Cellulose nanomaterials-incorporated emulsion coatings for controlling physiological activity, modifying surface morphology, and enhancing storability of postharvest bananas (*Musa acuminate*)

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Highlights

- Cellulose nanofiber (CNF) emulsion coatings were developed and validated on bananas.
- CNF emulsion coating had superior hydrophobicity and wettability onto fruit surface.
- CNF emulsion coating controlled physiological activity of bananas.
- CNF emulsion coating modified fruit surfaces for providing more uniform coverage.
- CNF emulsion coating reduced ripening and enhanced storability of bananas.

Abstract

Cellulose nanomaterials (CNs)-incorporated emulsion coatings with improved moisture barrier and wettability onto fruit surfaces were developed for controlling physiological activity and enhancing storability of postharvest bananas during ambient storage. Cellulose nanofiber (CNF)-based emulsion coatings (CNFC: 0.3% CNF/1% oleic acid/1% sucrose ester fatty acid (w/w wet base)) minimized chlorophyll degradation, weight loss, and firmness of fruit while ensuring the properly ripening of fruit during 10 d of ambient storage. CNFC coating had low contact angle, high spread coefficient onto banana surfaces, and lower surface tension (ST, 25.4 mN/m) than the critical ST (35.2 mN/m) of fruit surface, exhibiting good wettability onto banana surfaces. CNFC coating also delayed the ethylene biosynthesis pathway and reduced ethylene and CO_2 production, thus delaying fruit ripening. This study demonstrated the effectiveness of CNs incorporated emulsion coatings for improving the storability of postharvest bananas.

Key words

Cellulose nanomaterials, emulsion coatings, physiological activity, storability, postharvest banana

1. Introduction

Cavendish banana (*Musa acuminate*) is a rich source of vitamins and bioactive compounds (e.g. dietary fiber and phenolic compounds), and one of mostly consumed fruit worldwide (Singh, Singh, Kaur & Singh, 2016). As a climacteric fruit, however, bananas have a relatively short shelf-life, regarding physiological disorder, postharvest diseases, and senescence (Kader, 2002). Several postharvest technologies, such as low temperature, edible coatings, and hypobaric and controlled atmosphere storages, have been applied to delay the ripening and quality deterioration of fruit during postharvest storage (Maqbool, Ali, Alderson, Zahid & Siddiqui, 2011). However, low temperature storage might cause chill injury and physiological damage on banana fruit (Jiang, Joyce, Jiang & Lu, 2004), and hypobaric and controlled atmosphere storages are capital intensive and expensive (Pesis, Arie, Feygenberg & Villamizar, 2005; Burg, 2004). Edible coatings have been widely applied as a cost efficient, environmentally-friendly postharvest technology for fruit and vegetables. It can generate a modified atmosphere by creating a semipermeable barrier against oxygen, carbon dioxide, moisture, and solute movement. Although lipid and/or hydrocolloid-based coatings have been utilized for extending the shelf-life of postharvest bananas (Dhall, 2013; Lin & Zhao, 2007; Martínez-Romero et al., 2006), there exist a number of challenges, such as insufficient moisture and gas barrier and poor adhesion onto fruit surfaces (Lin & Zhao, 2007). This study attempted to enhance moisture and gas barrier and wettability of coatings onto fruit surface through incorporating emulsion system into cellulose nanomaterials (CNs)-based matrix, and to investigate the influence of developed coatings on the physiological activity, surface characteristics, and storability of bananas during ambient storage.

Previously, hydrocolloid-based emulsion coatings incorporated with lipid was evaluated to reduce superficial dehydration of moist fruit. As an oil phase in emulsion coatings,

monounsaturated oleic acid (OA) has been widely used as a hydrophobic and antioxidant agent (Khalifa, Barakat, El-Mansy & Soliman, 2016; Ramana Rao, Baraiya, Vyas & Patel, 2016). For creating stable emulsion with OA, it is important to select a compatible surfactant that can satisfy two critical performances: 1) tailor the hydrophobic oil droplets into the emulsion system and bring them to the hydrophilic polymer matrix (Anarjan & Tan, 2013), and 2) decrease the surface tension of coating materials for improving stability, uniformity, and spreadability of emulsion coatings (Jung, Deng, Simonsen, Bastías & Zhao, 2016). Therefore, in this study two types of non-ionic surfactant, including Tween 80 and sucrose ester of fatty acid (SEFA) that possess different hydrophilic heads (carbohydrate for Tween 80 and ethoxylate for SEFA) were investigated since their compatibility with OA depends on the composition and structure of the surfactant.

