Cellulose Nanocrystal Reinforced Chitosan Coatings for Improving the Storability of Postharvest Pears Under Both Ambient and Cold Storages

Zilong Deng, Jooyeoun Jung, John Simonsen, Yan Wang, and Yanyun Zhao

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 wk of ambient storage (20 ± 2 °C and 30 ± 2% RH) or 5 mo of cold storage (–1.1 °C and 90% RH), respectively. Ethylene and CO_2 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 (Semperfresh™, Pace International, Wapato, Wash., U.S.A.). 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.

Keywords: ambient storage, cellulose nanocrystal, chitosan, cold storage, edible coatings, postharvest quality of pears

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 fruits such as bananas, apples, or mangos.

Introduction

Postharvest losses of fruits and vegetables in 2011 were 40% to 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 is 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 2 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

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(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 (for example, 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 approximately 90%) for several months 1st, and then moved to ambient conditions for retail (15 to 21 °C, RH approximately 50% to 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 Methods

The laboratory 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 Dept. of Food Science & Technology, Oregon State Univ. (Corvallis, Oreg., U.S.A.). 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 (Hood River, Oreg., U.S.A.). 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 2 studies were conducted at 2 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 Univ. of Maine (Orono, Maine, U.S.A.). 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 (Solon, Ohio, U.S.A.). Acetic acid was acquired from J. T. Baker (Phillipsburg, N.J., U.S.A.). For the ambient bench-scale storage study, organic green D'Anjou pears (*Pyrus communis* L.) (Wenatchee Wash., U.S.A.) with no visual defects were purchased from a local market (Corvallis Oreg., U.S.A.) 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, Oreg. were coated after being stored under controlled atmosphere storage at –1 °C for 3 wk. The initial flesh firmness 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 2nd d.

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., Glen Allen, Va., U.S.A.) 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 to 60 Hz], Danbury, Conn., U.S.A.) 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 Pneumatic, Camarillo, Calif., U.S.A.) at 0.28 to 0.31 psi to achieve uniform surface coatings. Coated fruits were dried at ambient temperature under forced airflow for 1 h, and then stored at ambient conditions (20 ± 2 °C and 30 ± 2% RH) without packaging for up to 3 wk. 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. Fruits were then packed into wooden boxes (50 pears in each box), and stored at –1.1 °C and 90% RH for up to 5 mo. For both ambient and cold storage studies, noncoated fruits 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, Semperfresh^{TM} (SEMP) (Pace International, Wapato, Wash., U.S.A.). Sucrose ether-based Semperfresh 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 (TA), and total soluble solid (TSS) content, as well as ethylene and CO_2 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 × 170 mm), and dried at ambient conditions (20 ± 2 °C and 30 ± 2% RH) for 2 d. Films were then conditioned in a custom built chamber (Versa, Philadelphia, Pa., U.S.A.) at 25 °C and 50% RH for 2 d before evaluation. Film thickness was measured using a micrometer (NR 293-776-30, Mitutoyo Manufacturing Ltd., Aurora, Ill, U.S.A) at 10 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 and oxygen permeability 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 E96-16 (ASTM 2016) was used to measure WVTR (Park and Zhao 2004). A film sample (75 × 75 mm) was sealed by vacuum grease on the top of a Plexiglas test cup (57 × 15 mm) filled with 11 mL of distilled (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, Stanwood Wash., U.S.A.) 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 (m^2). Three films per treatment were evaluated, and means and standard deviation values were reported.

For OTR, oxygen permeation of the film sample (120 × 120 mm) was measured following the GB/T1038 method using a gas permeability tester (VAC-VBS, Labthink Instrument Co., Jinan China) at 26 ± 0.5 °C and 55 ± 5% RH. OTR was measured 3 times with 9 films in total (a single run of the instrument required 3 films), and the mean and standard deviation values of 3 replications were reported for each type of film.

