AN ABSTRACT OF THE DISSERTATION OF

Zilong Deng for the degree of Doctor of Philosophy in Food Science and Technology presented on November 29, 2018.

Title: <u>Cellulose Nanomaterial Incorporated Edible Coatings for Improving</u> Storability of Postharvest Fruit: Mechanisms, Development and Validation

Abstract approved:

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The overall objective of this dissertation study was to develop and validate the performance of cellulose nanomaterials (CNs) incorporated fruit coatings for improving the storability of postharvest pears and bananas under various storage conditions. Four specific studies were conducted to fulfill the research objectives as stated below.

First, cellulose nanocrystal (CNC, 0, 0.1% and 0.2%, w/w) reinforced chitosan (CH, 2% w/w) coatings were evaluated for delaying ripening and quality deterioration of postharvest D'Anjou pears (*Pyrus communis*) during ambient $(20 \pm 2 \,^{\circ}C \text{ and } 30 \pm 2\% \text{ RH})$ and cold storage (-1.1 $\,^{\circ}C$ and 90% RH), respectively. The concentration of incorporated CNC affected coating performance. The 0.1% CNC reinforced CH coating (CH-CNC) decreased chlorophyll degradation of pear peels, and reduced weight and firmness loss as well as change of total soluble solid content of pear flesh during 3-weeks of ambient storage. However, 0.2% CNC incorporated CH coating induced surface speckling and pithy brown though it provided similar control on weight and firmness loss compared to 0.1% CNC reinforced CH coating.

In the second study, CNC Pickering emulsion incorporated CH coating (CH-

PCNC, 0.1% CNC/3% oleic acid/2% CH, w/w) was developed to provide better stability under high relative humidity (RH) cold storage (90% RH and 1.7 °C) for Bartlett pears. For evaluating coating performance, CH-PCNC films derived from coating formulations and stored under 43% and 83% RH were subjected to FTIR analysis. Results showed no large absorption band for –OH stretching, whereas that of previously developed CH-CNC films stored at 83% RH exhibited an elevated peak in comparison with film conditioned at 43% RH. These results indicated that CH-PCNC held more stable matrix than CH-CNC under high RH conditions. Water vapor permeability (WVP) of CH-PCNC film was four times lower than that of CH-CNC film, indicating more hydrophobic and stable coating against high RH conditions. Moreover, CH-PCNC coatings delayed pear ripening and reduced senescent scalding incidence of pears during 3-months of cold storage.

In the third study, cellulose nanofiber (CNF) based emulsion coating (0.3% CNF/1% OA/1% sucrose ester fatty acid, w/w) was studied to improve the storability of postharvest bananas (*Musa acuminate*) under ambient conditions (20 ± 2 °C and 50 \pm 5% RH). Properties of coating suspensions and derived films were investigated to understand the mechanism of improved hydrophobicity, wettability and surface adhesion onto fruit surfaces. The developed emulsion coatings had low contact angle and surface tension, as well as high spread coefficient onto fruit surfaces, indicating good adhesion with banana skin. Coated bananas showed hindered ethylene biosynthesis pathway and reduced ethylene and CO₂ production, as well as reduced fruit skin chlorophyll degradation, weight and firmness loss, thus enhancing the marketability of fruit.

Finally, CNFs derived from different raw materials and production methods were evaluated and their performance by incorporating to previously developed CNF emulsion coatings on bananas were validated. A fine CNF without mineral incorporation had lower particle size, effective diameter and better dispersion, presenting better compatibility with the emulsion coating system. When incorporating into CNF based emulsion coating, it exhibited more uniform coating coverage without introducing visible white substances on the fruit peels and less peel spots at the ripening stage together with similar impact on overall appearance, chlorophyll degradation and firmness reduction. This study indicated that different CNFs with varied intrinsic properties could impact coating performance of CNF based emulsion coatings.

The results from this dissertation study demonstrated that CNs could be used as a coating forming matrix or carrier of emulsion droplets, reinforcing agent by incorporating to other biopolymers, and/or Pickering emulsifier to improve gas and moisture barrier as well as adhesion properties due to their superior intrinsic properties and compatibility with other polymers. The CNs incorporated coating improved the storability of postharvest pears and bananas under different storage conditions by reducing hydrophilicity of the biocomposite coating materials, providing stronger barrier to water and gas exchange through a modified atmosphere within fruit, and improving wettability and adhesion between coating suspension and fruit skins. Developed coatings could have great potential for reducing food waste of postharvest fruit.

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Cellulose Nanomaterial Incorporated Edible Coatings for Improving Storability of Postharvest Fruit: Mechanisms, Development and Validation

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Zilong Deng

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Zilong Deng, Author

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CONTRIBUTION OF AUTHORS

Dr. Yanyun Zhao and Dr. Jooyeoun Jung assisted with the experimental design, data analysis, and writing of each chapter. Dr. John Simonsen assisted with data collection of FTIR and manuscript revisions. Dr. Yan Wang assisted with data collection of pear studies and revision of Chapter 3.

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CHAPTER 1

INTRODUCTION TO CURRENT WORK

Fruit obtain great nutritional values and consumption of fresh fruit can decrease the risk of several chronic diseases (Kerch, 2015). However, fresh fruit suffer postharvest physiochemical changes (e.g. color degradation, weight loss, softening and senescence) and nutritional loss (e.g. ascorbic acid and polyphenols reduction) (Dhall, 2013; Lin and Zhao, 2007). Several postharvest technologies including refrigerated temperature, controlled atmosphere (CA) storage, application of 1-Methylcyclopropene (1-MCP), and edible coating have been commercially utilized to improve storability of many postharvest fruit (Valero et al., 2016). However, each of these technologies has either limited functions or drawbacks. For examples, refrigerated temperature (cold storage) alone would not provide sufficient control on postharvest physiological change together with poor color development and reduced flavor (Paull, 1999); CA storage is expensive and may also eventually cause severe senescent core breakdown; and 1-MCP irreversibly impairs ripening capacity of some fruit through binding with ethylene receptors in fruit cells (Deng et al., 2018; Zhi et al., 2018).

Edible coating is a minimally processed technology that forms a semipermeable thin layer through edible materials on fruit surfaces. The thin edible layer provides barriers against surrounding moisture and gases and creates a modified atmosphere, thus delaying fruit ripening and quality deterioration (Baldwin et al., 2011). However, current coating technologies have limited functionalities in regarding to insufficient water-resistance, gas barrier, mechanical and thermal properties as well as instable coating matrix under high RH storage conditions (Hassan et al., 2018; Vieira et al., 2011). Hence, continuous efforts in developing more effective coating technologies for postharvest fruit, through identifying new coating matrix materials, reinforcing biopolymer matrices, and/or incorporating hydrophobic compounds or other functional substances into coating matrix are necessary and important.

Cellulose nanomaterials (CNs) are emerging substances obtained from cellulose sources which comprises at least one dimension of nanoscale cellulose fibrils with highly ordered cellulosic chains aligned along the bundle axis (Azeredo et al., 2017; Moon et al., 2016). Derived from similar cellulose sources (e.g. trees and plants), there are two major categories of CNs, cellulose nanofibers (CNFs) produced through strong mechanical shear and cellulose nanocrystals (CNCs) processed through acid/enzymatic hydrolysis. CNs provides unique intrinsic properties including low density, abundant surface accessible hydroxyl groups, and high transparency, specific surface area and mechanical properties (Bharimalla et al., 2017; Moon et al., 2016). Many studies have demonstrated that CNs could potentially play significant roles in biocomposite food films and coatings as matrix, carrier of emulsion droplets, reinforcing agent by incorporating to other biopolymers, and/or Pickering emulsifier for introducing hydrophobic compound, leading to enhanced mechanical, barrier, thermal and rheological properties (Fernandes et al., 2010; Liou et al., 2017; Pereda et al., 2014). Therefore, it was hypothesized that 1) CNs incorporated biocomposite coatings can improve storability of postharvest fruit by

providing superior gas and moisture barrier and better water resistance together with compatibility with other biopolymers, 2) coating formulation should be customized for different fruit because of varied fruit physiological properties, surface characteristics, and required storage conditions, and 3) different CNs might impact coating effectiveness due to varied intrinsic properties and compatibility with other coating ingredients.

The overall objective of this dissertation research was to improve the storability of postharvest fruit under various storage conditions through CNs incorporated biocomposite coatings using a systematical approach by considering 1) fruit physiology alteration during postharvest life, 2) properties of coating materials, 3) adhesive interactions between fruit surface and coating suspension, and 4) storage conditions of the fruit. The work was divided into seven chapters and one appendix. Following this introduction to the current work, Chapter 2 provided a comprehensive literature review on postharvest fruit physiology and quality deterioration, edible coatings and films, and mechanism and principle of CNs incorporated coatings. Chapter 3 developed and evaluated CNC reinforced CH coating (CH-CNC) for improving storability of postharvest pears during both ambient and cold storage conditions. The manuscript was published in Journal of Food Science in 2017. Chapter 4 developed and validated CNC Pickering emulsion CH coating for improving the storability of postharvest pears against high RH long-term cold storage conditions. The manuscript was published in Food *Hydrocolloid* in 2018. Chapter 5 developed and demonstrated CNF based emulsion coating for improving storability of postharvest bananas under ambient storage. The

manuscript was published in *Food Chemistry* in 2017. Chapter 6 further investigated the intrinsic properties of different CNFs and the impact on banana coating performance when incorporating to CNF based emulsion coatings. Chapter 7 summarized key findings of the whole dissertation, suggested criteria for the development of CNs incorporated postharvest fruit coating technology, and provided future prospective. Appendix developed, characterized and evaluated CH adsorbed CNF films as food contact material. The manuscript was published in *LWT-Food Science and Technology* in 2017. It was expected that this dissertation study helped understand the mechanism and principle of CNs incorporated fruit coatings and filled up the gap in today's coating technology for postharvest fruit.

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CHAPTER 2

LITERATURE REVIEW

2.1. Introduction

Fruit have significant nutritional value due to rich content in vitamins, minerals and phytochemicals (Rico et al., 2007). There were evidences that fruit consumption may lead to the alleviation of cancers, diabetes and cardiovascular diseases (Kaur and Kapoor, 2001). Unfortunately, most fruit are highly perishable, and are subjected to postharvest quality losses, such as dehydration, discoloration, softening, and loss of flavor and nutritional value. In 2018, postharvest losses cover more than 50% of total loss of the freshly harvested fruit worldwide (Porat et al., 2018). Although more attentions have been paid in the last decade, issues in postharvest fruit loss during storage, distribution and retail market still remain due to responsive physiological alteration and quality deterioration (Porat et al., 2018).

Fruit are divided into two respiratory patterns: climacteric and non-climacteric, with distinct responses towards ripening process (Paul et al., 2012). Non-climacteric fruit possess a continuous but gradual postharvest process, in which ethylene is not considered as essential to induce ripening. Climacteric fruit are more sensitive to ethylene and have a drastic increase in CO₂, heat and autocatalytic ethylene production triggered by the exogenous ethylene application during postharvest ripening stage (Pech et al., 2008). As soon as climacteric fruit initiate the ripening process, rapid physiological alternation starts, pushing fruit to the market for consumption within a few days before reaching

significant postharvest deterioration and spoilage (Baldwin et al., 2011). Due to varied physiological natures between two respiratory patterns, different strategies may be required for postharvest quality extension depending on the type of fruit.

Derived from the major causes of fruit physiological alteration including respiration and transpiration, fruit suffer quality deterioration, such as dehydration, color degradation, softening, loss of nutritional value and flavor, and physiological disorders during their postharvest storage (Baldwin et al., 2011). Dehydration is mainly caused by the transpiration of moisture from the pulp to the external atmosphere. Color degradation, for cultivars with green skin color, takes place due to the function of chlorophyllase on chlorophyll, allowing the visibility of the yellow carotenes (Yamauchi et al., 2008). Fruit softening is determined by the integration level of plant cell walls due to pectin solubilization and depolymerization, because pectolytic enzymes defunctionalize the interspersed pectin and weaken the cellulose/hemicellulose network (Broxterman and Schols, 2018; Zdunek et al., 2016). Nutritional values including vitamin C, minerals and dietary fiber continuously decrease during storage owning to the degradation of integrated cell wall structure (Lin and Zhao, 2007). Flavors are also altered due to increased permeation of the fruit skin during ripening together with the accumulation of the aroma compounds (Deng et al., 2017). Physiological disorders are induced by non-pathological factors including improper controlled storage conditions or gas compositions. Fruit present

symptoms such as scald, internal core breakdown, pitting and chilling injury depending on the cultivars (Baldwin et al., 2011).

Several technologies including refrigerated temperature (cold storage), controlled atmosphere (CA) storage, 1-Methylcyclopropene (1-MCP) treatment, and edible coating have been commercially applied for improving storability of postharvest fruit (Valero et al., 2016). However, cold storage alone may not provide sufficient effectiveness together with poor color development and reduced flavors (Paull, 1999). CA storage suppresses the senescent disorder incidence of many fruit by regulating the O₂, CO₂ and N₂ concentrations without impairing the ripening capacity. However, CA may eventually cause severe senescent core breakdown leading to pathological disease and off-flavor, and the technology is very expensive (Thompson, 2016; Zhi et al., 2018). Although 1-MCP treatment can delay ripening and reduce the incidence of physiological disorders of some fruit, fruit suffer resistance to normal ripening due to its irreversible binding with ethylene receptors in the fruit cells (Villalobos-Acuña et al., 2011). Therefore, based on the great needs on preserving postharvest fruit and limitations of current postharvest technologies, a cost effective postharvest technology without impairing fruit ripening capacity and inducing unexpected physiological issues is required for different types of fruit under varied storage conditions.

As one of the minimal processed technologies, edible coating is the application of a thin edible layer on fruit surface to improve the storability postharvest fruit by providing moisture and gas barrier (Lin and Zhao, 2007). While several edible coating products are commercially available, each has limited functions, such as insufficient barrier, lack of stability under varied temperature and humidity conditions, and absence of integrity and mechanical strength.

This review described the conventional biocomposite edible coatings and derived films; introduced cellulose nanomaterials (CNs) as a new component in postharvest fruit coatings; discussed the principles and roles of CNs incorporated biocomposites as potential coating formulation for postharvest fruit, and evaluated previous studies on CNs incorporated coating applications for postharvest fruit.

2.2. Edible coatings and films

2.2.1. Definition, principle and functions of edible coatings

Edible coating technology for postharvest fruit is defined as applying a semipermeable thin membrane of edible material on fruit surface to provide barrier against water and gas exchange and create a modified atmosphere in fruit with multifunction. Coating may be applied through spraying, dipping and/or brushing methods (Dhall, 2013). Figure 1 illustrates the principle mechanism of edible coatings. The required properties of edible coatings may include (Dhall, 2013; Hassan et al., 2018; Lin and Zhao, 2007; Otoni et al., 2017):

1) To restrict gas exchange through fruit peel and form modified atmosphere within fruit by the reduction of oxygen and accumulation of carbon dioxide;

2) To provide additional moisture barrier for reducing weight loss of postharvest

fruit;

3) To carry functional ingredients, such as antimicrobial and antioxidant agents for enhancing antimicrobial and antioxidant property;

4) To retain fruit internal quality and secure developed volatile compounds; and5) To improve appearance, maintain integrity and prevent mechanical damage or UV attack.

2.2.2. Types of edible coatings

An edible coating formulation generally comprises one or a few of ingredients including polymer matrix, hydrophobic compound, and other functional compounds like filler, plasticizer, surfactant, nutrient, antimicrobial and antioxidant agents (Hassan et al., 2018). Fillers, such as cellulose and chitosan nanoparticles, are incorporated to polymer matrix in coating formulations for decreasing molecular mobility and relaxation behavior, thus enhancing thermal, mechanical and barrier properties (Otoni et al., 2017). Plasticizer, most commonly glycerol, is used to improve the mobility of polymer through reducing the intermolecular attractions between polymer chains, leading to more flexible matrix with less cracking and porous structure (García et al., 2000; Karbowiak et al., 2006). However, elevated chain mobility may result in weakened barrier properties due to the less tortuous diffusion pathway of gas and vapor (Belbekhouche et al., 2011; Han, 2014). Surfactant, such as Tween 80, sucrose fatty acid esters and lecithin, is applied to decrease the surface tension and achieve the uniform dispersion of coating suspension, thus improving the wettability of coatings on the designated fruit skin (Cisneros-Zevallos and Krochta, 2002). Edible coatings are excellent vehicles to deliver nutrients (e.g. vitamin E and calcium source), antimicrobial (e.g. potassium sorbate and essential oil) and antioxidant (e.g. ascorbic acid, essential oils) agents, which are absent, short of and/or of great needs in specific postharvest fruit, for enhancing the nutritional values, antimicrobial and antioxidant properties of the coating formulation.

Depending on the type of polymer matrix and hydrophobic compounds in the coating formulation, edible coatings and films are divided into three major categories: polysaccharides, proteins and lipids. Polysaccharides extracted from marine and plant sources including cellulose, chitosan, starch, alginate, gums and pectin, have been widely used in the edible coating and film formulations for decades. Polysaccharides based coatings are able to modify fruit internal atmosphere and reduce postharvest respiration, thus slowing down ripening and quality deterioration. However, due to the hydrophilic nature of the polysaccharides, the impact on reducing moisture loss may not be efficient (Flores-López et al., 2016). Two different categories of proteins, including fibrous proteins (whey protein, casein, gelatin and collagen) from animal tissues and globular proteins (zein and soy protein) from plant source, have also been used to form coatings and films (Dhall, 2013; Hassan et al., 2018). Protein chains are interacted through not only H-bonds but also covalent and ionic bonds, resulting in strengthened mechanical property. However,

proteins are more sensitive to acid, base, salt and heat, which may lead to the denaturation of the quaternary, tertiary and secondary structures, in turns impairing film and coating matrix (Krochta and De Mulder-Johnston, 1997). Moreover, surface hydrophilic nature of the proteins lead to poor moisture barrier property though providing good gas barrier at low RH (Dhall, 2013). Lipid based coatings mainly consist of neutral lipids (e.g. cholesterol), waxes (e.g. carnauba wax), fatty acids (e.g. oleic acid) and resins (e.g. shellac). Lipids are always incorporated with other biopolymers in order to achieve required functionalities. Lipid incorporated coatings provide good moisture barrier for fresh fruit with additional shininess (Baldwin et al., 2011). However, lipid based films have poor mechanical and optical properties and derived coatings bring about the greasy surface, rancidity and unexpected organoleptic sensory attributes for postharvest fruit (Arnon-Rips and Poverenov, 2016). None of polysaccharide, protein or lipid alone possesses all required functionalities. Therefore, composite coatings, by a combination of multiple types of materials compatibly, have been developed to accumulate the beneficial properties from different materials (Dhall, 2013).

Table 1 summarizes commonly applied polysaccharides, proteins, lipids and their composite edible coatings from the recent publications on postharvest fruit including apples (Mehyar et al., 2011; Sahraei et al., 2016), bananas (Maqbool et al., 2011; Soradech et al., 2017; Suseno et al., 2014), citrus (Arnon et al., 2014; Saberi et al., 2018), grapes (Fakhouri et al., 2015; Kanetis et al., 2017), mangos (Cissé et al., 2015; Moalemiyan et al., 2012), pears (Cruz et al., 2015; Kou et al., 2014; Nandane et al., 2017) and strawberries (Muñoz-Labrador et al., 2018; Ribeiro et al., 2007). These studied demonstrated that biocomposite edible coatings could effectively delay fruit ripening and prevent quality deterioration, leading to the improved storability of various postharvest fruit.

2.2.3. Limitations of conventional biocomposite based edible coatings

Although several edible coating products achieved some of the required functionalities and have been commercially utilized in postharvest fruit industry, each of them has certain constrains and limitations (Dhall, 2013; Fernandes et al., 2010; Hassan et al., 2018; Lin and Zhao, 2007), such as:

1) Insufficient moisture barrier and water resistance due to hydroscopic nature;

2) Limited gas barrier especially under high RH or over-restricted respiratory gas barrier leading to off-flavor and accumulation of acetaldehyde and ethanol within fruit;

3) Poor mechanical properties;

4) Lack of efficient wettability, adhesion and homogeneous dispersion due to different nature between hydrophilic coating materials and hydrophobic fruit surface; and

5) Potential allergic issue, especially with protein based coating material.

To meet the required coating performance and fill up the gaps of current coating technology, cellulose nanomaterials (CNs), was introduced as a new biodegradable

component in developing effective edible coatings throughout this dissertation research.

2.3. Cellulose nanomaterials (CNs)

2.3.1. Definition and production of CNs

Cellulose, as a naturally occurred biopolymer existed in plant cell walls, presents tightly packed polymer chain structure with the continuous repetition of D-glucose units covalently linked through β -1,4 glycosidic bonds (Hassan et al., 2018). CNs is defined as light cellulosic substances obtained from cellulose sources and comprises at least one of external dimension in nanoscale (1-100 nm). CNs are available from plants (e.g. wood pulp, cotton and algae), microbes (e.g. bacteria and fungi) and sea animals (e.g. tunicate). The large-scale CNs production are commonly produced from plant source including recycled paper products and biomass residuals (Mishra et al., 2018).

Mechanically delaminated cellulose nanofibers (CNFs) and hydrolytically extracted cellulose nanocrystals (CNCs) are two major types of CNs. CNFs and CNCs can be obtained by two different top-down methods from the same raw cellulose material. Production of CNs consists of two steps including pretreatment followed by refinement (Vilarinho et al., 2018). Pretreatment processing purifies and homogenizes the raw material to the individual cellulosic fibers through opening their structure, which are capable for further facilitation. Refinement, generally called fibrillation, is the secondary step to fragment the prepared individual fibers into CNF and CNC. For producing CNF, fragmented cellulosic structures were further tore apart through mechanical shear such as high pressure homogenization, grinding and ultrasonication with or without chemical and biological treatments. For manufacturing CNC, either acid (e.g. sulfuric acid and hydrochloric acid) or enzymatic hydrolysis is utilized to eliminate the amorphous regions of cellulosic fibers and obtain highly crystalline CNC (Khalil et al., 2016; Vilarinho et al., 2018). CNF, with nanofibrillar structure and entangled network structure, has diameter of 5-70 nm and length of 200 nm to several micrometers. CNC, as rod-like nanocrystalline cellulose in whisker shape, possesses 3-50 nm in diameter and 100-250 nm in length (Table 2) (Bharimalla et al., 2017; Mishra et al., 2018).

2.3.2. Intrinsic properties of CNs and their derived film properties

Generally CNs present distinguished intrinsic properties including low density, high aspect ratio, optical transparency, high mechanical property, superior gas and moisture barrier, and good thermal stability (Dumanli et al., 2014; Kümmerer et al., 2011; Zhu et al., 2013).

The presence of interfibrillar hydrogen bonds and entangled structure provide CNs with film forming property (Azeredo et al., 2017). The optical transparency of CNs film can be explained by their small interstices between densely packed cellulosic chains to avoid light scattering (Iwamoto et al., 2007). As a result of their nanodimension, CNs obtained high specific surface area between 50 and 200 g/m² with low density at 0.02 g/cm². The large surface area of CNs can increase their interactions with secondary material, resulting in enhanced functionality of the polymer blend (Stenstad et al., 2008). Well-stacked cellulose chains with high specific surface area bring about rigidity, durability and resilience of CNs (Moon et al., 2016). Compared to CNF (1.5-5.5 GPa for tensile strength and 150-500 GPa for elastic modulus), CNC showed higher tensile strength (7.5-7.7 GPa) and low elastic modulus (110-220 GPa, Table 2) (Mishra et al., 2018). In respect to thermal properties, CNF has higher degradation temperature at 350 °C while CNC possesses lower value between 200-300 °C (Table 2), indicating their better thermal stability compared to other lignocellulosic materials (Mishra et al., 2018).

Due to the highly crystalline nature and formed stiff network, both CNF and CNC films have high water resistance and low water permeability (Bharimalla et al., 2017). In comparison with CNF, CNC films are more restricted to water vapor transmission because of the further erased amorphous regions (Table 2) (Fujisawa et al., 2011; Guo et al., 2017). Although the surface water affinity is high for both CNF and CNC, they provide superior oxygen barrier under low RH. More importantly, the permeation of CNF and CNC films is lower than not only common biopolymers but also many petroleum polymers, especially for CNF (Lavoine et al., 2012). This is because the gas molecules have to penetrate through longer diffusion pathway with significantly elevated tortuosity due to the presence of the highly entangled nanocellulosic fibrils and low porous crystalline domains (Belbekhouche et al., 2011). Different from the trend of moisture barrier, CNC film is more gas-permeable for both O₂ and CO₂ than CNF film due to less

presence of entanglement (Table 2) (Belbekhouche et al., 2011). However, CNC can be embedded to other polymer matrices to slow down the transportation of gas molecules by reinforcing porous structures (Fortunati et al., 2012).

2.3.3. Principles, roles and potential applications of CNs in edible films and coatings

Based on their unique intrinsic properties, CNF and CNC can potentially play beneficial roles once incorporated to edible films and coatings. Due to presence of highly accessible hydroxyl groups on cellulosic backbone, both CNF and CNC are compatible with other surface-hydrophilic biopolymers through noncovalent linkages (e.g. adsorption, surfactant and electrostatic) for altering the dispersion, alignment, surface chemistry, interfacial properties, and percolation (Mishra et al., 2018; Moon et al., 2016), leading to great potential for incorporating to edible films and coatings. Specifically, CNF may be used as 1) film/coating forming matrix with excellent mechanical, moisture and gas barrier, 2) carrier of functional agents (e.g. antimicrobial and antioxidant agent, nutrients, and emulsion droplets), and 3) reinforcing agent to other biopolymers; CNC may be functioned as 1) filler to other biopolymers for enhancing mechanical, moisture and gas barrier properties, and 2) Pickering emulsifier to substitute conventional emulsifier (Figure 2) (Bharimalla et al., 2017; Dhall, 2013; Mishra et al., 2018; Otoni et al., 2017).

According to the highly crystalline nano-fibrillated structure, CNF can form

film/coating matrix due to strong tensile property and excellent barrier for preventing the penetration and diffusion of gas and water molecules (Xu et al., 2016), which may potentially reduce mechanical handling issue, restricted moisture transpiration and gas exchange as matrix of postharvest fruit coatings. Entangled nanocellulosic polymeric matrix with huge surface area and biocompatibility brings CNF as carrier to efficiently deliver the antimicrobial and antioxidant agents, such as organic acid, bacteriocins, essential oil and plant extract at small molecular weight (Khan et al., 2014). Moreover, CNF can reinforce other biopolymers through entanglement by providing enhanced mechanical and thermal properties (Table 2) (Nair and Yan, 2015).

CNC can also be utilized to reinforce other biopolymers through different mechanism by acting as filler. Different from CNF, the reinforcing effect of CNC not only improves mechanical property, but also limits gas and water vapor permeation by its highly crystalline nature and ability to form a dense percolating network with other amorphous biopolymers (Mariano et al., 2014). CNC can also be applied as Pickering emulsifier to produce surfactant-free highly stable emulsion due to the amphiphilic characteristic derived from the surface heterogeneity and the arrangement of crystalline cellulose chains at nanodimension (Kalashnikova et al., 2012). Rod-like non-spherical cellulosic CNC is located at oil-water interface as stabilizer and emulsifier due to the selfassembly ability leading to ultra-stable Pickering emulsion system (Capron, 2018). The longer-term stability and stronger adsorption compared to conventional emulsifier bring CNC to be a more advantageous approach for incorporating hydrophobic active compounds, resulting in improved moisture barrier and better delivery of hydrophobic functional agents for edible films and coatings.

