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Cellulose nanocrystals Pickering emulsion incorporated chitosan coatings for improving storability of postharvest Bartlett pears (*Pyrus communis*) during long-term 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 ~3300–3400 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/m^2·d·Pa) of CH-PCNC film was significantly ($P < 0.05$) lower than that of CH-CNC film (0.251 g mm/m^2·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 Semperfresh™ 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.

1. 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, Jung, Simonsen, Wang, & Zhao, 2017; Villalobos-Acuña et al., 2011; Wang & 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 & Sugar, 2015; Xie, Song, Wang, & Sugar, 2014), CA storage is expensive, though it does provide efficacy (East, Smale, & Trujillo, 2013; Lum et al., 2017), and wax coating gives an artificial appearance and chalking, along with the potential for off-flavor development (Chen & 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, Schueneman, & Simonsen, 2016; Perdones, Vargas, Atarés, & Chiralt, 2014; Tang, Sisler, Grishkewich, & Tam, 2017; Vilarinho, Silva, Vaz, & Farinha, 2017; Zou, Guo, Yin, Wang, & Yang, 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 (Fig. 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,

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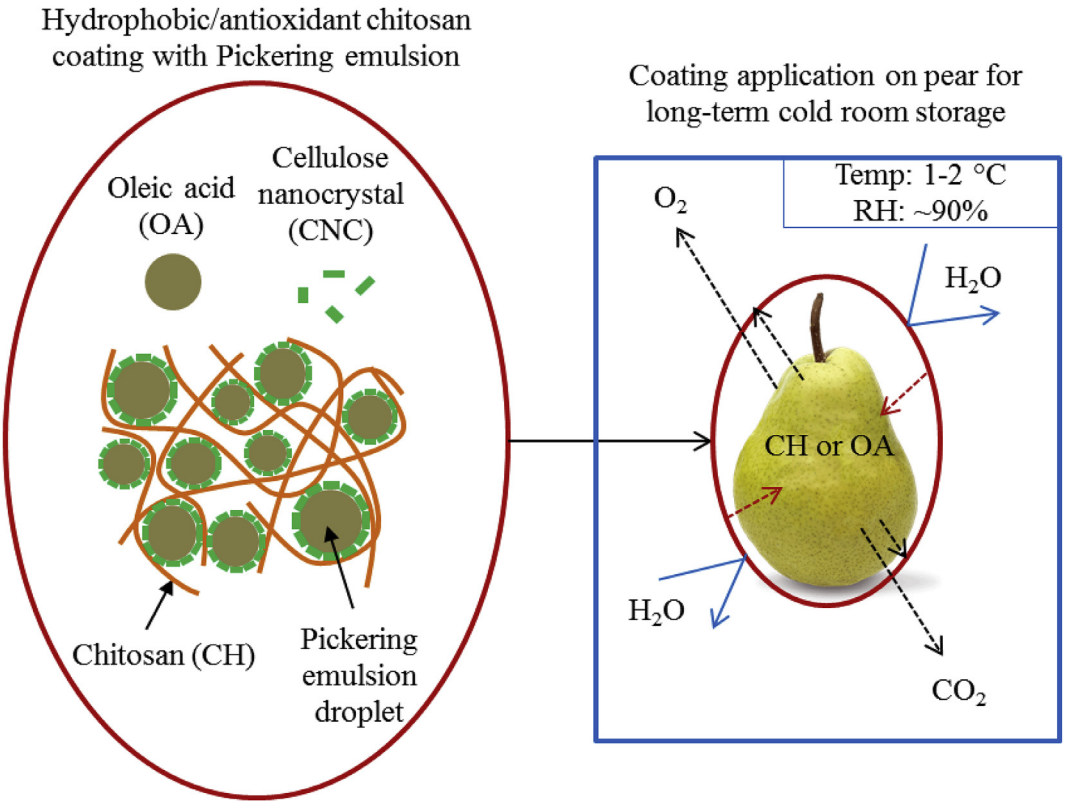


Fig. 1. Schematic diagram illustrating the formation of cellulose nanocrystal (CNC) Pickering emulsion incorporated chitosan (CH) coating (CH-PCNC) and its effect on improving the storability of Bartlett pears during long-term of high humidity cold storage.

