

RESEARCH ARTICLE

Preparation of fibroin/chitosan based preservatives for longan postharvest preservation

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Hanoi University of Mining and Geology, Grant/Award Numbers: Key Research group, HUMG-GCP

Abstract

During the last decades, the clean food regulation in the E.U. and the U.S.A., concerning the contents of toxic pesticides and preservatives in fruits has become increasingly strict, therefore, an organic preservation method is needed. Fibroin/chitosan based composite preservatives (FCCPs) for longan postharvest preservation were prepared from silk fibroin. The tensile stress at break and water uptake, which govern the efficiency of FCCPs, were adjusted by controlling the amount of polyvinyl alcohol (PVA) and beeswax additives. To preserve longans, thin films of FCCPs were coated on the fruit peels. The results showed that fibroin/chitosan films extended shelf life by about 30 days, reduced browning index and spoilage rate percentage, retarded weight loss and respiratory rate, inhibited the decrease of vitamin C, total acid and total sugar content of fresh longan fruit. The lowest loss weight and spoilage rate were observed in the longan sample coated with composite (Fib–Chi–PVA–BW) coating. Its loss weight and spoilage rate after 20 days were 3.63% and 1.71%; after 30 days were 5.82% and 7.26%, respectively whereas, loss weight and spoilage rate of control sample after 20 days were 11.48% and 23.29%. The presence of PVA (high biocompatibility, low tendency for protein adhesion, and low toxicity) and beeswax (a natural wax produced by honey bees) was found to increase the preservation potential of FCCPs. It is proposed that PVA increases the tightness and durability of the film while beeswax increases the hydrophobicity of FCCPs and reduces moisture loss of fruits. The results also showed that FCCPs can extend the shelf-life of longan fruits up to 30 days of storage at 5 °C and relative humidity of 90–95%, indicating their potential as an attractive alternative to improve the quality of fresh fruits during storage.

KEYWORDS

chitosan, composite preservatives, Longan postharvest, silk fibroin

1 | INTRODUCTION

According to the Food and Agriculture Organization of the United Nations, up to 30% of harvested fruits and vegetables are lost along supply chains, mostly due to poor handling, storage and transport of perishable goods.¹ Longan (*Dimocarpus longan* Lour.) is one of tropical and subtropical fruit trees, widely grown in many countries around the world, especially in China, Thailand, Vietnam, India, Pak-

istan, Australia, and South Africa.² However, fresh longan fruit has a very short shelf life, rotting within a few days at ambient temperature depending on the cultivar. The main factors that reduce longan shelf life and marketability are pericarp browning and microbial decay. Many postharvest preservation techniques, including refrigeration, chemical preservatives, edible coatings, packaging, and modified atmosphere packings have been studied.^{3,4} Recently, the most common commercial methods to prevent longan fruit

from rot and browning have been sterilization with SO_2 and dipping in fungicides because they are effective and inexpensive.⁵ However, sterilization with SO_2 and fungicides leave sulfide residues and toxic components that can have adverse effects on human health.

Edible coating provides a promising method for improving the quality and extending the shelf life of fruits and vegetables.⁶ To date, polysaccharides, proteins, resins, lipids and their combinations are the commonly used for coating formulations.^{7,8} Chitosan is a natural biopolymer having some special characteristics such as biocompatibility, biodegradability, antimicrobial and antifungal activities, membrane forming capacity, and non-toxicity.^{9,10} Chitosan coating on fruit and vegetable could help lessen moisture loss and slow respiration by reducing fruit oxygen uptake from the environment.¹¹ Therefore, it can inhibit the growth of pathogens, delay the senescence processes, extend the shelf-life, and enhance the storability and quality of postharvest fresh products.^{12–15} Chitosan coating has been successfully applied in many post harvested fruits and vegetables, such as green asparagus, sweet potatoes, Chinese cherries, guavas, litchis, mangoes, strawberries, nectarines, and longans.^{5,10,16–22} Although chitosan films can prolong storage time of many fruits, but modifications of chitosan including combination of chitosan-limonene and of chitosan-nanosilica can increase the physical properties such as strength, water permeability and gas permeability of chitosan films and improve storability further.^{22,23}

Silk fibroin is a fibrous material derived from certain spiders or insects. Silk fibroin has been investigated for textile, biomedical, photonic, and electronic applications.^{24,25} Fibroin is colorless and odorless, nontoxic, biodegradable, therefore, it is suitable for food packaging applications or for creating edible coatings to protect post-harvest fruits. Recently, fruits such as strawberries and bananas have been preserved effectively by fibroin coating.²⁶ The formation of micrometer-thin silk fibroin films around the fruits helps managing postharvest physiology of the fruits. Silk fibroin coatings enhance fruits' shelf life at ambient conditions by reducing cell respiration rate and water evaporation. The water-based processing and edible nature of silk fibroin makes this approach a promising alternative for food preservation with a naturally derived material.²⁶ While silk fibroin films have suitable water uptake and moderate mechanical properties, they are often brittle which reduce the mechanical stability of coatings.

Recently, Fernandez et al. has successfully prepared laminate and blend membranes from silk fibroin and chitosan.^{27,28} Due to the linkage between the functional groups of chitosan and fibroin, the laminate membranes have high mechanical strength and elasticity while blend membranes with suitable ratio have rather high mechanical strength. These results showed a new trend of biodegradable polymer production with special properties.²⁷

The present work focuses on preparation of fruit preservatives from fibroin, chitosan and some additives. To find the suitable ingredients for fruit preservatives, membranes were prepared from fibroin/chitosan based fruit preservatives and their properties such as tensile stress at break and water uptake were determined. PVA and bee wax (hydrophobic lipid) were introduced to increase the mechanical strength and control the water uptake of membranes formed by fibroin/chitosan based composite preservatives (FCCPs). To preserve longans, obtained FCCPs were coated on longans' peels using dipping technique. The protective effectiveness of the coated coatings was evaluated by measurement of nutritional and physiological characteristics of the fruits.