CNs possess crystalline, nanosized structure (diameter <100 nm and high aspect ratio with 5-100), and can provide low gas barrier and high mechanical strength of biocomposite derived from themselves or as reinforcing agent in other polymer matrix. CNs have low-to-minimal adverse health effect, and can enhance the stability of bioactive compounds and some delivery systems, such as emulsion and/or encapsulation, through non-covalent adsorption (Aulin, Gällstedt & Lindström, 2010; Belbekhouche et al., 2011; Moon, Schueneman & Simonsen, 2016). Cellulose nanofibers (CNF) with high flexibility and absorption ability can form coating/film matrix, while cellulose nanocrystals (CNC) with highly-rigid, rod-like structure and negative surface charge can function as reinforcing agent for chitosan matrix (Belbekhouche et al., 2011; Favier, Chanzy & Cavaille, 1995). Our previous fruit coating studies demonstrated that CNF based coating is suitable for preventing cherry cracking during fruit production, while CNC-reinforced chitosan coating is ideal for extending the storability of postharvest pears (Jung

et al., 2016; Deng et. al., 2017). The effectiveness of CNs coatings was selective, depending on the types of fruit (surface characteristics, postharvest physiological activity, etc.) and storage conditions (temperature and relative humidity). Hence, the current study investigated the effectiveness of both CNs based coating matrix systems (CNF emulsion and CNC-incorporated chitosan) on postharvest bananas. It was hypothesized that CNs-based coatings can improve the storability of postharvest bananas by improving the adhesion of coatings onto fruit surfaces, enhancing moisture barrier of coatings, controlling physiological activity, and modifying surface morphology of fruit.

For achieving the goal, a two-step experimental approach was employed in this study: 1) development of emulsion coatings showing the best performance based upon the coating formulations (fruit adhesion) and derived films (hydrophobicity), as well as external fruit quality parameters (chlorophyll degradation, weight loss, and fruit marketability) and 2) investigation of the developed coatings on the surface characteristics (critical surface tension of fruit peels and cell morphology) and physiological activity (ethylene biosynthesis pathway and ethylene and CO_2 production) of fruit, and validation of the coatings for enhancing storability of fruit by monitoring the internal fruit quality (starch degradation, firmness, soluble solid contents, and titratable acidity) during ambient storage of fruit. It is anticipated that this study will provide the scientific insight of CNs-based coatings as a simple and effective postharvest technology for improving the storability of banana fruit.

2. Materials and methods

2.1. Materials

CNF and CNC, derived from softwood Kraft pulp with solid content of 2.95% and 11.8%,

116 respectively, were produced from the Process Development Center at the University of Maine
117 (ME, USA). Chitosan (97% degree of deacetylation, 149 kDa Mw) was purchased from Premix
118 (Iceland), Tween 80 from Amresco (OH, USA), SEFA from TCI American (OR, USA), OA and
119 glycerol from Alfa Aesar (MA, USA), and acetic acid from J. T. Baker (NJ, USA). The
120 1-aminocyclopropane-1-carboxylic acid (ACC) and N-(2-hydroxyethyl)
121 piperazine-N'-3-propanesulfonic acid (EPPS) were purchased from Chem Impex Internation, Inc.
122 (IL, USA), $HgCl_2$ from MP biomedicals (CA, USA), pyridoxal phosphate from TCI American
123 (OR, USA), dithiothreitol (DTT) from Sigma (MO, USA), and trichloroacetic acid (TCA) and
124 NaOCl from JT Baker (NJ, USA).
125 Organic Cavendish bananas (Piura, Peru) at the ripeness stage of 2 (green with trace of
126 yellow) without visual defects were purchased from a local supermarket (OR, USA) at the day of
127 their arrival at the store, and coated on the same day of purchase.
128
129 2.2. Preparation of coating formulations and fruit coatings
130 Coating formulations were prepared based on wet base (w/w), and the range of concentration
131 of each component was determined based upon our preliminary studies (data not shown). Each
132 coating matrix, including 0.3% CNF and 0.2% CNC-reinforced 2% chitosan, was formulated
133 with surfactants (Tween 80 or SEFA) and/or OA, and derived into six different types of emulsion
134 coatings as reported in Fig. 1. Emulsion systems with different surfactant types were first
135 prepared as followed: 1% Tween 80 was suspended in water at the ambient temperature, and
136 SEFA was dispersed at 70 °C to enhance water solubility. Then, 1% OA (1%, w/w) was added to
137 surfactant solution, and homogenized for 1 min. Coating formulation with only Tween 80 (10%,
138 w/w dry base) was also prepared as a positive control. For CNF-based emulsion coating