Many studies have been conducted to evaluate the potential of incorporating CNs to biocomposite food packaging, and some of them could be eventually utilized as fruit coatings (Table 3). Reinforcement by incorporating CNF (Bilbao-Sainz et al., 2011; Fernandes et al., 2010; Mondragon et al., 2015; Tomé et al., 2013) and CNC (Alves et al., 2015; Bilbao-Sainz et al., 2011; Chaichi et al., 2017; Fortunati et al., 2012; Huq et al., 2012; Khan et al., 2012; Mandal and Chakrabarty, 2018) to other biopolymers especially polysaccharides was the major application to enhance the barrier, mechanical and thermal properties of the biocomposite films. Some studies also demonstrated the use of CNF as film forming matrix and carrier of S-nitroso-Nacetyl-D-penicillamine (SNAP) with improved antibacterial properties against E. faecalism, S. aureus and L. monocytogenes (Sundaram et al., 2016), while CNC as Pickering emulsifier for the incorporation of olive oil to improve the water vapor permeability and tensile properties of the biocomposite chitosan film (Pereda et al., 2014).

2.3.4. CNs incorporated edible coatings for postharvest fruit

The demonstrated barrier and mechanical properties of CNs incorporated biocomposites revealed their feasibility as effective coatings for postharvest fruit to meet the criteria of 1) improved stability under varied temperature and RH
conditions; 2) enhanced barrier to moisture/gas compared with conventional biopolymer based coatings; 3) controllable release of incorporated antimicrobial and nutritional agents; 4) better integrity and mechanical strength; and 5) improved overall coating appearance (Otoni et al., 2017; Zambrano-Zaragoza et al., 2018). Studies of developing and evaluating CNs incorporated biocomposite coatings to improve the storability of postharvest fruit are summarized in Table 4 and discussed below.

Several studies evaluated the role of CNF as reinforcing agent to other biopolymers for postharvest fruit coatings. Andrade et al. (2015) investigated the interactions between banana epicarps and CNF reinforced gelatin coating through monitoring drop behavior of the suspension on the banana skin. CNF improved the viscosity and surface tension of the gelatin coating and 5% CNF (5 g/100 g of gelatin in the dry base) incorporation maximized the spread factor, leading to enhanced adhesive interactions between coating suspension and fruit surface. However, fruit quality parameters during postharvest storage were not evaluated in this study, thus being unable to determine whether developed coating actually improving fruit storability or not. In another study conducted by Nabifarkhani et al. (2015), CNF (0.1%, w/w) was incorporated to 1% (w/w) chitosan suspension along with addition of tween 80 and thyme oil. The developed emulsion coating reduced weight loss and retained total soluble solid (TSS), sugar and anthocyanins contents for postharvest cherries under cold storage. Resende et al. (2018) further reported the effectiveness of incorporating different concentrations of CNFs (3 or 5 g/100 g of chitosan in the dry base) to 1% (w/w) chitosan for strawberries under cold

storage. The 5% CNF incorporation helped retain fruit firmness and decrease weight loss as the result of increased coating thickness and reduced water vapor permeability (WVP) of the derived films, compared to those with 3% CNF incorporation. Unfortunately, the presence of CNF was unable to show significant impact on fruit color degradation, TSS, ascorbic acid, total phenolics, and anthocyanins contents. Silva-Vera et al. (2018) followed up with another study by evaluating a composite coating formulation with 1% (w/w) of CNF, 4% (w/w) of hydroxypropyl methyl cellulose (HPMC), and 0.2% (w/w) of *k*-carrageenan on postharvest table grapes under refrigerated storage by applying different spray coating methods. Due to the stable adhesion of developed coatings, coated fruit showed less weight loss and higher fruit firmness than those of uncoated grapes.

Coating applications by using CNC as filler to reinforce other biopolymers for postharvest fruit was also studied. Azeredo et al. (2012) investigated the performance of CNC reinforced alginate and acerola puree coatings for postharvest acerola fruit stored at 6 °C, and found that the developed nanocomposite coating helped retain higher vitamin C content compared to the ones without CNC incorporation. However, no significant difference was observed on fruit weight loss, decay incidence and ripening rates. Dong et al. (2015) studied 5% CNC (5 g/100 g of chitosan in the dry base) reinforced 1% (w/w) chitosan coating on strawberries. CNC-chitosan coating significantly decreased weight loss and decay rate, and preserved TSS, total phenols and total anthocyanins contents of strawberries. Dong et al. (2016) further investigated the coating performance of the same formulation on cucumbers. Developed coating improved fruit overall appearance, retained weight loss, chlorophyll and TSS, and firmness. The retained fruit firmness was further explained by reduced enzyme activities, including catalase (CAT) and peroxidase (POD) activity. Moreover, Fakhouri et al. (2014) examined CNC reinforced gelatin coating on postharvest strawberries stored under refrigerated temperature. The coating was able to reduce weight loss and decay ratio, and retain ascorbic acid of fruit.

Note that above discussed CNs incorporated fruit coating studies mostly took place within the past four years. Hence, the area of research and development in CNs incorporated edible coatings for postharvest fruit is relatively new. Supported by the results from those studies, it was no doubt that CNs could lead to promising applications in edible films and coatings for improving storability of postharvest fruit. However, more studies are necessary to further understand the principle and mechanism of CNs as key components in biocomposite edible films and coatings, especially targeting the real fruit system. A systematical approach should be employed to develop effective CNs incorporated coatings specifically for different postharvest fruit with varied postharvest physiology, surface characteristics and designated storage conditions.

2.3.5. Limitations of current CN-incorporated coatings for postharvest fruit applications

Although CNs provide superior functionalities, it is still noticed that some distinguished functional properties of CNs are not fully understood and inherited once incorporated to biocomposite coating formulations for postharvest fruit.

As discussed above, one of the most important functions of CNs is to strengthen and reinforce other biopolymer matrices. However, inadequate dispersion and agglomeration strongly impaired the capability of CNs as reinforcement or filler (Mishra et al., 2018). This is because the stability of CNs are reduced in aqueous system due to their high specific surface area and thermodynamic potential in nanoscale, resulting from the hydrogen bonding between active -OH groups in neighborhood (Zhang et al., 2013; Zhou et al., 2013).

For postharvest fruit quality extension, moisture and gas barrier as well as the stability of coating under varied temperature and humidity conditions are essential. CNs meet the requirement with superior water vapor and gas barriers based on its size and swelling constraints from formed rigid network (Lavoine et al., 2012; Nair et al., 2014). However, most postharvest fruit require storage under high relative humidity (RH) conditions. Therefore, high water adsorption ability, due to the presence of active hydroxyl groups at the C-2, C-3 and C-6 atoms, leads to severely impaired moisture barrier under high RH (Vilarinho et al., 2018). Moreover, superior gas barrier property at low RH cannot be retained under high RH, due to the disrupted networks as water molecules enter and high swelling takes place (Spence et al., 2010).

In addition, difficulties to incorporate other hydrophobic functional ingredients and limited compatibility with other hydrophobic biopolymers restrict the application of CNs on postharvest fruit coatings, due to the hydrophilic nature of cellulosic surface structures and well-aligned highly crystalline regions (Khan et al., 2014).

Therefore, the distinguished properties of CNs should be fully utilized and the limitation of current CN-incorporated coatings should be overcome to meet the needs of edible coating for specific postharvest fruit. By incorporating CNF as matrix and/or carrier and CNC as filler and/or Pickering emulsifier, more efficient coatings can be developed for improving the storability of different postharvest fruit under varied storage conditions.

2.3.6. Safety and regulations about use of CNs in food system

In addition of the technical barriers, safety of CNs in food related applications should be considered. Several safety related concerns have been raised, including toxicity to human and animals, ecotoxicity through exposure to environment, and potential migration of CNs into food system through direct or indirect contact with food (Souza and Fernando, 2016). CNs incorporated fruit coating as a direct food contact material may result in potential migration of CNs through fruit skin into flesh, depending on the structural properties and functional roles of CNs incorporated coatings, skin characteristics of fruit, and interactions between coating and fruit skin (Azeredo et al., 2017). Although many studies have been done in evaluating the safety and toxicity of CNs, neither CNF and nor CNC is regulated as generally recognized as safe (GRAS) by Food and Drug Administration (FDA) or as food additive by Europe Union (EU) for its commercial application in food yet (Tan et al., 2019).

In respect to toxicity of CNs, inconsistent results have been reported. Some studies indicated that CNs majorly presented low or non-toxic impact based on oral ingestion and dermal contact, but others found negative results from pulmonary (e.g. respiratory) and cytotoxicity (e.g. cell viability) assay, indicating the potential risks on toxicological aspect (Anne Shatkin and Kim, 2015; Moon et al., 2016). Regarding exposure to the environment, studies founds that CNs exhibited none or less ecotoxicity and low environmental risks due to their short biodegradation period (Souza and Fernando, 2016). Although some potential adverse impacts were reported on growth and reproduction ability of luminescent bacteria and several aquatic species including rainbow trout, zebrafish embryos, thresholds of detected CNs for those reported adverse impact were at very high concentration (Anne Shatkin and Kim, 2015; Li et al., 2015),indicating their low-to-minimal environmental risks.

In general, there are still limited studies on the migration, toxicity and ecotoxicity of CNs. Moreover, a majority of the studies pointed to the safety and environmental concerns of CNs themselves through both *in vivo* and *in vitro* assays, but once incorporated to fruit coating, little research has been done. Hence, overall conclusions on safety and environmental impact of CNs incorporated coatings for postharvest fruit cannot be drawn comprehensively yet. It requires more knowledge of the interactions between CNs and other coating ingredients and the physiochemical changes of CNs that may occur during coating application, fruit storage, consumer oral intake and digestion (Szakal et al., 2014). Therefore, the toxicity and ecotoxicity of CNs, level of migration from the applied CNs incorporated coating into fruit, and acquisition of the GRAS status are the prior concerns for the implementation of CNs incorporated fruit coatings commercially (Huang et al., 2015).

2.4. Conclusions

Limitations of conventional polysaccharides, protein and lipid based coatings result in the introduction of cellulose nanomaterials (CNs) to fill up the gaps for the postharvest fruit coating technology. CNs are unique biodegradable materials with low density, high aspect ratio, superior barrier, mechanical and thermal properties, and compatibility with other biopolymers. They may be incorporated to fruit coatings as coating forming matrix, carrier of antimicrobial, antioxidant and nutritional compounds, reinforcement for other biopolymer matrices, and/or Pickering emulsifier to deliver hydrophobic compounds to meet the needs of improving storability of postharvest fruit.

However, several gaps exist on the application of CNs incorporated coating for postharvest fruit under required storage conditions, including

- Lack of sufficient understanding of intrinsic properties of CNs and their compatibility with other biopolymers;

- Limited studies in CNF and CNC incorporated fruit coatings on postharvest fruit and impaired moisture and gas barrier under specific postharvest storage conditions;
- Little knowledge on the CNs penetration and/or migration after being incorporated to formulations applied on postharvest fruit.

Therefore, a systematic approach is necessary for developing effective coating formulations for postharvest fruit by considering 1) fruit physiological characteristics, 2) type of coating matrix and other functional substances, 3) adhesive interactions between coating and fruit surface, and 4) storage conditions. The following chapters in this dissertation investigated the feasibility of CNs as component in fruit coatings for improving the storability of two major climacteric fruit, pears and bananas. The studies were focused on understanding the mechanism of CNF/CNC incorporated fruit coatings, developing coating formulations by implementing summarized systematic approach, and evaluating coating performance under required storage conditions. It is expected that this dissertation study will provide scientific guideline for developing CNs incorporated fruit coatings to fill up the gap in postharvest fruit edible coating technology.

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* Coating application provides functionalities through forming semipermeable barrier to restrict gas, moisture and aroma exchange, carrying functional ingredients like antimicrobial and antioxidant agents, and providing additional protection against external mechanical stress and UV attack during postharvest practices.



Fig. 2.2 Principles and roles of cellulose nanofibers (CNFs) as coating matrix/carrier of functional ingredients [A] and reinforcement to other biopolymers [B], and cellulose nanocrystals (CNCs) as fillers into other biopolymers [C] and Pickering emulsifier to incorporate hydrophobic compounds [D] in postharvest fruit coatings.

Type of fruit	Type of food polymers	Evaluated fruit quality parameters with positive effect	References
	Chitosan	\downarrow WL, \downarrow CD, \uparrow firmness, \downarrow peroxidase, \downarrow ethylene	Sahraei et al., 2016
Apple		production	
	Guar gum and pea starch	\downarrow microbial growth, \downarrow fungal growth	Mehyar et al., 2011
	Chitosan and gum arabic	\downarrow WL, \downarrow CD, \uparrow firmness, \uparrow total carbohydrates, \uparrow reducing	Maqbool et al., 2011
Banana		sugar, \downarrow respiration rate, \downarrow ethylene production	
Dallalla	Chitosan	\downarrow WL, \uparrow ascorbic acid, improved sensory quality	Suseno et al., 2014
	Shellac and gelatin	\downarrow WL, \uparrow firmness, \downarrow TSS, \uparrow TA	Soradech et al., 2017
Citrus	Chitosan and CMC	\uparrow Glossiness, \uparrow firmness, $↓$ TSS, \uparrow TA, \uparrow flavor, improved	Arnon et al., 2014
		sensory quality	
	Starch and guar gum	\uparrow Total polyphenol content, \uparrow vitamin C, \uparrow total carotenoid,	Saberi et al., 2018
		↑ total antioxidant activity	
Grana	Starch and gelatin	Improved overall appearance, ↓ WL, improved sensory quality	Fakhouri et al., 2015
Grape	Chitosan and acetonic extract	\downarrow WL, \downarrow TSS, \uparrow TA, \downarrow decay, improved sensory quality	Kanetis et al., 2017
	Pectin and beewax	\downarrow WL, \downarrow CD, \uparrow firmness, \downarrow respiration rate,	Moalemiyan et al.,
Mango			2012
	Chitosan	\downarrow WL, \uparrow firmness, \downarrow pH, \uparrow TA, \downarrow decay	Cissé et al., 2015
Pear	Soy protein isolate, HPMC	\downarrow WL , \downarrow TSS, \downarrow pH, \uparrow TA,	Nandane et al., 2017
	and olive oil		
	Chitosan and pullulan	↑ Total phenolic and flavonoid contents, ↑ chlorogenic acid,	Kou et al., 2014
		↑ catechin, ↑ caffeic acid, ↓ peroxidase	
	Candelilla wax, gum arabic,	\downarrow WL, \uparrow firmness, \downarrow pH,	Cruz et al., 2015
	jojoba oil		
Strawberry	Starch, carrageenan and	\downarrow WL, \uparrow firmness, \downarrow microbial growth, \downarrow deterioration,	Ribeiro et al., 2007
	chitosan	improved sensory evaluation	

 Table 2.1 Polysaccharide, protein, and lipid based composite edible coatings studied on major postharvest fruit.

Pectin	\downarrow WL , \downarrow TSS, \uparrow TA, \uparrow ascorbic acid, \downarrow respiration rate,	Muñoz-Labrador et
	\downarrow ethylene production, \downarrow fungal growth	al., 2018

↑ : Retained higher value by the application of biocomposite edible coating; ↓ : Retained low value by the application of biocomposite edible coating; WL: Weight loss; CD: Color degradation; TSS: Total soluble solid; TA: Titratable acidity; CMC: Carboxymethyl cellulose; HPMC: Hydroxypropyl methylcellulose.

 Table 2.2 Intrinsic properties of cellulose nanofiber (CNF)/ cellulose nanocrystal (CNC) and their roles and functionalities in edible films and coatings.

Source and properties	CNF	CNC	
Source	Wood, cotton, wheat, rice straw	Wood, sugar beet, potato tuber	
Structure	Long flexible nanofibrillar network	Rod-like nanocrystalline whiskers	
Diameter (nm)	5-70	3-50	
Length (nm)	200 - 2000	100-250	
Crystallinity (%)	51 - 69	54-88	
Tensile strength (GPa)	1.5-5.5	7.5-7.7	
Elastic modulus (GPa)	150-500	110-220	
Equilibrium moisture contents (%)	33.2-38.9 21.4-28.6		
O ₂ permeability coefficient	140.7	0.09	
CO ₂ permeability coefficient	118.8	0.10	
Thermal degradation temperature (°C)	350	200-300	
Roles in edible films and coatings	1) Matrix	1) Filler	
	2) Carrier	2) Pickering emulsifier	
	3) Reinforcing agent		
Functions in edible films and coatings	1) Deliver functional ingredients	1) Reinforce mechanical, barrier and	
	2) Provide/reinforce mechanical, barrier	thermal properties	
	and thermal properties	2) Incorporated hydrophobic compounds	

Type and functions of Evaluated film properties with significant enhancement Type of References through CNF/CNC incorporation biopolymers CNs Alginate CNC: filler [†] Moisture barrier, [†] tensile strength, [†] thermal property Huq et al., 2012 † crystallinity Chitosan \downarrow Water absorption, \uparrow moisture barrier, \uparrow tensile Khan et al., 2012 CNC: filler strength, † crystallinity, improved surface morphology **CNF:** reinforcement [†] Optical property, [†] Young's modulus, [†] thermal Chitosan Fernandes et al., 2010 stability CNF: matrix, carrier [†] Moisture barrier, [†] Young's modulus, [†] antimicrobial Sundaram et al., 2016 Chitosan property, improved surface morphology \downarrow Water absorption, \uparrow moisture barrier, \uparrow tensile Chitosan, olive CNC: filler, Pickering Pereda et al., 2014 oil emulsifier strength, *†* thermal property, *†* adhesion CNF: reinforcement ↑ Tensile strength, ↑ Young's modulus, ↑ thermal Chitosan, starch Tomé et al., 2013 stability CMC, starch CNC: filler \uparrow Moisture barrier, \uparrow tensile strength, \downarrow elastic modulus, Mandal and † thermal stability Chakrabarty, 2018 ↑ Gas barrier, ↑ thermal stability Mondragon et al., 2015 Gelatin CNF/CNC: reinforcement, filler HPMC **CNF/CNC**: [†] Optical property, [†] moisture barrier, [†] tensile strength, Bilbao-Sainz et al., reinforcement, filler † Young's modulus 2011 ↑ Moisture barrier, ↑ tensile strength, ↑ crystallinity, Pectin CNC: filler Chaichi et al., 2017 improved surface morphology PLA CNC: filler ↑ Moisture barrier, ↑ gas barrier Fortunati et al., 2012 Starch, gelatin CNC: filler \uparrow Tensile strength, \downarrow elastic modulus, \uparrow thermal Alves et al., 2015 property, † adhesion, improved surface morphology

Table 2.3 Summary of cellulose nanomaterials (CNs) including cellulose nanofiber (CNF) and cellulose nanocrystal (CNC) incorporated biocomposite films as potential postharvest coating applications, and improved film and coating properties.

 \uparrow : Retained higher value by the application of CNs incorporated film; \downarrow : Retained low value by the application of CNs incorporated film; HPMC: Hydroxypropyl methylcellulose; PLA: Polylactic acid.

Type of fruit	Type and functions of CNs	Other incorporated polysaccharides, proteins or lipids	Evaluated fruit quality parameters with positive effectiveness	References
Acerola fruit	CNC: filler	Alginate, extracted acerola puree	† Vitamin C	Azeredo et al., 2012
Banana	CNF: reinforcement	Gelatin	_	Andrade et al., 2015
Cherry	CNF: reinforcement	Chitosan, thyme oil	\downarrow WL, \downarrow TSS, \uparrow total sugar	Nabifarkhani et al.,
			content, † anthocyanin	2015
Cucumber	CNC: filler	Chitosan	↑ Firmness, ↓ catalase,	Dong et al., 2016
			↓ peroxidase	
Grape	CNF: reinforcement	HPMC, k-carrageenan	↓ WL, ↑ firmness	Silva-Vera et al., 2018
Strawberry	CNC: filler	Chitosan	\downarrow WL, \downarrow TSS, \uparrow anthocyanin,	Dong et al., 2015
			↓ decay	
Strawberry	CNF: reinforcement	Chitosan	↓ WL, ↑ firmness	Resende et al., 2018
Strawberry	CNF: reinforcement	Gelatin	\downarrow WL, \uparrow ascorbic acid, \downarrow decay	Fakhouri et al., 2014

Table 2.4 Summary of cellulose nanomaterials (CNs) including cellulose nanofiber (CNF) and cellulose nanocrystal (CNC) incorporated edible coatings for improving quality of postharvest fresh fruit.

 \uparrow : Retained higher value by the application of CNs incorporated coating; \downarrow : Retained low value by the application of CNs incorporated coating; WL: Weight loss; TSS: Total soluble solid; HPMC: Hydroxypropyl methylcellulose

CHAPTER 3

CELLULOSE NANOCRYSTAL REINFORCED CHITOSAN COATINGS FOR IMPROVING THE STORABILITY OF POSTHARVEST PEAR UNDER BOTH AMBIENT AND COLD STORAGES

Deng, Z., Jung, J., Simonsen, J., Wang, Y., Zhao, Y. Published in *Journal of Food Science* **2017**, 82, 453-462.

ABSTRACT

Cellulose nanocrystal (CNC, 0, 5%, and 10% w/w, in chitosan, dry basis) reinforced 2% chitosan aqueous coatings were evaluated for delaying the ripening and quality deterioration of postharvest green D'Anjou (Pyrus communis L.) and Bartlett (Pyrus *communis* L.) pears during 3 weeks of ambient storage (20 ± 2 °C and 30 ± 2 % RH) or 5 months of cold storage (-1.1 °C and 90% RH), respectively. Ethylene and CO₂ production, color, firmness, and internal fruit quality were monitored during both storage conditions. Moisture and gas barrier, antibacterial activity, and surface morphology of the derived films were also evaluated to investigate the mechanisms of delayed fruit ripening and quality deterioration. In the ambient storage study, the 5% CNC reinforced chitosan coating significantly (P < 0.05) delayed green chlorophyll degradation of pear peels, prevented internal browning, reduced senescence scalding, and improved retained fruit firmness. During cold storage, the 5% CNC reinforced chitosan coating showed a competitive effect on delaying fruit postharvest quality deterioration compared to a commercial product (SemperfreshTM). The 5% CNC coating strongly adhered to the pear surface, provided a superior gas barrier and a more homogenous matrix in comparison with the other coatings tested. Hence, it was effective in delaying ripening and improving the storability of postharvest pears during both ambient and cold storage.

PRACTICAL APPLICATION

Cellulose nanocrystal (CNC) reinforced chitosan coatings strongly adhered to the pear surface, and showed superior gas barrier and antibacterial properties. Such coatings have successfully delayed ripening and quality deterioration (weight loss, color, and texture) of postharvest pears during both ambient and cold storage. CNC reinforced chitosan coatings are easy to prepare and apply, and are stable under various conditions. They should thus be suitable to improve the postharvest storability of other climacteric fruit, such as bananas, apples, or mangos.

KEYWORDS

Postharvest quality of pears, edible coatings, cellulose nanocrystal, chitosan, ambient storage, cold storage

INTRODUCTION

Postharvest losses of fruit and vegetables in 2011 was 40-50% worldwide. Pears as a highly perishable crop experience very fast quality deterioration, such as shriveling, softening, and peel color degradation from green to yellow and yellow to brown during postharvest cold and ambient storage. This quality deterioration is usually described as ripening and senescence of the fruit, and decreases the shelf-life and marketability of postharvest fresh pears. Hence, there is a need for new and innovative storage strategies to delay fruit ripening and quality deteriorations in postharvest pears during both ambient and cold storage.

Several approaches, including cold temperature, controlled atmosphere storage, chemical treatment, and edible coatings, have been attempted to delay quality deterioration and ripening of fresh fruit during postharvest storage (Visakh and others 2013). Among them, edible coatings have shown great potential to reduce weight loss and delay quality deterioration by creating a moisture and/or gas barrier on the fruit surface and modifying the internal gas atmosphere within the coated fruit (Lin and Zhao 2007). Edible coatings can also be cost efficient and environmentally friendly (Dhall 2013). In addition, the functional properties and efficacy of the coatings can be improved by adding antimicrobial and antioxidant agents, surfactants, and reinforcing fillers into the coating matrix. While wax based coatings are commercially applied on pears, their capability for preventing peel browning and shriveling of postharvest pears are limited due to their insufficient gas barrier property, inflexibility, and weak resistance to applied mechanical stress as well as poor stability (Diab and others 2001).

Chitosan (1, 4-linked 2-amino-deoxy- β -d-glucan) has been of great interest as a polysaccharide coating material over the last two decades. In addition to its excellent film forming ability, the presence of the positively charged amino groups in chitosan provides a strong antimicrobial activity (Chen and Zhao 2012; Jung and others 2014). However, it forms a relatively poor moisture barrier and this has limited its effectiveness in controlling moisture transfer and providing physical protection from mechanical injury in postharvest fruit (Rhim and Ng 2007; Elsabee and Abdou 2013). As a result, there have been many attempts to improve the functionality of chitosan based coatings by incorporating other functional substances into the chitosan coating matrix. Cellulose nanocrystal (CNC) has been used as a filler for cellulose, silk and lignin or as crosslinking agent (Zhou and Wu 2012; Xu and others 2014) to enhance barrier and mechanical properties of these polymers through the formation of a percolated network (Favier and others 1995; Khan and others 2012). CNC possesses a highly ordered crystalline structure and negatively charged sulfate ester groups through the sulfuric acid hydrolysis process (Lin and Dufresne 2014). CNC reinforcement in the chitosan polymeric matrix has produced films with a superior moisture barrier property and tensile strength (Azeredo and others 2010; Pereda and others 2014), which triggered our interest in developing such coatings for delaying the postharvest ripening and quality deterioration of fruit that has a high postharvest respiration rate, such as pears.

Although several previous studies had investigated the use of CNC as the filler for polysaccharide based films, no study has actually evaluated the effect of CNC reinforced chitosan coatings on postharvest fruit. The development of fruit coatings is much more complicated and challenging than that of films since the coatings have to respond to various postharvest physiological changes of the fruit (e.g. ripening, respiration, or senescence) as well as storage conditions (temperature and relative humidity) in order to effectively delay postharvest fruit ripening and quality deterioration. Especially, coatings on postharvest pears that possess high respiration rate and ethylene production require an effective moisture and gas barrier for reducing weight loss and for delaying ripening and senescence scalding during storage. Meanwhile, for prolonging storage, fresh pears are usually subjected to a period of cold storage (-1 to 0 °C, RH ~90%) for several months first, and then moved to ambient conditions for retail (15 to 21 °C, RH ~50-60%) (USDA 2014). Hence, the coatings for fresh pears should not only provide sufficient moisture barrier and proper gas exchange, but also be stable when subjecting to variations in temperature and relative humidity.