Antibacterial activity

Two nonpathogenic bacterial strains, including Gram-positive strain *Listeria innocua* (ATCC 51742, American Type Culture Collection) and Gram-negative strain *Escherichia coli* (ATCC 25922, American Type Culture Collection) were cultured on brain heart infusion (BHI) agar (Becton, Dickinson and Co., Franklin Lakes, N.J., U.S.A.) and tryptic soy agar (Becton, Dickinson and Co. Franklin Lakes, N.J., U.S.A.), respectively, and stored at 4 °C during the course of the study. Prior to a given microbiological assay, a single typical colony of 2 bacteria was inoculated in tubes of appropriate broth (BHI broth [Becton, Dickinson and Co.] for *L. innocua* and tryptic soy broth (TSB) [Becton, Dickinson and Co.] for *E. coli*) and incubated at 37 °C for 16 to 24 h (Lab-Line Orbit shaker bath model 3527, Alpha Multiservices Inc., Melrose Park, Ill., U.S.A.). A film specimen (1 × 1 mm) was immersed into a test tube with 10 mL of sterilized BHI or TSB, respectively, and then inoculated with 100 μL of activated bacterial suspension. Inoculated test tubes (approximately 10^7 CFU mL^{-1}) without film treatment were used as controls. The optical density at 600 nm (OD_{600}) 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, Kyoto Japan) for evaluating the antibacterial effect of the derived films. The mean values and standard deviations of 3 replications with 2 measurements for each replication were reported for treatments and control.

Surface morphology

Surface morphology of the derived films was analyzed using an SEM (600F, FEI Quanta, Hillsboro, Oreg., U.S.A.). Prepared film pieces were placed on an aluminum stub and coated by gold palladium alloy sputter coater (Cressington Scientific Instruments Ltd., Watford 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, Parsippany, N.J., U.S.A.). Fruit diameter was monitored by a Vernier caliper (Spi2000, Swiss Precision Instrument, Garden Grove, Calif., U.S.A.) 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 wk) from the initial weight and diameter at 0 wk, and dividing by the initial weight and diameter, respectively. Six fruits 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 mo of storage was measured following the same method as stated above for 15 fruits per treatment and control.

Color and overall appearance. For the ambient storage test, fruit color was measured using a colorimeter (LabScan XE, HunterLab, Reston, Va., U.S.A.) 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 dia circle was marked on the surface of each fruit, and the same area was observed during 3 wk 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 L_0^*, a_0^*, and b_0^* represented the color values at 0 wk, and L^*, a^*, and b^* referred to the color values at different sampling times (1, 2, and 3 wk). 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-mo 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.

Table 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[a]	Film thickness (10^{-3} m)	WVTR (10^{-3} g/m^2·s)	OTR (10^{-12} m^3/m^2·s·Pa)
0CNC	0.069 ± 0.004[a]	11.81 ± 0.61[ab]	30.40 ± 2.89[a]
5CNC	0.078 ± 0.005[b]	12.52 ± 0.76[a]	15.16 ± 15.74[ab]
10CNC	0.077 ± 0.006[b]	12.41 ± 0.66[a]	4.17 ± 3.24[b]

[a]0CNC, 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.
[b]Means followed by the same upper letter in a column were not significantly different ($P > 0.05$).

Mean values were obtained from triplicate measurements for 15 pears in total.

Firmness, pH, titratable acidity, and total soluble solid

Quality parameters associated with fruit ripening including firmness, pH, TA, and TSS were measured for pears at the end of 3 wk of ambient storage. Fruit firmness was measured by a texture analyzer (TA-XT2 Texture Analyzer, Texture Technologies Corp., Scarsdale, N.Y., U.S.A.). Pears were cut in the stem-calyx axis, and 2 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 6 fruits with 2 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 dia peel disks. Ten fruits were measured for each replicate, and mean value was generated from triplicate measurements.

For the 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 DI water using a blender (Proctor Silex, NACCO Industry Inc., Glen Allen, Va., U.S.A.), 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, Southern Pines, Mass., U.S.A.), or titrated with 0.1 mol L^{-1} NaOH to reach pH 8.3 using a digital titrator (Brinkmann, Wixom, Tex., U.S.A.) (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 (Model 6001 Sierra Madre, Calif., U.S.A) and the juice was filtered with a uniform strip of milk filter. TSS and TA of the juice were determined using the same methods as described above except a different digital titrator (Model T80/20, Schott-Gerate, Hofheim, Germany) was employed.