formulations, 0.3% CNF was mixed with surfactants and/or OA (CNFA: 0.03% Tween 80 only, CNFB: 1% Tween 80 with 1% OA, and CNFC: 1% SEFA with 1% OA), and homogenized for 1 min (Polytron PT10-35, Luzernerstrasse, Switzerland). For CNC-reinforced chitosan emulsion coating formulations, 2% chitosan (w/w) was dissolved in 1% acetic acid solution (w/v), and homogenized with 0.2% CNC and 0.4% glycerol for 1 min. The prepared coating formulations were mixed with surfactants and/or OA (CNCA: 0.2% Tween 80 only, CNFB: 1% Tween 80 with 1% OA, and CNFC: 1% SEFA with 1% OA), homogenized for 3 min, and then degassed using a self-build water flow vacuum system (Chen & Zhao, 2012).

When applying coatings on the fruit surface, three different coating application methods (dipping, spraying, and dipping) were evaluated, and no significant difference on fruit storability was observed (data not shown). This study selected brushing method for further improving the spreadability of coatings over fruit surface (Njombolwan et al., 2013). Each emulsion coating formulation was manually brushed onto bananas using a paint brush (width: 25 mm) to achieve uniform coating. Fruit were dried under forced airflow for 1 h. Non-coated and coated fruit were stored for 10 d in the ambient conditions under the florescent light without packaging (20 ± 2 °C and 50 ± 5% RH). Fruit coated with Semperfresh^{TM} (Semp, 1.2%, w/w, Pace International, LLC, WA, USA) was used as a positive control. Semperfresh^{TM} is a commercial coating product containing sucrose ester of fatty acid, mono- and di-glycerides, and carboxymethyl cellulose (Dhall, 2013) and has been used for coating various fruit and vegetables, including bananas.

2.3. Wettability of coating formulations

Coating performance is strongly influenced by the wettability of coating formulation associating to the surface characteristic of fruit (Choi, Park, Ahn, Lee & Lee, 2002). Previously,

limited efforts have been made to understand the correlation of coating wettability with fruit surfaces. This study measured contact angle (CA) of coating formulation and spread coefficient (W_s) of coating formulations on banana surface, and also investigated the surface tension (ST) of coating formulations to meet the critical ST of banana surfaces for ensuring sufficient adhesion of coatings on the banana surfaces.

CA was determined using a video contact angle system (FTA 32, First Ten Angstroms, Inc., USA) equipped with a face contact angle meter. A 10 μL of coating formulation was dropped from 10 mm height to a horizontal surface of banana surface. CA was recorded after 30 sec for all samples excluding the influence of dispersing time on spreadability (Zhong, Li & Zhao, 2012). ST of coating formulations was determined by using a FTÅ model T10 (First Ten Ångstroms, Portsmouth, VA) equipped with a Du Nuöy ring (CSC Scientific Co, Fairfax, VA) (Ryder, Wu, McKelvey, McGuire & Schilke, 2014). All data were collected within 5 min to reach the steady state of ST. The spreadability of coating formulations was calculated and expressed as the spreading coefficient ($W_s = W_a - W_c$) derived from adhesion coefficient ($W_a = \gamma_{SV} + \gamma_{LV} - \gamma_{SL}$, impacting the spreading) and cohesion coefficient ($W_c = 2\gamma_{LV}$, impacting the contraction), where γ_{SV}, γ_{SL}, and γ_{LV} represented solid-vapor, solid-liquid, and liquid-vapor of interfacial tensions of a coating formulation (Casariego et al., 2008).

For ensuring sufficient and uniform adhesion of coating formulations on coated fruit surface, ST of the developed coating formulations should be lower or close to the critical ST(γC) of that fruit surface. The critical ST of banana surface was obtained by extrapolation from the Zisman's plot, which was built using water, formamide and 1-methyl naphthalene as reference liquids (Fowkes & Zisman, 1964; Tzoumaki, Biliaderis & Vasilakakis, M., 2009). It is worthwhile to

mention that the critical ST of fruit surfaces depends on the texture and composition of that fruit (Casariego et al., 2008).

