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Delaying ripening of 'Bartlett' pears (*Pyrus communis*) during long-term simulated industrial cold storage: Mechanisms and validation of chitosan coatings with cellulose nanocrystals Pickering emulsions

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ABSTRACT

Chitosan edible coatings with cellulose nanocrystals Pickering emulsions were investigated at different oleic acid concentrations as coatings to extend storage of 'Bartlett' pears by delaying ripening. Pear coatings contained 10 or 20 g oleic acid/kg emulsion (1OA, 2OA). A 7-month simulated industrial storage trial (−1.1 °C) was conducted against carnauba wax-based coating, controlled atmosphere storage (CAS), and uncoated control. The 2OA maintained the highest peel chlorophyll ($P < 0.05$) when ripened after 3 months, significantly higher than control and carnauba wax throughout the 7 months. Respiration rate and ethylene production peak of 2OA and CAS pears while ripening in ambient conditions were delayed 2 months compared with control pears, and ethylene production peak of 2OA pears had the lowest intensity (31.91 μL/Lgh), significantly ($P < 0.05$) lower than CAS and control pears. When ripened after 7 months, 2OA and 1OA pears maintained equivalent titratable acidity (~0.23 g/100 mL) and total soluble solids (12.8 g/100 g) to each other, significantly ($P < 0.05$) different from all other treatments. The 2OA emulsion coating therefore preserved 'Bartlett' pears better than 1OA and carnauba wax coatings and competitively with CAS, providing a feasible and accessible postharvest preservation alternative.

1. Introduction

While 'Bartlett' pears (*Pyrus communis* L.) are a multi-million dollar market in the United States, they are also particularly susceptible to quality changes and disorders that shorten the supply chain and increase postharvest losses, such as senescent and superficial scalding (Jung, Deng, & Zhao, 2019). 'Bartlett' pears also possess naturally high skin permeability to gas and water vapor compared to other pear varieties, leaving them vulnerable to quicker ripening and degradation (Amarante, Banks, & Ganesh, 2001; Zhi, Dong, & Wang, 2019). Minimizing these changes is essential to raise the value of 'Bartlett' pears in retail and export markets. A variety of postharvest preservation technologies have been developed to maintain pear appearance, decrease respiration and transpiration, and reduce senescence disorders (Wang & Sugar, 2015; Whitaker, Villalobos-Acuna, Mitcham, & Mattheis, 2009; Zhi et al., 2019). Controlled atmosphere storage (CAS) is an effective way to extend shelf-life of fruit by lowering environmental O_2 and increasing CO_2 concentrations, thereby suppressing ripening and development of senescence disorders (Sugar, 2002; Thompson, Prange, Bancroft, & Puttongsiri, 2018). While many 'Bartlett' pears are kept in CAS for up to 6 months, the system is demanding of both money and space, therefore not universally feasible (Bai, Mattheis, & Reed, 2006; Mitcham & Elkins, 2007). Edible coatings are a promising area of development in postharvest preservation of pears, as they do not require such storage equipment. Carnauba wax edible coating is currently commercially applied to some pears, but it does not have optimally efficient gas and moisture barriers attuned to the specific respiration and ripening characteristics of 'Bartlett' pears (Dhall, 2013). For this reason, a stable cellulose nanocrystals (CNC) Pickering emulsion incorporated chitosan (CH) coating (CH–PCNC) was developed and tailored to 'Bartlett' pears in low temperature and high relative humidity conditions (Deng, Jung, Simonsen, Wang, & Zhao, 2017; Deng, Jung, Simonsen, & Zhao, 2018). This coating proved successful due to incorporation of CNC, which has unique oxygen barrier properties and the capacity to stabilize a Pickering emulsion by adhering to the surface of incorporated oleic acid (OA) droplets (Capron & Cathala, 2013). With the hydrophobic component of 30 g OA/kg emulsion, CH–PCNC coating reduced senescent scalding of 'Bartlett' pears in a lab-scale trial

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for up to 3 months with good wettability, hydrophobicity, and stability (Deng et al., 2018). A subsequent study (Jung et al., 2019) explored the effectiveness of the same coating on pears in a commercial cold storage scale trial at −1.1 °C, but its performance was not competitive with commercial treatments such as CAS beyond 5 months. To improve longevity, concentrations of OA and CNC were then experimentally optimized in an accelerated cold storage at 1.7 °C. These studies established the effectiveness and limitations of the CH–PCNC coatings, leaving room for validation of the optimized CH–PCNC formulations in industrial storage conditions as well as their capacity to limit ripening once the pears move to ambient conditions.

The specific objectives of this study were, therefore, 1) to verify optimal OA concentration in the CH–PCNC coatings based on properties of emulsions and derived films, 2) to validate the effectiveness of the optimized CH–PCNC coatings for delaying ripening of postharvest 'Bartlett' pears in simulated long-term commercial cold storage and when moved to ambient conditions, and 3) to compare the effectiveness of the developed CH–PCNC coatings with a commercial wax-based coating and CAS in industrial storage conditions. It was expected that this study would validate the feasibility of using the optimized CH–PCNC edible coatings in replacing CAS for extending long-term storage of commercial 'Bartlett' pears.

2. Materials and methods

2.1. Materials

Emulsion coating solutions were comprised of chitosan (149 kDa, 97% degree of deacetylation, Primex, Siglufjordur, Iceland), cellulose nanocrystals (104 g/kg slurry, Process Development Center of the University of Maine, Orono, ME, USA), oleic acid (Alfa Aesar, Haverhill, MA, USA), and acetic acid (J. T. Baker, Phillipsburg, NJ, USA). 'Bartlett' pears (*Pyrus communis L.*) at the mature green stage with firmness of 80 N were harvested from the Oregon State University Mid-Columbia Research and Extension Center (Hood River, OR) in August 2018. Carnauba wax-based commercial coating (6 g solids/kg solution, US Syntec, Yakima, WA, USA) was provided by a local fruit packing company.