2 | MATERIALS AND METHODS

2.1 | Materials

Chitosan (molecular weight of 100,000 Daltons and deacetylation degree of 90%) was supplied by Chitosan Vietnam Manufacturing and Trading Co., Ltd. Waste silkworm cocoons was purchased from Phung Xa commune, My Duc district, Hanoi city, Vietnam. NaOH (99.5%), glycerol (99.5%), ethanol (99.5%), acetic acid (98%), Na_2CO_3 (99%), CaCl_2 (99.6%), gelatin, and poly vinyl alcohol (PVA) were supplied by Guangdong Guanghua Chemical Factory, China. Beeswax was purchased from Vietnam Bee Development Joint Stock Company.

2.2 | Silkworm cocoons degumming and fibroin fiber dissolution

2.2.1 | Degumming silkworm cocoons

Waste silkworm cocoons were cleaned from impurities such as dust and cut into small pieces. Silkworm cocoons pieces were then boiled in 0.02 M Na_2CO_3 solution for 30 min, followed by rinsing with deionized water three times, and drying overnight at 70 °C.²⁹

2.2.2 | Fibroin fiber dissolution

Degummed silkworm cocoons were dissolved in a mixture containing CaCl_2 , ethanol, and water (1:2:8 molar ratio) at 80 °C for 25–30 min until the silk was completely dissolved.²⁹ The ratio of the silk fibers and solution was 1:20 (m/v). Resulting homogeneous mixture was filtered to remove the insoluble fraction. Excess amounts of glycerol and ethanol were added to precipitate fibroin. The resulting mixture was centrifuged to separate fibroin.

2.3 | Preparation of fibroin/chitosan based composite membranes

Mixture of fibroin in water (5%) contained in a 15 cm × 12 cm glass tray was dried at 37 °C for 12 h to create a fibroin film.

Chitosan was dissolved in 1% acetic acid solution (solid/liquid ratio is 2% w/v) to form a homogeneous solution. Then obtained mixture was dried overnight at 37 °C to evaporate the solvent.

To create fibroin-chitosan membranes, different amounts of fibroin were dispersed to the 2% chitosan solution and then membrane preparation process was performed as described above.

Similarly, PVA was added to the mixture of fibroin and chitosan before preparing Fib-Chi-PVA membrane; PVA and beeswax were added to the mixture of fibroin and chitosan before preparing Fib-Chi-PVA-BW membrane.

2.3.1 | Membrane FT-IR and SEM measurements

FT-IR spectra of membranes were measured using a 6700—Thermo Nicolet—Thermo, SEM images of membranes were measured by a Joel 6490 JED 2300. Force at break of membrane was measured using a Universal Testing Machine, AG-5kNXPlus according to ISO 527 (2012) standard. Tensile stress at break (MPa) is calculated by the following Formula (1):

$$\sigma_b = \frac{F_B}{A} \quad (1)$$

σ_b = Tensile stress at break (MPa)

F_B = Force at break (N)

A = Initial cross section of the specimen (mm²)

2.3.2 | Water uptake

Squared (2 cm × 2 cm) membranes were kept under stable conditions of temperature and humidity (23 ± 2 °C, 50 ± 5%) for 24 h.³⁰ Then membranes were weighed (m_1) and soaked in water for 1 h. The water excess was blotted from the surface of the squares using filter paper, and after that, membranes were weighed (m_2). Three replicates were carried out.

$$W_c = \frac{(m_1 - m_2)}{m_1} \quad (2)$$

W_c is the water uptake of the membrane (%);

m_1 , m_2 are the weights of the membrane before and after immersing in water (mg).

2.4 | Preparation of preservatives

To evaluate the protective effectiveness of preservatives made of different ingredients, 6 preservatives were prepared by the following ways:

-15 g of fibroin was added to a beaker containing 1000 mL of 2% acetic acid solution, the mixture was continuously stirred until fibroin was completely dispersed. 15 grams of chitosan was slowly added to the mixture while continuously stirring at 350 rpm. Then the mixture was stirred for 2 h to get homogeneous gel labeled by Chi-Fib sample.

-1 of PVA was slowly introduced to 1000 mL of Chi-Fib sample while stirring continuously, the resulting mixture was stirred until the PVA is completely dissolved to get Chi-Fib-PVA sample.

-0.15 g of surfactant was dispersed in 15 mL of water and then 0.6 g of molten beeswax was added to form a homogeneous mixture. The resulting mixture was then dispersed into the 1000 mL of Chi-Fib sample and stirred for 30 min to obtain the Chi-Fib-BW sample.

-All the above steps were performed completely to obtain the composite sample FCCP.

2.5 | Coating and storage of longan fruit

Selected fruit samples were distributed randomly into groups of 300 fruits. Seven different treatments were used: (1) control (acetic acid coating); (2) fibroin coating; (3) chitosan coating; (4) Chi-Fib coating; (5) Chi-Fib-PVA coating, (6) Chi-Fib-BW coating, (7) composite sample (Fib-Chi-BW-PVA) coating. All the treatments were performed in triplicate. The fruits were dipped into the 6 corresponding preservatives for 4 min. An aqueous solution of glacial acetic acid (2%, v/v) was used for the coating control samples. Each group was placed in a perforated polyethylene bag (300 fruits/bag). All the samples were stored at room temperature (20–22 °C) with 90–95% relative humidity.⁵

The changes in the quality of the longan samples during the storage period were monitored every 5–10 days (until the spoilage rate is greater than 20%) in order to evaluate the preservation effectiveness of the preservatives.