2.4. Hydrophobicity of coating formulation and derived film

Contact angle (CA) of coating formulation and WVP of derived film were determined for measuring the hydrophobicity. CA of coating formulations onto the surface of silicon wafer was determined by using the same method mentioned above. Films were derived from developed coating formulations. Briefly, 60 mL of coating formulations were uniformly casted onto 150 mm diameter polystyrene petri dish (Falcon, PA, USA), and dried at room temperature for 2 d. Derived films were then conditioned at 25 °C and 50% RH in a self-assembled chamber before measurement (Versa, PA, USA) (Jung et al., 2016). WVP of the films were measured using a cup method based on ASTM Standard E96-87 (ASTM 2000; Park & Zhao, 2004). Each film sample (75 x 75 mm) was sealed with vacuum grease between the lid and the Plexiglas test cup (57 x 15 mm) filled up with 11 mL of distilled (DI) water, and the seal ring was closed tightly using rubber bands. Test cup assemblies were stored in the self-assembled chamber at 25 °C and 50% RH and weighed hourly for 6 h. Data were reported as the mean value and standard deviation of three replications.

2.5. Evaluation of fruit quality during ambient storage

Chlorophyll content of banana peels, weight loss (%), and marketability (%) of uncoated (control) and coated fruit samples were evaluated and used as the scientific basis for selecting the ideal coating formulations to improve the storability of fruit. Eighteen bananas were randomly assigned into three groups (6 fruit/group), with each group as one replication and three

replications per treatment. Chlorophyll content of banana peels was measured using a DA meter (Sinteleia, Bolonga, Italy), and the percentage of chlorophyll degradation was reported as chlorophyll content change at different sampling times (1-10 d) from the initial chlorophyll content (Xie, Song, Wang & Sugar, 2014). The fruit weight loss (%) was calculated as weight change at different sampling times from the initial weight and multiplied by 100. The marketability (%) of fruit was determined based upon the visual observation of brown spots on banana peels, in which fruit was considered unmarketable when about 20% of the fruit peels was covered with brown spots (Ahmed & Palta, 2016). The marketability (%) was then calculated as the number of marketable fruit at different sampling times (1-10 d) divided by total number of fruit per treatment (18 ea), and multiplied by 100.

Coating formulation (CNFC in this study) showing the best performance based upon above measured parameters was further validated by coating a fresh set of fruit. Starch degradation, firmness, titratable acidity (TA), and total soluble solid (TSS) of uncoated and coated (CNFC and Semp) fruit were evaluated and photos of fruit were taken at various sampling times (0, 3, 7 and 10 d) during the same storage conditions as tested above. Pulp starch content was determined using the iodine dyeing method for estimating the conversion of starch to sugar as the result of fruit ripening (Blankenship, Ellsworth & Powell, 1993). Iodine solution was freshly made using 2.5 g/L iodine and 10 g/L potassium iodide. The cross-section cut of banana was dipped into the iodine solution for 5 s at each sampling time, and visually observed for six cross-section cut randomly selected from six fruit for each treatment. Fruit firmness was determined as the maximum penetration force (N) using a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Corp., NY, USA), in which individual banana was penetrated by a P/6 stainless cylinder probe with 7 mm depth at a speed of 10 mm/s (Ahmed & Palta, 2016). Three

measurements at different locations for each individual fruit were conducted as one replication per treatment. Mean values and standard deviations were reported with six replications. For TSS and TA, 40 g of banana flesh was mixed with 160 mL of DI water using a blender (Proctor Silex, NACCO Industry Inc., VA, USA). The mixture was filtered using a qualitative filter paper with the pore size of 2.5 μm (Whatman, GE Healthcare Bio-Sciences, PA, USA). TSS of the filtrate was measured using a refractometer (RA250-HE, KEM, Tokyo, Japan). The filtrate was then titrated with 0.1 N NaOH until pH 8.3 using a pH meter (Orion 410A, Fisher scientific, MA, USA) and digital titrator (Brinkmann, TX, USA) (Cavender, Liu, Hobbs, Frei, Strik & Zhao, 2014). TA was reported as the equivalent percentage of malic acid as the predominant acid in ripen banana. One measurement was conducted for each fruit as a replication per treatment, and mean values and standard deviations were reported with six replications.

2.6. Effect of coatings on physiological activity and surface characteristics of fruit

Fruit physiological activity and surface characteristics of uncoated and coated fruit were investigated for understanding the mechanisms of effective coating.