In this study, it was hypothesized that CNC reinforced chitosan coatings could successfully provide the needed moisture and gas barrier and also modify the atmospheric conditions within the coated fruit, thus controlling ethylene production and delaying fruit ripening and quality deterioration during postharvest storage (Zhao and others 2014). Postharvest studies of pears were conducted in both ambient and cold storage conditions. The coatings were targeted for delaying fruit ripening and senescence scalding, reducing weight loss, and reducing quality deteriorations at ambient storage and for delaying fruit ripening and quality deterioration at cold storage and then properly ripened at ambient storage. For understanding the functional and microstructural properties of the developed coatings, the coating formulations were cast into films, and the moisture and gas barrier properties, antibacterial activity, and surface morphology of the derived films were investigated. It was anticipated that this study could provide new insights about the effectiveness of CNC reinforced chitosan coatings for the improved storability of postharvest pears under both cold and ambient storage conditions.

MATERIALS AND METHOD

The lab bench-scale, ambient storage study was conducted on pears coated by various CNC reinforced chitosan coating formulations to evaluate the effectiveness of the coatings for delaying postharvest quality deterioration and ripening of pears at the Department of Food Science & Technology, Oregon State University (OR, USA). The coating formulation that resulted in the least quality deterioration and the slowest ripening of pears at the ambient conditions was then applied for large-scale, cold storage study in the Mid-Columbia Agriculture Research and Extension Center (MCAREC) (Hood River, OR, USA). It should be pointed out that some analytical methods and instruments used were different between the ambient and cold storage studies, due to instrument accessibility since the two studies were conducted at two different locations.

Materials

Chitosan (97% degree of deacetylation, 149 kDa Mw) was purchased from Premix (Iceland). CNC was purchased from the Process Development Center at the University of Maine (ME, USA). It was derived from softwood Kraft pulp with a final concentration of 11.8% (Choi and Simonsen 2006). Surfactants including Tween 80 and Span 80 were obtained from Amresco (OH, USA). Acetic acid was acquired from J. T. Baker (NJ, USA). For the ambient bench-scale storage study, organic green D'Anjou pears (*Pyrus communis* L.) (WA, USA) with no visual defects were purchased from a local market (OR, USA) at the day they arrived at the grocery store, and subjected to coating treatment on the same day. For the large-scale cold storage study, green Bartlett pears (*Pyrus communis* L.) harvested from mature trees in an orchard in Hood River, OR were coated after being stored under controlled atmosphere storage at -1 °C for 3 weeks. The initial flesh firmness (FF) of the fruit was 79.0 N, meeting the recommended commercial harvest maturity. The fruit was held overnight under cold storage conditions (-1 °C) after harvest, and applied with coating treatments on the second day.

Preparation of coating formulations and fruit coatings

Chitosan (2%, w/w) was dissolved in aqueous acetic acid solution (1%, w/v). CNC at 5.0% and 10% (w/w chitosan, dry basis) was dispersed in the prepared chitosan solution using a blender (Proctor Silex, NACCO Industry Inc., VA, USA) for 60 s. The mixture of Tween 80 and Span 80 at a ratio of 1:1 (w/w) was added into the above mixture (10%, w/w chitosan, dry basis) for improving the wettability of coatings onto the hydrophobic fruit surfaces and for increasing the stability of prepared coating formulations. The mixture was thoroughly blended by a homogenizer (Polytron PT10-35, Luzernerstrasse, Switzerland) for 120 s, sonicated (Branson B-220H (50-60Hz), CT, USA) for 60 s, and then degassed using a custom water flow vacuum system (Chen and Zhao 2012).

For the ambient storage test, 15 mL of freshly prepared coating formulation was spray-coated on each individual fruit using an air-spray gun (Central Pmeumatic, CA, USA) at 0.28-0.31 psi to achieve uniform surface coatings. Coated fruit were dried at ambient temperature under forced airflow for 1 h, and then stored at ambient conditions $(20 \pm 2 \text{ °C} \text{ and } 30 \pm 2\% \text{ RH})$ without packaging for up to 3 weeks. For the cold storage

study, the dipping method was chosen to apply a more uniform coating on the fruit in the large-scale experiment (185 pears for each treatment). Fruit was dipped in the coating formulation for 60 s and then dried at the ambient conditions for 2 h. Fruit were then packed into wooden boxes (50 pears in each box), and stored at -1.1 °C and 90% RH for up to 5 months. For both ambient and cold storage studies, non-coated fruit were included as controls.

Three different coating formulations (0, 5%, and 10% CNC reinforced 2% chitosan, represented as 0CNC, 5CNC, and 10CNC) were selected based on preliminary studies (data not shown). The coating formulation that resulted in the minimum quality change and ripening of fruit from the ambient storage study was then selected for the cold storage study in comparison with a commercial coating product, SemperfreshTM (SEMP) (Pace International, LLC, WA, USA). Sucrose ether-based SemperfreshTM has been widely used in the fresh pear industry to reduce bruising and weight loss and preserve the green color in postharvest storage. Fruit quality parameters including weight loss, color change, firmness, pH, titratable acidity, and total soluble solid content, as well as ethylene and CO₂ production rate, and ripening capacity (cold storage fruit only) were monitored during storage studies.

Film preparation and evaluation

To investigate the functional and microstructural properties of the coatings, coating formulations (0, 5%, and 10% CNC reinforced 2% chitosan) were cast into films. All coating formulations contained 10% surfactant mixture to simulate the same formulations applied on pear coatings. Each coating formulation was uniformly distributed onto a

leveled Teflon-coated glass plate (170 x 170 mm), and dried at ambient conditions (20 ± 2 °C and 30 ± 2 % RH) for 2 days. Films were then conditioned in a custom built chamber (Versa, PA, USA) at 25 °C and 50% RH for 2 days before evaluation. Film thickness was measured using a micrometer (NR 293-776-30, Mytutoyo Manufacturing Ltd., Japan) at ten randomly selected locations on each film, and represented as the mean value and standard deviation for each film formulation.

Water vapor transmission rate (WVTR) and oxygen transmission rate (OTR)

WVTR and OTR of the films were measured instead of water vapor permeability (WVP) and oxygen permeability (OP) to investigate the coating barrier effect on fruit when coated by the same amount of coating formulations regardless of coating thickness. A cup method according to ASTM Standard E96-87 (ASTM 2000) was used to measure WVTR (Park and Zhao 2004). A film sample (75 x 75 mm) was sealed by vacuum grease on the top of a Plexiglas test cup (57 x 15 mm) filled with 11 mL of DI water, and the seal ring was tightly closed by using rubber bands. Test cup assemblies were stored in a controlled environment chamber (T10RS 1.5, Hyland Scientific, WA, USA) at 25 °C and 50% RH. Each cup assembly was precisely weighed hourly for up to 6 h, and WVTR (g m⁻² s⁻¹) was calculated from the slope of the straight line for weight loss per unit time (g s⁻¹) divided by test film area (m²). Three films per treatment were evaluated, and means and standard deviation values were reported.

For OTR, oxygen permeation of the film sample (120 x 120 mm) was measured following the GB/T1038 method using a gas permeability tester (VAC-VBS, Labthink Instrument Co., China) at 26 ± 0.5 °C and $55 \pm 5\%$ RH. OTR was measured three times

with 9 films total (a single run of the instrument required 3 films), and the mean and standard deviation values of three replications were reported for each type of film.

Antibacterial activity

Two non-pathogenic bacterial strains, including Gram-positive strain L. innocua (ATCC 51742, American Type Culture Collection) and Gram-negative strain E. coli (ATCC 25922, American Type Culture Collection) were cultured on brain heart infusion (BHI) agar (Becton, Dickinson and Co., USA) and tryptic soy agar (TSA) (Becton, Dickinson and Co., USA), respectively, and stored at 4 °C during the course of the study. Prior to a given microbiological assay, a single typical colony of two bacteria was inoculated in tubes of appropriate broth (brain heart infusion (BHI) broth (Becton, Dickinson and Co., USA) for L. innocua and tryptic soy broth (TSB) (Becton, Dickinson and Co., USA) for *E. coli*) and incubated at 37 °C for 16 – 24 h (Lab-Line Orbit shaker bath model 3527, Melrose Park, IL, USA). A film specimen (1 x 1 mm) was immersed into a test tube with 10 mL of sterilized BHI or TSB respectively, and then inoculated with 100 μ L of activated bacterial suspension. Inoculated test tubes (~10⁷ CFU mL⁻¹) without film treatment were used as controls. The optical density at 600 nm (OD₆₀₀) indicating bacterial growth was measured at 0, 2.5, 5, 7.5 and 10 h by using the UV-vis spectrophotometer (UV-1800, UV-Vis Spectrophotometer Shimadzu Corporation, Japan) for evaluating the antibacterial effect of the derived films. The mean values and standard deviations of three replications with two measurements for each replication were reported for treatments and control.

Surface morphology

Surface morphology of the derived films was analyzed using an SEM (FEI Quanta 600F, OR, USA). Prepared film pieces were placed on aluminum stub and coated by gold palladium alloy sputter coater (Cressington Scientific Instruments Ltd., UK) to improve the interface conductivity. Digital images were collected at an accelerating voltage of 5 kV.

Fruit quality evaluation

Weight loss and shrinkage

Fruit weight was measured using an electronic balance (SP402, Ohaus Scout, NJ, USA). Fruit diameter was monitored by a Vernier caliper (Spi2000, Swiss Precision Instrument, CA, USA) to investigate the shrinkage in fruit size. For the ambient storage test, the percentage of fruit weight loss (%) and shrinkage (%) was calculated by subtracting the weight and diameter at different sampling times (1, 2, and 3 weeks) from the initial weight and diameter at 0 week, and dividing by the initial weight and diameter, respectively. Six fruit per treatment and control were evaluated, and the mean values and standard deviations were reported. For the cold storage study, the percentage of fruit weight loss (%) after 2.5-month of storage was measured following the same method as stated above for 15 fruit per treatment and control.

Color and overall appearance

For the ambient storage test, fruit color was measured using a colorimeter (LabScan XE, HunterLab, Reston, USA) calibrated with a standard white plate ($L^* = 93.87$; $a^* = -$

0.92; $b^* = 0.14$). Due to the color variation on the surface of individual fruit, a 3 cm diameter circle was marked on the surface of each fruit, and the same area was observed during 3 weeks of storage in the reflectance mode (L*: lightness, a*: redness, and b*: yellowness). Total color difference was calculated as

 $(\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2})$, where L₀*, a₀*, and b₀* represented the color values at 0 week, and L*, a* and b* referred to the color values at different sampling times (1, 2, and 3 weeks). Color values were obtained from 6 individual pears for each treatment and control, and mean values and standard deviations were reported. Photos were also taken weekly to report their overall appearance. For the cold storage study, peel chlorophyll content was measured using a DA meter (Sinteleia, Bolonga, Italy) and the percentage of chlorophyll degradation was calculated by subtracting the chlorophyll content value at the 2.5-month storage from the initial one, and dividing by the initial value. Two measurements were obtained from each side of the equator of an individual fruit. Mean values were obtained from triplicate measurements for 15 pears in total.

Firmness, pH, titratable acidity (TA), and total soluble solid (TSS)

Quality parameters associated with fruit ripening including firmness, pH, TA, and TSS were measured for pears at the end of 3 weeks of ambient storage. Fruit firmness was measured by a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Corp., NY, USA). Pears were cut in the stem-calyx axis, and two opposite unpeeled sides at the widest diameter of the pear were punctured by a P/6 stainless cylinder probe at a speed of 1.0 mm/s with the travel distance of 50% of the fruit height (Karlsen and others
1999). The maximum force was measured as firmness of fruit, and mean values and standard deviations of six fruit with two measurements for each fruit were reported for each treatment and control. For the cold storage study, fruit firmness was measured by another texture analyzer (GS-14, Guss Manufacturing Ltd., Strand, South Africa) using an 8 mm probe at a speed of 1.0 mm/s and the travel distance of 9 mm. Two measurements were conducted for each fruit by evaluating both sides on the equator of fruit after removing 20 mm diameter peel discs. Ten fruit were measured for each replicate, and mean value was generated from triplicate measurements.

For analysis of fruit pH, TA and TSS under ambient conditions, 10 g of fruit flesh excluding peel and core was blended with 90 mL of distilled (DI) water using a blender (Proctor Silex, NACCO Industry Inc., VA, USA), and filtered using the Whatman No. 1 filter paper. The filtrate was directly used for measuring TSS content using a refractometer (RA250-HE, KEM, Tokyo, Japan), but diluted 10 times with DI water for measuring pH and TA using a pH meter (Orion 410A, Fisher scientific, MA, USA), or titrated with 0.1 mol L⁻¹ NaOH to reach pH 8.3 using a digital titrator (Brinkmann, TX, USA) (Cavender and others 2014), respectively. TA was reported as the equivalent percentage of malic acid. Six pears per treatment and control were evaluated, and the mean values and standard deviations were reported. For the cold storage test, 100 g of flesh tissue was ground for 3 min in a juice extractor (Acme Model 6001) and the juice was filtered with a uniform strip of milk filter. TSS and TA of the juice was determined using the same methods as described above except a different digital titrator (Model T80/20, Schott-Gerate, Hofheim, Germany) was employed.

Ethylene and CO₂ production of pears

For the ambient storage test, ethylene production of the pears was evaluated using a gas chromatograph (GC-2014, Greenhouse gas analyzer, Shimadzu, Japan) with a flame ionization detector (FID). An individual pear was sealed in a 300 mL air-tight glass jar with a 10 mm rubber septa attached on the lid for the sampling of headspace gas. Noncoated and coated fruit were packed in the tightly closed glass jar for 1 day at ambient temperature (20 ± 2 °C), and ethylene production was compared between jars containing non-coated and coated fruit. Note that the pear samples used for ambient storage study were obtained from a local market which had already been stored at refrigerated conditions for several months. Hence, the fruit had much less ethylene production rate compared with those freshly harvested pears. Therefore, much longer incubation time of pears in the jar was required for detecting the production of ethylene (1-day) in order to provide more convincing comparison between control and coated pears. For each jar, 1 mL of headspace gas was collected using an air tight syringe (Series A, Valco Instrument Co., USA) and then injected into the GC fitted with three kinds of packed columns: 80/100 HAYESEP D, 8/100 HAYESEP N, and 60/80 molecular sieve column (Supelco, Bellefonte, PA, USA). Helium was used as the carrier gas at a pressure of 350 kPa and flow rate of 21.19 mL min⁻¹. The temperature of injector, column, and FID detector were set at 150, 90, and 250 °C, respectively. The ethylene standard gas was purchased from Air Liquide (ScottTM, PA, USA), and GC solution software (Shimadzu, Japan) was used for calculating the amount of ethylene production. For the cold storage study, ethylene production and the respiration rate of pears were determined by incubating 5 fruit from each treatment inside a 3.8 L jar at 20 °C for 1 h. Gas samples were withdrawn through a

self-made septum on the top using a 1 mL gas-tight syringe. Gas chromatography (Shimadzu GC-8A, Kyoto, Japan) was used to analyze the concentration using 0.8 mL·s⁻¹ nitrogen as carrier gas. The injector and detector port temperature were set up at 90 and 140 °C, respectively. The headspace gas was evaluated for the concentration of CO₂ by an O₂ and CO₂ analyzer (Model 900151, Bridge Analyzers Inc., CA, USA). The ethylene production and respiration rate were expressed as μ L·kg⁻¹·h⁻¹ and μ g·kg⁻¹·h⁻¹, respectively.

Ripening capacity

After cold storage for 2.5 months, fruit ripening capacity was evaluated by measuring fruit firmness (N) (Wang and Sugar 2015). Ripening capacity of pear is defined as the ability of the fruit to soften below 18 N. The pears were taken out from the cooler and left at 20 °C for 5 days. Fruit firmness was measured using the method and instrument as for the ambient storage study described above. Each replication included 10 fruit samples. Mean values were obtained from triplicate measurements for 30 fruit samples in total.

Experimental design and statistical analysis

A completely randomized design with a single treatment factor (3 coating formulations of 0CNC, 5CNC, and 10CNC for ambient storage and 2 coating formulations of 5CNC and 0.5% SemperfreshTM for cold storage, respectively) was applied for the fruit coating study. Non-coated fruit were used as controls. Both the fruit and film study were conducted in triplicate. An one-way ANOVA was carried out to determine the significant differences among treatments and control, and a *post hoc* least

significant difference (LSD) was conducted by means of statistical software (SAS v 9.2, The SAS Institute, USA). Results were considered to be significantly different at P<0.05.

RESULTS AND DISCUSSION

Properties of derived films

The films derived from 0CNC, 5CNC and 10CNC coating formulations were evaluated on their moisture and gas barrier properties, antibacterial activity, and surface morphology (Table 3.1). Thickness of 5CNC and 10CNC films were significantly (P < 0.05) higher than that of 0CNC film, whereas no significant difference in WVTR was observed among different films (Table 3.1). The thicker 5CNC and 10CNC films were probably the result of the higher total solids in the formulations in comparison with 0CNC film (chitosan only). Although the thicker film could absorb more moisture through hydrogen bonds with the hydroxyl groups of chitosan or CNC, no significant increase in WVTR was observed in 5CNC and 10CNC films in comparison with 0CNC films, which might be because of the strong electrostatic and hydrogen bonding interactions between chitosan and CNC (Khan and others 2012) that reduced the moisture absorption in the CNC reinforced chitosan films. CNC is composed of a crystalline polymeric structure with anionic sulfate surface groups could function as both a crosslinking agent and filler in the chitosan matrix. It was thus concluded that CNC reinforced chitosan could form a stronger film matrix and thus an improved moisture barrier.

For OTR, CNC reinforcement in chitosan significantly (P<0.05) reduced O₂ permeation of chitosan films, with an inverse relationship (Table 3.1). The same

mechanism worked here as for WVTR (Favier and other 1995; Khan and other 2012; Pereda and other 2014). The CNC reinforced chitosan matrix also increased tortuosity, which led to slower gas diffusion (Azeredo and other 2010).

The antibacterial potential of the derived films against both Gram-positive (*L. innocua*) and Gram-negative (*E. coli*) bacteria was evaluated by measuring the optical density changes of enriched broth as an indication of microbial growth from 5 h to 24 h (Fig. 3.1). For both *L. innocua* and *E. coli*, all treatments showed great suppression on the microbial growth compared to the control. This was consistent with the results from our previous study, which showed that the interactions between the protonated amino groups from chitosan and the negatively charged bacterial cell membrane resulted in a strong antibacterial property (Jung and Zhao 2013). A previous study reported that CNC reinforced chitosan coatings and films possessed a strong antibacterial property and extended the shelf-life of ground meat (Dehnad and other 2014). It should be also noted that the antibacterial effect of CNC reinforced chitosan towards *E. coli* was weaker during the first 7 h than that of chitosan only film. This was probably because of the affinity of the chitosan backbone on the CNC surface.

SEM images of the surfaces of 0CNC, 5CNC, and 10CNC films illustrated the distribution of CNC on the surface of chitosan films (Fig. 3.2). The surfaces of the 5CNC and 10CNC films were rougher than that of the 0CNC film, probably because of the formation of a polyelectrolyte-macroion complex (PMC) between CNC and chitosan, as observed by Wang and others (Wang and Roman 2011; Khan and others 2012). The 5CNC film exhibited a more homogeneous and dense structure with less CNC

agglomerates or PMC crystals on the film surface than that of 10CNC film, indicating a better dispersion of CNC into chitosan matrix at the lower CNC concentration (Khan and others 2012). The surface of the 10CNC film showed more crystals on the film surface, probably because the CNC aggregated and/or there were increased PMCs.

Effectiveness of coatings on delaying fruit ripening and quality deterioration during ambient storage

A coating system with well controlled gas and moisture barrier functionality should effectively delay the physiological changes (i.e. ripening, respiration, and senescence) as well as quality deterioration of fruit during postharvest storage (Arvanitoyannis and Gorris 1999; Arnon and others 2013; Dhall 2013). In this study, non-coated and coated pears were examined for ethylene production and important quality parameters during 3 weeks of ambient storage.

Pear ripening

Ethylene production in pears accelerates the ripening process of fruit during postharvest storage (Alexander and Grierson, 2002). The studied coating treatments significantly (P<0.05) reduced ethylene production (0-12 μ L·kg⁻¹·h⁻¹) compared to controls (~52 μ L·kg⁻¹·h⁻¹) (Fig. 3.3). This result was consistent with the OTR and WVTR results (Table 3.1). It was assumed that the atmosphere inside the coated fruit was modified by a lowered gas transmission through the coating, thus possibly slowing down ethylene production and delaying fruit ripening.

Fruit firmness, TSS, pH, and TA were measured to evaluate the ripening status of fruit during storage. During fruit ripening, cell wall degradation can decrease fruit firmness, and modify both pectin and hemicellulose, which further soften fruit texture (Hiwasa and others 2004). Meanwhile, TSS is increased during ripening of fruit as a result of starch hydrolysis into sugars, while is *vice versa* for TA owing to the degradation of organic acids (Chaimanee and Suntornwat 1994; Makkumrai and others 2014). It should be also noted that TA of climacteric fruit may increase after fruit harvest to reach a climacteric peak as observed in mango, banana, and guava (Vazquez-Salinas and Lakshminarayana 1985; Bashir and Abu-Goukh 2003). Hence, fruit firmness, TSS, pH, and TA were utilized as the fruit ripening indicators to compare between non-coated and coated pears at the end of 3 weeks of ambient storage (Table 3.2). Firmness of 5CNC and 10CNC coated pears were significantly (P < 0.05) higher than that of non-coated and 0CNC coated ones. The 5CNC and 10CNC coated pears showed significantly (P < 0.05) lower TSS values than that of non-coated and 0CNC coated ones, which demonstrated delayed fruit ripening by preventing the hydrolysis of starch into sugars (Afshar and Rahimi 2010). There was no significant difference in TA between control and 5CNC coated fruit, but TA of 0CNC and 10CNC coated pears was significantly (P < 0.05) lower than that of control. It was speculated that the decrease in TA of 0CNC and 10CNC coated fruit may be related to the anaerobic conditions inside the coated fruit, in which organic acids might be used as energy production/reserves under this condition (Liu and others 2010; Tariq and others 2001). Although the film study showed that oxygen transmission of 0CNC was higher than that of 5CNC (Table 3.1), their performance as coating on pears could be altered depending on the interactions of coating formulation

with the surface character of fruit and surrounding humidity conditions. The 5CNC coating incorporating the hydrophilic CNC could respond more sensitively to the surrounding humidity condition, and the coating matrix might moderately expand to reach an ideal gas permeability for preventing ripening and anaerobic status of pears. However, 0CNC coating with less amount of hydrophilic compound could be relatively resistant against humidity condition, probably inducing an anaerobic condition in coated pears that resulted in low TA (Table 3.2) and scalding of pears (Fig. 3.4). On the other hand, 10CNC coating with excessive CNC incorporation enhanced the strength of coating matrix, even led to aggregated CNC or polyelectrolyte-macroion complex (PMC) particles on the surface (as shown on the surface morphology of films in Fig. 3.2), thus inducing the anaerobic disorder (superficial scalding) of coated pears (Fig. 3.4). Therefore, based on the results on ethylene production, firmness, and internal quality of pears, it might be concluded that the 5CNC coating was the optimal formulation.

Color and appearance

Color change (ΔE) and surface appearance (photos) of non-coated and coated fruit during 3 weeks of ambient storage are illustrated in Fig. 3.4. Photos of internal flesh and cores in pears were also taken at the end of 3 weeks to investigate fruit tissue browning caused by CO₂ injury (Franck and others 2007). Coated fruit remained significantly (*P*<0.05) lower in ΔE values (<6.0) in comparison with that of non-coated samples (~12) during 3 weeks of ambient storage (Fig. 3.4). Photos of the pears also showed that 5CNC coated pears retained green pigments much longer than non-coated and other coated fruit (Fig. 3.4). The retained green chlorophyll pigment in 5CNC coated pears was clear evidence of delayed fruit ripening as the result of reduced ethylene production. It might be that the gas composition inside the coated fruit was modified with increased CO_2 , which in turn interacted with ethylene binding sites, thus reducing ethylene production (de Wild and others; Li and others 2013; Mattheis and others 2013). This result was supported by the relatively higher O_2 barrier in CNC reinforced chitosan films (Table 3.1). However, 10CNC coated pears showed skin speckling and pithy brown core (Fig. 3.4), indicating CO_2 injury as a physiological disorder (Mattheis and others 2013). Again, this result was supported by the lower O_2 permeation in 10CNC film compared to that of 5CNC film (Table 3.1). Therefore, the 5% CNC reinforced 2% chitosan coating was effective to control gas atmosphere conditions (CO_2 and O_2 levels) inside coated pears, thus retaining green pigment and delaying fruit ripening without causing internal tissue browning.

Weight loss and shrinkage

Both coated and non-coated pears showed increasing trends of weight loss (%) during storage, but coated pears had significantly (P<0.05) lower weight loss than that of non-coated, while no difference was observed in weight loss between 5CNC and 10CNC coated pears (Fig. 3.5A). It could be concluded that the coatings adhering to the hydrophobic pear surface formed good gas and moisture barriers, thus slowing down the physiological transformations from carbohydrates and O₂ into sugar, CO₂, and moisture, thus reducing weight loss (Quamme and Gray, 1985). However, no significant difference was observed in shrinkage between non-coated and coated pears (Fig. 3.5B).

Hence, the 5CNC coating was a superior gas barrier and gave a homogenous distribution of CNC in the coating matrix, which effectively delayed the ripening and improved the storability of postharvest pears without physiological disorder of fruit during storage. This coating formulation was thus selected for the cold storage study.

Effect of coatings on delaying fruit ripening and quality deterioration during cold storage

During 2.5 months of cold storage, 5CNC coated pears lost 34% of their chlorophyll content, whereas SEMP and non-coated fruit lost 39% and 46% of their chlorophyll content, respectively (Fig. 3.6A). These results were clearly reflected in the fruit photos, where more green pigments were retained in 5CNC coated pears in comparison with SEMP and non-coated fruit (Fig. 3.6B). By the end of 5 months of storage (Fig. 3.6B), green chlorophyll pigments in both 5CNC and SEMP coated pears further degraded in comparison with fruit from 2.5 months of storage, but both batches of fruit still maintained good quality. However, non-coated fruit showed significant decay with large surface areas of senescence scalding and fruit softening. The 5CNC coated pears had significantly lower weight loss (1.64%) than that of non-coated pears (2.71%), but no significant difference from that of SEMP coated fruit (1.97%). No difference (P>0.05) in ethylene production and respiration rates were observed between coated and non-coated fruit throughout the 5 months of cold storage. This could probably be explained as due to the high RH condition during cold storage, which weakened the CNC reinforced chitosan coating matrix due to the plasticizing effect of water, compared to the low RH environment at ambient storage. Similarly, it was reported that OTR of a biocomposite

film at 95% RH was about 90 times higher than that at 50% RH (Liu and others 2011). In addition, there was no significant difference in fruit firmness, TSS content, and TA values between non-coated and coated pears, which was also probably caused by the moisture weakened performance of 5CNC coating during cold storage.