Ethylene and CO_2 production of pears

For the ambient storage test, ethylene production of the pears was evaluated using a gas chromatograph (GC-2014, Greenhouse gas analyzer, Kyoto, 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 fruits were packed in the tightly closed glass jar for 1 d at ambient temperature (20 ± 2 °C), and ethylene production was compared between jars containing noncoated and coated fruits. 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 fruits 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 d) 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., Poulsbo, Wash., U.S.A.) and then injected into the GC fitted with 3 kinds of packed columns: 80/100 HAYESEP D, 8/100 HAYESEP N, and 60/80 molecular sieve column (Supelco, Bellefonte, Pa., U.S.A.). 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 injector, column, and FID detector were set at 150, 90, and 250 °C, respectively. The ethylene standard gas was purchased from Air Liquide (Scott^{TM}, Pa., U.S.A.), and GC solution software (Shimadzu) 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 fruits 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 temperatures were set up at 90 and 140 °C, respectively. The headspace gas was evaluated for the concentration of CO_2 by an O_2 and CO_2 analyzer (Model 900151, Bridge Analyzers Inc., Alameda, Calif., U.S.A.). The ethylene production and respiration rate were expressed as μL·kg^{-1}·h^{-1} and μg·kg^{-1}·h^{-1}, respectively.

Ripening capacity

After cold storage for 2.5 mo, 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 d. 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%

Semperfresh for cold storage, respectively) was applied for the fruit coating study. Noncoated fruits were used as controls. Both the fruit and film study were conducted in triplicate. A one-way ANOVA was carried out to determine the significant differences among treatments and control, and a *post hoc* least significant difference was conducted by means of statistical software (SAS v 9.2, The SAS Institute, Cary, N.C., U.S.A.). 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 1). Thickness of 5CNC and 10CNC films was significantly ($P < 0.05$) higher than that of 0CNC film, whereas no significant difference in WVTR was observed among different films (Table 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 strengthened chitosan matrix as crosslinking agent and filler due to its anionic sulfate surface groups and crystalline polymeric structure. 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 O_2 permeation of chitosan films, with an inverse relationship (Table 1). The same mechanism worked here as for WVTR (Favier and others 1995; Khan and others 2012; Pereda and others 2014). The CNC reinforced chitosan matrix also

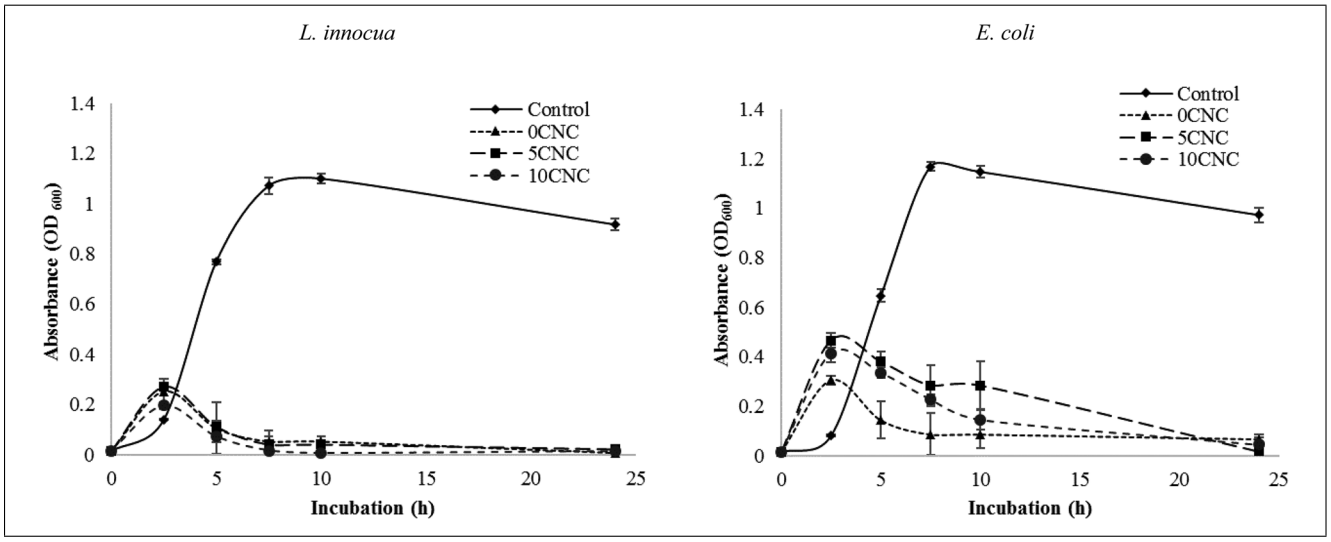

Figure 1–Inhibition of microbial growth (absorbance at 600 nm) against *L. innocua* and *E. coli* enrichment broth treated with films derived from 2% chitosan containing different concentrations of cellulose nanocrystal (CNC); 0CNC, 5CNC, and 10CNC represented films derived from 2% chitosan containing 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 enrichment broth without any added film.