2.6.1. Respiration and ethylene production

The respiration (O_2 and CO_2) and ethylene production of bananas was measured using a gas chromatograph (GC-2014, Greenhouse gas analyzer, Shimadzu, Japan) with a flame ionization detector (FID, ethylene and CO_2) and thermal conductivity detector (TCD, O_2) (Deng et al., 2016). Five bananas were randomly selected, weighted, placed inside a 1.5 L of air-tight glass jar with lid holding a 10 mm rubber septa for sampling headspace gas, and stored at the ambient temperature (20 ± 2 °C). The O_2 and CO_2 productions were monitored after 24 h, while ethylene

253 production was measured after 48 h due to the low amount of ethylene production. For each jar,
254 1 mL of headspace gas was collected using an air tight syringe (Series A, Valco Instrument Co.,
255 USA) and then injected into the GC fitted with three kinds of packed columns: 80/100
256 HAYESEP D, 8/100 HAYESEP N, and 60/80 molecular sieve column (Supelco, Bellefonte, PA,
257 USA). Helium was applied as the carrier gas at a pressure of 350 kPa and flow rate of 21.19 mL
258 min^{-1}. The temperature of injector, column, and FID detector was set at 150, 90, and 250 °C,
259 respectively. The O_2, CO_2, and ethylene standard gases were purchased from Air Liquide
260 (Scott^{TM}, PA, USA), and GC solution software (Shimadzu, Japan) was used to calculate the
261 amount of O_2, CO_2, and ethylene.
262
263 2.6.2. ACC and ACS activity
264 As illustrated in Fig. 1, the coatings could impact the ethylene biosynthesis pathway of fruit
265 by generating modified atmosphere condition. This study measured ACC as the precursor of
266 ethylene and ACS activity as an enzyme that catalyzes the synthesis of ACC from S-Adenosyl
267 methionine (SAM) (Kato & Hyodo, 1999).
268 For measuring ACC and ACS, banana flesh samples were collected at different sampling
269 times (0, 3, 7, and 10 d), and stored at -80 ºC prior to analysis. For extracting ACC, 2 g of
270 freshly-thawed banana flesh in 10 mL of 9% TCA was homogenized for 60 s and incubated at
271 4 °C for 24 h. The extract was centrifuged at 10,000 x g for 30 min, and the supernatant was
272 adjusted to pH 7-8 with 1N NaOH. Two of sample reaction mixtures were prepared with 500 µL
273 of supernatant, 100 µL of 10 mM $HgCl_2$ (100 µL), and 300 µL of DI water in capped 10 mL vials.
274 One of them was spiked using internal standard ACC (50 µL of 0.05 mM ACC). Both of them
275 were incubated for 3 min at 4 °C after adding 100 µL of saturated NaOH and 5.25% NaOCl for

hydrolysis of ACC into ethylene (Hoffman & Yang, 1982). Then, 5 mL gas sample was taken for ethylene measurements, and quantified by using GC. ACC concentration was expressed as pmol/g fresh sample.

For measuring ACS, 5 g of freshly-thawed banana flesh was homogenized in 10 mL of buffer with 100 mM N-(2-hydroxyethyl) piperazine-N'-3-propanesulfonic acid (EPPS), 0.5 µM pyridoxal phosphate, and 4 mM dithiothreitol (DTT) for 60 s, and adjusted to pH 8.5 with KOH. The extract was centrifuged at 10,000 x g for 30 min, and the supernatant was dialyzed overnight at 4 °C in dialysis buffer solution (pH 8.5) containing 2 mM EPPS, 0.2 µM pyridoxal phosphate, and 0.1 mM DTT. Likewise, two of reaction mixtures containing 400 µL of enzyme solution, 50 µL of 600 mM EPPS (pH 8.5), and 90 µL DI water were prepared in capped 10 mL vials. One of them was spiked using internal standard ACC (50 µL of 0.05 mM ACC). After adding 60 µL of 0.5 mM SAM, both reaction mixtures were incubated for 3 h at 30 °C, and then mixed with 100 µL of 10 mM $HgCl_2$ and 200 µL of DI water. The reaction mixture was finally hydrolyzed by adding 100 µL of saturated NaOH and 5.25% NaOCl. A 5 mL of headspace gas was then collected after incubation at 4 °C for 3 min, and ethylene production was measured by using GC (Hoffman & Yang, 1982). ACS activity was expressed as pmol ethylene/g fresh sample.

2.6.3. Surface characteristics of bananas

Effect of coatings on the surface morphology of bananas was investigated by a scanning electron microscope (SEM) (FEI Quanta 600, Cressington Scientific Instruments Ltd., UK). Non-coated, Semp-coated, and CNFC-coated banana peels were cut into 5 mm pieces and placed in a modified Karnovsky fixative for 2 h. Samples were rinsed in 0.1 M sodium cacodylate buffer and dehydrated in a graded series of acetone (10%, 30, 50, 70, 90, 95,

100-100%), 10-15 min each. Samples were dried in an EMS 850 critical point drier, mounted on the SEM stub skin side up, and coated with gold and palladium. Digital images were acquired at an accelerating voltage of 5 kV.