Fruit ripening capacity after long-term cold storage is usually evaluated by measuring the firmness of fruit after being moved into ambient conditions and stored for 5 days (Calvo and Sozzi, 2009). All coated fruit samples ripened similarly to the controls, but the 5CNC coated pears (6.55 N) retained significantly (P<0.05) higher firmness, compared to non-coated (5.28 N) and SEMP (4.79 N) coated fruit. This result indicated that 5CNC coating delayed fruit ripening and senescence in comparison with SEMP coating. The results from the cold storage study implied that the 5CNC coating was also effective in delaying fruit ripening and quality deterioration, and had a competitive result with a commercial product (SemperfreshTM). However the performance of CNC reinforced chitosan coating was weakened at high RH cold storage conditions, which will be the subject of future research.

CONCLUSION

CNC reinforced chitosan coatings demonstrated their effectiveness in delaying ripening and quality deterioration of green D'Anjou pears during postharvest storage at ambient conditions. The 5% CNC (w/w in chitosan, dry basis) reinforced 2% (as applied) chitosan coatings successfully retained green chlorophyll pigments on the peels along with delayed fruit quality deterioration (i.e. reduced changes in weight loss, fruit firmness, and soluble solid content) during 3 weeks of ambient storage. Ethylene production was significantly reduced in both 5% and 10% CNC reinforced chitosan coated pears in comparison with chitosan only and non-coated fruit, but the lowest O_2 permeability in 10% CNC reinforced chitosan coating may cause CO₂ injury, thus resulting in surface speckling and pithy brown cores. During cold storage, 5% CNC reinforced chitosan coating delayed fruit ripening and reduced weight loss as well as providing better fruit ripening capacity in comparison with non-coated and SemperfreshTM coated fruit. However, the effectiveness of CNC reinforced chitosan coatings under cold storage was weakened in comparison with ambient storage. In addition, CNC reinforced chitosan films provided superior antibacterial property against both Gram-positive and Gram-negative bacteria. This study indicated that the performances of CNC reinforced chitosan coatings depend on the amount of CNC reinforcement, the fruit postharvest response, and the storage conditions. For future studies, CNC reinforced chitosan or other polymer based coating formulations need to be further improved to provide more hydrophobicity under high RH storage conditions. These studies should correlate the coating performance with fruit physiological responses, peel structure, and storage conditions to optimize the formulation for each individual variety of fruit.

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Fig. 3.1 Inhibition on microbial growth (absorbance at 600 nm) against *L. innocua* and *E. coli* enrichment broth treated with films derived from 2% chitosan containing different concentrations of cellulose nanocrystal (CNC); 0CNC, 5CNC and 10CNC represented films derived from 2% chitosan containing 0%, 5% or 10% (w/w chitosan in dry base) CNC, respectively; Each formulation contained 5% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80; Control was measured for enrichment broth without any film treatment.



Fig. 3.2 Scanning electron microscope (SEM) images of derived films from coating formulations containing different concentrations of cellulose nanocrystal (CNC); 0CNC, 5CNC and 10CNC represented films derived from 2% chitosan containing 0%, 5% or 10% (w/w chitosan in dry base) CNC, respectively; Each formulation contained 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80.



Retention time (min)

Fig. 3.3 Effect of 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC) on ethylene production of pears after 1 day of ambient storage (20 ± 2 °C and $30\pm2\%$ RH); Control represented non-coated fruit; 0CNC, 5CNC, and 10CNC represented fruit coated with 2% chitosan containing 0%, 5% or 10% (w/w chitosan in dry base) CNC, respectively; Each formulation contained 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80; FID: flame ionization detector.



Fig. 3.4 Effects of 2% chitosan coating dispersions containing different concentrations of cellulose nanocrystal (CNC) on surface color change (ΔE) and appearance of pears during 3 weeks of ambient storage ($20\pm2^{\circ}C$ and $30\pm2^{\circ}$ RH); Control represented non-coated fruit; 0CNC, 5CNC and 10CNC represented fruit coated with 2% chitosan containing 0%, 5% or 10% (w/w chitosan in dry base) CNC, respectively; Each formulation contained 5% or 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80 for pears. $\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}$, where L₀*, a₀*, and b₀* represented the values at 0 day and L*, a*, and b* represented the values at different sampling times during storage.



Fig. 3.5 Effect of 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC) on weight loss (A) and shrinkage (B) of pears during 3 weeks of ambient storage ($20\pm2^{\circ}$ C and $30\pm2^{\circ}$ RH); The bar chart with least significant difference (LSD) *Post hoc* multiple comparison test was represented for weight loss at the end of 3 weeks; The same letters placed above each column were not significantly different (p > 0.05); Control represented non-coated fruit; 0CNC, 5CNC, and 10CNC represented fruit coated with 2% chitosan coating containing 0%, 5%, and 10% (w/w chitosan in dry base) CNC, respectively; Each formulation contained 5% or 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80 for pears, respectively; Fruit weight loss was calculated by subtracting the weight at different sampling times from the initial weight, dividing by the initial weight.

[A]					[B]		
	Control ¹	SEMP	5CNC		0 1	2.5 1	
Chlorophyll degradation (%)	46.4±2.7 ^{a2}	38.6±3.6 ^b	34.3±3.3 ^b			2.5 months	5 months
Weight loss (%)	2.71±0.21 ª	$1.97{\pm}0.19^{\mathrm{b}}$	1.64±0.20 ^b	Control			
Firmness (kg·m/s ²)	78.7±3.1 ª	74.7±1.3 °	76.5±4.4 ª				
Total soluble solid content (TSS, %)	14.2±0.4 ^a	14.0±0.7 ^a	14.8±0.7 ^a			AD	100 2
Titratable acidity (TA, %)	0.36±0.02 ª	0.35±0.06ª	0.33±0.02 ª	SEMP			2010
Ethylene production rate (μL·kg ⁻¹ ·h ⁻¹)	136±33 ª	106±11 ª	129±30 ª			aat	0440
Respiration rate (µg·kg ⁻¹ ·h ⁻¹)	1.53±0.10ª	1.12±0.30 ª	1.28±0.21 ª	5CNC	HAL-	Star C	RRA
Ripening capacity ³ (firmness, kg·m/s ²)	5.28±0.05 ^a	4.79±0.16 ^a	6.55±0.66 ^b			Here a	A CAR

Fig. 3.6 Comparisons of color degradation, weight loss, internal quality, ripening capacity, and gas production among non-coated pears, pears coated with either commercial Semperfresh or cellulose nanocrystal reinforced chitosan coating at refrigerated storage condition (-1.1°C and 90% RH) for 2.5 months [A], and illustration of fruit status depending on coating formulations for 2.5 and 5 months, respectively [B];

¹Control: non-coated; SEMP: 0.5% Semperfresh commercial coatings; 5CNC: 2% chitosan coating containing 5% (w/w chitosan in dry base) of CNC and 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80.

²Means followed by the same upper letter in a column were not significantly different (p > 0.05).

³Firmness showing ripening capacity was measured after 5 days stored at ambient condition; All other measurements were conducted at the same day pears taken out from cold room.

Table 3.1 Thickness, water vapor transmission rate (WVTR), and oxygen transmission rate (OTR) of films derived from 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC)

The type of films ¹	Film thickness (10 ⁻³ m)	WVTR $(10^{-3} \text{ g/m}^2 \cdot \text{s})$	OTR (10 ⁻¹² m ³ /m ² ·s·Pa)
0CNC	$0.069{\pm}0.004^{a}$	11.81 ± 0.61^{a2}	30.40±2.89ª
5CNC	$0.078{\pm}0.005^{b}$	$12.52{\pm}0.76^{a}$	15.16±15.74 ^{ab}
10CNC	$0.077 {\pm} 0.006^{b}$	12.41 ± 0.66^{a}	4.17 ± 3.24^{b}

¹0CNC, 5CNC and 10CNC represented films derived from 2% chitosan coating formulations added with 0%, 5% and 10% (w/w chitosan in dry base) of CNC, respectively; Each formulation contained 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80.

²Means followed by the same upper letter in a column were not significantly different (p > 0.05).

Coating treatment ¹	Firmness (kg·m/s ²)	Total soluble solid content (TSS, %)	рН	Titratable acidity (TA, %)
Control	3.15 ± 1.55^{c2}	$14.0\pm0.9^{\rm a}$	$4.29\pm0.05^{\text{b}}$	$0.17\pm0.02^{\rm a}$
0CNC	$10.73\pm6.02^{\text{b}}$	$13.5\pm1.4^{\rm a}$	4.79 ± 0.43^{ab}	$0.11\pm0.02^{\rm c}$
5CNC	$16.08\pm6.77^{\mathrm{a}}$	$11.7 \pm 1.5^{\mathrm{b}}$	4.69 ± 0.20^{ab}	0.14 ± 0.03^{ab}
10CNC	$20.71\pm2.83^{\mathrm{a}}$	10.8 ± 1.0^{b}	$4.93\pm0.68^{\rm a}$	$0.13\pm0.03^{\text{bc}}$

Table 3.2 Comparisons of firmness, total soluble solid, pH, and titratable acidity between non-coated pears and pears coated with cellulose nanocrystal (CNC) reinforced 2% chitosan coatings at the end of 3 weeks of ambient storage ($20\pm2^{\circ}$ C and $30\pm2\%$ RH)

¹Control represented non-coated fruit; 0CNC, 5CNC and 10CNC represented 2% chitosan coatings containing 0%, 5% and 10% (w/w chitosan in dry base) of CNC, respectively; Each coating formulation contained 10% (w/w chitosan in dry base) surfactant mixture at a 1:1 ratio of Tween 80 and Span 80.

²Means followed by the same upper letter in a column were not significantly different (p > 0.05).

CHAPTER 4

CNC PICKERING EMULSION INCORPORATED CHITOSAN COATINGS FOR IMPROVING STORABILITY OF POSTHARVEST BARTLETT PEARS (*PYRUS COMMUNIS*) DURING LONG-TERM OF COLD STORAGE

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ABSTRACT

Cellulose nanocrystal (CNC) Pickering emulsion was prepared and incorporated into chitosan (CH) coatings (CH-PCNC, 0.1% CNC/3% oleic acid/2% CH) for improving hydrophobicity of coatings on Bartlett pears during long-term cold storage (1.7 °C and 90% RH). FTIR analysis of CH-PCNC films stored at both 43% and 83% RH showed no large absorption band at ~3,300-3,400 cm⁻¹ (–OH stretching), whereas that of CNC reinforced CH film (CH-CNC) stored at 83% RH exhibited a large increase compared to film stored at 43% RH. Water vapor permeability (0.06 g·mm/m²·d·Pa) of CH-PCNC film was significantly (*P*<0.05) lower than that of CH-CNC film (0.251 g·mm/m²·d·Pa). These results suggested that CH-PCNC matrix is more stable than CH-CNC at high RH. CH-PCNC coatings significantly (*P*<0.05) delayed ripening and reduced senescent scalding of Bartlett pears compared to SemperfreshTM coating during 3 months of storage. This study demonstrated the possibility of using CNC Pickering emulsions for enhancing the stability of hydrophilic chitosan-based coatings.

KEY WORDS

Cellulose nanocrystal Pickering emulsion, chitosan, fruit coating, Bartlett pears, longterm cold storage

INTRODUCTION

Bartlett (Pyrus communis) is one of the predominant pear cultivars produced in the U.S. Northwest region. Freshly harvested Bartlett pears are highly susceptible to ripening and senescence scalding, and are usually stored under refrigerated temperature (-1.1 °C and 90-94% RH) for extending fruit shelf-life up to 3 months (Deng et al., 2017a; Villalobos-Acuña et al., 2011; Wang and Sugar, 2013). Several postharvest treatments, including 1-methylcyclopropene (1-MCP), controlled atmosphere (CA) storage, and wax coating have been applied to further improve storability of postharvest pears. Unfortunately, each of these technologies reported some limitations that have prevented their application. For example, 1-MCP restricts fruit ripening due to its irreversible binding with ethylene receptors of fruit cells (Wang and Sugar, 2015; Xie et al., 2014), CA storage is expensive, though it does provide efficacy (East et al., 2013; Lum et al., 2017), and wax coating gives an artificial appearance and chalking, along with the potential for off-flavor development (Chen and Nussinovitch, 2001). Hence, this study was aimed at developing pear coatings that overcome the liabilities of the above mentioned postharvest technologies.

Our previous study developed cellulose nanocrystals (CNC) reinforced chitosan (CH) (CH-CNC) coatings with good gas barrier and antibacterial and antioxidant functions (Deng et al., 2017; Jung, Simonsen & Zhao, 2018; Zhao, Simonsen, Cavender, Jung, & Fuchigami, 2017). The CH-CNC coating significantly improved the storability of fresh pears during ambient storage, but its effectiveness was weakened at cold storage with high RH due to the presence of the hydrophilic components (i.e., CH and Tween 80) and the absence of a hydrophobic agent. It has been known that CNC with its high surface area and high aspect ratio could form stable Pickering emulsions, sometimes more stable than conventional emulsions using surfactants (Moon et al., 2016; Perdones et al., 2014; Tang et al., 2017; Vilarinho et al., 2017; Zou et al., 2015). This study thus utilized CNC as both a Pickering emulsion agent and a reinforcing agent in a chitosan coating formulation to enhance the hydrophobicity and stability of the hydrophilic CH matrix without using hydrophilic surfactants (Figure 4.1). The hypothesis of this study was that a CH coating containing a CNC Pickering emulsion (PCNC) (CH-PCNC) would improve the storability of coated pears under high RH cold storage conditions owning to the improved coating hydrophobicity and stability.

The specific objectives of this study were to 1) compare physical properties among CNC, CH-CNC, and CH-PCNC coating formulations, 2) evaluate and compare the hydrophobicity of CH-CNC and CH-PCNC coatings by studying a) water vapor permeability (WVP), b) the polymeric structures of prepared films conditioned at 43% and 83% RH, and c) ethylene production of coated pears stored at 43% and 83% RH, 3) investigate the interactions of coatings with pear surfaces, and 4) validate the effectiveness of CH-PCNC coatings for improving storability of pears during high RH cold storage. It is anticipated that this study will reveal the mechanisms how CNC Pickering emulsion system may improve moisture barrier, hydrophobicity, and stability of CH based coatings at high RH environment for satisfying its application on pears.

MATERIALS AND METHODS

Materials

CNC (11.8% slurry) was produced at the Process Development Center of the

University of Maine (ME, USA). Oleic acid (OA), CH (149 kDa Mw, 97% degree of deacetylation), and acetic acid were purchased from Alfa Aesar (Ward Hill, MA), Premix (Iceland), and J. T. Baker (Phillipsburg, NJ), respectively. All chemicals were reagent grade. Organic green Bartlett pears without visual defects were purchased from a local supermarket (Corvallis, OR) right after they arrived in the store, and were subjected to coating treatments on the same day.

Preparation of coating suspensions and films

For preparing CH-PCNC suspension, 0.1% CNC (w/w wet basis), 3% OA (v/w wet basis), and 2% CH (w/w wet basis) were used based upon our preliminary studies (data not shown). For preparing the CNC Pickering emulsion, 3% OA was slowly added into 0.1% CNC aqueous suspensions and homogenized for 3 min (PT10-35, Polytron, Luzernerstrasse, Switzerland). A 2% CH (dissolved in 1% acetic acid (w/w) solution) was then incorporated into the CNC Pickering emulsion and homogenized for 1 min. A CH-CNC suspension without the Pickering emulsion was prepared as a positive control based on our previous study (Deng et al., 2017a). A 0.5% commercial SemperfreshTM (SEMP, Pace International, Wapato, WA) coating suspension was used as another positive control. SEMP is a commercial coating product consisting of sucrose esters of fatty acids, mono- and di-glycerides, and carboxymethyl cellulose. All coating suspensions were degassed using a self-assembled water flow vacuum system before measurements (Chen and Zhao, 2012).

CNC (1%, w/w wet basis) and prepared coating suspensions were cast into films (Deng et al., 2017c). Briefly, the prepared suspension (60 mL) was uniformly cast onto a

150 mm diameter polystyrene petri dish (Radnor, VWR, PA), and dried at room temperature for 48 h. The derived films were conditioned at 25 °C and 50% RH for 48 h in an environmental test chamber (Versa 3, Tenney Environmental, Williamsport, PA) before evaluation.

Physicochemical properties of CNC and coating suspensions

Particle size, polydispersity, and Zeta-potential of CNC (0.1%, w/w wet basis) and the coating suspensions were measured using a phase analysis light scattering (DLS) zeta potential analyzer (NanoBrook ZetaPALS, Brookhaven Instrument Corporation, Holtsville, NY) at a 90° scattering angle (Zhang et al., 2016).

Water vapor permeability of derived films

WVP of the films was measured using a cup method (Jung et al., 2016). Briefly, film specimen (75 mm x 75 mm) was sealed using vacuum grease between the lid and the Plexiglas test cup that contained 11 mL of distilled water, and the seal ring was tightly closed with rubber bands. Test cups were stored at 25 °C and 50% RH in controlled environment chamber (T10RS 1.5, Hyland Scientific, Stanwood, WA) and weighed every hour for 6 h. Data (n=3) were reported as the mean value and standard deviation.

Fourier transform infrared (FTIR) spectroscopy

The influence of RH on the polymeric structure of the films was investigated using an iS 50 FTIR (Nexus 470 FTIR Spectrometer, GMI, Ramsey, MN) equipped with a Smart ITR attenuated total reflection attachment (ATR) (Thermo Scientific/Nicolet Ltd, UK). The self-assembled RH controlled glass jars were constructed using saturated K_2CO_3 (43% RH) and KCl (83% RH). Prepared films were conditioned at 43% and 83% RH glass jars under ambient temperature, respectively. The absorbance between 800 and 4,000 cm⁻¹ with 32 scans was collected at 4 cm⁻¹ resolution and the FTIR spectrum was reported in the region of 2,000-3,800 cm⁻¹ (i.e. –OH and –CH stretching) (Deng et al., 2017c).

Ethylene production of coated pears

The influence of RH on the ethylene production of uncoated and coated pears was determined using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID). The self-assembled RH controlled glass jars (3.5 L) with Vaseline sealed lid holding a 10 mm rubber septa for sampling headspace gas were prepared and controlled at 43% and 83% RH using the method stated above, respectively. Pears were dipped in coating suspension for 60 s and then dried under the forced air circulation at ambient conditions for 4 h. Five replicates were precisely weighed, placed inside a RH controlled glass jar, and stored at the ambient temperature for 3 h. A 1 mL sample of headspace gas was collected using an air tight syringe (Series A, Valco Instrument Co., Poulsbo, WA) and injected into the GC equipped with three packed columns, including 80/100 HAYESEP D, 8/100 HAYESEP N, and 60/80 molecular sieve column (Supelco, Bellefonte, PA). Helium was used as the carrier gas at a pressure of 350 kPa and flow rate of 21.19 mL min⁻¹. The temperatures of the injector, column, and FID detector were adjusted to 150, 90, and 250 °C, respectively. Standard ethylene gas was purchased from Air Liquide (ScottTM, PA), and GC solution software (Shimadzu,

Kyoto, Japan) was used for data analysis (Deng et al., 2017b). Measurements were conducted in duplicate. Data (ppm/kg) from the 1st and 2nd trials were separately reported due to the variability in the results.

Contact angle (CA) and surface tension (ST) of the coating suspension

Contact angle (CA) of the coating suspensions on pear surfaces and the correlation of surface tension (ST) of the coating suspension with the critical ST (γ_c) of fruit surfaces were determined using a video contact angle system (FTA 32, First Ten Angstroms Inc., Portsmouth, VA) equipped with a face contact angle meter (Casariego et al., 2008; Ramírez et al., 2012). ST was measured by a FTÅ model T10 (First Ten Ångstroms, Portsmouth, VA) equipped with a Du Nuöy ring (CSC Scientific Co, Fairfax, VA). The γ_c of pear surfaces was estimated using the extrapolation from the Zisman plot with water, formamide, and 1-methyl naphthalene as reference liquids (Casariego et al., 2008). Data (n=3) were reported as the mean value and standard deviation.

Scanning electron microscopy (SEM)

Interactions between coatings and fruit surfaces were also investigated by evaluating the adhesion of coating suspensions onto fruit surfaces using scanning electron microscopy (SEM, FEI Quanta 600, Cressington Scientific Instruments Ltd., Watford, UK). Both cross-section and surface images of coated fruit surfaces were obtained. Uncoated and coated pear 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 for 10–15 min in a graded series of acetone (10, 30, 50, 70, 90, 95, 100– 100%), respectively. Samples were dried in an EMS 850 critical point drier, mounted on the SEM stub cross section or surface up, and coated with gold and palladium. Digital images were collected at an accelerating voltage of 5 kV. The most representative image based on extensive observation was reported.

Coating evaluation study

The developed coating suspensions were investigated on Bartlett pears stored at 1.7 °C and 90% RH for 3 months. The applied storage temperature of 1.7 °C was higher than the commercially recommended cold storage temperature of -1.1 °C in order to accelerate fruit ripening and senescence of Bartlett pears. Senescent scalding (SS) and core breakdown (SCB) were visibly evaluated and illustrated using photos after 2 and 3 months of storage (Wang and Sugar, 2015). The ratio (%) of SS was also assessed for 18-20 pears in each group and data (n=3) were presented as the mean value and standard deviation.

Physicochemical properties and internal qualities of pears were evaluated at the end of 3 months of storage. Chlorophyll content of pear peels was measured on opposite sides of the equator of each individual fruit using a delta absorbance (DA) meter (Sinteleia, Bolonga, Italy) (Xie et al., 2014).(Xie et al., 2014) Chlorophyll degradation (%) was calculated as the reduced amount of chlorophyll at the sampling date in comparison with the initial value. The fruit weight loss (%) was calculated as weight change from the initial weight. Fruit firmness was determined using a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Corp., Hamilton, MA) for measuring the maximum penetration force (N) using an 8 mm diameter cylinder at 9 mm distance and test speed of 10 mm/s (Wang and Sugar, 2015). For measuring total soluble solid (TSS) and titratable acidity (TA), 40 g of pear flesh was mixed with 160 mL of distilled water using a blender (Proctor Silex, Nacco Industry Inc., Glen Allen, VA). The mixture was filtered using a qualitative filter paper with the pore size of 2.5 μ m (Whatman, GE Healthcare Bio-Sciences, Issaquah, WA). TSS of the filtrate was measured using a refractometer (RA250-HE, KEM, Tokyo, Japan). For pH, 30 mL of filtrate was titrated with 0.1 N NaOH using a digital titrator (Brinkmann, Missouri City, TX) to pH 8.3 (pH meter, Orion 410A, Fisher scientific, Pittsburgh, PA). TA was reported as the equivalent percentage of malic acid (Deng et al., 2017a). Data (n=3) were reported as the mean value and standard deviation.

Superficial scald of pears occurs due to oxidative stress during storage (Whitaker et al., 2009). As an indicator, α -farnesene and reactive conjugated trienols (CTols) were determined for pear peels (Rowan et al., 1995). Two segments (1 cm diameter) of the peel were obtained from opposite sides of each pear, immersed in 15 mL of hexane in transparent Falcon tubes, and kept at ambient temperature for 15 min. The mixture was then centrifuged (Sorvall Centrifuges, Dupont Co., Wilmington, DE) for 10 min at 9,000 g. Absorbance of α -farnesene at 232 nm (A₂₃₂) and CTols at 281 and 290 nm (A₂₈₁-A₂₉₀) was determined using a UV/Vis spectrophotometer (Model UV-1800, Thermo Fisher Scientific, Inc., Pittsburgh, PA). According to their molar extinction coefficients (ε =27,440 for α -farnesene and ε =25,000 for CTols), α -farnesene and CTols were calculated via $\frac{A_{232}x \ extract \ volume \ x \ 10^6}{27,440 \ x \ sample \ weight}$ and $\frac{(A_{281} - A_{290}) \ x \ extract \ volume \ x \ 10^6}{25,000 \ x \ sample \ weight}$, respectively (Xie et al., 2014). Data (n=3) were reported as the mean value and standard

deviation.

Experimental design and statistical analysis

A completely randomized design was applied in this study. A one-way ANOVA was used to determine the significant differences among different treatments (uncoated, SEMP, CH-CNC, and CH-PCNC). A *post hoc* least significant difference (LSD) was conducted by means of statistical software (SAS v 9.2, The SAS Institute, Cary, NC). Results were considered to be significantly different at P<0.05.

RESULTS AND DISCUSSION

Since Bartlett pears are stored at high RH and low temperatures, we hypothesized that an effective coating would need to be hydrophobic in nature. It would also have to wet the surface during application and dry to an attached thin film on the pear surface. The formulation reported here confirmed our hypothesis. Thus, Figure 4.1 illustrates the proposed enhanced storability mechanism of the CH-PCNC coating. A CNC Pickering oil-in-water emulsion system was effectively formed by the CNC particles encapsulating oleic acid droplets. These emulsion droplets then reinforced the CH matrix. This formulation allowed the formation of a hydrophobic coating without the use of traditional surfactants. We observed that the CH-PCNC coating delayed postharvest ripening and senescence of pears during long term/high RH cold storage.

Physical properties of CNC and coating suspensions

Physical properties of CNC and CH-PCNC are reported in Table 4.1. The particle size of CNC was 156 nm with 0.219 of polydispersity and -38.1 mV of Zeta-potential. The

particle size, polydispersity, and Zeta-potential of CH-CNC suspension were reported as 4901 nm, 0.361, and 20.9 mV, respectively, whereas those of CH-PCNC suspension were 888 nm, 0.005, and +5.4 mV, respectively. It was seen that the particle size of CH-PCNC was significantly ($P \le 0.05$) smaller than that of CH-CNC which demonstrated that CNC could potentially form a stable Pickering emulsion against coalescence and deemulsification by the assembled solid particles of nanometric size around near-micro sized Pickering emulsion droplets (Chevalier and Bolzinger, 2013; Han et al., 2013). The significantly lower polydispersity of CH-PCNC suspension than that of CH-CNC suspension indicated homogeneous dispersion of Pickering emulsion droplets with less coalescence over CH matrix (Kassama et al., 2015). The zeta-potential of the CH-PCNC suspension was changed to a positive value of +5.4 mV due to the introduction of $-NH_3^+$ groups on CH (Zeta-potential of +34.9 mV), which neutralized the SO₃⁻ groups on the CNC surface. These results support our hypothesis that CNC interacted with the CH in the formulation and successfully reinforced it (Capron and Cathala, 2013; Pereda et al., 2014). Hence, we conclude that the CNC Pickering emulsion was incorporated into the CH matrix to form a well-dispersed, homogenous, and stable CH-PCNC suspension.

Hydrophobicity of films and coatings

To evaluate the stability of the films (not on the pear surface as a coating) at high RH, FTIR spectra in the region of 2,000-3,800 cm⁻¹ (i.e. –OH and –CH stretching) was compared between films from the same batch conditioned (stored) at 43% and 83% RH (Figure 4.2). For the CH-CNC film, a larger absorption band in the region of 3,300-3,400 cm⁻¹ referring to –OH stretching was observed on films conditioned at 83% RH in comparison with the film at 43% RH. It might be because the hydrophilic compounds, such as CH, CNC, and surfactant, were plasticized, and/or the mobility of the polymer (with hydrophilic surface) chain was enhanced at high RH, due to significantly increased penetration of water molecules and formation of hydrogen bonds in the polymer matrix (Azizi et al., 2013; Kurek et al., 2014; Salam et al., 2013). In comparison with CH-CNC film, no particular change in the absorption band was observed in CH-PCNC film at both 83% RH and 43% RH, indicating the increased hydrophobicity and stability of film structure against high RH.