2.6 | Evaluation of quality of longan fruits during storage

2.6.1 | Measurement of respiration rate

Ten longan fruits were sampled for measuring the fruit respiration rate according to the method of Lin et al.³¹ The respiratory rate was determined using a ICA250 (UK) analyzer. In detail, ten coated longan fruits (approximately 100 g) were placed in glass jar and incubated at room temperature (28 °C) for 1 h. The glass jar had air-tight

screw caps and the percentages of carbon dioxide were recorded. The results were calculated and expressed as mL CO₂ kg⁻¹ h⁻¹.

2.6.2 | Measurement of weight loss

For each treatment, the measurement of weight loss of ten fruits in each replication was carried out. The weight loss was determined gravimetrically using analytical balance (Kern ALJ 250–4A). The percentage of weight loss of longan fruits during storage was calculated via comparison to the weight of the fruits on storage day 0.³¹

2.6.3 | Total soluble solids, titratable acid, and ascorbic acid measurements

Tissue (20 g) from 5 fruit pulp was homogenized in a grinder and then centrifuged for 20 min using 15 000 g Beckman J20-2). The supernatant phase was collected for analyses for: total soluble solids, using a hand refractometer (PAL-1, Japan, 0–53 °Brix); titratable acid was determined with 0.1 M NaOH, and ascorbic acid by 2,6-dichlorophenolindophenol titration.³² Total acid and total soluble solids were presented in unit of %, while the vitamin C content was presented as the unit of mg%.

2.6.4 | Determination of percentage of spoilage

The percentage of spoilage is expressed as the ratio of the damaged fruit mass to the total fruit mass of the sample. Fruit is considered to be rotten when the surface of the rind appears mould or the rind turns dark brown.

2.6.5 | Measurement of the color change

The color change of the pods was determined using a Color Tec PCM Meters that measure 3 values *L*, *a*, *b*. The peel color of ten individual fruits was measured for each treatment. Readings were taken from stem end, mid region, and blossom end of each fruit. The chromatic coordinates were defined as: *L** (lightness), *a** (–greenness to +redness) and *b** (–blueness to +yellowness). Reading of *L* value was taken to represent the lightness of the fruit (0 = black; 100 = white).³¹ The color change (ΔE_{ab}) was then determined using the following equation:

$$\Delta E_{ab} = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \quad (3)$$

3 | RESULTS AND DISCUSSION

3.1 | Properties of membranes

3.1.1 | Tensile stress at break and water uptake

The results show that the fibroin membranes are less stable than chitosan membranes (Table 1), however, they have lower water uptake which can decrease the moisture loss of fruits and increase their durability. Chitosan membranes have higher tensile stress at break and high-water uptake. Therefore, it is recommended to use both fibroin and chitosan for preparing membranes that have better mechanical properties.

Blend membranes have higher water uptake than single membranes because of the formation of cross-linking between the chitosan and fibroin macromolecules. When the chitosan/fibroin ratio (chitosan content) in the membranes increased, the tensile stress at break increased from 26.11 MPa (ratio of 0.5:1) to 38.96 MPa (ratio of 2:1) and then gradually decreased to 24.31 MPa (ratio of 4:1). Water uptake increases continuously from 88% (ratio of 0.5:1) to 685% (ratio of 4:1).

The increase of water uptake with increasing content of chitosan in the membrane can be due to the interactions of –OH groups in chitosan and –NH₂ groups in fibroin. These interactions create a three-dimensional polymer network with large space to contain water molecules. The membrane with Fib:Chi ratio of 1:2 has a rather high tensile stress at break (sample 5) but it can be swollen when contacting to water or high humidity because of its high-water uptake (460%). From the tensile stress at break and water uptake, chitosan–fibroin mixture with 1:1 ratio was selected to prepare preservatives. When 3 wt% PVA is used as an additive for Fib–Chi membrane with 1:1 ratio, the tensile stress at break of the membrane increased from 33.60 to 55,290 MPa but membrane's water permeability also increased from 138% to 257% (samples 4,8). To reduce water permeability without changing the strength of the membrane, 2 wt% BW is added to the membrane (sample 9).

3.2 | FT-IR

FT-IR spectra of synthesized membranes are shown in Figure 1. As seen in fibroin spectrum, the peak of wave numbers of 3285 cm⁻¹ showed O–H and N–H stretching vibrations.^{33,34} The bands at wave numbers of 2979–2923 cm⁻¹ indicated the presence of the aliphatic asymmetric and symmetric (C–H) (sp³) stretching vibrations.^{35–37} The bands around wave numbers of 1652 cm⁻¹ and 1540 cm⁻¹ were assigned to the absorption peaks of the peptide backbone of amide I (C=O stretching) and amide II (N–H bending), the bands around 1236 and 1448 cm⁻¹ were assigned to amide

TABLE 1 Tensile stress at break and water uptake membranes.