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.

2. Materials and methods

2.1. 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.

2.2. 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, Jung, Simonsen, Wang, et al., 2017). A 0.5% commercial Semperfresh™ (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 (J. L. Chen & Zhao, 2012).

CNC (1%, w/w wet basis) and prepared coating suspensions were cast into films (Deng et al., 2017). 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 Enviromental, Williamsport, PA) before evaluation.

2.3. 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, Jung, & Zhao, 2016).

2.4. Water vapor permeability of derived films

WVP of the films was measured using a cup method (Jung, Deng, Simonsen, Bastías, & Zhao, 2016). Briefly, film specimen (75 mm × 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.

2.5. Fourier transform infrared (FTIR) spectroscopy

The influence of RH on the polymeric structure of the films was investigated using a 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 K2CO3 (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 4000 cm^{-1} with 32 scans was collected at 4 cm^{-1} resolution and the FTIR spectrum was reported in the region of 2000–3800 cm^{-1} (i.e. –OH and –CH stretching) (Deng et al., 2017).

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

2.7. 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, Gallegos, Ihl, & Bifani, 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.

2.8. 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.

2.9. 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 & 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). 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 & 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 μm (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, Jung, Simonsen, Wang, et al., 2017). Data (n = 3) were reported as the mean value and standard deviation.

Superficial scald of pears occurs due to oxidative stress during storage (Whitaker, Villalobos-Acuña, Mitcham, & Mattheis, 2009). As an indicator, α-farnesene and reactive conjugated trienols (CTols) were determined for pear peels (Rowan, Allen, Fielder, Spicer, & Brimble, 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 9000 g. Absorbance of α-farnesene at 232 nm (A_{232}) and CTols at 281 and 290 nm (A_{281}-A_{290}) was determined using a UV/Vis spectrophotometer (Model UV-1800, Thermo Fisher Scientific, Inc., Pittsburgh, PA). According to their molar extinction coefficients ($\varepsilon = 27{,}440$ for α-farnesene and $\varepsilon = 25{,}000$ 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.

2.10. 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 $P < 0.05$.

3. 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, Fig. 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.

3.1. Physical properties of CNC and coating suspensions

Physical properties of CNC and CH-PCNC are reported in Table 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 ($P < 0.05$) smaller than that of CH-CNC, which demonstrated that CNC could potentially form a stable Pickering emulsion against coalescence and de-emulsification by the assembled solid particles of nanometric size around near-micro sized Pickering emulsion droplets (Chevalier & Bolzinger, 2013; Han, Zhou, Wu, Liu, & Wu, 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, Kuponiyi, & Kukhtareva, 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 SO_3^- groups on the CNC surface. These results support our hypothesis that CNC interacted with the CH in the formulation and successfully reinforced it (Capron & Cathala, 2013; Pereda, Dufresne, Aranguren, & Marcovich, 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.

3.2. 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 2000–3800 cm^{-1} (i.e. –OH and –CH stretching) was compared between films from the same batch conditioned (stored) at 43% and 83% RH (Fig. 2). For the CH-CNC film, a larger absorption band in the region of 3300–3400 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, Ahmad, Mahdavi, & Abdolmohammadi, 2013; Kurek, Galus, & Debeaufort, 2014; Salam, Lucia, & Jameel, 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/m^2·d·Pa, four times lower ($P < 0.05$) than that of CH-CNC film (0.251 g mm/m^2·d·Pa) (Table 2). This result was consistent with the FTIR analysis, showing that the CH-PCNC film was more hydrophobic than the CH-CNC film.