The WVP of CH-PCNC films was ~0.060 g·mm/m²·d·Pa, four times lower (P<0.05) than that of CH-CNC film (0.251 g·mm/m²·d·Pa) (Table 4.2). This result was consistent with the FTIR analysis, showing that the CH-PCNC film was more hydrophobic than the CH-CNC film.

The hydrophobicity of the coatings was also evaluated by comparing the ethylene production of coated fruit conditioned (stored) at 43% and 83% RH at room temperature (Table 4.2). At 43% RH, the ethylene production of pears coated with SEMP (10.1 and 19.1 ppm/kg in 1st and 2nd trials, respectively), CH-CNC (8.6 and 17.1 ppm/kg), and CH-PCNC (6.8 and 8.1 ppm/kg) was all lower than that of uncoated fruit (15.1 and 20.8 ppm/kg). This indicated that the coatings were effective at this RH. However, at 83% RH the ethylene production of fruit coated with SEMP (12.3 and 20.0 ppm/kg in 1st and 2nd trial) and CH-CNC (10.3 and 19.7 ppm/kg) tended to increase in comparison with those at 43% RH. This could be due to increased absorption of water into the coating, reduced hydrophobicity, and thus resulting in increased permeability to gas and water vapor transporting through the weakened films. On the other hand, ethylene production of fruit
coated with CH-PCNC and stored at 83% RH (5.6 and 6.8 ppm/kg in 1st and 2nd trials) showed no significant increase in comparison with those stored at 43% RH (6.8 and 8.1ppm/kg). This result demonstrated that CH-PCNC coating was more hydrophobic and stable at high RH than that of SEMP and CH-CNC coatings, and could effectively suppress ethylene production and delay fruit ripening. These data were also consistent with the results of the film studies.

Interactions between coatings and fruit surfaces

In order for the coating to provide protection to the fruit, it must wet the surface, i.e. spread across the surface of the fruit and also adhere to the surface so that it does not "flake off," or sluff after application. Wetting can be investigated by measuring the contact angle of the applied solution on the surface of the pear (Table 4.1). A lower contact angle (CA) value represents better wetting (Jung et al., 2016; Seo and Lee, 2006). The CH-PCNC coating suspension had a significantly lower CA value (\sim 34.7 °) than that of CH-CNC suspension (~38.1 °), demonstrating better wettability onto fruit surface. While these values do not represent spontaneous wetting, the ideal, they do show good wetting behavior of the formulations. Further evidence for wetting was obtained by measuring the ST of the coating suspensions, which should be equal to or lower than that of the critical ST of the pear surface to provide for good wetting (Deng et al., 2017b). The critical ST of the pear surface was 36.5 mN/m, extrapolated from the Zisman plot (Table 4.2). Both CH-CNC and CH-PCNC (~33.2 and ~30.3 mN/m) coatings had lower ST values than the critical ST of the fruit surface (Table 4.2), thus we conclude there should be good wetting ability onto pear surfaces. Moreover, CH-PCNC coating suspension had

significantly (P < 0.05) lower ST than that of CH-CNC coating. Improved wetting also implies improved adhesion to the surface since it results from attractive forces between the surface and the formulation.

SEM micrographs were obtained for both cross-section and surface samples of uncoated and coated pear peels. In the cross-sections (Figure 4.3), the CH-PCNC coating was slightly detached from the fruit surface and showed cracks in comparison with other coatings. This behavior of CH-PCNC coating might indicate its rigid and dense matrix that was unable to tolerate the disturbance occurred during the drying process of sample for the SEM analysis (Cheng, Abd Karim, & Seow, 2008). It was thus unrelated to the actual effectiveness of coating on the fruit surface. With respect to the SEM micrographs of the fruit surfaces (Figure 4.4), the CH-PCNC coating was smoother and showed fewer voids than the SEMP and CH-CNC coatings. The CH-PCNC coating also showed spherical and small globules, perhaps emulsion droplets, with fewer "humps" and craterlike holes, suggesting an improved gas and moisture barrier (Bosquez-Molina et al., 2003). Hence, the CH-PCNC coating with a rigid and dense matrix and good dispersion of emulsion droplets over the fruit surface was further evaluated on pears during long term/high RH cold storage, with uncoated and SEMP coated as base and positive controls.

Evaluation of the coatings on Bartlett pears during storage

Senescent scald (SS) and senescent core breakdown (SCB) are commonly found in Bartlett pears during cold storage (Porritt, 1982). The appearance of uncoated and SEMP and CH-PCNC coated Bartlett pears were observed at the end of 2 and 3 months of storage and SS and SCB were determined (Figure 4.5). At the end of 2 months of storage, SEMP and CH-PCNC coatings both reduced SS and SCB of pears in comparison with uncoated fruit. In comparison with SEMP, the CH-PCNC coating retained more green pigments in the pear peels. At the end of 3 months of storage, CH-PCNC coating further delayed SS and SCB of pears without a major presence of browning spots in comparison with SEMP coated and uncoated samples. Senescent scald (SS, %)) was also assessed for 18-20 pears in each group (n=3) at the end of 3 months of storage (Table 4.3). The CH-PCNC coated pears showed no senescent scald, whereas the uncoated and SEMP coated fruit had ~88% and ~66% SS, respectively.

The physicochemical properties and internal qualities of uncoated and coated pears were also investigated at the end of 3 months of storage (Table 4.3). For chlorophyll degradation, CH-PCNC coated pears (~62%) were significantly (P<0.05) lower than that of SEMP coated (~81%) and uncoated one (~93%). CH-PCNC coatings significantly (P<0.05) reduced the weight loss of fruit in comparison with SEMP coated and uncoated samples. CH-PCNC coated pears had higher fruit firmness (~59 N) than SEMP coated (~49 N) and uncoated fruit (~42 N), presented lower TSS and higher TA values than that of SEMP coated samples. These data indicated delayed fruit ripening. Superficial scalding induced by conjugated trienes (CTols) as a result of oxidation of naturally occurring α -farnesene in fruit is one of the severe issues for Bartlett pears during longterm cold storage (Chen et al., 1990; Whitaker et al., 2009). Both SEMP and CH-PCNC coatings resulted in significantly (P<0.05) lower CTols and α -farnesene, compared to uncoated samples, representing less accumulation and oxidation of α -farnesene and production of CTols in pear peel tissue after 3 months of storage (Table 4.3). These results supported our hypothesis that a CH-PCNC coating could effectively delay fruit ripening and improve storability of postharvest pears during long-term, high RH cold storage.

CONCLUSION

A CNC Pickering emulsion was developed and combined with a CH matrix to produce a coating with enhanced hydrophobicity and stability under high RH conditions. This coating can improve the postharvest quality of Bartlett pears during long-term, cold storage. The CNC Pickering emulsion incorporated CH (CH-PCNC) coating also was shown to be more hydrophobic and stable than a CNC reinforced CH coating (CH-CNC) without introducing Pickering emulsion system, especially at high RH conditions.

A coating evaluation study on Bartlett pears stored at 1.7 °C and 90% RH for 3 months demonstrated that the CH-PCNC coating delayed fruit ripening and reduced senescence scalding in comparison with a commercial coating (SemperfreshTM). This study also indicated that the CH-PCNC coating adhered well to and covered the fruit surface with a smoother texture and fewer voids than that of SemperfreshTM or the CH-CNC coatings. Therefore, the CH-PCNC coating show potential for commercialization by the pear industry for the improvement of postharvest storability during long term/high RH cold storage of Bartlett pears. A pilot-plant scale study for freshly harvested pears is underway.

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Fig. 4.1 Schematic diagram illustrating the formation of cellulose nanocrystal (CNC) Pickering emulsion incorporated chitosan (CH) coating (CH-PCNC) and its effect on improving the storability of Bartlett pears during long-term of high humidity cold storage.



Fig. 4.2 Fourier-transform infrared spectroscopy (FTIR) of films derived from CNC or coating suspensions. CNC: 1% (w/w/ wet basis) cellulose nanocrystals; CH-CNC: CNC reinforced chitosan (CH) coating suspension without Pickering emulsion; CH-PCNC: CNC Pickering emulsion (PCNC) incorporated chitosan (CH) coating; Films were conditioned (stored) at 43% and 83% RH, respectively, prior to FTIR analysis.



Fig. 4.3 Scanning electron microscopy (SEM) micrographs of cross-sections of uncoated and coated pear peels; Digital images were collected at an accelerating voltage of 5 kV.



Fig. 4.4 Scanning electron microscopy (SEM) micrographs of the surfaces of uncoated and coated pear peels. Digital images were collected at an accelerating voltage of 5 kV.



Fig. 4.5 Effect of cellulose nanocrystal Pickering emulsion (PCNC) incorporated chitosan (CH) coating (CH-PCNC) on senescent scalding (SS) and senescent core breakdown (SCB) of Bartlett pears during storage at 1.7 °C and 90% RH.

Physical properties									
CNC and coating suspensions		Particle size (nm) Polydispersity		Zeta-potential (mV)					
CNC*		$156 \pm 4^{c,+}$	0.219 ± 0.012^{10}	$-38.1 \pm 2.4^{\circ}$					
CH**-CNC ***		4901 ± 109^{a}	0.361 ± 0.026	20.9 ± 1.2^{a}					
CH-PCNC		888 ± 127^{b}	0.005 ± 0.000	5.4 ± 4.6^{b}					
Critical surface tension (γ_c) of pear peels and surface characteristics of coating suspensions									
			Contact angle (°)	Surface tension (mN/m)					
1	•••••••	SEMP	43.7 ± 1.3^{a}	30.4 ± 0.5 ^b					
		CH-CNC	38.1 ± 2.6 ^{ab}	33.2 ± 0.3 ^a					
0.8 •		CH-PCNC	$34.7\pm5.3^{\:b}$	$30.3\pm0.5^{\ b}$					
	I-methyl .	····.							
	napinalene	Formamide							
	····								
Coi	······································								
0.4	Critical surface	tension (γ_{I}) of	f ^{*•} •••						

•••••

 $\begin{array}{l} y = -0.0223x + 1.8121 \\ R^2 = 0.9949 \end{array}$

65

Water

75

Table 4.1 Physical properties of CH-PCNC suspensions and correlations of coating suspensions with pear surface.

*CH-CNC: CNC was prepared at 0.003% (w/w wet basis).

Bartlett pear skin

= 36.4mN/m

45

0.2

0 35

** ZP of 2% chitosan dissolved in 1% acetic acid was $+34.9 \pm 1.1$ mV.

*** CNC reinforced CH coating without Pickering emulsion.

⁺ Means followed by different superscript letters within each column were significantly different according to the Least Significant Difference (LSD) test (P < 0.05).

55

 $\gamma_L \,(\underline{mN/m})$

Table 4.2 Effect of CH-PCNC coatings on ethylene production of Bartlett pears and water vapor permeability (WVP) of the derived films

		Ethylene production of fruit				
Assembled controlled humidity vessel	Coating treatments	1 st trial (ppm/kg)		2 nd trial (ppm/kg)		$= WVP \text{ of films}$ $(10^{-2} \text{ g·mm/m}^2 \cdot \text{d} \cdot Pa)$
		43% RH	83% RH	43% RH	83% RH	(10 g min in a ra)
	Control	15.1	13.5	20.8	19.0	N/A
	Semperfresh TM	10.1	12.3	19.1	20.0	N/A**
	CH-CNC *	8.6	10.3	17.1	19.7	25.1±1.0 ^{a, ***}
	CH-PCNC	6.8	5.6	8.1	6.8	6.0±1.1 ^b

* CH-CNC: CNC reinforced CH coating without Pickering emulsion.

** N/A: WVP of SemperfreshTM was not available because films could not be formed from SemperfreshTM suspension.

*** Means followed by different superscript letters within each column were significantly different according to the least significant difference (LSD) test (P < 0.05).

quanties of Bartiett pears after 5 months of storage at 1.7 °C and 5070 fth								
	Uncoated	Semperfresh TM	CH-PCNC					
Chlorophyll degradation (%)	$92.5\pm 4.5^{a,*}$	$80.6\pm9.4^{\text{ b}}$	$61.8\pm14.2^{\text{ c}}$					
Weight loss (%)	$4.41\pm0.82^{\:ab}$	$4.70\pm0.97^{\text{ a}}$	$4.02\pm0.64^{\ b}$					
Firmness (N)	42.3 ± 1.2^{c}	$49.2\pm2.6^{\:b}$	$58.6\pm3.5^{\ a}$					
Total soluble solid (°Brix)	$10.4\pm0.4^{\text{ a}}$	$10.2\pm0.1~^{\rm a}$	$9.0\pm0.3^{\ b}$					
Titratable acidity (%)	0.169 ± 0.009^{b}	$0.183 \pm 0.003^{\ b}$	$0.203 \pm 0.013~^{a}$					
Senescent scald (SS, %)	88.6 ± 3.9^{a}	$65.9\pm4.5^{\ b}$	0 °					
α-farnesene (µmol/g)	$92.9\pm8.1~^{a}$	$59.3\pm6.1^{\text{ b}}$	$50.6\pm1.1^{\text{ b}}$					
Conjugated trienols (µmol/g)	$37.9\pm3.2^{\rm \ a}$	$30.4\pm0.8^{\text{ b}}$	26.9 ± 1.4^{b}					

Table 4.3 Effect of CH-PCNC coatings on the physicochemical properties and internal qualities of Bartlett pears after 3 months of storage at 1.7 °C and 90% RH

* Means followed by different superscript letters within each column were significantly different according to the least significant difference (LSD) test (P < 0.05).

CHAPTER 5

CELLULOSE NANOMATERIALS EMULSION COATINGS FOR CONTROLLING PHYSIOLOGICAL ACTIVITY, MODIFYING SURFACE MORPHOLOGY, AND ENHANCING STORABILITY OF POSTHARVEST BANANAS (*MUSA ACUMINATE*)

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ABSTRACT

Cellulose nanomaterials (CNs)-incorporated emulsion coatings with improved moisture barrier, wettability and surface adhesion onto fruit surfaces were developed for controlling postharvest physiological activity and enhancing storability of bananas during ambient storage. Cellulose nanofiber (CNF)-based emulsion coating (CNFC: 0.3% CNF/1% oleic acid/1% sucrose ester fatty acid (w/w wet base)) 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 banana peels, and exhibited good wettability onto banana surfaces. CNFC coating delayed the ethylene biosynthesis pathway and reduced ethylene and CO₂ production, thus delaying fruit ripening. As the result, CNFC coating minimized chlorophyll degradation, weight loss, and firmness of bananas while ensuring the properly fruit ripening during 10 d of ambient storage. This study demonstrated the effectiveness of CNF based emulsion coatings for improving the storability of postharvest bananas.

KEY WORDS

Cellulose nanomaterials, emulsion coatings, physiological activity, surface morphology, storability, bananas, postharvest storage

INTRODUCTION

Cavendish banana (*Musa acuminate*) is a rich source of vitamins and bioactive compounds (e.g. dietary fiber and phenolic compounds), and one of the mostly consumed fruit worldwide (Singh et al., 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 et al., 2011). However, low temperature storage might cause chill injury and physiological damage on banana fruit (Jiang et al., 2004), and hypobaric and controlled atmosphere storages are capital intensive and expensive (Pesis et al., 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 fruit (Dhall, 2013; Lin and 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 and 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 based matrix, and to investigate the influence of developed coatings on the physiological activity, surface morphology, and storability of bananas during ambient storage (Jung, Simonsen and Zhao, 2016).

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 et al., 2016; Ramana Rao et al., 2016; Scramin et al., 2011). 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 and Tan, 2013), and 2) decrease the surface tension of coating materials for improving stability, uniformity, and spreadability of emulsion coatings (Jung et al., 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 may vary due to their composition and structure.

Cellulose nanomaterials (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 et al., 2010; Belbekhouche et al., 2011; Moon et al., 2016). Cellulose nanofibers (CNF) with high flexibility and absorption ability can form coating and 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 et al., 1995). Our previous fruit

coating studies demonstrated that CNF based coating is suitable for preventing cherry rain cracking during fruit production, while CNC-reinforced chitosan coating is ideal for extending the storability of postharvest pears (Jung et al., 2016). The effectiveness of CNs coatings is selective, depending on the types of fruit (surface characteristics, postharvest physiological activity, etc.) and storage conditions (temperature, relative humidity, etc.). 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 banana surfaces, enhancing moisture barrier of coatings, controlling physiological activity, and modifying surface morphology of bananas.

For achieving the goal, a two-step experimental approach was employed in this study: 1) development of emulsion coatings by evaluating the performance of coating formulations (wettability and adhesion onto fruit surfaces) and derived films (hydrophobicity), as well as the external fruit quality parameters (chlorophyll degradation, weight loss, and fruit marketability) when coating bananas, and 2) investigation of the developed coatings on the physiological activity (ethylene biosynthesis pathway and ethylene and CO₂ production), surface characteristics (critical surface tension of fruit peels and cell morphology), and the internal fruit quality (starch degradation, firmness, soluble solid contents, and titratable acidity) of bananas during ambient storage. It is anticipated that this study will provide the scientific insight of CNsbased coatings as a simple and effective postharvest technology for enhancing the storability of banana fruit.

MATERIALS AND METHODS

Materials

CNF and CNC, derived from softwood Kraft pulp with solid content of 2.95% and 11.8%, respectively, were produced from the Process Development Center at the University of Maine (ME, USA). Chitosan (97% degree of deacetylation, 149 kDa Mw) was purchased from Premix (Iceland), Tween 80 from Amresco (OH, USA), SEFA from TCI American (OR, USA), OA and glycerol from Alfa Aesar (MA, USA), and acetic acid from J. T. Baker (NJ, USA). The 1-aminocyclopropane-1-carboxylic acid (ACC) and N-(2-hydroxyethyl) piperazine-N'-3-propanesulfonic acid (EPPS) were purchased from Chem Impex Internation, Inc. (IL, USA), HgCl₂ from MP biomedicals (CA, USA), pyridoxal phosphate from TCI American (OR, USA), dithiothreitol (DTT) from Sigma (MO, USA), and trichloroacetic acid (TCA) and NaOCl from JT Baker (NJ, USA).

Organic Cavendish bananas (Piura, Peru) at the ripeness stage of 2 (green with trace of yellow) without visual defects were purchased from a local supermarket (OR, USA) at the day of their arrival at the store, and coated on the same day of purchase.

Preparation of coating formulations and fruit coatings

Coating formulations were prepared based on wet base (w/w), and the range of concentration of each component was determined based upon our preliminary studies (data not shown). Each coating matrix, including 0.3% CNF and 0.2% CNC-reinforced 2% chitosan, was formulated with surfactants (Tween 80 or SEFA) and/or OA, and derived into six different types of emulsion coatings as reported in Fig. 5.1. Emulsion systems with different surfactant types were first prepared as followed: 1% Tween 80

was suspended in water at the ambient temperature, and SEFA was dispersed at 70 °C to enhance water solubility. Then, 1% OA (1%, w/w) was added to surfactant solution, and homogenized for 1 min. Coating formulation with Tween 80 at the level of 0.03% for CNF-based coating and 0.2% for CNC-reinforced chitosan coating was also prepared as the positive control. From our previous studies, surfactant at 10% (w/w dry basis) level to the concentration of polymeric matric materials produced good wettability of coating formulations to the surfaces of fruit (Deng et al., 2017; Jung et al., 2017). In this study, two different polymeric materials (0.3% CNF for CNF-based coating and 2% chitosan for CNC-reinforced chitosan coating) were used as coating matrix for bananas, thus Tween 80 at the level of 0.03% (10% of 0.3% CNF) and 0.2% (10% of 2% CH) were added to CNF-based and CNC-reinforced chitosan coatings, respectively. 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, CNCB: 1% Tween 80 with 1% OA, and CNCC: 1% SEFA with 1% OA), homogenized for 3 min, and then degassed using a self-build water flow vacuum system (Chen and Zhao, 2012).

When applying coatings on the fruit surface, three different coating application methods (dipping, spraying, and brushing) 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 (Njombolwana 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 $50 \pm 5\%$ RH). Fruit coated with SemperfreshTM (Semp, 1.2%, w/w, Pace International, LLC, WA, USA) was used as a positive control. SemperfreshTM 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.

Wettability of coating formulations

Coating performance is strongly influenced by the wettability of coating formulation associating to the surface characteristic of fruit (Park, 1999). 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 et al., 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 et al., 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; Lima et al., 2010).

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 ($\gamma_{\rm C}$) 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 1methyl naphthalene as reference liquids (Fowkes et al., 1964). 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).

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 and 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.

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. Six fruit were randomly assigned into each replication per treatment. Mean values and standard deviations were reported with triplicates during the storage. 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 et al., 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 and 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 et al., 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 and 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 triplicates. 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 et al., 2014). TA was reported as the equivalent percentage of malic acid as the predominant acid in ripen banana. Three fruit were randomly selected for one measurement and considered as one replication per treatment, and mean values and standard deviations were reported with three replications.

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.

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 thermal conductivity detector (TCD, O_2 and CO_2) (Deng et al., 2017). 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 ($20 \pm 2 \, ^{\circ}$ C). The O_2 and CO_2 productions were monitored after 24 h, while ethylene production was measured after 48 h due to the low amount of ethylene production. The fruit incubation time (24 or 48 h) was determined based upon our preliminary study (2-48 h) providing the significant difference between control and treatments. It was assumed that banana samples obtained from a local market had already been stored for several days, which might have much lower ethylene production rate compared with freshly harvested ones. For each jar, 1 mL of headspace gas was collected using an air tight syringe (Series A, Valco Instrument Co., USA) and then injected into the GC fitted with three kinds of packed columns: 80/100 HAYESEP D, 8/100 HAYESEP N, and 60/80 molecular sieve column (Supelco, Bellefonte, PA, USA). Helium was applied as the carrier gas at a pressure of 350 kPa and flow rate of 21.19 mL min⁻¹. The temperature of injector, column, and FID detector was set at 150, 90, and 250 °C, respectively. The O₂, CO₂, and ethylene standard gases were purchased from Air Liquide (ScottTM, PA, USA), and GC solution software (Shimadzu, Japan) was used to calculate the amount of O₂, CO₂, and ethylene.

ACC and ACS activity

As illustrated in Fig. 5.1, the coatings could impact the ethylene biosynthesis pathway of fruit by generating modified atmosphere condition. This study measured ACC as the precursor of ethylene and ACS activity as an enzyme that catalyzes the synthesis of ACC from S-Adenosyl methionine (SAM) (Kato and Hyodo, 1999).

For measuring ACC and ACS, banana flesh samples were collected at different sampling times (0, 3, 7, and 10 d), and stored at -80 °C prior to analysis. For extracting ACC, 2 g of freshly-thawed banana flesh in 10 mL of 9% TCA was homogenized for 60 s and incubated at 4 °C for 24 h. The extract was centrifuged at 10,000 x g for 30 min, and the supernatant was adjusted to pH 7-8 with 1N NaOH. Two of sample reaction mixtures were prepared with 500 μ L of supernatant, 100 μ L of 10 mM HgCl₂ (100 μ L), and 300 μ L of DI water in capped 10 mL vials. One of them was spiked using internal standard ACC (50 μ L of 0.05 mM ACC). Both of them were incubated for 3 min at 4 °C after adding 100 μ L of saturated NaOH and 5.25% NaOCl for hydrolysis of ACC into ethylene (Hoffman and 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), $0.5 \,\mu$ M 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 μ L of enzyme solution, 50 μ L of 600 mM EPPS (pH 8.5), and 90 μ L 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 μ L of 10 mM HgCl₂ and 200 μ L 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 and Yang, 1982). ACS activity was expressed as pmol ethylene/g fresh sample.

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.

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.

RESULTS AND DISCUSSION

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 5.1). 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 5.1). 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, 35.2 mN/m, derived from the Zisman plot (Fig. 5.1), 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 the 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 et al., 2009). Above results indicated 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 onto 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 5.1), which could be attributed to the more hydrophobic SEFA in comparison with Tween 80, thus reducing the oil-water interfacial tension and improving the hydrophobicity of the coatings (Ariyaprakai et al., 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/m² d Pa) had the lowest value among all coating formulations, indicating a superior moisture barrier (Table 5.1). It was hypothesized that OA/SEFA emulsion could be well dispersed and/or absorbed 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 et. al., 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 the 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 that are critical for enhancing storability of fresh banana fruit.

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. 5.2. CNFC coating resulted in the least and slowest chlorophyll degradation of banana peels among all coating formulations (Fig. 5.2A). 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. 5.2B). Furthermore, CNFC coated fruit retained the highest marketability compared to fruit coated by both coating formulations over the storage period (Fig. 5.2C). 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 better performance of CNFC emulsion coating could be explained that the

hydrophobic OA/SEFA emulsion system was well dispersed and/or absorbed in CNF matrix with flexible structure compared to the CNC-reinforced chitosan matrix with high crystallinity, thus deriving uniform coating coverage onto fruit surface and generating a modified atmosphere condition inside the fruit. This modified internal atmosphere delayed fruit physiological activity and ripening, in turn prevented moisture loss, reduced chlorophyll degradation, and improved marketability of fruit during storage. It is worth to mention that a sensory evaluation is under the way to evaluate the flavor and other sensory attributes of coated fruit potentially associated with the modified internal atmosphere condition, and ethanol and acetaldehyde compounds as the indicators of anaerobic respiration will also be analyzed in our future studies.

Validation of CNFC emulsion coating for controlling physiological activity and surface characteristics of bananas during ambient storage

Uncoated, Semp-coated, and CNFC-coated fruit were further validated for their effect on the physiological activity (Fig. 5.3) and surface characteristics (Fig. 5.4) 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 (2.38 ppm/g) ones (Fig. 5.3A). The CNFC coated fruit also produced lower CO₂ and retained higher O₂ in headspace of the jar in comparison with non-coated one, while was similar to that of Semp-coated fruit (Fig. 5.3B). Fruit respiration (O₂ and CO₂) and ethylene production are the main physiological indexes tracking the change of ripening and senescence over the storage period (Ahmed and Palta, 2016). These data supported that CNFC coating suppressed the respiration and ethylene production of bananas, 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. 5.1). As shown in Fig. 5.3C, 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 the hydrolysis of ACC into ethylene, thus generating less ethylene with accumulated ACC in fruit (Ketsa et al., 2013). This result was consistent with the lower ethylene production in CNFC-coated fruit in comparison with uncoated and Semp-coated ones (Fig. 5.3B). In the future study, the conjugated ACC will be measured to fully understand the potential influence of both free and conjugated forms of ACC on fruit physiological process and correlation with ACS activity. 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. 5.3D). The initial higher ACS activity could be associated with the onset of subsequent peel yellowing of obtained fruit samples (Ketsa et al., 2013). It was possible that the banana fruit obtained from the local market might already reach the onset of subsequent peel yellowing. Unlike uncoated fruit which overly had brown spots at 7 d of storage (Fig. 5.4), Semp and CNFC-coated fruit showed the increasing trend of ACS activity after 7 d of storage. This might be associated with the postponed ripening stage of fruit, which stimulated its activity for suitable ripening process at the late stage of storage. It was also previously reported that once the fruit started to ripen, ACS activity

increased, and then declined when ethylene production had reached its maximum, but ACS activity could be increased again at the end of ripening and during subsequent postharvest storage (Poel et al., 2012). 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 CO₂ and less ACS activity for delaying fruit ripening.