2.7. Experimental design and statistical analysis

A completely randomized two factorial design considering two treatment factors (types of coating matrix: CNF and CNC-reinforced chitosan; types of emulsions: Tween 80 only, Tween 80 with OA, and SEFA with OA) was applied for analyzing the performance of coating formulations and derived films. PROC GLM was used to identify significant differences and interactions among each factor using the SAS program (SAS v 9.2, The SAS Institute, USA), and *post-hoc* least significant difference (LSD) was used for the multiple comparisons. All measurements were conducted in triplicates and results were considered to be significantly different at $P<0.05$.

A completely randomized design with a single treatment factor (type of coating formulations: non-coated, Semp-coated, and CNFC-coated) was then applied for further in-depth study on internal fruit quality, physiological activity and surface characteristics of bananas. All measurements were taken in either duplicates or triplicates. A one-way ANOVA was carried out to determine the significant differences among the treatments, and a *post-hoc* LSD was conducted using statistical software (SAS v 9.2, The SAS Institute, USA). Results were considered to be significantly different at $P<0.05$.

3. Results and discussion

3.1. Properties of developed emulsion coating formulations and derived films

The effectiveness of fruit coatings for reducing water loss and controlling postharvest respiration rely on the sufficient wettability and adhesion of coating formulations onto the fruit surfaces and the hydrophobicity of formed coatings. In this study, wettability and hydrophobicity of coating formulations were evaluated by measuring the wettability (contact angle and spread coefficient) of coating formulations onto fruit surface and the correlation of surface tension (ST) of the coating formulations with the critical ST of fruit surface, hydrophobicity (contact angle) of coating formulations onto hydrophobic silica wafer, and WVP of the derived films.

The type of emulsions incorporated into coating formulations had significant (P<0.05) impact on CA on banana surfaces, showing lower CA in coating formulation containing OA/Tween 80 (36.8 °) or OA/SEFA (31.2 °) than that with Tween 80 only (44.8 °) (Table 1). The spread coefficient (W_s) of coating formulations was significantly (P<0.05) affected by the interactive effect between the type of coating matrix and emulsion, with the higher W_s in emulsified coating formulations (CNCB, CNCC, CNFB, and CNFC) than those without emulsion (CNCA and CNFA). For ST, the two treatment factors (coating matrix and emulsion) had significant (P<0.05) interactive effect on ST of coating formulations, showing the lowest ST in CNCC and CNFC coating formulations (26.0 mM/m and 25.4 mM/m, respectively) among all treatments (Table 1). These results supported that emulsified coating formulations improved the wettability of coatings onto hydrophobic banana surfaces composing of cutin and wax in cell wall (Soradech et al., 2017). In addition, the ST of developed coating formulations was lower than the critical ST of fruit surfaces, derived from the Zisman plot, was 35.2 mN/m (Fig. 1), indicating that banana surfaces carried low surface energy (< 100 mN/m). Many fruit surfaces have low surface tension due to the presence of natural wax layer. While this nature wax layer is protective for fruit, it requires high wettability of aqueous coatings to be uniformly adhered on

fruit surfaces (Viña et al., 2007). To enhance wettability of coatings onto fruit surface, the ST of coating formulations should be closer and/or lower than the critical ST of the fruit surface (Tzoumaki, Biliaderis & Vasilakakis, 2009). Above results supported that all coating formulations developed from this study except CNFA had lower ST than the critical ST of banana surface, thus ensuring sufficient adhesion of coatings on the banana surfaces.

In respect to the hydrophobicity, coating formulation with OA/SEFA had a significantly (P<0.05) lower CA onto hydrophobic silicon wafer than that with OA/Tween 80 (Table 1), which could be attributed to the more hydrophobic SEFA in comparison with Tween 80, thus reducing the oil-water interfacial tension and improving hydrophobicity of the coatings (Ariyaprakai, Limpachoti & Pradipasena, 2013). Meanwhile, the type of coating matrix and incorporated emulsion had significant (P<0.05) effect on WVP of derived films, in which WVP of CNFC film (0.03 g mL/m^2 d Pa) had the lowest value among all coating formulations, indicating a superior moisture barrier (Table 1). It was hypothesized that OA/SEFA emulsion could be well dispersed into continuous CNF phase with slight surface charges and flexible structure in comparison with CNC-reinforced chitosan coating, thus preventing moisture diffusion throughout the hydrophobic CNF emulsion matrix (Ruíz-Ramos, Pérez-Orozco, Báez-González, Bósquez-Molina, Pérez-Alonso & Vernon-Carter, 2006). CNC-reinforced chitosan matrix could be less compatible with OA/SEFA emulsion system as shown by the reduced surface charges due to the electrostatic interaction between positively charged chitosan and negative surface charges of CNC and high crystallinity of continuous phase. Therefore, the emulsion system composed of OA and SEFA in CNF-based coating matrix could derive hydrophobic coatings with improved moisture barrier function. However, further studies should

be conducted to investigate differences in surface chemistry and interactions amongst various components between the two coating matrix systems to validate the suggested hypothesis.