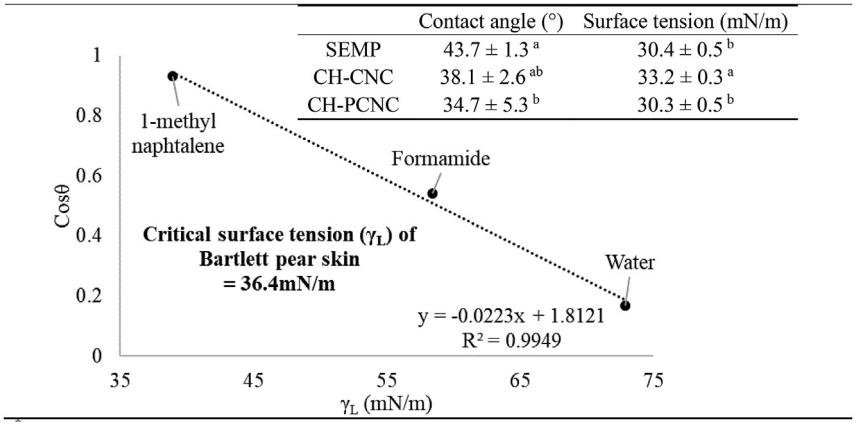
The hydrophobicity of the coatings was also evaluated by comparing the ethylene production of coated fruit conditioned (stored) at 43% and 83% RH at room temperature (Table 2). At 43% RH, the

Table 1
Physical properties of CH-PCNC suspensions and correlations of coating suspensions with pear surface.

Physical properties			
CNC suspensions	Particle size (nm)	Polydispersity	Zeta-potential (mV)
CNC *	156 ± 4 [c, +]	0.219 ± 0.012 [b]	−38.1 ± 2.4 [c]
CH**-CNC ***	4901 ± 109 [a]	0.361 ± 0.026 [a]	20.9 ± 1.2 [a]
CH-PCNC	888 ± 127 [b]	0.005 ± 0.000 [c]	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)
SEMP	43.7 ± 1.3 [a]	30.4 ± 0.5 [b]
CH-CNC	38.1 ± 2.6 [ab]	33.2 ± 0.3 [a]
CH-PCNC	34.7 ± 5.3 [b]	30.3 ± 0.5 [b]



*CH-CNC: CNC was prepared at 0.003% (w/w wet basis).
**ZP of 2% chitosan dissolved in 1% acetic acid was +34.9 ± 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 ($P < 0.05$).

