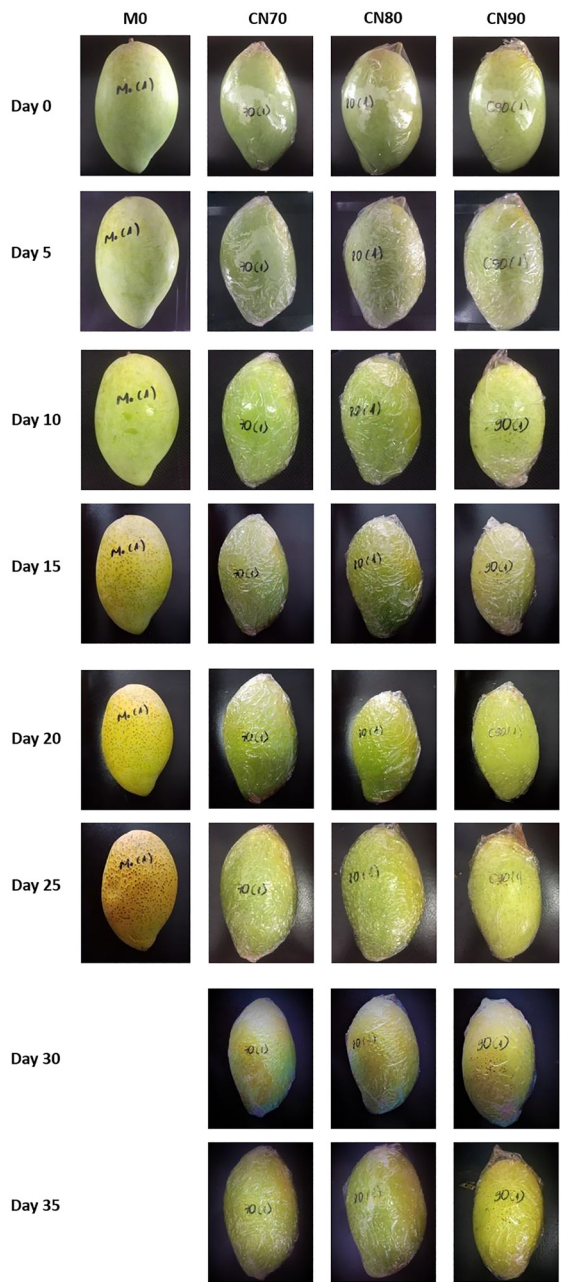


Fig. 6. Colour changes of the peel of Hoa Loc mango during storage



during storage. As observed, until the 5th day, no black spot was noted for any control and coated mangoes. However, at the 10th day, the black spots first time showed up on the peel of some control mangoes (M0), accounting for 7.8% of the uncoated samples. Continuously from the 10th day, black spot level of the control samples increased significantly in a continuous manner, expanding with storage duration, and reached the value of 100% at the 25th day. In contrast to the control, black spots did not show up on the peel of any of the three coated samples (CN70, CN80, and CN90) until the 35th day. However, after the 35th day of storage, the black spots started to show up rapidly on the peel of all three coated samples, accounting for spoilage levels of 38.2, 25.6, and 19.1% for the coated samples CN70, CN80, and CN90 at the 40th day, respectively (Table 1). The data in Table 1 show that the higher the deacetylation degree of the chitosan-based nano-silver film the lower the spoilage level of the coated samples. With the application of the chitosan-based nano-silver films coating, mango fruits can be stored and/or transported and/or marketed at 12 °C for a total period of 35 days.

3.4. Change in peel colour

The colour of mango peel is one of selection criteria for consumers as it can indicate the status of fruit ripeness. During the course of storage, the mango peels of all samples were photographed once every 5 days to keep track of their changes. Figure 6 presents the photos of all mango samples during storage.

During the storage period, the peel colour of the uncoated and coated mangoes changed from green to orange-yellow. From our observation, the peel colour of the control mangoes (M0) changed quickly from green to yellow (Fig. 6) in concurrence with the formation of the ethylene peak. For the coated mangoes, the peel colour turned from green to yellow very slowly. It was clear that the chitosan-based nano-silver films of different DDs (CN70, CN80, and CN90) used as coating materials helped delay the fruit ripening and the colour change of the peel. As shown in Fig. 6, at the 35th day, the peel colour of the coated mangoes mostly remained yellow-green. During the ripening, chlorophyll is broken down and other pigments are synthesised. The orange-yellow colour of the mango is the dominant carotenoid group, which made the green colour of the mango disappeared and formed its characteristic yellow colour (Medlicott et al., 1990).

4. CONCLUSIONS

Coating Hoa Loc mango fruits using the chitosan-based nano-silver films of three degrees of deacetylation of 70, 80, and 90% is able to extend the shelf-life of the fruits up to 35 days, compared with about 10 days of the control. The coating using the chitosan-based nano-silver films does not induce significant negative alterations on most of the physical characteristics and chemical constituents of the fruits. In addition, the coating using those materials helps reduce the respiration rate, limit the ethylene gas production, retard the ripening process of mango fruits, and make very positive effect on the inhibition of black spots development on the skin of the fruits. Higher deacetylation degree chitosan-based films (CN80 and CN90) better moderate the respiration of mango. It reveals that the chitosan-based films with high deacetylation degree (80–90%) possess a high potential in preservation of mango fruits, which belong to the climacteric fruit family.



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