Based on the performance of developed coating formulations and derived films, it might be concluded that CNF-based coating with OA/SEFA emulsion (CNFC) could provide superior moisture barrier and good wettability onto banana surfaces for extending storability of fresh banana fruit.

3.2. Effect of developed emulsion coatings on fruit quality during ambient storage

The effect of coating formulations on the chlorophyll degradation, weight loss, and marketability of bananas during 10 d of ambient storage is reported in Fig. 2. CNFC coating resulted in the least and slowest chlorophyll degradation of banana peels among all coating formulations (Fig. 2A). CNFC coating also caused the lowest weight loss (~17%) of fruit at the end of 10 d ambient storage in comparison with uncoated (~24%) and other treatments (~19-23%) (Fig. 2B). Furthermore, CNFC retained the highest fruit marketability compared to other coating formulations over the storage period (Fig. 2C). About 50% of uncoated (control) fruit lost marketability after 5 d of storage, whereas about 90% of CNFC-coated fruit were still marketable at 8 d of storage. The effectiveness of CNFC coating could be attributed the wekk dispersed OA/SEFA emulsion in the CNF coating matrix that closely interacted with fruit surfaces to provide uniform coating coverage and good moisture barrier, thus preventing moisture loss, reducing chlorophyll degradation, and improving marketability of fruit during storage. Hence, CNFC coating was selected for further validation and in-depth studies to understand the mechanism of the coating on the physiological activity and surface characteristics of banana fruit.

3.3. Effect of developed CNFC coating on the physiological activity and surface characteristics of banana fruit

Uncoated, Semp-coated, and CNFC-coated fruit were further studied for their effect on the physiological activity (Fig. 3) and surface characteristics (Fig. 4) of bananas during ambient storage. The CNFC coating significantly reduced ethylene production of fruit (0.82 ppm/g), compared to non-coated (4.41 ppm/g) and Semp-coated fruit (2.38 ppm/g) ones (Fig. 3A). The CNFC-coated fruit also contained lower CO_2 and higher O_2 in comparison with non-coated one, while had similar CO_2 and O_2 to the Semp-coated fruit (Fig. 3B). Fruit respiration (O_2 and CO_2) and ethylene production are the main physiological indexes tracking the change of ripening and senescence over the storage period (Ahmed & Palta, 2016). These data supported that CNFC coating suppressed the respiration and ethylene production of bananas by forming modified internal atmosphere within fruit, thus delaying fruit ripening and senescence.

Postharvest climacteric fruit produces ethylene through autocatalytic ethylene biosynthesis, in which ACC as the precursor of ethylene and ACS as the catalytic enzyme synthesizing ACC from SAM (Fig. 1). As shown in Fig. 3C, CNFC coating resulted in significantly higher ACC concentration of fruit in comparison with uncoated and Semp-coated ones. This result indicated that CNFC coating modified the internal atmosphere of fruit, which limited hydrolysis of ACC into ethylene, thus generating less ethylene production with accumulated ACC in fruit (Ketsa, Wisutiamonkul & van Doorn, 2013). This result was consistent with the lower ethylene production in CNFC-coated fruit in comparison with uncoated and Semp-coated ones (Fig. 3B). Meanwhile, ACS activity was peaked at 0 d of storage, then gradually reduced during the first 4-5 d of storage, but increased again for CNFC and Semp-coated fruit during the rest of storage

(Fig. 3D). The initial higher ACS activity could be associated with the onset of subsequent peel yellowing of obtained fruit samples (Ketsa, Wisutiamonkul & van Doorn, 2013). It was possible that the banana fruit obtained from the local market might already reach to the onset of subsequent peel yellowing prior to the local store. The increased ACS activity of Semp and CNFC-coated fruit after 7 d of storage might be associated with the delayed ripening in the postponed ripening stage of fruit. CNFC coating resulted in lower fruit ACS activity than that of Semp coating, showing slower ripening process. Hence, CNFC coating could control the physiological activity of bananas as shown by the lower production of ethylene and CO_2 and less ACS activity, thus delaying fruit ripening.