No.	Membranes	Fib:Chi ratio (w:w)	Tensile stress at break (MPa)	Water up take W_c (%)
1	Fibroin	1:0	24.22	82
2	Chitosan	0:1	39.74	230
3	Fib-Chi	1:0.5	26.11	88
4	Fib-Chi	1:1	33.60	138
5	Fib-Chi	1:2	38.96	460
6	Fib-Chi	1:3	35.28	535
7	Fib-Chi	1:4	24.79	585
8	Fib-Chi-PVA	1:1	45.29	257.2
9	Fib-Chi-PVA-BW	1:1	44.50	143.4

III (C–N stretching), and the bands at 670 cm^{-1} were assigned to amide IV.³⁸ The typical vibration of C–O group in fibroin was shown by a band of wave numbers of 1059 cm^{-1} .³⁴

Due to the presence of amine groups in chitosan, bands around 1540 cm^{-1} were assigned to amide II (N–H bending), the bands around 1407 and 666 cm^{-1} were assigned to amide III (C–N stretching) and 670 cm^{-1} were assigned to amide IV, respectively. The weak band at 1650 cm^{-1} showed the small number of C=O bonds due to the incomplete deacetylation of chitin.³⁴

Similarly, Fib-Chi and Fib-Chi-PVA blend membrane have all characteristic peaks of individual materials (fibroin, chitosan, PVA). In Fib-Chi-PVA spectrum, the band around 1140 cm^{-1} showed the C–O stretching vibrations in alcohol³⁹ and the high intensity band at 3262 cm^{-1} was due to high water uptake of PVA component in the membrane.³⁴ Because of the presence of beeswax the intensity of broad band around 3280 cm^{-1} in Fib-Chi-PVA-BW becomes weaker.

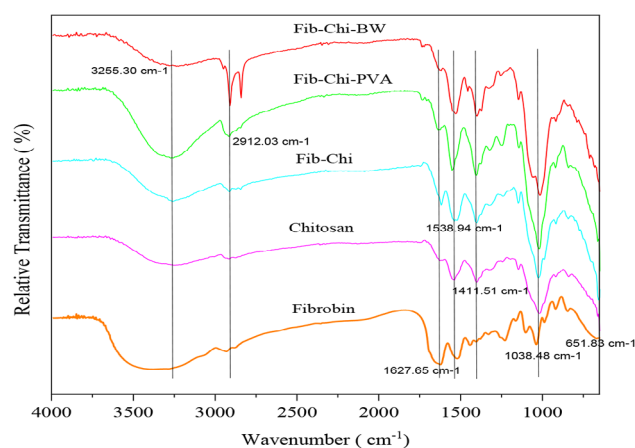
3.3 | Morphology

SEM photographs of chitosan and Fib-Chi membranes are given in Figure 2. The surface of chitosan membranes was homogeneous while that of Fib-Chi membranes show that chitosan and fibroin molecules are dispersed to each other.

3.4 | Preservation of the coatings during longan storage

3.4.1 | Influence of coating composition on the respiration rate

Respiration behavior is one of the parameters affecting the storage time of fruits and vegetables. High respiration rates may result in a high consumption of metabolic

**FIGURE 1** FT-IR spectra of prepared fibroin, chitosan, Fib-Chi, Fib-Chi-PVA, and Fib-Chi-PVA-BW membranes.

substrates and heat generation, therefore, the higher the respiration rate during storage, the serious symptom of decay of fruits and vegetables. Chitosan and/or fibroin coating was one of effective ways to reduce the respiratory rate and therefore, to prolong the time of commercial fruits and vegetables.^{5,23,40}

As shown in Figure 3a, the coatings dramatically affect the respiratory rate of the longans during storage time. A rapid increase in respiration rate of the control sample group was observed within the initial ten storage days (from 24.25 to 29.35 $\text{mL CO}_2\text{ kg}^{-1}\text{ h}^{-1}$), followed by a sharp raising in the next 10 days of storage (to 52.11 $\text{mL CO}_2\text{ kg}^{-1}\text{ h}^{-1}$). The treatments of longan with fibroin/chitosan based coatings reduce respiration rate and increased the storage time. The respiratory rate of treated longans decreased gradually in the first 10–15 storage days, then gradually increased within the next 15th and 20th days of storage, and increased rapidly at 30 and 40 days. Similar results were observed by Jiang and Lin et al. when longans were coated with chitosan and treated chitosan in order to prolong storage time.^{40,41} Influence of the composition of the coatings on the longan storability was also observed. The samples coated with Fib-Chi