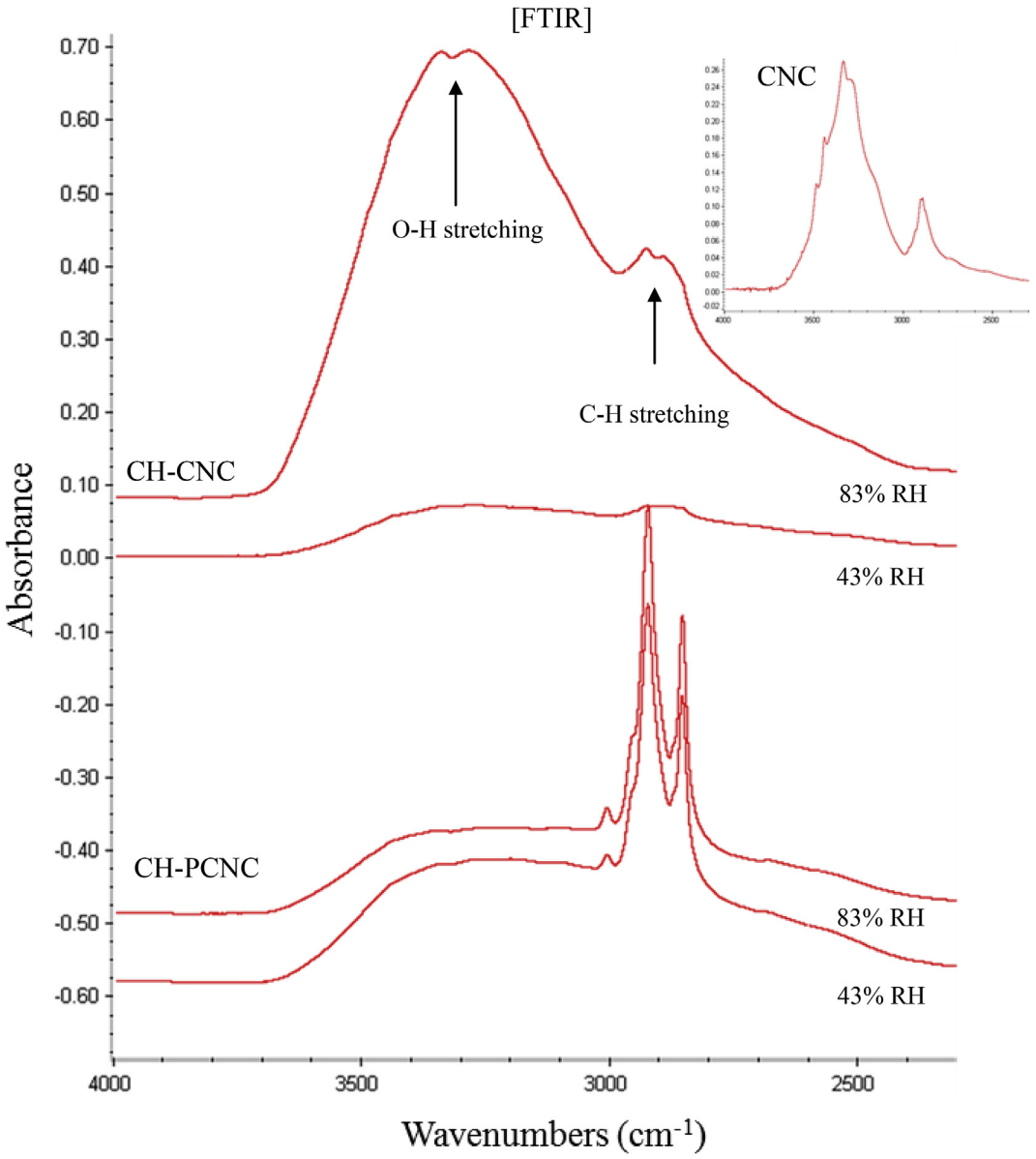


Fig. 2. Fourier-transform infrared spectroscopy (FTIR) of films derived from CNC or coating suspensions. CNC: 1% (w/w/wet basis) cellulose nanocrystals; CH-CNC: CNC reinforced chitosan (CH) coating suspension without Pickering emulsion; CH-PCNC: CNC Pickering emulsion (PCNC) incorporated chitosan (CH) coating; Films were conditioned (stored) at 43% and 83% RH, respectively, prior to FTIR analysis.

Table 2
Effect of CH-PCNC coatings on ethylene production of Bartlett pears and water vapor permeability (WVP) of the derived films.

Assembled controlled humidity vessel	Coating treatments	Ethylene production of fruit				WVP of films (10^{-2} g mm/m^2·d·Pa)
		1st trial (ppm/kg)		2nd trial (ppm/kg)		
		43% RH	83% RH	43% RH	83% RH	
	Control	15.1	13.5	20.8	19.0	N/A
	Semperfresh™	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 Semperfresh™ was not available because films could not be formed from Semperfresh™ suspension.
***Means followed by different superscript letters within each column were significantly different according to the least significant difference (LSD) test ($P < 0.05$).