The influence of coatings on the fruit surface characteristics through SEM analysis is illustrated in Fig. 4. CNFC coating uniformly covered the pericarp surface without cleavage among epidermal cells, whereas some cracks and/or cleavage between the cells were appeared for non-coated and Semp-coated fruit. These insufficient coverage might potentially accelerate moisture loss, respiration, and fungus invasion (Amarante, Banks & Ganesh, 2001). In addition, the size and shape of epidermal cells of CNFC-coated fruit were altered as marked in Fig. 4, which was probably due to the interactions between the fibrous CNF matrix and the epidermal cells of banana peels. Hence, the surface morphology of the fruit further ensured that the fibrous, hydrophobic CNFC coating could be well associated with the banana surfaces to provide effective coating performance.

3.4. Validation of CNFC coating formulation on fruit quality during ambient storage

Validation study was conducted for uncoated, Semp-coated, and CNFC-coated fruit. Visual appearance of fruit was monitored at 3, 7, and 10 d of ambient storage (Fig. 5). During

436 green-yellow life of banana (0-3 d of storage), both Semp and CNFC coatings slowed down the
437 chlorophyll degradation. During the yellow-brown life (7-10 d of storage), CNFC coating further
438 reduced the incidence of browning spots on the fruit surfaces in comparison with uncoated and
439 Semp-coated ones. At yellow stage, banana fruit continue to ripen, the presence of polyphenol
440 oxidase (PPO) further promotes the changes of phenol into quinine and increase in
441 macromolecules by polymerization, thus leading to the accumulation of brown pigment
442 (Soradech, Nunthanid, Limmatvapirat & Luangtana-anan, 2017). CNFC coating reduced the
443 enzymatic browning in the yellow stage of fruit by delaying banana ripening and scencence.
444 The startch test showed the high content of starch in CNFC-coated bananas as reflected by
445 the darker blue/black color on the cross-cut fruit surfaces from iodine reaction in comparison
446 with uncoated and Semp-coated fruit (Fig. 3). Similar trend was observed from TSS at 3 and 7 d
447 of storage, showing CNFC coating resulted in the lowest TSS of fruit in comparison with
448 non-coated and Semp-coated ones (Fig. 3). These results proved that CNFC coating delayed
449 banana ripening by preventing starch hydrolysis and conversion into soluble sugars (Prabha &
450 Bhagyalakshmi, 1998). Meanwhile, TSS of CNFC-coated bananas had no significant difference
451 from that of non-coated one at 10 d of storage, indicating that proper ripening process continued
452 in bananas during the storage. This result was also supported by the increased ACS activity after
453 7 d of storage, indicating that the proper ripening continued in CNFC-coated bananas.
454 Both Semp-coated and CNFC-coated bananas retained higher firmness than uncoated sample
455 at 3 and 7 d of storage (Fig. 3). Firmness is an important parameter to determine the ripening
456 stage and quality of banana fruit. While ripening, pectinesterase and polygalacturonase
457 hydrolyzed the pectin and starch, leading to the destruction and deterioration of the cell wall
458 structure, in turn softened the fruit (Singh, 1993; Yaman & Bayoındırlı, 2002). Based on the

results reported and discussed above, CNFC coating with uniform surface coverage onto fruit surface through the interactions between CNF and epidermal cells of banana skin was able to delay physiological activity and ripening of coated fruit, thus retaining fruit firmness during storage.

TA of CNFC coated fruit was significantly ($P<0.05$) higher than uncoated and Semp-coated fruit throughout the 10 d of storage (Fig. 5). It was assumed that CNFC coating reduced the consumption of organic acids as the primary substrate for respiration process during the storage due to controlled physiological activity of fruit (Maqbool, Ali, Alderson, Zahid & Siddiqui, 2011). The validation study confirmed that CNFC coating was effective to delay ripening, retard quality deterioration, and extend the storability of postharvest bananas during ambient storage.

4. Conclusion

This study developed cellulose nanofiber (CNF)-based emulsion coatings for enhancing postharvest storability of banana fruit during ambient storage through controlling the physiological activity and improving the adhesion of coatings onto the fruit surfaces. The emulsion system with oleic acid (OA) and sucrose ester fatty acid (SEFA) enhanced the hydrophobicity, stability and wettability of coatings onto fruit surfaces. The CNF emulsion coatings also delayed the ethylene biosynthesis pathway and reduced the production of ethylene and CO_2 of the fruit, as well as modified the fruit surface morphology to provide more uniform coating coverage. CNF emulsion coating demonstrated its effectiveness for reducing chlorophyll degradation of banana peels and weight loss and firmness of fruit, thus enhancing the marketability and storability during ambient storage. Further studies for investigating the

481 influence of coatings on sensory attributes and consumer acceptance are under the way for
482 promoting the potential commercial applications of developed coating technology.

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