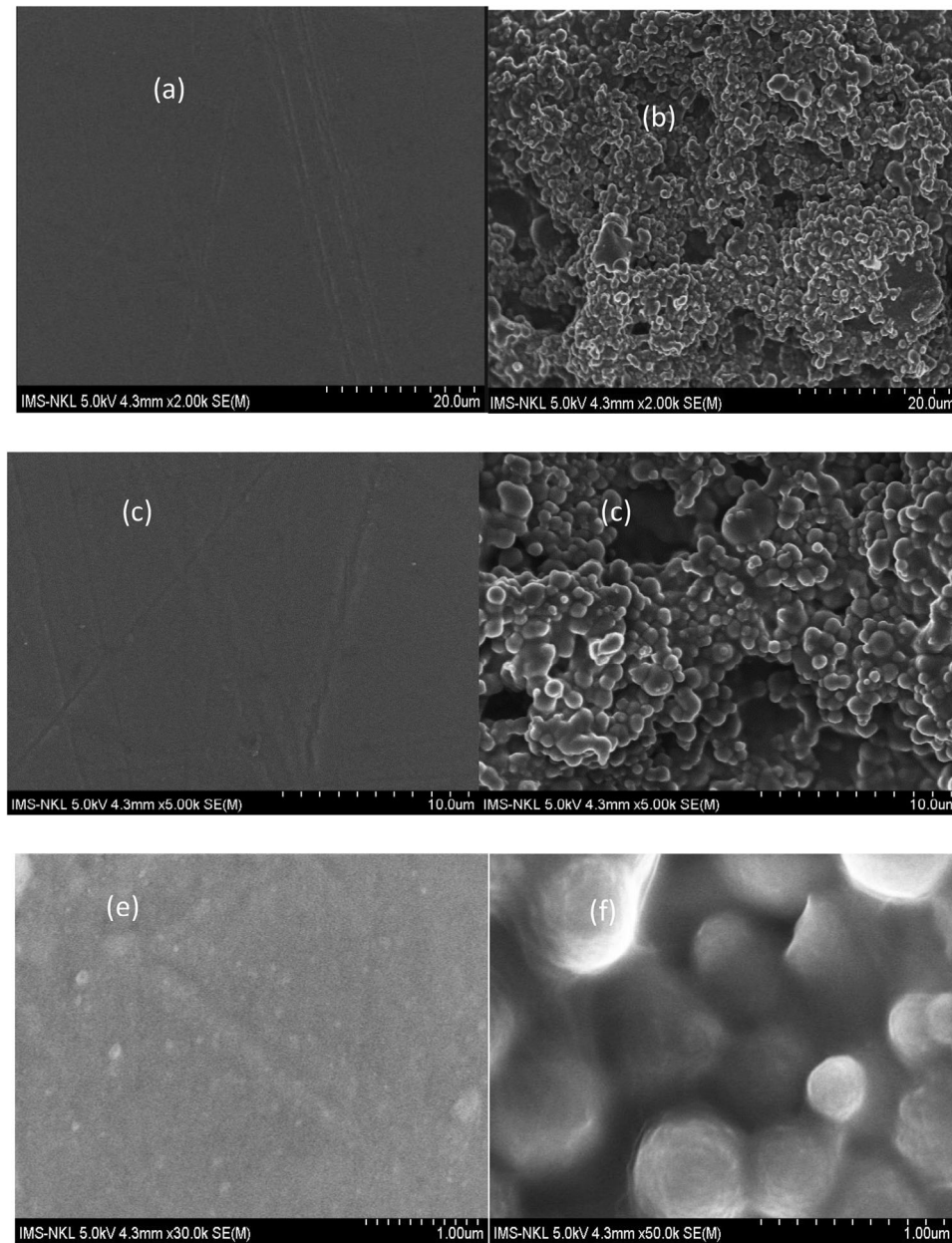


FIGURE 2 SEM photographs of membranes (a, c, e) chitosan; (b, d, f) Fib-Chi.

films have lower respiration rates than samples coated with single chitosan or fibroin films. The addition of PVA and beeswax additives increase further the storage ability of films and the Fib-Chi-PVA-BW (composite) film has the best storage ability. After 40 days of storage, the respiratory rate of longan fruits treated with chitosan reaches $44.72 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$ while samples treated with composite membrane increase to $34.21 \text{ mL CO}_2 \text{ kg}^{-1} \text{ h}^{-1}$. These can be due to the increase in tightness and the hydrophobicity of the films containing PVA and beeswax additives.

3.4.2 | Influence of coating composition on the weight loss

Natural weight loss is an inevitable physiological phenomenon during the storage of fresh fruits and vegetables. This is due to the transpiration (evaporative water loss) and respiration processes of the fruits leading to a decrease in the natural weight and dry matter content of the fruits.⁴¹ One of the effects of the coating is to limit the transpiration process of fresh fruits. To evaluate the effectiveness of the coatings, change in the rate of natural weight loss of the

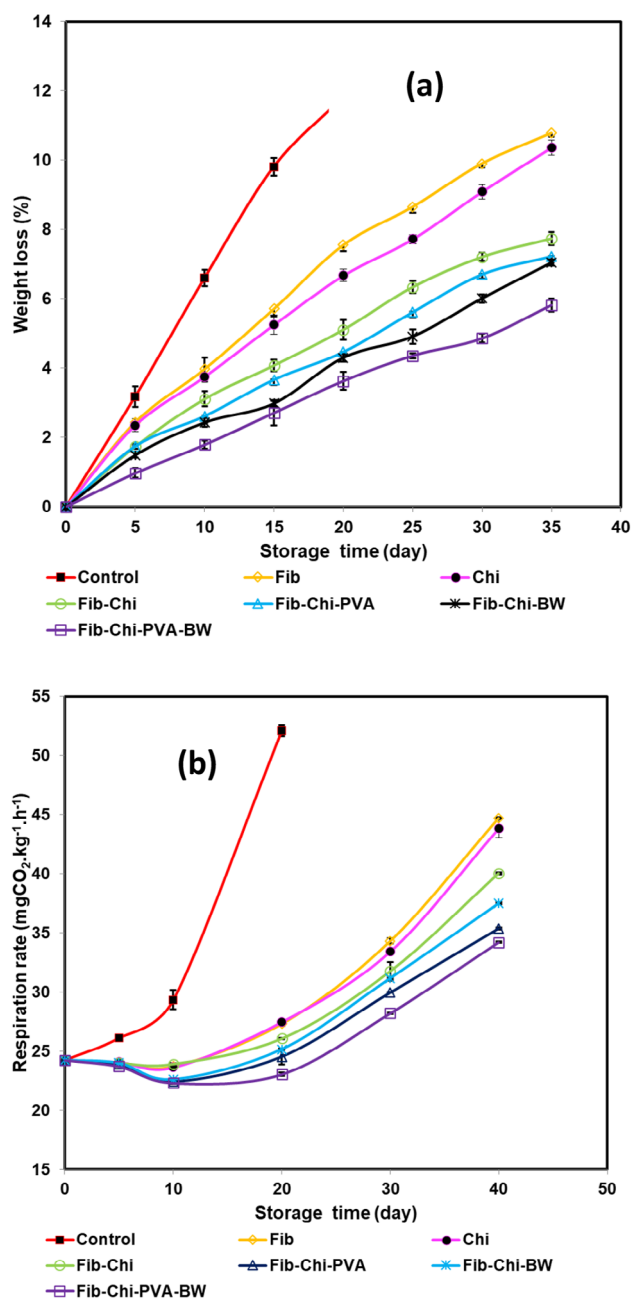


FIGURE 3 Weight loss (a) and respiration rate ($\text{mgCO}_2 \text{ kg}^{-1} \text{ h}^{-1}$), (b) of longan during storage (at room temperature ($20\text{--}22^\circ\text{C}$) with 90–95% relative humidity).