The influence of coatings on the fruit surface characteristics through SEM analysis is illustrated in Fig. 5.4. 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. The insufficient coverage might potentially accelerate moisture loss, respiration, and fungus invasion (Amarante et al., 2001). In addition, the size and shape of epidermal cells of CNFC-coated fruit were altered as marked in Fig. 5.4, 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 superior coating performance.

Validation of CNFC emulsion coating on appearance and internal quality of bananas during ambient storage

Visual appearance of fruit was monitored at 3, 7, and 10 d of ambient storage (Fig. 5.5). During green-yellow life of banana (0-3 d of storage), both Semp and CNFC coatings slowed down the chlorophyll degradation. During the yellow-brown life of bananas (7-10 d of storage), CNFC coating further reduced the incidence of browning
spots on the fruit surfaces in comparison with uncoated and Semp coated ones. At yellow stage, banana fruit continued to ripen, the presence of polyphenol oxidase (PPO) further catalyzed the hydrolysis of phenolic compounds into o-quinone, which was then oxidized and polymerized to form dark brown melanins (Soradech et al., 2017). As shown on the SEM images of the coated fruit surfaces, CNFC coating provided more uniform coverage onto fruit surfaces without cracks and/or cleavage, thus better controlling the internal atmosphere inside fruit for reducing physiological activity in comparison with Semp coating. Hence, CNFC coating was more effecive to delay fruit ripening and postponed fruit senesence as demonstrated by the less browning pigments in the yellow stage of fruit.

The startch test showed the high content of starch in CNFC-coated bananas as reflected by the darker blue/black color on the cross-cut fruit surfaces from iodine reaction in comparison with uncoated and Semp coated fruit (Fig. 5.5). Similar trend was observed from TSS at 3 and 7 d of storage, showing CNFC coating resulted in the lowest TSS of fruit in comparison with non-coated and Semp coated ones (Fig. 5.5). TSS value is a good indicator of fruit ripening since starch is hydrolyzed into soluble sugars during ripening (Prabha and Bhagyalakshmi, 1998). The results from both starch test and TSS value proved that CNFC coating further delayed fruit ripening in comparison with Semp coating. Meanwhile, TSS of CNFC coated bananas had no significant difference from that of non-coated one at 10 d of storage, indicating that proper ripening process continued in bananas during the storage. This result was also supported by the increased ACS activity after 7 d of storage, indicating that the proper ripening continued in CNFC-coated bananas.

Both Semp and CNFC coated bananas retained higher firmness than uncoated fruit at 3 and 7 d of storage (Fig. 5.3). Firmness is an important parameter to determine the ripening stage and quality of banana fruit. While ripening, pectinesterase and polygalacturonase hydrolyzed the pectin, leading to the destruction and deterioration of the cell wall structure, in turn softened the fruit (Seymour, 1993; Yaman and Bayoundurlu, 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 Sempcoated fruit throughout the 10 d of storage (Fig. 5.5). 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 et al., 2011). The validation study confirmed that CNFC coating was effective to delay ripening, retard quality deterioration, and enhance the storability of postharvest bananas during ambient storage.

CONCLUSION

This study developed cellulose nanofiber (CNF)-based emulsion coatings for enhancing postharvest storability of banana fruit during ambient storage through improving the water barrier and adhesion of coatings onto the fruit surfaces and controlling the fruit postharvest physiological activity. 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. To assure the success of the developed coating technology for commercial application, the influence of coatings on sensory attributes and consumer acceptance will be studied as the continuous effort following the current coating development study.

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Fig. 5.1 Development mechanisms of cellulose nanomaterial-incorporated emulsion coatings with improved water and gas barrier functions, wettability onto fruit surface, and their effects on delaying ethylene biosynthesis of postharvest bananas; Surfactant types for forming oleic acid (OA) emulsion included Tween 80 and sucrose ester of fatty acid (SEFA); CNF: cellulose nanofiber; CNC: cellulose nanocrystal; CH: chitosan; θ : Contact angle of reference liquid on banana surface; γ_L : Surface tension of reference liquids on banana skin; γ_c : Critical surface tension of banana skin.



Fig. 5.2 Effect of different coating formulations on banana chlorophyll degradation [A], weight loss [B], and marketability [C] during 10 days storage period at 25±2°C and 50±5% relative humidity (RH); Ctrl: Uncoated; Semp: 1.2% commercial SemperfreshTM solution; Coating formulations were investigated in two different categories: the series of CNC made of 2% (w/w wet base) chitosan with 0.2% (w/w wet base) cellulose nanocrystal (CNC) and 0.4% (w/w wet base) glycerol and the series of CNF made of 0.3% (w/w wet base) cellulose nanofiber (CNF); A, B, and C were distinguished by using different emulsion systems; A: Tween 80 only; B; Tween 80 and oleic acid; C: sucrose ester of fatty acid and oleic acid.



Fig. 5.3 Comparisons of 1-aminocyclopropane-1-carboxylic acid (ACC) [A], ACC synthase (ACS) activity [B], ethylene [C], and O_2 and CO_2 production [D] between uncoated and coated (SemperfreshTM and cellulose nanofiber (CNF)) banana; SemperfreshTM prepared in 1.2%; CNFC prepared in 0.3% CNF, 1% sucrose ester and 1% oleic acid; Ethylene production was measured after 48 h of banana storage in 1.5 L jar; O_2 and CO_2 production values were evaluated after 24 h of banana storage in 1.5 L jar; ACC concentration and ACS activity were monitored during 10-day storage period at 25±2°C and 50±5% relative humidity (RH)



Fig. 5.4 Surface morphology of uncoated and coated (SemperfreshTM and CNFC) bananas at different resolutions (100 μ m above and 5 μ m below); SemperfreshTM prepared in 1.2%; CNFC prepared in 0.3% CNF, 1% sucrose ester of fatty acid and 1% oleic acid.



[Firmness]

[Total soluble solid]

[Titratable acidity]



Fig. 5.5 Comparisons of appearance, starch degradation, firmness, total soluble solid (TSS), and titratable acidity (TA) among uncoated and coated (SemperfreshTM and cellulose nanofiber (CNF)) banana for 10 days storage at 25±2°C and 50±5% relative humidity (RH); SemperfreshTM prepared in 1.2%; CNFC prepared in 0.3% CNF, 1% sucrose ester of fatty acid and 1% oleic acid.

Table 5.1 Analysis of variance (ANOVA) results and *Post-hoc* LSD multiple comparison tests for investigating main effects of matrix and emulsion and their interactions (matrix x emulsion) on spread coefficient, contact angle (CA) onto silicone and banana skin, surface tension of coating formulations and water vapor permeability (WVP) of the derived films

			Coating formulations				Derived films
			CA (°) onto	CA (°) onto	Spread coefficient	Surface tension	WVP
			banana skin	silicon wafer	$(W_s, mN/m)$	(ST, mN/m)	(g ml/m ² d Pa)
Factors			Analysis of variance (ANOVA) results				
Matrix			0.8800	0.4213	0.0812	0.0006	< 0.0001
Emulsion			< 0.0001	0.0002	< 0.0001	< 0.0001	< 0.0001
Matrix x emulsion			0.2405	0.2714	0.0140	0.0006	< 0.0001
Levels			Post-hoc LSD multiple comparison test ⁺				
Matrix	Emulsion	Interaction	Emulsion	Emulsion	Emulsion	Interaction	Interaction
A: CNC [*]	A: Tween 80 only	CNCA	44.8±4.1 ^a	64.9±5.8 ^a	-17.6±0.4 ^b	33.7±2.1 ^b	0.364±0.015 ª
B: CNF ^{**}	B: Tween 80 with OA ⁺⁺	CNCB	36.8±3.9 ^b	42.8±5.0 ^b	-8.1±1.0 ª	27.0 ± 1.5 ^{cd}	0.268±0.016 ^b
	C: SEFA ⁺⁺⁺ with OA	CNCC	31.2±3.9 °	40.1±6.6 ^b	-6.2±1.6 ^a	26.0 ± 0.8 ^d	0.156±0.008 °
		CNFA			-25.6±4.7 °	40.6±1.0 ^a	0.107 ± 0.016^{d}
		CNFB			-6.8±2.0 ^a	28.6±0.3 °	0.153±0.003 °
		CNFC			-6.1±2.6 ^a	$25.4{\pm}0.7^{\text{ d}}$	0.031±0.001 e

* CNC was made of 2% chitosan with 0.2% cellulose nanocrystal (CNC) and 0.4% glycerol.

** CNF was made of 0.3% cellulose nanofiber (CNF).

⁺ *Post-hoc* LSD multiple comparison test was only conducted for the factor showing the significant (P<0.05) effect based upon ANOVA results.

⁺⁺OA indicates oleic acid.

⁺⁺⁺ SEFA indicates sucrose ester of fatty acid.

CHAPTER 6

CHARACTERIZATION OF INTRINSIC PROPERTIES OF DIFFERENT CELLULOSE NANOFIBERS AND THEIR PERFORMANCE IN OIL-IN-WATER EMULSION COATING ON POSTHARVEST BANANAS (*MUSA ACUMINATE*)

ABSTRACT

The intrinsic properties of different cellulose nanofibers (CNF1: fine grade with CaCO₃; CNF2: coarse grade without CaCO₃; CNF3: fine grade without CaCO₃) were evaluated in respect to their physical, morphological and structural aspects. Each CNF was individually incorporated to the previously developed emulsion coating formulation (0.3% CNF/1% oleic acid/1% sucrose ester fatty acid), followed by the evaluation of coating properties through the same aspects and performance of each coating on post-harvest bananas stored under ambient conditions (20 ± 2 °C and $50 \pm 5\%$ RH). CNF3 exhibited lower effective diameter (43 nm) and polydispersity (0.380), more negative surface charges (-21.94 mV), and better distributed fibrillar structure compared to those of CNF0 (containing 2.95% solid without CaCO₃, used as a standard for comparison), CNF1 and CNF2. Once incorporated to emulsion coatings, CNF3 incorporated formulation (CF-CNF3) consistently presented low particle size (121 nm) and polydispersity (0.376), indicating the formation of stable and homogeneous emulsion coating. Bananas coated with CF-CNF3 showed more uniform coverage on the fruit skin together with similar chlorophyll content, texture and peel brown spotting incidence to those coated with CNF0 incorporated formulation. This study demonstrated that the finegraded CNF without addition of CaCO₃ lead to expected coating performance for improving the storability of postharvest bananas, owning to its low effective diameter, high surface charge, well-distributed fibrills and compatibility with developed emulsion system.

KEY WORDS

Intrinsic properties, different cellulose nanofibers, coating performance, storability,

postharvest bananas

INTRODUCTION

Cellulose nanofiber (CNF) possessing superior mechanical, barrier and thermal properties has been applied as either reinforcing phase or matrix in food packaging, as well as incorporated in fruit coating in the past few years (Azeredo et al., 2017; Deng et al., 2017a; Jung et al., 2016). Previous study in our lab demonstrated that CNF incorporated emulsion coating with 0.3% CNF, 1% oleic acid (OA) and 1% sucrose ester fatty acid (SEFA) significantly improved the storability of postharvest bananas under ambient conditions. The developed coating showed hydrophobic material property, superior gas and moisture barrier, and good adhesion onto the fruit skin, thus delaying physiological alternation and quality deterioration of postharvest bananas (Deng et al., 2017b).

CNF can be manufactured from different raw materials (e.g. wood pulp, cotton fibers, straw, hemp and algae) and/or using different production methods (e.g. microfluidization, microgrinding, high pressure homogenization, and high-intensity ultrasonication) through a top-down method including pretreatment and refinement steps to fragment cellulosic structures through strong mechanical shear (Abdul Khalil et al., 2016; Vilarinho et al., 2018). Different raw materials and manufacturing methods could lead to different intrinsic properties of CNFs, including shape, size, surface charge, morphology, crystallinity and dispersion (Mishra et al., 2018; Vilarinho et al., 2018). Previous studies found that CNFs with different intrinsic properties could have varied compatibility with the emulsion system (Moon et al., 2016; Vilarinho et al., 2018), which in turn might

impact the performance of developed coatings. Hence, it was hypothesized that CNFs processed from different raw materials and/or production methods with addition of functional minerals may possess varied compatibility with the developed OA-SEFA oil-in-water emulsion system, thus leading to diverse coating performance on coated fruit.

The objectives of this study were 1) to evaluate the intrinsic properties of different CNFs and their incorporated emulsion coating suspensions, and 2) to validate the effectiveness of different CNFs incorporated emulsion coatings and identify the best performed CNF for improving storability of bananas under ambient storage. The results generated from this study could provide guideline to select CNF with ideal intrinsic properties for acquiring the good compatibility with OA-SEFA emulsion system that leads to the optimal coating performance on postharvest bananas.

MATERIALS AND METHOD

Materials

Three types of CNFs were provided with potential different physical properties and chemical compositions due to varied manufacturing methods and addition of mineral content (Calcium Carbonate (CaCO₃)). CNF1 was in a fine-graded slurry form with 5.80% CNF solid and addition of CaCO₃ (CNF1:CaCO₃=1:1, w/w in the dry based). CNF2 and CNF3, both in the slurry form without mineral incorporation, consisted of coarse-graded 5.00% and fine-graded 2.80% CNF (w/w), respectively. Noted that fine and coarse grade of CNFs were determined by the degree of mechanical shearing during

their production at higher and lower levels, respectively. CNF, derived from softwood Kraft pulp in aqueous slurry form with 2.95% (w/w) solids and produced by the Process Development Center in University of Maine (ME, USA), was used as a positive control and referred as CNF0. Oleic acid (OA) and sucrose ester fatty acid (SEFA) were obtained from Alfa Aesar (MA, USA) and TCI American (OR, USA), respectively. All chemicals were reagent grade. Organic Cavendish bananas (Piura, Peru) at the ripeness stage of green with trace of yellow were purchased from a local supermarket (Corvallis, OR) right after they arrived in store, and subjected to coating treatments on the same day.

Characterization of different CNFs and derived coating formulations

Particle size, polydispersity, and zeta-potential (ZP) of four different CNFs (0.003%, w/w wet basis) and their incorporated emulsion coating suspensions were analyzed by a phase analysis light scattering (DLS) zeta potential analyzer (NanoBrook ZetaPALS, Brookhaven Instrument Corporation, Holtsville, NY) at a 90° scattering angle (Deng et al., 2018). 0.003% (w/w) concentration CNFs was applied to characterize the CNF physical and morphological properties for ensuring the sufficient dispersion of cellulose fibrils in the aqueous system.

For evaluating the morphology, CNFs were diluted to 0.003% (w/w wet basis), homogenized at 8,000 rpm for 2 min (PT10-35, Polytron, Luzernerstrasse, Switzerland) and degassed through a self-assembled vacuum system. A 200 μ l of 0.3% CNF suspensions was dropped on aluminum stubs, dried under ambient conditions (20 ± 2 °C and $50 \pm 5\%$ RH) for 48 h and coated with gold palladium alloy sputter coater (Cressington Scientific Instruments Ltd., UK) before taking the measurement to improve

the interface conductivity. Digital images at different magnifications were collected at an accelerating voltage of 5 kV (Deng et al., 2017b). The liberated CNF diameter to indicate effective diameter of cellulosic bundles was obtained by measuring at least five individual values illustrated from the SEM images (Chen et al., 2013).

Fourier-transform infrared spectroscopy (FTIR) spectra of both 0.3% CNF suspensions and derived CNF incorporated emulsion coating formulations (see following section on the preparation of coating formulations) were analyzed by Nexus 470 FTIR (Nicolet iS50 FT-IR, Thermo Scientific, WI, USA) equipped with attenuated total reflection (ATR) using diamond crystal with ZnSe focusing element (Nicolet Smart Golden Gate, Specac Ltd, UK). Drips of suspension were transferred through Pasteur pipette by covering the crystal on ATR-FTIR and the absorbance between 1,000 and 4,000 cm⁻¹ at a resolution of 4 cm⁻¹ with accumulation of 32 scans was monitored (Jung et al., 2014).

Preparation and application of CNF incorporated emulsion coating formulation

CNF incorporated emulsion coating formulations were prepared following the procedures described in Deng et al. (2017b) based on wet base (w/w). A 1% SEFA was dispersed in DI water at 70 °C to enhance solubility. Emulsion systems were formed by slowly adding 1% OA to prepared 1% SEFA aqueous suspension and homogenized at

8,000 rpm for 2 min (PT10-35, Polytron, Luzernerstrasse, Switzerland). A 0.3% of different CNFs (CNF0, CNF1, CNF2 and CNF3) were incorporated into the prepared emulsions and homogenized at 8,000 rpm for another 2 min to obtain the final coating suspensions, referred as CF-CNF0, CF-CNF1, CF-CNF2 and CF-CNF3.

For the coating application on bananas, brushing coating method was chosen to achieve uniform coating on the banana surfaces. Freshly prepared coating suspensions were manually brushed onto the banana surface using a painting brush (width: 25 mm) to ensure the full coverage of coating, and the coated fruit were air-dried for 1 h, and then stored for 10 days under the ambient conditions (20 ± 2 °C and $50 \pm 5\%$ RH) and the florescent light without packaging.

Evaluation of CNFs incorporated emulsion coating performance on fresh bananas stored under ambient conditions

Fruit overall appearance, chlorophyll degradation (CD), weight loss (WL), firmness and total soluble solid content (TSS) content were evaluated during 10 days of ambient storage to investigate the varied coating performance between CF-CNF1, CF-CNF2 and CF-CNF3 in comparison with CF-CNF0.

Fruit overall appearance was presented by taking photos of bananas with different treatments after 3, 5 and 10 days of storage. CD was determined using a Delta absorbance (DA) meter (Sinteleia, Bolonga, Italy) and reported as the reduced amount of chlorophyll content after 10 days divided by the initial value. WL was calculated based on the weight alteration divided by the initial weight of fruit (Wang and Sugar, 2015). Fruit firmness was determined through maximum compression using a texture analyzer (TA-XT2, Texture Technologies Corp., NY, USA) equipped with a P/6 stainless cylinder probe having 7 mm depth penetration into fruit skin at a speed of 10 mm/s (Deng et al., 2017a). For measuring TSS, 40 g of banana flesh was blended (Proctor Silex, NACCO Industry Inc., VA, USA) with 160 mL of DI water. Mixed suspensions were filtered through filter paper at the pore size of 2.5 μ m (Whatman, GE Healthcare Bio-Sciences, PA, USA). TSS values of fruit was obtained by evaluating the filtrate with a refractometer (RA250-HE, KEM, Tokyo, Japan) and reported by multiplying the dilution factors to obtain the original value of the flesh (Maqbool et al., 2011). All mean values and standard deviations were reported in triplicates (n=3).

Experimental design and statistical analysis

All experiments were conducted in triplicates. A completely randomized design was applied with a single treatment factor (different types of CNFs). The treatments included uncoated fruit as a control (CTRL), and different types of CNFs incorporated emulsion coating treatments (CF-CNF0, CF-CNF1, CF-CNF2 and CF-CNF3) on banana fruit. A *post hoc* least significant difference (LSD) was applied by using statistical software (SAS v 9.2, The SAS Institute, Cary, NC). Results were determined to be significantly different at P < 0.05 through one-way ANOVA.

RESULTS AND DISCUSSION

Intrinsic properties of CNFs

Table 6.1 reported physical properties, including particle size, polydispersity and zeta-potential of CNF aqueous suspensions and their incorporated emulsion coatings. Although diluted to low concentrations, all CNFs had detected particle size higher than 1,000 nm, probably due to the strong tendency of self-association between surface hydroxyl groups inducing agglomeration (Dufresne, 2013). It was demonstrated that particle size obtained from DLS were only reliable parameter for particle size determination when nanomaterials were spherical, in dispersed form, and with narrow distribution (Pabisch et al., 2012). As highly entangled long-chain nanofibrils with the presence of both amorphous and crystalline regions resulting less narrow distribution, size and shape of liberated CNFs might be better evaluated through direct SEM images later in Fig. 6.1 compared to the particle size values above. For polydispersity, two finegraded CNFs (CNF1 and CNF3) presented lower values (0.390 and 0.380, respectively) than that of the coarse-graded CNF2 (0.466), probably due to the further mechanical shearing of CNF1 and CNF3 to a finer grade through their production. For ZP, CNF3 obtained the highest negative charge (-21.94 mV) compared to other CNFs (-18.27 to -13.37 mV) showing its optimal dispersion due to the repulsion between highly charged cellulosic chains in the aqueous system (Lin et al., 2015), which might in turn act as better carrier and induce better compatibility with the emulsion system. CNF2 had the lowest ZP value (-13.37 mV) probably because of its lower surface area derived from weaker mechanical shearing. CNF1 also exhibited significantly lower ZP value than that of CNF3 due to the dissociation of the Ca²⁺ from incorporated CaCO₃ in the aqueous suspension, leading to the screening of the negatively charged carboxylate groups through cation-carboxylate interactions (Dong et al., 2013). Therefore, CNF3 possessing lower polydispersity and higher ZP may result in better compatibility for further incorporation compared to those of CNF1 and CNF2.

SEM image and effective diameter of liberated CNF bundles in Fig. 6.1 were applied to investigate the morphology of different CNFs. Noted that the magnifications were varied for different CNFs for better illustrating their morphology due to their significantly different size and shape. Consistent with the trend in the physical properties reported in Table 6.1, CNF3 exhibited a more completely-distributed entangled fibrillar structure based on the observation of its SEM image. This was probably because the application of strong mechanical shear for achieving fine-graded CNF3 further tore apart the individual cellulose fibers more completely without impairing the entanglements during the production (Vilarinho et al., 2018). CNF1 exhibited some aggregations and less entangled fibrils due to the added CaCO₃ probably owing to the low water solubility of CaCO₃ and ionic crosslinking between Ca²⁺ and carboxylate groups (Chahal et al., 2015). CNF2 presented coarser nanofibrillar structure due to the application of weaker mechanical shearing during manufacturing resulting in less breakdown of the amorphous regions in cellulosic chains (Mishra et al., 2018). For the effective diameters derived from SEM images which better illustrated the size and shape of CNFs, coarse-graded CNF2 consistently possessed significantly higher value than other CNFs. Although no

significant difference among CNF0, CNF1 and CNF3, CNF3 (43 nm) had slightly lower value of effective diameter than those of CNF0 and CNF1 (69 and 83 nm, respectively), probably resulted from stronger mechanical shearing during production and absent interference of additional mineral compounds, which might in turns lead to a significantly enhanced compatibility for further incorporation (Moon et al., 2011). Therefore, the morphology of CNFs illustrated that CNF3 possessed well-distributed highly entangled fibrillar matrix as well as the lowest effective diameter, which may act as an optimal carrier for the incorporation of the emulsion system.

FTIR analysis is conducted to investigate the structural properties of CNFs and their incorporated emulsion coating suspensions (Fig. 6.2). For CNFs (Fig. 6.2A), all CNFs presented typical bands for O-H stretching from the hydrogen bonds between hydroxyl groups and C=O stretching for cellulose and residue of hemicellulose at around 3,400 cm⁻¹ and 1,646-1,607 cm⁻¹, respectively (Jonoobi et al., 2011). The addition of CaCO₃ in CNF1 led to the intense bands at 1,435 cm⁻¹ due to the fundamental vibrational mode (v₄) of carbonite ions in bulk calcite (Al-Hosney and Grassian, 2005). Probably derived from the similar original source, no other distinct peak was observed based on FTIR spectrum of CNFs. Therefore, characterization of intrinsic properties of CNFs indicated that CNF3 might be optimal carrier for developed OA-SEFA emulsion system due to its welldistributed highly-entangled fibrillar structure.

Properties of different CNFs incorporated emulsion coating suspensions

As shown in Table 6.1, once CNFs incorporated to developed emulsion system, particle size determined the size of formed emulsion droplets. CF-CNF3 came up with the smallest particle size (121 nm) due to its further separation of fibrils, indicating the formation of the most stable emulsion droplets among all. This was consistent with the most uniform dispersion of CF-CNF3 suspension observed during the preparation of coating suspensions, which could in turns lead to more uniform coverage and prolonged functionality of coating performance when applied on banana. For polydispersity, the trend was consistent with the individual CNFs that CF-CNF2 had highest polydispersity (0.381) among CNFs incorporated coatings, due to the less homogeneous dispersion of developed emulsion resulting from coarse-graded CNF2, which could potentially lead to poor coating uniformity compared to other three coating formulations. Regarding to ZP, only CF-CNF1 switched to a positive charges (6.66 mV) while the others remained negative with no significant difference among each other. In CF-CNF1, the presence of Ca²⁺ cations could ionically cross-link with the highly negatively charged CNF surface through electrostatic interactions, in turns hindered the interactive compatibility between CNF and developed emulsion system (Dong et al., 2013; Zander et al., 2014). This might cause less efficient incorporation of the emulsion system to CNF1 based coating matrix, leading to impaired delivery of OA-SEFA emulsion from CF-CNF1 in comparison with other formulations. Hence, results from the physical properties indicated the advantageous compatibility of CNF3 with the developed emulsion system based on the formation of more stable emulsion droplets at lower particle size, more homogeneous and uniform suspension explained by the lower polydispersity, and more efficiently incorporation of the emulsion system due to the lower ZP, compared to those of CNF1 and CNF2.

FTIR spectrum (Fig. 6.2B) show new peaks in CF-CNF suspensions in comparison with CNFs only at 2,925 and 2,854 cm⁻¹ referred to the fundamental vibrations (v_a and v_s) of -CH₂ and -CH₃, illustrating the successful incorporation of OA for all coating formulations (Lee and Condrate, 1999). CF-CNF1 presented unique absorption bands at the wavelength of 3,400, 1,730, 1,506 and 1,435 cm⁻¹. At 3,400 cm⁻¹, CF-CNF1 obtained the smallest O-H stretching band among all, probably because the incorporation of CaCO₃ impaired the water hold capacity of CNF matrix due to the hindered surface hydroxyl groups on the cellulose backbones (Rong et al., 2006). Hindrance of the hydrophilic CNF surface could result in low compatibility between CNF1 and developed emulsion system, which in turns might impair the integrity and stability of coating as well as the efficiency of carrying the developed emulsion droplets. At 1,730 cm⁻¹, the stretching band of carbonyl groups was weakened due to combination bands by the incorporation of CaCO₃ (Al-Hosney and Grassian, 2005). At 1,435 cm⁻¹, CaCO₃ presented its typical peak leading to elevated absorption band of CF-CNF1 at 1,506 cm⁻¹ due to the combination with C=C stretching. These results including physical, morphological and structural properties of CNFs and their incorporated coating suspensions showed that CF-CNF3, with the formation of stable and well incorporated emulsion system and homogeneous coating, might lead to the best coating performance

on postharvest fruit.