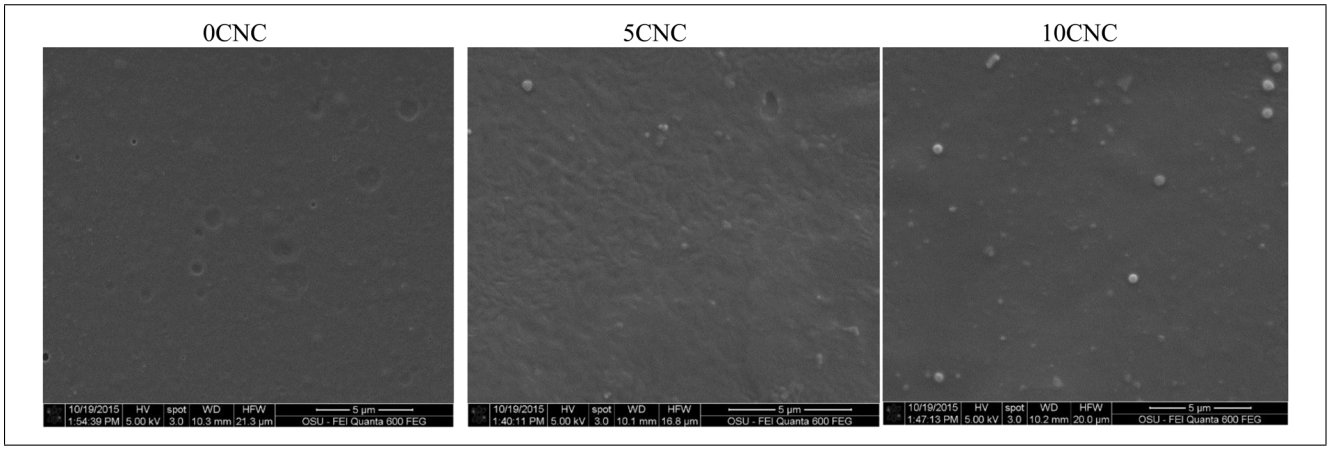

Figure 2–Scanning electron microscope (SEM) images of derived films from coating formulations containing different concentrations of cellulose nanocrystal (CNC); 0CNC, 5CNC, and 10CNC represented films derived from 2% chitosan containing 0%, 5%, or 10% (w/w chitosan in dry base) CNC, respectively. Each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

Table 2–Comparisons of firmness, total soluble solid, pH, and titratable acidity between noncoated pears and pears coated with cellulose nanocrystal (CNC) reinforced 2% chitosan coatings at the end of 3 wk of ambient storage (20±2 °C and 30±2% RH).

Coating treatment[a]	Firmness (kg·m/s^2)	Total soluble solid content (TSS, %)	pH	Titratable acidity (TA, %)
Control	3.15 ± 1.55^{cb}	14.0 ± 0.9^{a}	4.29 ± 0.05^{b}	0.17 ± 0.02^{a}
0CNC	10.73 ± 6.02^{b}	13.5 ± 1.4^{a}	4.79 ± 0.43^{ab}	0.11 ± 0.02^{c}
5CNC	16.08 ± 6.77^{a}	11.7 ± 1.5^{b}	4.69 ± 0.20^{ab}	0.14 ± 0.03^{ab}
10CNC	20.71 ± 2.83^{a}	10.8 ± 1.0^{b}	4.93 ± 0.68^{a}	0.13 ± 0.03^{bc}

[a]Control represented noncoated 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.
[b]Means followed by the same upper letter in a column were not significantly different ($P > 0.05$).

increased tortuosity, which led to slower gas diffusion (Azeredo and others 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 to 24 h (Figure 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 others 2014). It should be also noted that the antibacterial effect of CNC reinforced chitosan toward *E. coli* was weaker during the 1st 7 h than that of chitosan only film. This was probably because of the affinity of the chitosan amino groups for the negatively charged CNC surface and also adsorption 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 (Figure 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 (that is, ripening, respiration, and senescence) as well as quality deterioration of fruit during postharvest storage (Arvanitoyannis and Gorris 1999; Arnon and others 2014; Dhall 2013). In this study, noncoated and coated pears were examined for ethylene production and important quality parameters during 3 wk of ambient storage.