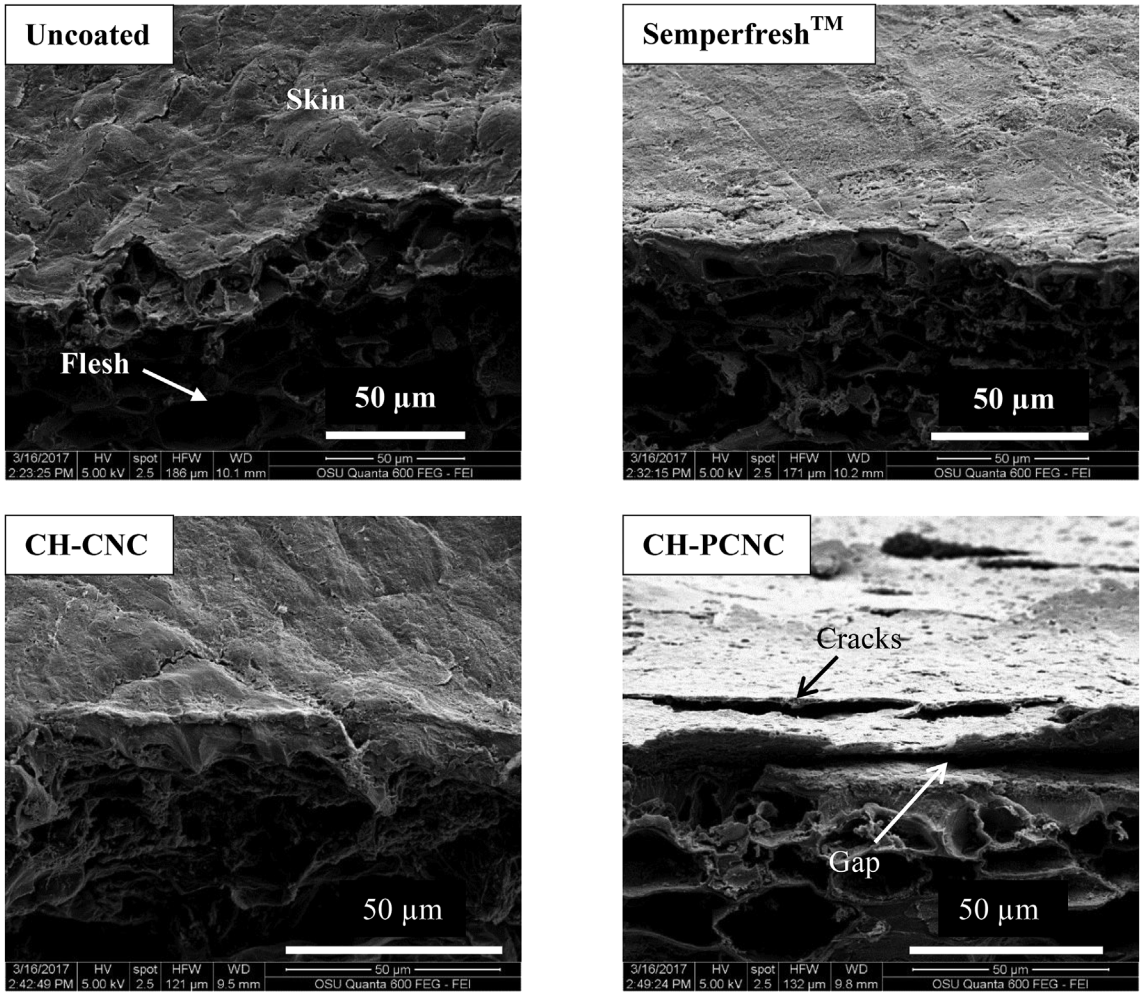


Fig. 3. Scanning electron microscopy (SEM) micrographs of cross-sections of uncoated and coated pear peels; Digital images were collected at an accelerating voltage of 5 kV.

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 8.1 ppm/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.

3.3. 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 1). A lower contact angle (CA) value represents better wetting (Jung et al., 2016; Seo & Lee, 2006). The CH-PCNC coating suspension had a significantly lower CA value (~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., 2017). The critical ST of the pear surface was 36.5 mN/m, extrapolated from the Zisman plot (Table 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 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 (Fig. 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