samples coated with 6 types of films were monitored. The results in Figure 3b show that the natural weight losses in all samples increase gradually with the storage time. However, during the same periods, the control sample has the highest natural weight loss (6.6% within the initial ten storage days and 11.81% in the next ten days of storage while the samples coated with preserved films have a significant reduction in the weight loss. For example, the weight loss within the initial ten storage days of longan treated with fibroin, and chitosan Fib–Chi coatings reaches 3.97; 3.77; 3.11%, with Fib–Chi–PVA and Fib–Chi–BW is 2.62 and 2.43%

while with FCCP is 1.79%. These results are in agreement with previous researches that weight loss of harvested longan treated with chitosan were lower than untreated fruit within the postharvest storage.^{23,40} This is explained by the fact that the control sample was not packaged, therefore, the transpiration process into the environment occurred strongly, the fruit quickly dried out. In addition, the high respiration rate leads to high loss of nutrients. Coating can restrict water transfer or dehydration, gas exchange, and nutrient loss by acting as a protective barrier, and thus reduces weight loss of postharvest fruit.⁴⁰ Fib–Chi film has better mechanical properties compared to Fib and Chi films that leads to a higher preservation ability of Fib–Chi film compared to Fib and Chi films. When 2 wt% of beeswax and 3 wt% of PVA are added to Fib–Chi film, the rate of moisture loss decreases. This is because beeswax is hydrophobic and PVA increases the tightness of the film. When both beeswax and PVA (composite sample) are used, the moisture loss is further reduced. The slower rate of moisture loss from the composite coated fruits may be attributed to the additional barrier against diffusion through stomata.²³

3.4.3 | Influence of coating composition on the percentage of spoilage

The percentage of spoilage is an important indicator to reflect the economic value of healthy fruit. It can be seen from Figure 4a that the cold storage method limits the damage of longan for a short time (spoilage was not observed all samples after 5 days). But after 10 days, the spoilage of the control sample was 5% and of samples coated with fibroin, chitosan, and Fib–Chi were 2.16%, 1.01%, and 1.05%, respectively, while no rot was observed in Fib–Chi–PVA, Fib–Chi–BW, and composite coated samples. These results show that the application of these preservatives have a significant effect in limiting the spoilage of longan at longer storage periods.

After 15 days of storage, high spoilage rate ($>12\%$) was observed at the control sample while the spoilage rates of samples coated with fibroin, chitosan, Fib–Chi increase to about 4–5%, whereas the spoilage rate of samples coated with Fib–Chi–PVA, Fib–Chi–BW is about 1–2%, and samples coated with composite has not been rotten. As shown in figure, after 30 days of storage, the spoilage rate of samples coated with fibroin film was 15.12% and with chitosan, Fib–Chi was from 11–14% ($>10\%$), while samples coated with Fib–Chi–PVA, Fib–Chi–BW, and composite samples showed spoilage rate lower than 10%—reaching the preservation goal. The effect of fibroin/chitosan films on reducing the spoilage rate of longan fruits is probably due to the decrease in respiration and metabolic activity, which retard the spoilage process. It was well documented that the films formed excellent semipermeable coatings around the fruits, modified the internal atmosphere by

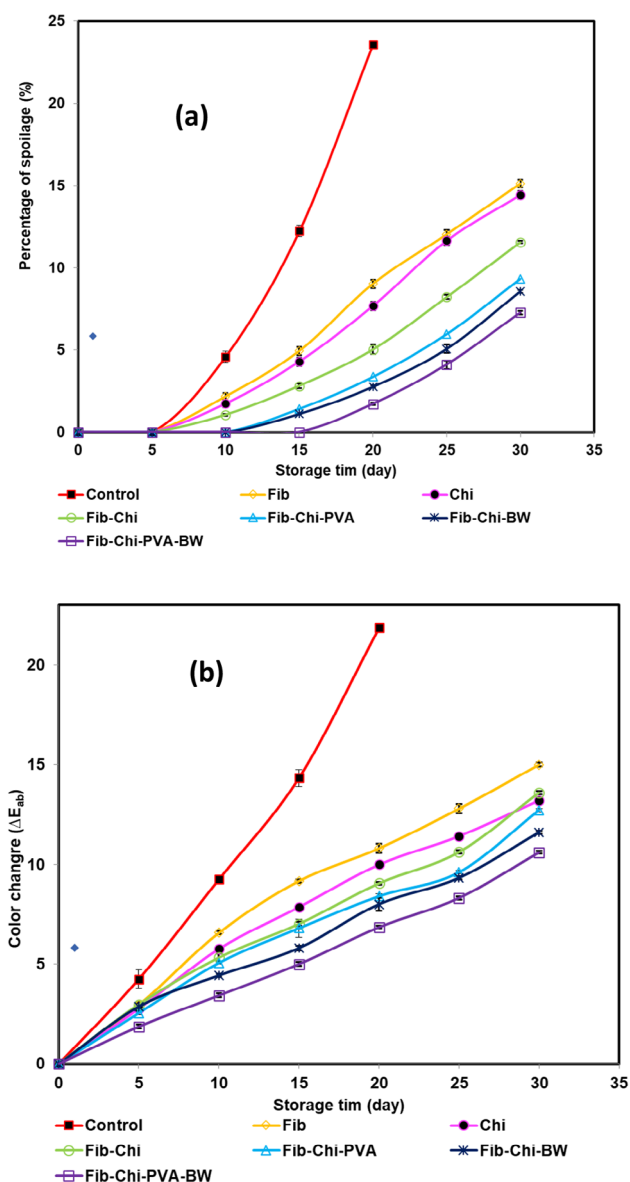


FIGURE 4 Percentage of spoilage (a) and color change (ΔE_{ab}) on the pods (b) of longan during storage (at room temperature (20–22 °C) with 90–95% relative humidity).

reducing O_2 and/or elevating CO_2 , and suppressing ethylene evolution.⁴⁰ The presence of additives such as PVA and beeswax improves the preservative properties of films. These can be due to the increase in tensile stress at break and hydrophobic property of the films by the presence of PVA and beeswax.