Pear ripening

Ethylene production in pears accelerates the ripening process of fruit during postharvest storage (Alexander and Grierson

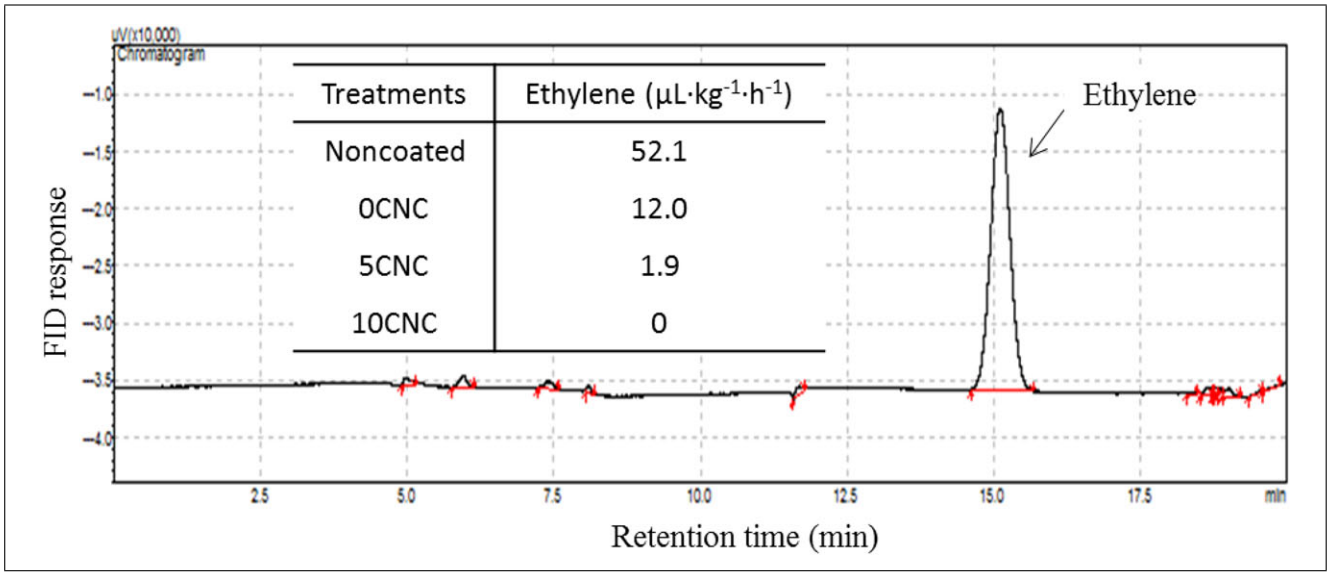

Figure 3–Effect of 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC) on ethylene production of pears after 1 d of ambient storage (20±2 °C and 30±2% RH). Control represented noncoated fruit; 0CNC, 5CNC, and 10CNC represented fruit coated with 2% chitosan containing 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.

2002). The studied coating treatments significantly ($P < 0.05$) reduced ethylene production (0 to 12 $\mu L \cdot kg^{-1} \cdot h^{-1}$) compared to controls (approximately 52 $\mu L \cdot kg^{-1} \cdot h^{-1}$) (Figure 3). This result was consistent with the OTR and WVTR results (Table 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 it is *vice versa* for TA owing to the degradation of organic acids (Chaimanee and Suntornwat 1994; Makkumrai and others 2014). It should also be 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 noncoated and coated pears at the end of 3 wk of ambient storage (Table 2). Firmness of 5CNC and 10CNC coated pears was significantly ($P < 0.05$) higher than that of noncoated and 0CNC coated ones. The 5CNC and 10CNC coated pears showed significantly ($P < 0.05$) lower TSS values than that of noncoated and 0CNC coated ones, which demonstrated delayed fruit ripening by preventing the hydrolysis of starch into sugars (Afshar-Mohammadian and Rahimi-Koldeh 2010). There