Performance of CNFs incorporated emulsion coatings on bananas

The effectiveness of different CNFs incorporated coatings was evaluated by comparing with previously developed CNF0 incorporated emulsion coating. Overall appearance, as one of the major attractions to influence consumer acceptance, was recorded after 3, 5 and 10 days of storage (Fig. 6.3). After 3-day storage at green-yellow life of bananas, all coated fruit remained greener skin color compared to the uncoated ones, indicating the delayed ripening of fruit due to barrier properties of CNF based emulsion coatings and good adhesion on fruit skin (Deng et al., 2017b). Among coating treatments, CF-CNF3 coated fruit retained the most greenness and the effectiveness was even slightly better than that of CF-CNF0 coated one. After 5 days, uncoated fruit started to produce senescent peel spotting due to the production of dark brown melanins, while coated fruit, especially CF-CNF0 and CF-CNF3 coated ones, were absent from major peel brown spots (Beltrán-García et al., 2014). After 10 days, all coated fruit reached normal ripened status and both CF-CNF0 and CF-CNF3 coated fruit consistently showed significantly less severe brown spot incidence than those of other treatments. The above distinct results from CF-CNF3 might be attributed to the low effective diameters, polydispersity, high surface negative charges and well-distributed fibrillar structure of CNF3. These intrinsic properties of CNF3 provided improved gas barrier as matrix and acted as compatible carrier of the hydrophobic and antioxidant OA. As a result, CF-CNF3 created a modified atmosphere within fruit and hindered the formation of brown pigments from free phenolics in the peel at the beginning of the cascade (Choehom et al., 2004). Moreover, more uniform coverage with better adhesion on the fruit skin and minimal residue of greasy white substances after coating application (picture not shown) were observed from CF-CNF3 coated bananas compared to that of CF-CNF0 coated ones. This result was probably due to the more completely fragmented fibrils and absence of CaCO₃ (as white colorant) of CNF3. Meanwhile, it was noted that CF-CNF1 and CF-CNF2 coated fruit were not as marketable as that of CF-CNF3 coated ones, probably due to the impaired hydrophilic CNF surface through crosslinking between Ca²⁺ and carboxylate groups leading to low compatibility between CNF1 and emulsion system, and less homogeneous distribution of emulsion system in CNF2 matrix resulting in limited barrier properties, respectively.

Consistent to the overall appearance, banana coated with both CF-CNF0 and CF-CNF3 presented significantly lower chlorophyll degradation ratio (63.0% and 64.0%, respectively) compared to fruit applied with other coating treatments after 10 days (Fig. 6.3), indicating delayed ripening and prolonged storability. The retained color may attribute to improved oxygen barrier of coatings, resulting in postponed chlorophyllase activity on chlorophyll (Yamauchi et al., 2008). The firmness of CF-CNF0, CF-CNF1 and CF-CNF3 coated fruit after 10 days storage remained at around 10 N while those of CTRL and CF-CNF2 fruit were less than 8 N (Fig. 6.3). This was probably because finegraded CNFs (CNF0, CNF1 and CNF3) with significantly lower effective diameters acted as more compatible coating forming matrix and carrier of the emulsion system, resulting in a better moisture and gas barrier of their incorporated emulsion coatings compared to that of CNF2. Therefore, applied coatings with enhanced barrier properties further slowed down the ripening and quality deterioration of banana, leading to less hydrolysis of pectin and in turns destruction of cell wall structure (Seymour, 1993). No significant coating effect was found in weight loss and total soluble solid probably due to the high variations among individual fruit. Hence, the incorporation of CNF3 to emulsion coating provided best coating performance among three tested CNFs in respect to better extension of green-yellow life, more uniform coating coverage and absence of white substances on fruit surface, together with similar retention of chlorophyll content and fruit firmness to those of CNF0.

CONCLUSION

CNFs derived from different production methods with/without addition of mineral may lead to their varied intrinsic properties. This study demonstrated that different CNFs incorporated emulsion coatings could exhibit diverse performance when coating bananas for improving their postharvest storability under the ambient conditions, due to varied intrinsic properties and compatibility between CNF and developed emulsion system. The fine-graded CNF without CaCO₃ (CNF3) exhibited low effective diameter and polydispersity, high surface charges, and well-distributed fibrillar matrix structure. CNF3 incorporated emulsion coating achieved imporved barrier properties by the formation of

stable and homogeneous emulsion system within CNF matrix, leading to better extension of green-yellow life, more uniform coverage, and absence of greasy white substances on coated fruit skin. CNF3 incorporated emulsion coating performed competitively with that of the standard CNF in respect to retaining chlorophyll content and firmness, and postponing the development of brown spots on fruit surface during yellow-brown stage. Therefore, CNF, with low effective diameter and polydispersity, high surface charges, and well-distributed highly entangled fibrillar matrix, should be selected for acquiring the compatibility with OA-SEFA emulsion for improving postharvest storability of bananas.

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Fig. 6.1 Scanning electron miscroscopy (SEM) images and librated diamters of cellulose nanofiber (CNF) derived from 0.003% CNF suspensions including CNF0 (standard CNF), CNF1 (fine-grade CNF with CaCO₃), CNF2 (coarse-grade CNF without CaCO₃), and CNF3 (fine-grade CNF without CaCO₃).


Fig. 6.2 Fourier-transform infrared spectroscopy (FTIR) of cellulose nanofiber (CNF) suspensions [A] and derived coating formulations [B]

* CNF0: standard CNF; CNF1: fine-grade CNF with CaCO₃; CNF2: coarse-grade CNF without CaCO₃; CNF3: fine-grade CNF without CaCO₃.

** CF-CNF0, CF-CNF1, CF-CNF2, and CF-CNF3 refer to 0.3% concentration of CNFs incorporated coating formulations with 1% oleic acid and 1% sucrose ester of fatty acid.

Day of storage	CTRL	CF-CNF0*	CF-CNF1	CF-CNF2	CF-CNF3
3					
5	and		au	MU	
10	JUNE.				
CD^{**}	86 0 ^a	63.0°	74 9 ^b	79 2 ^{ab}	64 0 °
(%)	00.0	02.0	7 11 9	17.2	0110
WL (%)	18.0 ^a	18.1 ^a	17.3 ^a	17.9 ^a	17.2 ^a
Firmness (N)	7.5 °	10.3 ^a	10.2 ^{ab}	7.9 ^{bc}	9.9 ^{ab}

Fig. 6.3 Comparisons of overall appearance, chlorophyll degradation (CD), weight loss (WL), firmness and total soluble solid (TSS) content among uncoated and different types of cellulose nanofibers (CNFs) coated bananas under ambient storage⁺

20.3 ^a

20.3 ^a

⁺Except appearance, all quality parameters were collected at 10 days of storage.

19.8^a

TSS (°)

20.3 ^a

* CF-CNF0, CF-CNF1, CF-CNF2 and CF-CNF3 referred to fruit coated by 0.3% concentration of CNFs incorporated coating formulations with 1% oleic acid and 1% sucrose ester of fatty acid. CTRL: uncoated fruit.

^{**} CD: chlorophyll degradation ratio of the banana skin; WL: weight loss ratio; TSS: total soluble solid of the peeled banana flesh.

19.9^a

	8		
	Particle size (nm)	Polydispersity	Zeta-potential (ZP, mV)
CNF0*	3309 ^a	0.321 ^c	-18.27 ^b
CNF1	1009 ^d	0.390 ^b	-16.94 ^b
CNF2	1541 ^c	0.466 ^a	-13.37 ^a
CNF3	2495 ^b	0.380 ^b	-21.94 °
CF-CNF0**	169 ^b	0.373 ^b	-5.38 ^b
CF-CNF1	199 ^a	0.374 ^b	6.66 ^a
CF-CNF2	136 °	0.381 ^a	-8.92 ^b
CF-CNF3	121 ^d	0.376 ^b	-3.01 ^b

Table 6.1 Particle size, polydispersity and zeta-potential of cellulose nanofiber (CNF) suspensions and derived coating formulations

* A 0.003% suspension of CNFs. CNF0: standard CNF; CNF1: fine-grade CNF with CaCO₃; CNF2: coarse-grade CNF without CaCO₃; CNF3: fine-grade CNF without CaCO₃.

** CF-CNF0, CF-CNF1, CF-CNF2 and CF-CNF3 refer to 0.3% concentration of above CNFs incorporated coating formulations with 1% oleic acid and 1% sucrose ester of fatty acid.

CHAPTER 7

OVERALL CONCLUSIONS

Cellulose nanomaterials (CNs), including cellulose nanofibers (CNFs) and cellulose nanocrystals (CNCs), have unique intrinsic properties, such as low density, high aspect ratio, superior gas and moisture barrier, high mechanical property, and thermal stability. They are highly compatible with other polysaccharides, hence can be incorporated to biocomposite coatings as matrix, carrier of emulsion droplets, reinforcing agent, and/or Pickering emulsifier to improve barrier and adhesion properties of fruit coatings.

Through four specific projects, this dissertation research demonstrated the feasibility and success of CNs incorporated biocomposite coatings for improving the storability of two major climacteric fruit, pears and bananas under varied storage conditions.

For postharvest pears (*Pyrus communis*), CNC reinforced chitosan (CH) coating (CH-CNC) and CNC Pickering emulsion incorporated CH coating (CH-PCNC) were developed and validated to improve fruit storability under ambient and cold storage conditions, respectively. CNC as reinforcement to CH matrix enhanced the moisture and gas barrier of CH coatings, which in turn created modified atmosphere within fruit to retain quality of pears under ambient conditions. However, the effectiveness of CH-CNC coating under the cold storage conditions (low temperature and high RH) was weakened due to the presence of hydrophilic components, such as CH and Tween 80, and the absence of hydrophobic compounds. Hence, CNC was utilized as a Pickering emulsifier to replace conventional emulsifiers (e.g. Tween 80) for the incorporation of oleic acid (OA) to further improve hydrophobicity and stability of coating formulation against the high RH cold storage conditions. CH-PCNC coating showed improved hydrophobicity and stability against high RH for coated pears during the long-term cold storage.

Meanwhile, cellulose nanofiber (CNF) based emulsion coating (CNF/OA/sucrose ester fatty acid (SEFA)) was developed to improve storability of bananas (Musa acuminate) under ambient conditions. The application of CNF was based on its flexible matrix structure, superior gas barrier properties, and especially compatibility with banana skin, resulting in enhanced interactions between nanofibrillar CNF and banana peels. The non-covalent adsorption of well-dispersed and adsorbed OA-SEFA emulsion system into the continuous CNF phase provided CNF based emulsion coating with superior gas and moisture barrier, and good adhesion onto the banana skin. The coating resulted in reduced ethylene production and respiration rate of fruit through regulating the ethylene biosynthesis pathway. Moreover, CNFs with varied intrinsic properties (particle size, effective diameter and dispersion derived from different raw materials and/or production methods) exhibited different barrier properties, adhesion as well as compatibility with developed emulsion system, thus impacting coating performance once incorporated to CNF based emulsion coating.

To fully understand the mechanisms and principle of the roles and functionalities of CNs and their incorporated coatings, a systematic approach was implemented to develop and validate CNs incorporated coatings for postharvest fresh fruit by considering 1) fruit physiological characteristics, 2) type of coating matrix and incorporated other functional substances, 3) adhesive interactions between coating and fruit surface, and 4) storage conditions. It is expected that CNs incorporation will lead to a new era for edible coating technology to preserve postharvest fruit, and the suggested research approach can be utilized for developing new coating technologies for various fruit with specific technical challenges in the future.

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APPENDIX

DEVELOPMENT, CHARATERIZATION, AND VALIDATION OF CHITOSAN ADSORBED CNF FILMS AS WATER RESISTANT AND ANTIBACTERIAL FOOD CONTACT PACKAGING

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ABSTRACT

Compatibility of CNF with three polysaccharides having different surface charges and backbones (chitosan, methyl cellulose, and carboxymethyl cellulose) was investigated. Chitosan (CH) incorporation reduced water absorption (WA) of CNF films (P < 0.05). CH molecular weight (Mw) (68, 181, 287 kDa) and amount (10 and 20g/100g CNF in dry basis) impacted moisture barrier, mechanical, antibacterial, thermal, and structural properties of CNF films. Regardless of Mw, CH incorporation (20g/100g CNF) decreased (P<0.05) WA of CNF films, and high Mw (287 kDa) CH (20g/100g CNF) incorporation resulted in lower film water solubility while increasing film water vapor permeability compared with low Mw CH (68 kDa) incorporation (P<0.05). CNF film with low Mw CH (20g/100g CNF) exhibited antibacterial activity against L. innocua and E. coli. Interaction mechanisms between CH and CNF were investigated through thermal, structural, and morphology analyses using DSC, FTIR, and SEM, respectively. CNF films with low or high Mw CH incorporation (20g/100g CNF) were further validated as surface contact films for fresh beef patties, showing effectiveness to prevent moisture transfer between the layered patties. This study demonstrated the potential of using CNF-CH composite films as water resistant and antibacterial packaging for foods with high moisture surfaces.

KEY WORDS

Cellulose nanofiber, chitosan, food contact packaging, water resistance, antibacterial activity

INTRODUCTION

Cellulose nanofiber (CNF) forms films with superior mechanical and gas barrier properties because of its nano-sized dimension, high aspect ratio, surface area, and flexibility (Azeredo, 2009; H.P.S et al., 2016). However, due to the hydrophilic surface property of CNF, those functional properties of the CNF films may be suppressed by direct moisture contact and/or exposure to high relative humidity (RH) environment (Liu et al., 2011) (McHUGH et al., 1993), thus limiting their application for packaging food products with wet surface, high moisture content, and/or stored at high RH environment. Incorporation of inorganic fillers (i.e. silver), chemical modification (i.e. plasma polymerization or derivatives), and adsorption of other film matrix materials (i.e. xyloglucan or guar gum) have been studied to enhance the water-resistance of CNF films (Eronen et al., 2011; Hernández-Hernández et al., 2010; Lavoine et al., 2014)2011). Among these methods, the adsorption of polymeric materials onto CNF surface avoids the use of strong or harmful chemicals, and is simple, safe and efficient, hence was investigated in this study to develop water resistant packaging films for food with wet and adhesive surfaces.

Polysaccharides are classified based on their surface charges and backbones. Both carboxymethyl cellulose (CMC) and methyl cellulose (MC) are composed of glucose monomers, but contain negatively-charged carboxymethyl (-CH₂COOH) and non-charged relatively hydrophobic methoxyl (-OCH₃) functional groups, respectively. Chitosan (CH) is composed of β -(1-4)-linked D-glucosamine and *N*-acetyl-D-glucosamine with positively-charged amino (-NH₂) groups in acidic solution. The chemical interactions (e.g. hydrogen bonds or electrostatic interactions) and/or physical

interactions (e.g. adsorption) of these polysaccharides onto CNF surface vary depending on their functional groups, surface charges, molecular weights (Mw), concentrations, and conformation (Lin and Dufresne, 2014). This study thus selected three types of polysaccharides (CH, MC, and CMC) for understanding their affinities onto CNF surface with different surface charges and backbones and possible impact on the film waterresistant property.

Our previous studies proved that the abundance and availability of the functional amino groups and spatial entanglement of CH vary depending on molecular weight (Mw) of CH (Jung and Zhao, 2012; 2013; Chen and Zhao, 2012). Hence, CH incorporation at different Mw and concentrations into CNF film was tested in this study based on the hypothesis that the affinity of CH with CNF depends on Mw and concentration of CH due to different abundance and availability of the functional amino groups, spatial entanglement and crystallinity of CH, which in turn could impact the physicochemical, mechanical, and antibacterial properties of CNF films. The derived CNF-based films were expected to have sufficient water-resistant and antibacterial properties that can be applied as food contact packaging film to interleave food products with high moist (e.g. meat pieces or patties) surface, thus preventing moisture transfer between layered products.

Therefore, the objectives of this study were to first identify the mostly compatible polysaccharide with CNF to develop water-resistant CNF films and then to validate their applications for contacting food items with wet surfaces and stored under high RH refrigerated temperature. The former objective was achieved through combined two experimental designs: (1) Taguchi design to select the mostly compatible polysaccharide (CMC, MC, or CH) with CNF and (2) completely randomized two factorial design to investigate the influences of Mw and concentration of CH on the properties of CNF films. The derived films were evaluated in physicochemical (color, thickness, haze), water-resistant (water absorption, water solubility, water vapor permeability), mechanical (tensile strength, elongation at break), thermal (differential scanning calorimetry (DSC)), structural (Fourier transform infrared spectroscopy (FT-IR), and morphological (scanning electron microscopy (SEM)) properties, as well as antibacterial activity against *Listeria* innocua (L. innocua) and Escherichia coli (E. coli). The latter objective was accomplished by applying CNF films with the best performance based upon the first part of studies to layered beef patties as the separation sheets, and evaluating water absorption of films after 1-week refrigerated storage. This study was thus anticipated to provide new insights on the strategies of incorporating functional polysaccharides into CNF for enhancing water resistance and antibacterial activity of CNF films to meet the critical needs of biodegradable antibacterial packaging films and for understanding the mechanisms of improved performance of CNF film based on Mw and concentration of incorporated CH.

MATERIALS AND METHOD

Materials

A CNF slurry (2.95 g/100 g wet basis) was obtained from the Process Development Center of the University of Maine (ME, USA). CNF was extracted from northern bleached softwood kraft pulp by using the Masuko MKZB15-50J super mass collider creating a high shear zone, thus liberating nanofibers present in natural lignocellulosic fibers with dimensions of 20-50 nm in width and up to several hundred microns in length (The Process Development Center, University of Maine, 2016). CMC (400-800 cPs), MC (400 cPs), and CH (97% degree of deacetylation, 287 kDa Mw) were purchased from Alfa Aesar (MA, USA), Sigma Chemical (MO, USA), and Premix (Iceland), respectively. Glycerol was acquired from Fisher Scientific (NJ, USA). Tween 80 and Span 80 were obtained from Amresco (OH, USA). Cellulase was gained from *Aspergillus niger* (TCI America, OR, USA). Ground beef (80% lean and 20% fat) was purchased from a local market on the day that experiment was conducted.

Chitosan depolymerization

CH was depolymerized to different Mw levels (high: 287 ± 43 kDa, med: 181 ± 18 kDa, and low: 68 ± 2 kDa) through enzymatic hydrolysis using the method from our previous study (Jung and Zhao, 2013). Briefly, high Mw CH (287 kDa) (1 g/100 g) was dissolved in acetic acid (1 g/100 mL distilled water) and adjusted to pH 5 using 10 g/100 mL NaOH. Cellulase (10 g/100 g CH in dry basis) was added to prepared chitosan solutions, and reacted at 50 °C for 5 min or 1.5 h to obtain med or low Mw CH, respectively. The hydrolyzates were boiled for 10 min to inactivate cellulase, and centrifuged at 8,500 x g for 30 min to remove denatured enzyme. Supernatant was then adjusted to pH 9 by NaOH (10 g/100 g in distilled water), and the precipitated portion was washed and collected by centrifugation at 8,500 x g for 30 min. Collected samples were dried at a 40 °C oven overnight. The viscosity-average Mw of CH (0.01 g/100 g 0.1 M CH₃COOH and 0.2 M NaCl) was measured by the Ubbelohde dilution viscometer (Cannon Instrument Co., PA, USA) with a capillary size of 0.58 nm. The intrinsic

viscosity was determined by the intercept between the Huggins (reduced viscosity) and Kraemer (relative viscosity) plots. The viscosity-average Mw of CH was calculated using Mark–Houwink–Sakurada (MHS) equation: $[\eta] = K (M_w)^a$, where $K = 1.81 \times 10^{-3}$ mL g^{-1} , a = 0.93, and $[\eta]$ represented the intrinsic viscosity (Jung and Zhao, 2013).

Preparation of CNF-based films

Development of film formulations

Film formulations were developed using two consecutive experimental designs, Taguchi design (the first part of study) and a completely randomized two factorial design (the second part of study). For Taguchi design (Table Appx.1), CNF (0.75 g/100 g distilled water) and glycerol (10 g/100 g CNF in dry basis) were formulated with different types (CMC, MC, and CH) and concentrations (0, 15, and 30 g/100 g CNF in dry basis) of polysaccharides, avocado oil (0, 15, and 30 g/100 g CNF) in dry basis), and surfactant mixture (1:1 of Tween and Span 80, 0, 20, and 40 g/100 CNF in dry basis). Mw of CH used for this part of the study was 287 kDa. Avocado oil was chosen to increase the hydrophobicity of the films, and surfactant was selected to improve the incorporation of hydrophobic compound and decrease the surface tension of the derived films. For a completely randomized two factorial design (Table Appx.2), CNF (0.5 g/100 g distilled water) for creating thinner film and glycerol (10 g/100 g CNF in dry basis) were used along with the addition of the mostly compatible polysaccharide identified from the Taguchi design. In this study, CH was identified as the mostly effective polysaccharide, and different Mw (68, 181, and 287 kDa) and concentrations (10 and 20 g/100 g CNF in dry basis) of CH were incorporated into CNF film formulations. For each concentration of CH, prepared CH solution (3 g/100 mL acetic acid (1 g/100 g distilled water)) was diluted 60 and 30 times, respectively. In this case, the concentration of acetic acid was < 0.05 g/100 g for both concentrations of CH. It was previously reported that antibacterial activity of acetic acid starts at 0.166 g/100 mL (Fraise et al., 2013). Hence, the influence of acetate on antibacterial activity of films was negligible in this study. Preparation of films

Prepared film formulations were homogenized (Polytron PT10-35, Luzernerstrasse, Switzerland) for 5 min, and degassed using a self-build water flow vacuum system (Chen and Zhao, 2012). A 60 mL of formulation was uniformly cast onto 150 mm diameter polystyrene petri dish (Falcon, PA, USA), and dried at room conditions (20 ± 2 °C and 30 $\pm 2\%$ RH) for 2 days. Dried films were then conditioned in a self-assembled chamber (Versa, PA, USA) at 25 °C and 50% RH for 2 days before all measurements (Chen and Zhao, 2012).

Evaluation of film properties

Transmission haze and color difference (ΔE)

The transmission haze of the films was measured using a ColorQuest spectrophotometer (HunterLab, VA, USA). Film specimens were placed in front of the sensor and all extraneous light was eliminated before reading. Results were reported in percentage of haze. Color difference (ΔE) of the films was measured using a LabScan XE colorimeter (HunterLab, VA, USA) that was calibrated with a standard white plate (L* = 93.87; a* = -0.92; b* = 0.14). ΔE was calculated as

 $\Delta E^* = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2}, \text{ where } L_0^*, a_0^*, \text{ and } b_0^* \text{ represented}$

the color values of 0.5% CNF and 10% glycerol film, and L*, a* and b* referred to the color values of CNF-based films incorporating other materials. Measurements were conducted in triplicates, and mean values were reported.

Mechanical property

Film thickness was measured using a micrometer (NR 293-776-30, Mytutoyo Manufacturing Ltd., Japan) at eight randomly selected locations on each film, and mean value was reported for each replication. Elongation at break (EB, %) and tensile strength (TS, MPa) of the films were determined using a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Corp., NY, USA) according to ASTM D882 standard (ASTM, 2001) with some modifications, in which the initial grip separation and crosshead speed were set at 40 mm and 0.4 mm/s, respectively. Film piece (250 x 700 mm) was mounted on a sample grip (TA 96). TS was calculated using maximum load (N) divided by film cross-sectional area (mm²), and EB (%) was calculated as distance at break divided by the initial length of the specimen and multiplied by 100%. All data were collected in triplicates.

Water absorption ability (WA), water solubility (WS), and contact angle (CA)

Each film specimen (30 x 30 mm) was precisely weighed, and placed in a petri dish with 30 mL of distilled water. After 2 h, samples were placed on paper tissue flatwise to absorb water from the film surface, and then weighed. WA was measured as the percentage weight gain of the films after suspending in water for 2 h. WS was determined by the percentage weight loss of the films after suspending in water for 2 h and drying at 40 °C for 24 h (Zhong et al., 2012). Both WA and WS were reported as the mean value of three replications.

Hydrophobicity of the film surfaces was evaluated by measuring the contact angle (CA) of the interface between water and film. A 10 μ L of distilled water was dropped from 10 mm height to a horizontal flat film specimen (30 x 30 mm) (Hou et al., 2012), and CA value was determined using CA goniometer (FTA 32, First Ten Angstroms, Inc., VA, USA). A high CA value represented a high water resistance (hydrophobicity) of the film, and data were reported as the mean value of three replications.

Water vapor permeability (WVP)

A cup method was used to measure WVP of the films according to ASTM Standard E96-87 (ASTM, 2000). Cups and lids used for WVP measurements were preconditioned at 25 °C and 50% RH for 24 h. Each film sample (75 x 75 mm) was sealed by vacuum grease on the top of a Plexiglas test cup (57 x 15 mm) filled with 11 mL of distilled water, and the seal ring was tightly closed by rubber bands. Test cup assemblies were stored in a temperature and humidity control chamber (T10RS 1.5, Hyland Scientific, WA, USA) at 25 °C and 50% RH. Each cup assembly was precisely weighed hourly for up to 6 h. Means values of three replications were reported.

Thermal property

Differential scanning calorimetry (DSC) measurements of each film specimen were performed with DSC Q2000 (TA Instruments, New Castle, DE). Sample $(11 \pm 0.5 \text{ mg})$ was placed into the hermetic aluminum pan (T131219, TA Instruments, DE, USA) and tested from 0 to 300 °C with a heating rate of 20 °C/min under a nitrogen atmosphere at a flow rate of 50 mL/min during all measurements.

Fourier Transform Infrared spectroscopy (FTIR)

FTIR spectra of the film was determined by Nexus 470 FTIR (Nicolet iS50 FT-IR,

Thermo Scientific, WI, USA) equipped with attenuated total reflection (ATR) using diamond crystal with ZnSe focusing element (Nicolet Smart Golden Gate, Specac Ltd, UK). Film specimen (20 x 20 mm) was placed onto ATR-FTIR and the absorbance between 800 and 4,000 cm⁻¹ with accumulation of 32 scans was collected at a resolution of 4 cm⁻¹.

Morphology of films

The cross-section morphology of CNF films was investigated using SEM (FEI Quanta 600F, OR, USA). The fractured sample obtained from the mechanical measurements was used for imaging the cross-section morphology. Prepared sample was mounted on aluminum stub with the cross-section oriented up and coated by gold palladium alloy sputter coater (Cressington Scientific Instruments Ltd., UK) to improve the interface conductivity. Digital images were collected at an accelerating voltage of 5 kV with a magnification of 20 µm.