3.4.4 | Influence of coating composition on the color change

The occurrences of pericarp browning, fruit disease as well as pulp breakdown are the main symptoms of postharvest problem for longans. The color of longan pericarp

is a main symptom affecting the consumption of longan, the color change (ΔE_{ab}) severely affects the quality and shortens the shelf-life of postharvest longans.⁴⁰ Therefore, color change measurement is necessary to control the storage process. It can be seen from the results in Figure 4b that the highest significant change in color was observed at control sample, after 20 days of storage the ΔE_{ab} value of control samples reached 21.86, the pericarps were browned. However, pericarp browning was obviously inhibited in treated samples, ΔE_{ab} value of treated samples were much lower (6.85–10.81). The retardation of color change in treated longan fruits could be due to the modification of internal atmosphere of the films that leads to low respiration and ethylene production rate. The treated samples have created a microclimate preservation environment with suitable temperature and humidity, which can partially limit water loss and physiological and biochemical activities, thereby inhibit activity of enzyme polyphenoloxidase existing in the cytoplasm of the pericarps.

3.4.5 | Influence of coating composition on the total soluble solids

Total soluble solids (TSS) in the fruits include mainly sugar, organic acids, soluble vitamins etc., which are important nutritional ingredients. TSS is the main substrates of respiration process and their content is usually used to indicate the postharvest quality and maturity of fruit.⁴² It can be seen from Figure 5a that the TSS content of samples decreases with different rates during storage due to the fruits are still a living organism after harvesting, therefore, respiration and metabolism still take place. TSS content of control samples decreased quickly from 19.49 to 16.12°Bx during 20 days of storage while the TSS content of treated groups decreased more slowly from 19.49°Bx to 17.41°Bx ÷ 18.96°Bx after 20 days of storage and from 19.49°Bx to 16.55°Bx ÷ 17.92°Bx after 30 days. The lowest level of TSS content was recorded in control fruit at the end of storage period. The effect of coatings on reducing the TSS contents of longan fruits were probably due to slowing down of respiration and metabolic activities, which retard the senescence process.⁵ Similar results were recorded in longan treated with chitosan and chitosan/nano-silica composite.^{5,40} It may be due to that excellent semipermeable films covered around the fruits, modified the internal atmosphere by reducing O_2 and/or elevating CO_2 , and suppressing ethylene evolution⁵ (Shi et al. 2013). In comparison to the chitosan/fibroin treated samples, Fib–Chi treated samples displayed a higher level of TSS in longan pulp during postharvest storage. When PVA or beeswax, especially both PVA and beeswax are added to the coating composition, the TSS was further higher. This is because PVA could increase mechanical properties of film that leads to the higher storage ability of Fib–Chi film. In addition, beeswax is hydrophobic and could reduce moisture loss of longan by evaporation.

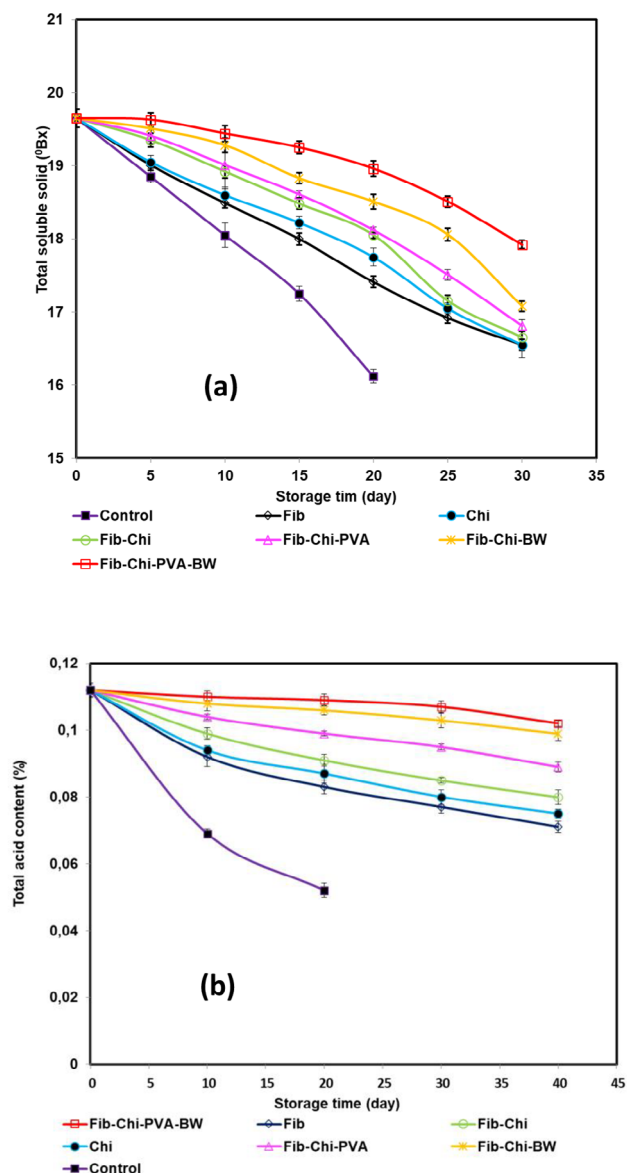


FIGURE 5 Total soluble solid ($^{\circ}\text{Bx}$) (a) and total acid (b) contents of longan during storage (at room temperature (20–22 °C) with 90–95% relative humidity).