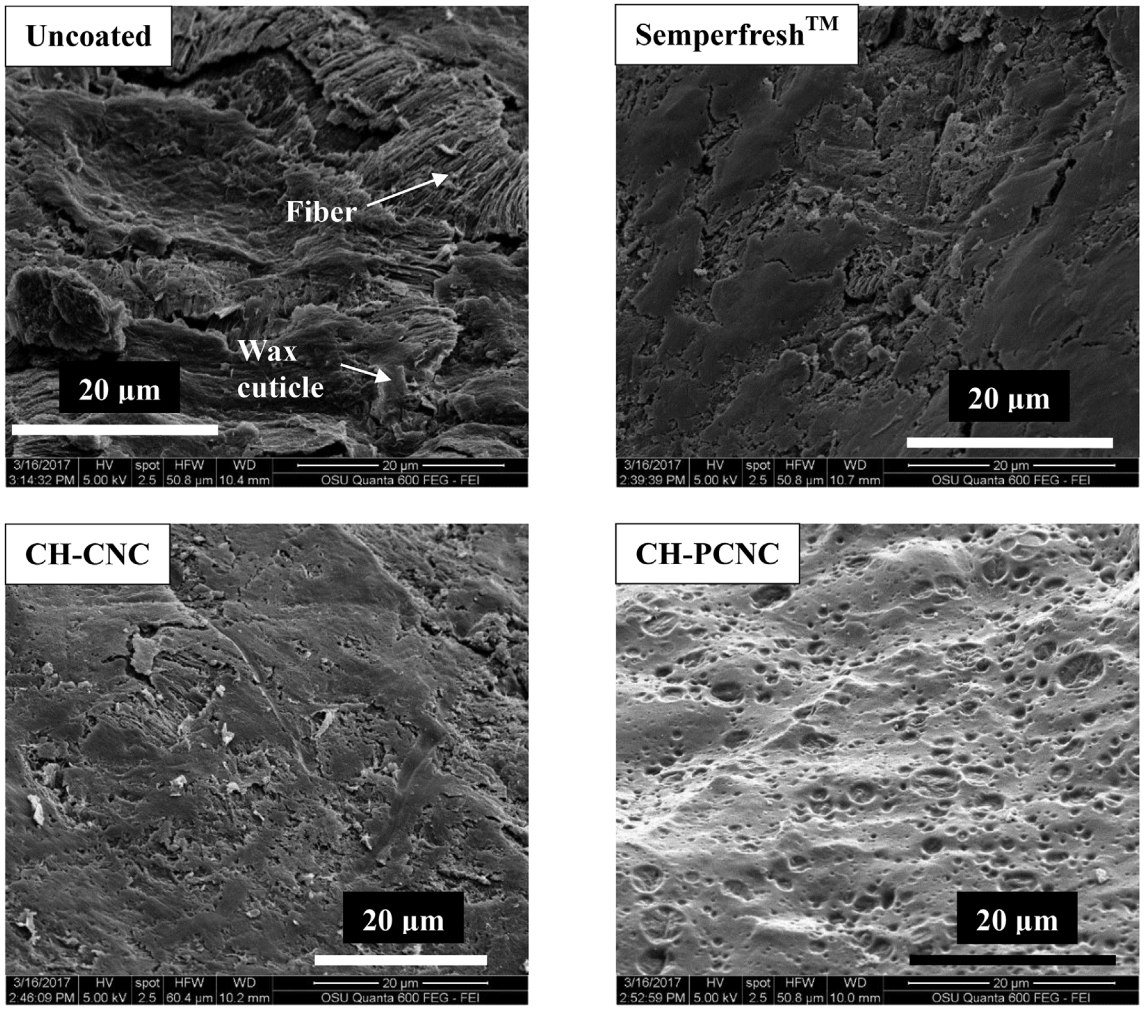


Fig. 4. Scanning electron microscopy (SEM) micrographs of the surfaces of uncoated and coated pear peels. Digital images were collected at an accelerating voltage of 5 kV.

(Fig. 4), 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 crater-like holes, suggesting an improved gas and moisture barrier (Bosquez-Molina, Guerrero-Legarreta, & Vernon-Carter, 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.

3.4. 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 (Fig. 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 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 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 long-term cold storage (Chen, Varga, Mielke, Facteau, & Drake, 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 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.