Antibacterial activity

Antibacterial activity of films was evaluated using two methods: 1) optical density measurement for quickly evaluating the number of bacteria in enriched culture broth and 2) total plate count method for confirming the data obtained from the optical density measurement. Two non-pathogenic bacterial strains, Gram-positive strain *L. innocua* (ATCC 51742, American Type Culture Collection, VA, USA) and Gram-negative strain *E. coli* (ATCC 25922, American Type Culture Collection), were cultured on brain heart infusion (BHI) agar (Becton, Dickinson and Co., NJ, USA, VA, USA) and tryptic soy agar (TSA) (Becton, Dickinson and Co., NJ, USA), respectively, and stored under 4 °C during the course of the study. Prior to a given microbiological assay, a single typical colony of two bacteria was inoculated in tubes of appropriate broth, and incubated at 37 °C for 24 h (Lab-Line Orbit shaker bath model 3527, IL, USA) with the approximately enriched culture of 10^7 CFU/mL. Two film specimens (10 x 20 mm) were immersed into test tubes with 10 mL of sterilized BHI and tryptic soy broth (TSB), and then inoculated with 100 µL of *E. coli* and *L. innocua*. For the method of optical density measurement, inoculated test tubes without film treatment were used as a negative control (Ctrln) and tubes with CNF only films were applied as positive control (Ctrl). The optical density at 600 nm (OD₆₀₀) indicating bacterial growth was measured at 0, 5, 10 and 24 h using the UV-Vis spectrophotometer (UV-1800, UV-Vis Spectrophotometer Shimadzu Corporation, Japan) to quickly evaluate the antibacterial effect of the derived

films (Zhang et al., 2016). The mean values of three replications were reported for treatments and controls.

For measuring the total plate counts of culture broth, 1 mL of sample was taken from the tested tubes at 24 h and added with 9 mL of sterilized peptone solution (0.1 g/100 g distilled water) for the 10-fold serial dilution. Then, 1 mL of sample was immediately transferred into petri dish (n=2), and plated for the enumeration. BHI agar and tryptic soy agar (TSA) were used for *L. innocua* and *E. coli* enumeration, respectively. Plates were incubated at 37 °C for 48 h, and the number of colonies was counted and reported as log₁₀ CFU/mL.

Validation study

To validate the performance of developed CNF-CH films, the films were applied as a separation sheet between multi-layered beef patties with high moist surface. A 90 x 90

mm film was precisely weighed and placed between ground beef patties (~80 mm diameter and ~150 g). Six beef patties were stacked together with total five pieces of CNF films, and tested for three stacks for both control and treatments (n=3). Samples without further packaging were stored at a 5-7 °C refrigerator. After 1-week refrigerated storage, each individual film was reweighed, and WA of film was calculated as the percentage of weight gain in comparison with the initial weight of the film. The mean values of three replications were reported for control and treatment samples.

Experimental design and statistical analysis

Taguchi design has been considered as the simple and systematic method for studying the contribution of factors on the measured parameters and for optimizing the treatment conditions with combined levels of each factor (Jung and Zhao, 2011). With nine trials (L₉), Taguchi design was applied to identify the mostly compatible polysaccharide (MC, CH, and CMC) with CNF based on low film WVP, WA and WS. Results were analyzed to investigate statistical significance via *post hoc* least significant difference (LSD) by SAS program (SAS v 9.2, The SAS Institute, USA) and considered to be significantly different at P<0.05.

A completely randomized two factorial design was then applied to the selected polysaccharide (CH in this study) to investigate the effect of CH molecular weight (CHM) and CH concentration (CHC) and their interactive effect on the film functionalities. PROC GLM was utilized to identify significant differences and interaction among each factor using the SAS program, and *post hoc* LSD was tested as the multiple comparisons. All film measurements were conducted in triplicate and results were considered to be significantly different at P < 0.05.

RESULTS AND DISCUSSION

Selection of the most compatible polysaccharide with CNF through Taguchi design

The most compatible polysaccharide with CNF was identified based on WVP, WA, and WS values of the films obtained from three respective Taguchi designs for each polysaccharide (MC, CH, and CMC) (Table Appx.1). For MC incorporation, MC concentration was the highest contributing factor impacting all WVP, WA, and WS values. Significant increase in WA and WS was observed in MC (30 g/100 g CNF) incorporation, indicating weaker affinity of MC to CNF. This might be because the presence of non-polar, hydrophobic methoxyl functional groups in MC impaired the interactions between MC and the hydrophilic CNF surface (Eronen et al., 2011).

For CH incorporation, CH concentration was also the highest contributing factor impacting all WVP, WA, and WS values (Table Appx.1). Although CH incorporation (30 g/100 g CNF) significantly increased WVP (0.045 g mm/m² d Pa) of the CNF films (P<0.05), compared to that of CNF only film (0.032 g mm/m² d Pa), WA was significantly (P<0.05) reduced in CH-incorporated CNF films. The CH incorporation at 15 g/100 g CNF decreased WS from 27.8% to 20.5% (P<0.05) of CNF films and also achieved a 3 times of reduction on WA compared with the CNF only film without significant impact on WVP. The reduced WA of CH-incorporated CNF films could be due to the partial elimination of the hydrophilic hydroxyl groups from CNF through physicochemical interactions between CH and CNF (Eronen et al., 2011; Nordgren et al., 2009). CH could be adsorbed and well-interacted with CNF owning to their similar conformation structures, and chemically interacted through the hydrogen bonding and electrostatic interaction between the slightly negative-charged CNF and positivelycharged CH as well as increasing crystallinity (Khan et al., 2012). The higher WVP of CNF film with CH incorporation could be due to increased crystallinity of film structure that could reduce moisture diffusion through film matrix (Zhong and Xia, 2008; Jahit et al., 2016). It was also noticed that the addition of different levels of avocado oil and surfactant in CNF films showed no significant difference and had less contribution compared to CH concentration. It was thus concluded that the incorporation of CH at 15 g/100 g CNF improved the water-resistant properties (WA and WS) of CNF film.

For CMC incorporation, the surfactant was the most contributing factor on WVP of the CNF films. WVP was significantly (*P*<0.05) higher in films with surfactant at 40 g/100 g CNF (0.043 g mm/m² d Pa versus 0.037 g mm/m² d Pa in film without the surfactant). WA and WS could not be analyzed since CMC-incorporated CNF films were solubilized while testing these parameters. It was probably because the slightly negativecharged CNF surface was not compatible with the negatively-charged CMC through potential electrostatic repulsions (Hatanaka et al., 2014; Ma et al., 2014), thus forming loosen matrix of films. Previous studies consistently indicated that CMC-incorporated CNF films was not as strong as both neutral MC and positively charged CH incorporation ((Abe et al., 2007; Eronen et al., 2011). This study confirmed that among 3 tested polysaccharides (CMC, MC, and CH), CH is mostly compatible with CNF in respect to improving water resistance of CNF film. The most suitable Mw and concentration of CH to be incorporated into CNF for improving moisture barrier, mechanical, antibacterial, thermal, structural, and morphological properties of CNF films were then further
identified. In addition, the mechanisms of CH incorporation at different Mw and concentrations for improving the performance of CNF film was studied.

Characterization of CH-incorporated CNF films

According to the results in ANOVA analysis (Table Appx.2 and Fig. Appx.1b), CHM (CH Mw) reported a significant (P<0.05) impact on color difference (ΔE), WS, and WVP of CNF films, whereas CHC (CH concentration) had the significant effect on color, thickness, TS, WA, and WVP of CNF films. There were significant (P<0.05) interactions between CHM and CHC on WVP. In the following sections, only those factors showing a significant effect on measured quality parameters were reported and discussed. Color difference (ΔE)

Color difference (ΔE) of med (181 kDa) and high (287 kDa) Mw of CH-incorporated CNF films was significantly (*P*<0.05) higher than that of low (68 kDa) Mw of CH-incorporated CNF films (Table Appx.2). The color change of low Mw CH was diminished, due to the degradation of carotenoid (i.e. astaxanthin) pigment while reducing Mw (Seo et al., 2007). The CH incorporation (20 g/100 g CNF) induced higher ΔE than that of CH incorporation (10 g /100 g CNF) in the films (Table Appx.2) because more carotenoid pigment appeared in high concentration of CH (Hong and Samuel, 1995). Hence, the color of CNF-CH films were affected by Mw and concentration of incorporated CH.

Mechanical property

CNF film was significantly (P<0.05) thicker with CH incorporation at 20 g/100 g CNF (0.031 mm) than that with CH incorporation at 10 g/100 g CNF (0.027 mm) (Table Appx.2), and CH incorporation at 20 g/100 g CNF decreased TS (24.9 MPa) of the CNF film in comparison with CH incorporation at 10 g/100 g CNF (29.5 MPa) (Table Appx.2). CNF with large surface area and high aspect ratio could enhance its mechanical property through physicochemical interactions with CH (Fernandes et al., 2010, 2009). However, CH incorporation at 20 g/100 g CNF could induce polymer agglomeration (Salehudin et al., 2014), thus impacting the compatibility with CNF. Therefore, the concentration of CH influenced the thickness and TS of CNF films.

Water-resistant property

All CH-incorporated CNF films showed significantly (P<0.05) higher CA in comparison with the CNF only film (Fig. Appx.1a). The adsorption of CH onto CNF led to less available hydroxyl groups of CNF, resulting in less hydrophilic nature of CNF films. For WA and WS, the CH incorporation at 20 g/100 g CNF significantly (P<0.05) reduced WA of the CNF film in comparison with CH incorporation at 10 g/100 g CNF due to the reduced available hydroxyl groups through interactions between CNF and CH. The incorporation of high Mw CH resulted in significantly (P<0.05) lower WS of the film than that of low Mw CH (Table Appx.2), also possibly due to the reduced available hydroxyl groups through interactions between CNF and CH with high Mw. Previous study also reported that CMC, MC, and CH could be irreversibly adsorbed onto the CNF matrix prepared from the kraft pulp (Eronen et al., 2011). These results were consistent with previous studies, showing physicochemical interactions between CNF and CH (Toivonene et al., 2015) and less swelling capacity of CNF films with CH incorporated compared to CNF film (Wu et al., 2014).

CHM, CHC and CHM x CHC showed to have a significant (P < 0.05) impact on WVP

(Fig. Appx.1b). At 10 g/100 g CHC, there was no significant difference among samples at different Mw; however at 20 g/100 g CHC, low Mw CH incorporation (0.036 g mm/m² d Pa) resulted in significantly (P<0.05) lower WVP than that of the high Mw CH (0.06 g mm/m² d Pa) due to the decreased moisture diffusion through film matrix. Less moisture diffusion of CNF film incorporated with low Mw CH could be due to free volume decrease, and consequently, moisture diffusion decrease through the films (Cao et al., 2007). Thus, it was concluded that low Mw CH formed tightly-packed and water-resistant CH-incorporated CNF films. The CH-incorporated CNF film with improved water-resistance could be applied to food with high moist surface.

Antibacterial property

Antibacterial property of the films was evaluated against both *L. innocua* and *E. coli* by measuring both the optical density of enriched broth at 5, 10 and 24 h and enumerating total bacterial number of broth at 24 h (Fig. Appx.2). For both *L. innocua* and *E. coli*, CNF film incorporated with low Mw CH at 20 g/100 g CNF had the lowest absorbance value after 24 h among all formulations, showing the least bacterial number in the enriched culture broth. This result was also confirmed by total bacterial counts, which was significantly reduced in CNF film with low Mw CH (5.29 log₁₀ CFU/mL) and med Mw CH (5.93 log₁₀ CFU/mL) at 20 g/100 g CNF against *L. innocua*, compared to CNF only film (6.28 log₁₀ CFU/mL). In respect to *E. coli*, bacterial count of broth added with CNF film incorporating low Mw CH at 20 g/100 g CNF was significantly (*P*<0.05) lower (5.24 log10 CFU/mL) than that with CNF only film (6.68 log₁₀ CFU/mL). These trends were most likely due to the higher availability and releasing of the functional groups (-NH₃⁺) in low Mw CH, which was more susceptible to bacteria growth,

compared to med and high Mw CH. These results and suggested hypothesis were supported by our previous studies, in which 74 kDa CH had significantly (*P*<0.05) higher suppression on *E. coli* growth in comparison with 111 kDa and 27 kDa CH (Jung and Zhao, 2013). Hence, the antibacterial activity of CNF films was enhanced by incorporation of low Mw CH at 20 g/100 g CNF. Such films may be utilized as antibacterial packaging for reducing surface contamination of foods. Structural, thermal, and morphological properties

Fig. Appx.3a shows the FTIR spectrum of CNF-based films. The strong and wide peak in the 3,500-3,300 cm⁻¹ range attributed to the hydrogen-bonded O-H stretching in both CH and CNF, and the overlapping N-H stretching from the primary amine and type II amide in CH appeared in both CNF film and CH-CNF films (Rafieian and Simonsen, 2014), indicating strong interactions between CNF and CH through hydrogen bonds. The spectral bands in the region of 1,650 cm⁻¹ (amide II carbonyl (–C=O) stretching) were more distinguished in CH-incorporated CNF films than that in CNF film, illustrating the presence of CH in CNF films. Moreover, the spectral band at 1,600 cm⁻¹ indicating –NH bending of amide I bend attributed from CH was only observed in CH-incorporated CNF films. These results indicated that CH adsorbed onto CNF through hydrogen bonds and/or electrostatic interactions support the forming of improved water-resistant CNF films (Khan et al., 2012).

Fig. Appx.3b illustrates the DSC curves along with the determined glass transition temperature (T_g) of CNF-based films. All compositions exhibited one single broad endothermic peak at slightly different positions, indicating the compatible interactions between the two polymer blends. T_g of CNF film incorporated with med (181 kDa) Mw

CH at 20 g/100 g CNF was higher (~133 °C) than that (~129 °C) of CNF only film. The shift of T_g could be the result of improved interactions between the two polymers, which led to a decreased free rotation of amorphous polymeric chains (Azizi Samir et al., 2004). T_g of CNF film incorporated with high (287 kDa) Mw CH at 20 g/100 g CNF was the lowest (112 °C) among all films. The reduced T_g might be because the incorporation of high Mw CH weakened the interactions (e.g. hydrogen bond) between CNF. However, further studies should be conducted to prove the influence of Mw CH on the polymeric structures of derived films to validate this hypothesis.

Fig. Appx.4 provides the cross-section morphology of CNF films incorporated with low and high Mw CH at 20 g/100 g CNF in comparison with CNF only film. Compared to CNF only (Fig. Appx.4a), CH at low Mw (Fig. Appx.4b) was well-adsorbed into CNF and tightly packed throughout the cross-section matrix possibly due to the strong electrostatic interactions and/or hydrogen bonds between CH and CNF in comparison with CNF only. This result could support the lower WA in CH-incorporated CNF film in comparison with CNF only (Table Appx.1). It was also seen that CNF film with high Mw CH had relatively less packed structures (Fig. Appx.4c), compared to that with low Mw CH (Fig. Appx.4b). This result could be related to the lower WVP in low Mw CHincorporated CNF films (Fig. Appx.1b) due to less free volumes within film matrix than CNF films with high Mw CH incorporation, allowing less moisture diffusion through film matrix.

Validation of applying CH-incorporated CNF films for packaging fresh beef patties Fig. Appx.5 illustrates the appearance of CH-incorporated CNF films as separation

sheets placed between beef patties that were stored in a refrigerator (5-7 °C) for 1-week along with measured film WA. CNF only film (Fig. Appx.5a) showed significant color change (pink to red), compared to CH-incorporated CNF films (Fig. Appx.5b and Appx.5c) because it absorbed more water and blood leaked from the beef patties. Consistently, CH incorporated CNF films had significantly lower liquid absorption (lower WA values) than that of CNF only film (Fig. Appx.5), indicating the enhanced water resistance of CH-incorporated CNF films. These results demonstrated that CHincorporated CNF films are durable against high moisture condition so that can be potentially applied to high moist surface food as separating sheet to prevent moisture transfer between the layered products. Hence, this study successfully validated that CHincorporated CNF films can be used as separation sheets between moist surfaces to minimize the moisture transfer between layered food products.

CONCLUSION

This study developed and characterized CNF based films with improved waterresistant and antibacterial properties through a simple and safe polysaccharide adsorption method. The type of polysaccharide played an important role in respect to improving the water-resistance of the CNF films. Chitosan incorporation resulted in significant reduction of water absorption, indicating its better compatibility with CNF than methyl cellulose and carboxymethyl cellulose. Mw and concentration of applied chitosan impacted water-resistant and antibacterial properties of chitosan incorporated CNF films. The incorporation of 287 kDa chitosan at 20 g/100 g CNF offered the least water absorption and water solubility of the CNF-CH films, while the incorporation of 68 kDa chitosan at 20 g/100 g CNF provided strong antibacterial property of the CNF films. The developed CNF-CH films as food contact packaging were validated by applying on food products with high moist and adhesive surfaces, and showed their success for reducing moisture loss and adhesion between layered food products. Such films may be utilized as food contact packaging for replacing waxed papers or other synthetic polymers. Future studies are necessary to improve the functional and sealable properties of CNF-CH films by using layer-by-layer assemble and/or other approaches, and also validate their applications in other food products.

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Fig. Appx.1 Effect of chitosan concentration (CHC) and molecular weight (CHM) on contact angle of water on the film surface [a] and water vapor permeability (WVP) [b] of cellulose nanofiber (CNF)-based films; All films were prepared by incorporating 0.5% (w/w water in wet base) CNF and 10% (w/w chitosan in dry base) glycerol; Ctrl=0% CH, A=10% CH at 68 kDa, B=10% CH at 181 kDa, C=10% CH at 287 kDa, D=20% CH at 68 kDa, E=20% CH at 181 kDa and F=20% CH at 287 kDa, respectively. ⁺ Means followed by the same letter were not significantly different (P > 0.05)



Fig. Appx.2 Inhibition on microbial growth (optical density, OD 600 nm) against *L. innocua* and *E. coli* enrichment broth treated with cellulose nanofiber (CNF)-based films; All films were prepared by incorporating 0.5% (w/w water in wet base) CNF and 10% (w/w chitosan in dry base) glycerol; Ctrl=0% CH, A=10% CH at 68 kDa, B=10% CH at 181 kDa, C=10% CH at 287 kDa, D=20% CH at 68 kDa, E=20% CH at 181 kDa and F=20% CH at 287 kDa, respectively. Ctrln represented enrichment broth without any film treatment.



Fig. Appx.3 Fourier transform infrared spectroscopy (FT-IR) [a] and differential scanning calorimetry (DSC) spectrum [b] of cellulose nanofiber (CNF)-based films; All films were prepared by incorporating 0.5% (w/w water in wet base) and 10% (w/w chitosan in dry base) glycerol; Ctrl=0% CH, A=10% CH at 68 kDa, B=10% CH at 181 kDa, C=10% CH at 287 kDa, D=20% CH at 68 kDa, E=20% CH at 181 kDa and F=20% CH at 287 kDa, respectively; Glass transition temperature (Tg) was determined and reported with DSC spectrum.



Fig. Appx.4 Cross-section SEM micrographs for CNF only film [a], and low Mw (68 kDa) of chitosan (20% w/w dry base) incorporated CNF film [b], and high Mw (287 kDa) of chitosan (20% w/w dry base) incorporated CNF film [c]; All films were prepared by incorporating 0.5% (w/w water in wet base) CNF and 10% (w/w chitosan in dry base) glycerol. Digital images were collected at an accelerating voltage of 5 kV and with a magnification of 20 µm.



Fig. Appx.5 Demonstration of film applications as separation sheets placed between layered beef patties stored at 5-7 °C for 1 week; [a]: CNF-based film; [b]: low Mw (68 kDa) of chitosan (20% w/w dry base) incorporated CNF-based film; [c]: high Mw (287 kDa) of chitosan (20% w/w dry base) incorporated CNF film; All films were prepared by incorporating 0.5% (w/w water in wet base) CNF and 10% (w/w chitosan in dry base) glycerol.

⁺Ground beef (80% lean and 20% fat) was purchased from a local market on the day when experiment was conducted. A 90 x 90 mm film was precisely weighed and placed between beef patties (\sim 80 mm diameter and \sim 150 g of each patty)

⁺⁺ Means followed by the same letter were not significantly different (P > 0.05)

Factors and levels*			els*	Methylcellulose (MC)**			Chitosan (CH)**			Carboxymethyl cellulose (CMC)**		
No.	А	В	С	WVP (g mm/m ² d Pa)	WA (%)	WS (%)	WVP (g mm/m ² d Pa)	WA (%)	WS (%)	WVP (g mm/m ² d Pa)	WA (%)	WS (%)
1	1	1	1	0.029	435.8	26.56	0.029	392.6	25.20	0.034	354.8	21.69
2	1	2	2	0.035	673.2	32.43	0.030	607.5	30.72	0.040	482.6	18.57
3	1	3	3	0.036	648.5	26.53	0.037	551.6	27.52	0.045	623.3	24.59
4	2	1	2	0.028	540.7	22.70	0.034	172.8	21.86	0.037	ND^{***}	ND
5	2	2	3	0.035	711.9	26.56	0.040	166.8	17.96	0.042	ND	ND
6	2	3	1	0.035	674.7	21.13	0.044	163.4	21.72	0.038	ND	ND
7	3	1	3	0.045	1129.4	27.59	0.044	157.6	26.34	0.041	ND	ND
8	3	2	1	0.036	988.4	36.23	0.045	153.7	22.79	0.038	ND	ND
9	3	3	2	0.038	899.8	30.17	0.045	170.6	23.10	0.043	ND	ND
Factor		Lev	vel			Me	an values of each	n factor at e	each level			
A		A1		$0.033{\pm}0.004^{a}$	586±131 ^a	$28.5{\pm}3.4^{ab}$	$0.032{\pm}0.005^{a}$	517±112 ^a	27.8 ± 2.8^{a}	$0.040{\pm}0.005^{a}$	NA	NA
		A2		$0.033{\pm}0.004^{a}$	642±90 ^a	23.5±2.8 ^a	$0.039{\pm}0.005^{ab}$	168±5 ^b	20.5 ± 2.2^{b}	0.039±0.003 ^a	NA	NA
		A3		$0.040{\pm}0.005^{a}$	1006±116 ^b	$31.3{\pm}4.4^{b}$	$0.045{\pm}0.001^{b}$	161±9 ^b	$24.1{\pm}2.0^{ab}$	$0.040{\pm}0.002^{a}$	NA	NA
		RA^{++}		0.007	420	7.9	0.013	357	7.3	0.002	NA	NA
	B1		1	$0.034{\pm}0.009^{a}$	702 ± 374^{a}	25.6±2.6ª	$0.036{\pm}0.008^{a}$	$241{\pm}132^a$	$24.5{\pm}2.3^{a}$	$0.037{\pm}0.003$ ^a	NA	NA
D		B2		$0.035{\pm}0.001^{a}$	791±172 ª	31.7±4.9 ^a	$0.038{\pm}0.008^{a}$	309±258ª	$23.8{\pm}6.4^{a}$	$0.040{\pm}0.002^{a}$	NA	NA
D	B3		3	$0.036{\pm}0.001^{a}$	$741{\pm}138^{a}$	25.9±4.6 ^a	$0.042{\pm}0.004$ ^a	295±222ª	24.1 ± 3.0^{a}	$0.042{\pm}0.004$ ^a	NA	NA
		RB	++	0.002	89	6.1	0.006	68	0.6	0.005	NA	NA
С	C C		1	$0.033{\pm}0.004^{a}$	$700{\pm}277^{a}$	$28.0{\pm}7.7^{\mathrm{a}}$	$0.039{\pm}0.009^{a}$	237±135 ^a	$23.2{\pm}1.8^{a}$	$0.037 {\pm} 0.002^{a}$	NA	NA

Table Appx.1 Effects of different polysaccharides (methylcellulose, chitosan, and carboxymethyl cellulose) on water-barrier properties (water vapor permeability (WVP), water absorption ability (WA), and water solubility (WS)) of cellulose nanofiber (CNF)-based films by comparing three respective Taguchi analyses

C2	$0.034{\pm}0.005^{a}$	705±182 ^a	$28.4{\pm}5.1^{a}$	$0.037{\pm}0.008^{a}$	317 ± 252^{a}	$25.2{\pm}4.8^{a}$	0.040 ± 0.003^{ab}	NA	NA
C3	$0.039{\pm}0.005^{a}$	830±261 ^a	26.9 ± 0.6^{a}	$0.040{\pm}0.004^{a}$	292±225 ^a	23.9 ± 5.2^{a}	$0.043{\pm}0.002^{b}$	NA	NA
RC^{++}	0.005	130	1.5	0.004	80	2.0	0.006	NA	NA
Rank ⁺⁺⁺	A>C>B	A>C>B	A>B>C	A>B>C	A>C>B	A>C>B	C>A>B	NA	NA

* A, B and C represented experimental factors, including concentration of polysaccharide (A₁: 0; A₂: 15%; A₃: 30%, w/w CNF dry base), avocado oil (B₁: 0; B₂: 15%; B₃: 30%, w/w CNF dry base), and surfactant mixture (C₁: 0; C₂: 20%; C₃: 40%, w/w CNF dry base) at a 1:1 ratio of Tween 80 and Span 80, respectively.

** Films were prepared by incorporating 0.75% (w/w water in wet base) CNF and 10% (w/w CNF in dry base) glycerol.

*** ND: Non-detected due to high film solubility; NA: Not applicable

⁺Means followed by the same upper letter in a column were not significantly different (P > 0.05).

⁺⁺ RA, RB, and RC were the largest difference between the highest and lowest values within each factor, indicating the most contributing factor on each measurement.

⁺⁺⁺ Ranks were based on the order of RA, RB, and RC values.

Table Appx.2 Effects of chitosan concentrations (CHC) and molecular weights (CHM) on physicochemical (color, thickness, and haze), water-resistant (water absorption (WA), and water solubility (WS)), and mechanical properties (tensile strength and elongation at break) of cellulose nanofiber (CNF)-based films

	Analysis of variance (ANOVA) results (P-value)*								
	ΔE^{**}	Thickness	ГS (MPa)*	WA (%)*	WS (%)*	EB (%)*	Haze (%)		
		(mm)							
Main factor									
CHC^+	<.0001	<.0001	0.0240	0.0331	0.6633	0.8312	0.0588		
CHM^+	0.0003	0.5884	0.8570	0.9261	0.0278	0.5938	0.0810		
Interaction factor									
CHC x CHM	0.8251	0.9343	0.8069	0.1281	0.0728	0.9415	0.7315		
			Post	hoc multiple co	omparison tests ⁺⁺				
Levels		ΔE	Thick	ness (mm)	TS (MPa)	WA (%)	WS (%)		
	CHC	CHM		CHC	CHC	CHC	CHM		
1	0.62±0.13 ª	$0.82{\pm}0.49$ a	$0.027{\pm}0.000$ a		29.5±0.9 ^a	94.4±10.4 ^a	31.3±3.3 ^a		
2	1.33±0.14 ^b	$1.04{\pm}0.50^{b}$	0.03	$\pm 0.001^{\text{b}}$ 24.9 $\pm 1.0^{\text{b}}$		77.2±9.2 ^b	$28.3{\pm}1.7^{\text{ ab}}$		
3		1.07±0.53 ^b					21.5±7.5 ^b		

* There was no significant effect of main factor and their interactions when *P*-value was higher than 0.5.

** ΔE , TS, WA, WS, and EB represented the color difference from CNF only film, tensile strength, water absorption ability, water solubility, and elongation at break of CNF films incorporating with different Mw and concentration of chitosan, respectively. ⁺ Two levels for CHC: 10% and 20% w/w CNF in dry base and three levels for CHM: 68, 181 and 287 kDa.

⁺⁺ *Post hoc* multiple comparison tests were selectively reported for results showing the significant effect of applied factors in ANOVA result.

All films were prepared by incorporating 0.5% (w/w water in wet base) CNF and 10% (w/w CNF in dry base) glycerol.