3.4.6 | Influence of coating composition on total acid content

Organic acids can participate in biochemical (respiration) cycles of fruits; therefore, their contents are also reduced during storage. Similar to TSS, TA contents in control fruits decreased significantly during storage time but more slowly (Figure 5b) in fruits coated with the fibroin, chitosan, and Fib–Chi films. Similar results were also reported, chitosan coating produced a small change in TA concentration throughout storage, and chitosan/nano-silica composite had a more efficiency in delaying the TA decline regardless of the storage time.⁴¹ PVA and beeswax additives contribute to delay the decrease in TA contents in fruits coated

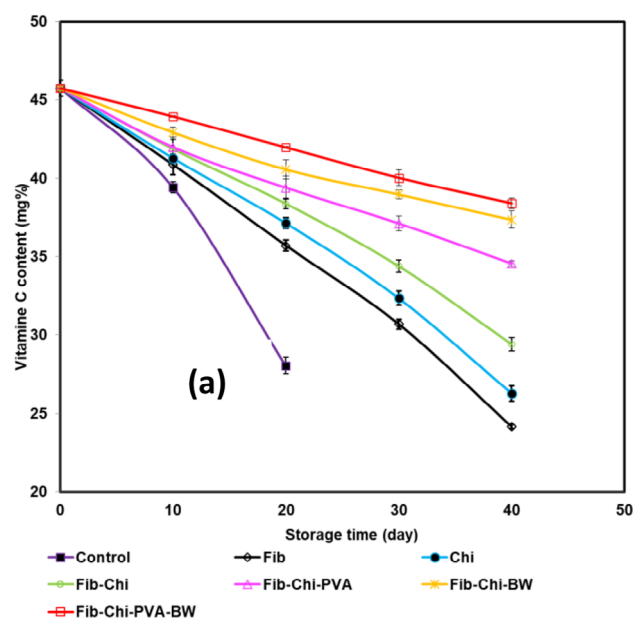


FIGURE 6 Vitamin C content of longan during storage (mg%) (at room temperature (20–22 °C) with 90–95% relative humidity).

with Fib–Chi–PVA and Fib–Chi–BW films; this could be due to the increase in tensile stress at break and hydrophobic property of the films. When a combination of both PVA and beeswax in Fib–Chi–PVA–BW film, the TA content of coated longans retained highest. Interestingly, up to 20 days, the TA content in the control sample decreased to 46% compared to the original while the acid content of fruit coated with composite film has remained about 91% after 40 days of storage. As shown in Figure 5b, it appears that postharvest longans treated with Fib–Chi–PVA–BW could retain the highest TA content.

3.4.7 | Influence of coating composition on the vitamin C content

Vitamin C is an important nutritional and antioxidant component of fruits but is easily degraded during storage. The results (Figure 6a) show that vitamin C content decreased rapidly in the control sample, from 45.75 to 39.42 mg/% g after 10 days and to 28.8 mg% after 20 days of storage. However, vitamin C content decreased much more slowly in the preserved samples. Chi–Fib films have better preservation ability than single chitosan and fibroin single films. After 20 days of storage, vitamin C contents in samples coated with chitosan, fibroin and Chi–Fib films were maintained in the levels of 38.38, 37.13, and 35.71 mg/%, respectively.

The addition of both PVA and beeswax additives reduce the rate of spoilage, thus reducing the decomposition of vitamin C of the fruits; the Fib–Chi–PVA–BW sample has the best preservation ability. After 20 days, the vitamin C contents of fruits coating with Fib–Chi–PVA and Fib–Chi–BW films were 39.39 and 40.47 mg%, respectively while vita-

min C content in fruits coating with composite film was 41.97 mg%.

4 | CONCLUSIONS

In order to find suitable composition for preservation films the tensile stress at break and water uptake of some fibroin and chitosan based different composition membranes were first determined. It can be seen from results that the properties of Fib–Chi membrane are better than those of single membranes because of chemical linkages between functional groups of chitosan and fibroin molecules. A small amount of PVA added to Fib–Chi membrane can increase the flexibility and tensile stress at break of the membranes and the addition of beeswax to membranes can adjust their swelling or water uptake. Therefore, 6 fibroin/chitosan based preservatives were prepared for storage of longan fruits by coating. Experimental results show that these fibroin/chitosan films can significantly prolong longan storage time, reduce browning index, retard weight loss and respiration and delay the reduction of vitamin C, total acid, and total sugar contents in fresh longan fruits. The addition of PVA and beeswax to Fib–Chi coating can lead to a further increase in preservation ability. The condensation reactions between –OH groups of PVA and –COOH, –NH₂ groups in chitosan and fibroin can enhance the tightness and durability of the films. In addition, the hydrophobicity of beeswax reduces moisture (water) loss of fruits through coatings. Our results indicated that, Fib–Chi–PVA–BW films bring the highest protective effect among investigated films and can extend the shelf life of longan fruit up to 30 days during storage at 5 °C. These results showed that the fibroin/chitosan coatings could provide an alternative approach for extending the storage-life of longans during postharvest storage. Storage studies at temperatures higher than 5 °C need to be performed in further investigation in order to reduce the consumption of energy during storage.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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How to cite this article: N.-C. Pham, H. T. N. Uong, T. T. L. Bui. Preparation of fibroin/chitosan based preservatives for longan postharvest preservation, *Vietnam J. Chem.* **2025**, *1*.
<https://doi.org/10.1002/vjch.70039>