4. Conclusion

A CNC Pickering emulsion was developed and combined with a CH

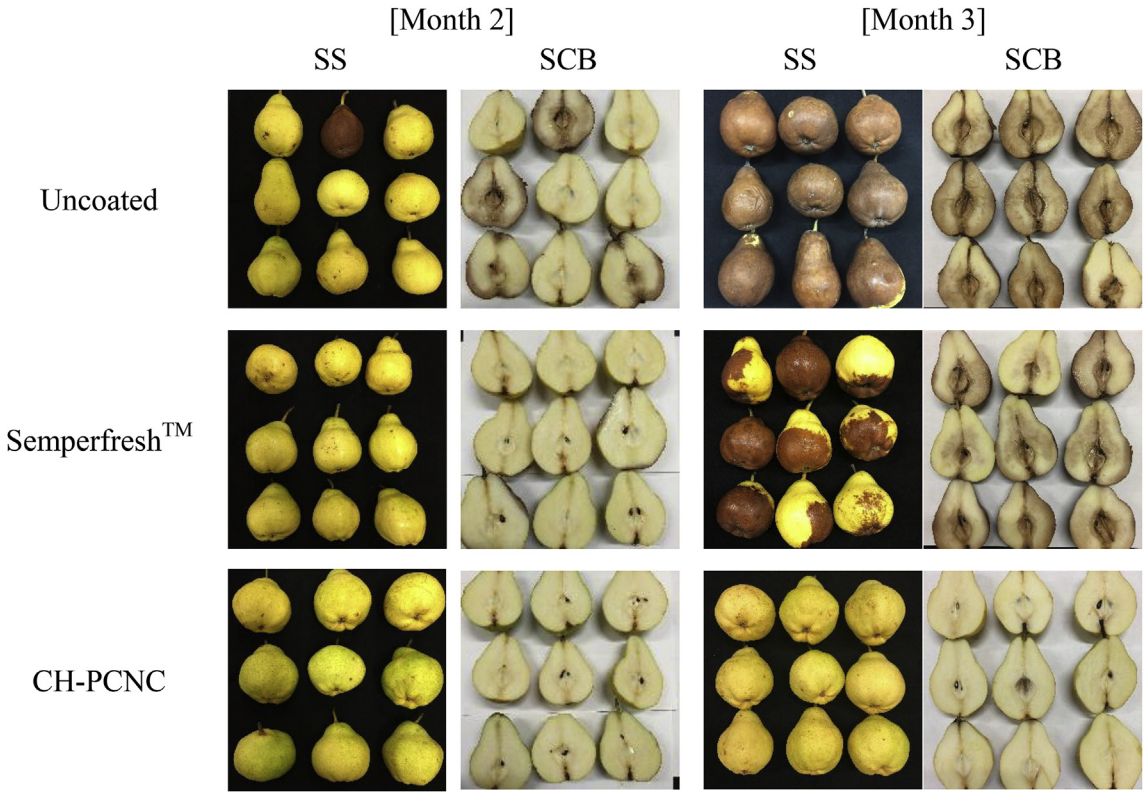


Fig. 5. Effect of cellulose nanocrystal Pickering emulsion (PCNC) incorporated chitosan (CH) coating (CH-PCNC) on senescent scalding (SS) and senescent core breakdown (SCB) of Bartlett pears during storage at 1.7 °C and 90% RH.

Table 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.

	Uncoated	Semperfresh™	CH-PCNC
Chlorophyll degradation (%)	92.5 ± 4.5 ^{a,*}	80.6 ± 9.4 ^{b}	61.8 ± 14.2 ^{c}
Weight loss (%)	4.41 ± 0.82 ^{ab}	4.70 ± 0.97 ^{a}	4.02 ± 0.64 ^{b}
Firmness (N)	42.3 ± 1.2 ^{c}	49.2 ± 2.6 ^{b}	58.6 ± 3.5 ^{a}
Total soluble solid (°Brix)	10.4 ± 0.4 ^{a}	10.2 ± 0.1 ^{a}	9.0 ± 0.3 ^{b}
Titratable acidity (%)	0.169 ± 0.009 ^{b}	0.183 ± 0.003 ^{b}	0.203 ± 0.013 ^{a}
Senescent scald (SS, %)	88.6 ± 3.9 ^{a}	65.9 ± 4.5 ^{b}	0 ^{c}
α-farnesene (μmol/g)	92.9 ± 8.1 ^{a}	59.3 ± 6.1 ^{b}	50.6 ± 1.1 ^{b}
Conjugated trienols (μmol/g)	37.9 ± 3.2 ^{a}	30.4 ± 0.8 ^{b}	26.9 ± 1.4 ^{b}

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

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 (Semperfresh™). 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 Semperfresh™ 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.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.foodhyd.2018.06.012.

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