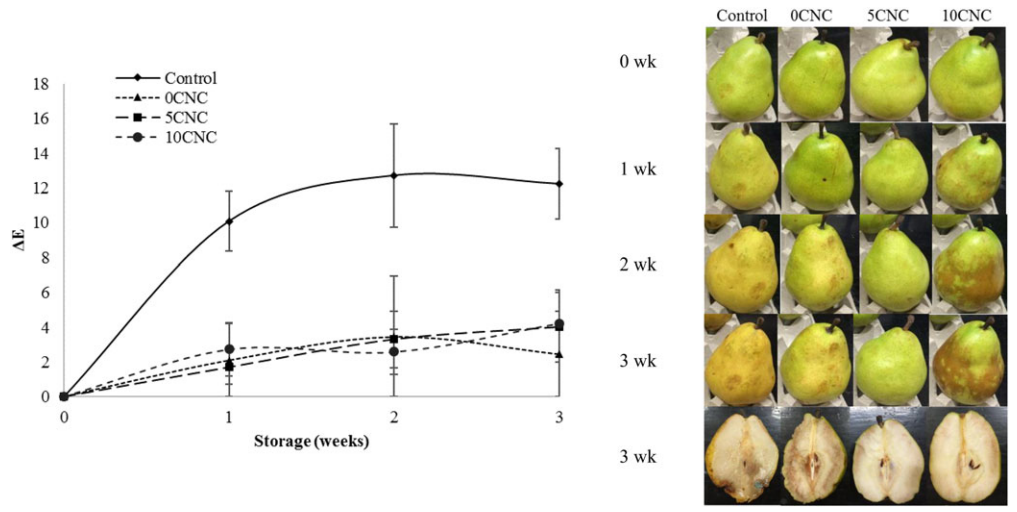

Figure 4–Effects of 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC) on surface color change (ΔE) and appearance of pears during 3 wk of ambient storage (20±2 °C and 30±2% RH). Control represented noncoated fruit; 0CNC, 5CNC, and 10CNC represented fruit coated with 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 L_0^*, a_0^*, and b_0^* represent the values at 0 d and L^*, a^*, and b^* represented the values at different sampling times during storage.

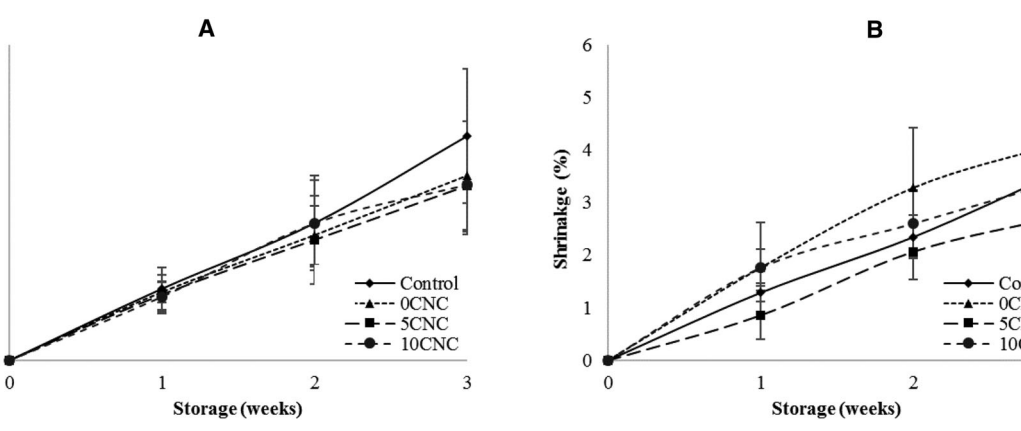

Figure 5–Effect of 2% chitosan coating containing different concentrations of cellulose nanocrystal (CNC) on weight loss (A) and shrinkage (B) of pears during 3 wk of ambient storage (20±2 °C and 30±2% RH); the bar chart with least significant difference (LSD). *Post hoc* multiple comparison test was represented for weight loss at the end of 3 wk. The same letters placed above each column were not significantly different ($P > 0.05$). Control was noncoated 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.

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 (Tariq and others 2001; Liu and others 2010). Although the film study showed that oxygen transmission of 0CNC was higher than that of 5CNC (Table 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 2) and scalding of pears (Figure 4). On the other hand, 10CNC coating with excessive CNC incorporation enhanced the strength of coating matrix, and even led to aggregated CNC or PMC particles on the surface (as shown on the surface morphology of films in Figure 2), thus inducing the anaerobic disorder (superficial scalding) of coated pears (Figure 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 noncoated and coated fruits during 3 wk of ambient storage are illustrated in Figure 4. Photos of internal flesh and cores in pears were also taken at the end of 3 wk to investigate fruit tissue browning caused by CO_2 injury (Franck and others 2007). Coated fruits remained significantly ($P < 0.05$) lower in ΔE values (<6.0) in comparison with that of noncoated samples (approximately 12) during 3 wk of ambient storage (Figure 4). Photos of the pears also showed that 5CNC coated pears retained green pigments much longer than noncoated and other coated fruits (Figure 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 2003; 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 1). However, 10CNC coated pears showed skin speckling and pithy brown core (Figure 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 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.

A

	Control[1]	SEMP	5CNC
Chlorophyll degradation (%)	46.4±2.7 [a2]	38.6±3.6 [b]	34.3±3.3 [b]
Weight loss (%)	2.71±0.21 [a]	1.97±0.19 [b]	1.64±0.20 [b]
Firmness ($kg \cdot m/s^2$)	78.7±3.1 [a]	74.7±1.3 [a]	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]
Titratable acidity (TA, %)	0.36±0.02 [a]	0.35±0.06 [a]	0.33±0.02 [a]
Ethylene production rate ($\mu L \cdot kg^{-1} \cdot h^{-1}$)	136±33 [a]	106±11 [a]	129±30 [a]
Respiration rate ($\mu g \cdot kg^{-1} \cdot h^{-1}$)	1.53±0.10 [a]	1.12±0.30 [a]	1.28±0.21 [a]
Ripening capacity[3] (firmness, $kg \cdot m/s^2$)	5.28±0.05 [a]	4.79±0.16 [a]	6.55±0.66 [b]

B

Figure 6–Comparisons of color degradation, weight loss, internal quality, ripening capacity, and gas production among noncoated pears, pears coated with either commercial Semperfresh or a cellulose nanocrystal reinforced chitosan coating at refrigerated storage condition (–1 °C and 90% RH) for 2.5 mo (A), and illustration of fruit status depending on coating formulations for 2.5 and 5 mo, respectively (B);
[1]Control: noncoated; 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.
[2]Means followed by the same upper letter in a column were not significantly different ($P > 0.05$).
[3]Firmness showing ripening capacity was measured after 5 d stored at ambient condition; all other measurements were conducted at the same day the pears were taken from the cold room.

Weight loss and shrinkage

Both coated and noncoated pears showed increasing trends of weight loss (%) during storage, but coated pears had significantly ($P < 0.05$) lower weight loss than that of noncoated, while no difference was observed in weight loss between 5CNC and 10CNC coated pears (Figure 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 O_2 into sugar, CO_2, and moisture, and thus reducing weight loss (Quamme and Gray 1985). However, no significant difference was observed in shrinkage between noncoated and coated pears (Figure 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 mo of cold storage, 5CNC coated pears lost 34% of their chlorophyll content, whereas SEMP and noncoated fruits lost 39% and 46% of their chlorophyll content, respectively (Figure 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 noncoated fruits (Figure 6B). By the end of 5 mo of storage (Figure 6B), green chlorophyll pigments in both 5CNC and SEMP coated pears further degraded in comparison with fruit from 2.5 mo of storage, but both batches of fruit still maintained good quality. However, noncoated fruits 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 noncoated 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 was observed between coated and noncoateds fruit throughout the 5 mo 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 noncoated 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 d (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 noncoated (5.28 N) and SEMP (4.79 N) coated fruits. 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 (Semperfresh). 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 (that is, reduced changes in weight loss, fruit firmness, and soluble solid content) during 3 wk of ambient storage. Ethylene production was significantly reduced in both 5% and 10% CNC reinforced chitosan coated pears in comparison with chitosan only and noncoated fruits, but the lowest O_2 permeability in 10% CNC reinforced chitosan coating may cause CO_2 injury, thus resulting in surface speckling and pithy brown cores. During cold storage, 5% CNC reinforced chitosan coating had better effect on improving the fruit storability in comparison with noncoated and Semperfresh 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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Author Contributions

Zilong Deng was responsible for formulation development, experiment implementation, data collection, and manuscript composition and revision. Dr. Jung worked on formulation development and manuscript revision. Dr. Zhao, Dr. Simonsen, and Dr. Wang provided instructions and facilities for this study, and Dr. Zhao supported the revision of the manuscript.

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