2.2. Preparation of coating formulations and derived films

Emulsion coatings were prepared according to Deng et al. (2018). First, a Pickering emulsion was formed by incorporating OA (10, 20, 30 g/kg emulsion) into an aqueous slurry of CNC (1 g/kg emulsion) and homogenizing for 3 min (Polytron PT10-35, Kinematica, Luzern, Switzerland). Next, chitosan (CH) solution (dissolved in 10 g acetic acid/kg solution) was added to a final concentration of 20 g CH/kg and homogenized for 1 min. Each emulsion was cast into films by distributing 25 mL onto 100 mm × 15 mm Petri dishes (VWR, Radnor, PA, USA). The films dried at 21 ± 1 °C and 45 ± 5% relative humidity (RH) for 3 days, then conditioned in an environmental chamber (Versa 3, Tenney Environmental, Williamsport, PA, USA) at 50% RH and 25 °C for 48 h.

2.3. Analysis of emulsions and derived films

The emulsions were observed for stability visually and photographed over 10 days. Film thickness was measured with a micrometer (NR 293-776-30, Mitutoyo Manufacturing Ltd., Aurora, IL, USA) at 6 locations on 3 films. Color was determined with 5 measurements on each of 2 films per formulation using a LabScan colorimeter (LabScan XE, HunterLab, Reston, VA, USA) and reported as lightness (L*), hue angle (h°, $h_{ab} = \tan^{-1}(\frac{b^*}{a^*})$) and chroma ($c^* = \sqrt{(a^*)^2 + (b^*)^2}$). For water vapor permeability (WVP), ASTM standard cup method was utilized by sealing a sample of each film over each of 3 Plexiglas cups containing 11 mL of deionized water (ASTM, 2000). The apparatuses were kept in an environmental chamber (Versa 3, Tenney Environmental, Williamsport, PA, USA) at 25 °C and 50% RH and weighed hourly for 6 h to calculate WVP in g mm m^{-2} day^{-1} kPa^{-1}.

2.4. Storability study of fruit

After harvest, the pears were stored at −1.1 °C for 24 h to remove field heat, then coated by dipping for 1 min, dried 2 h under forced air, and placed into large wooden crates (n = 60) lined with perforated polyethylene liners. Initial measurements of the pears were taken, and the remaining fruit stored at −1.1 °C and >90% RH. Pears in CAS were sealed into gas-tight cabinets and flushed with purified nitrogen generated from a membrane gas generator (CPA-5, Permea, St. Louis, MO, USA). The CAS conditions (18 mL/L O_2 and 90–94% RH) were achieved within 7 days and maintained throughout storage (Zhi et al., 2019). For each treatment, an additional small box of 21 pears was set up for non-destructive monthly comparisons. All treatments were sampled at 1, 2, 3, 5 and 7 months except CAS, which was sampled at 2, 3, 5 and 7 months.

2.5. Evaluation of fruit quality

2.5.1. Weight loss and color change

At each sampling time, the same 21 pears were weighed individually to an accuracy of 0.01 g, and weight loss was reported as the percentage difference from the initial weight . Chlorophyll content of the pear peels was taken with a Delta Absorbance meter (Sinteleia, Bologna, Italy) on both sides of each fruit and recorded as index of absorbance difference (I_{AD}) (Ziosi et al., 2008). Surface and internal photos of the pears were taken to document appearance.

2.5.2. Firmness, total soluble solids (TSS) and titratable acidity (TA)

At each sampling time, 1 large crate of each treatment (n = 60) was moved from cold storage to ambient conditions overnight, and the following analyses repeated on day 1 and day 5 with 30 pears each. Firmness was measured with a texture analyzer (GS-14, Güss 95 Manufacturing Ltd., Strand, South Africa) on either side of each pear with an 8 mm diameter probe to 9 mm depth at 9 mm s^{-1} (Zhi et al., 2019). Peeled segments from each pear (~10 g) were juiced (6001, Acme Juicer Manufacturing Co, Sierra Madre, CA, USA) in 3 replicates (n = 10) for 3 min each to collect samples for TSS and TA. The juice was diluted 1:4 with deionized water and a sample auto-titrated to pH 8.1 with 0.1 mol/L NaOH (T80/20, Schott-Gerate, Hofheim, Germany) to calculate TA as equivalent concentration of malic acid and reported as g/100 mL. A digital handheld refractometer (PAL-1, Atago, Tokyo, Japan) was used to measure TSS of the juice.

2.5.3. Respiration and ethylene production

Respiration and ethylene production rates of the pears were calculated at each sampling time on day 1 and day 5 in ambient storage. Glass jars (3.8 L) of 5 intact pre-weighed pears were prepared in triplicate for each treatment, and 1 mL headspace samples taken after 1 h. Samples were injected into a gas chromatograph (GC-8A, Shimadzu, Tokyo, Japan) equipped with a flame ionization detector for ethylene analysis. The injector and detector were set to 90 °C and 140 °C, respectively, and nitrogen employed as carrier gas at 0.8 mL s^{-1}. A gas analyzer (900,161, Bridge Analyzers Inc., Alameda, CA, USA) was utilized to quantify headspace concentration of CO_2 to calculate respiration rate.

2.6. Experimental design and statistical analysis

A one-way analysis of variance (ANOVA) was used to compare effects of different OA concentrations on the emulsion films. In the validation study on 'Bartlett' pears, a completely randomized experimental

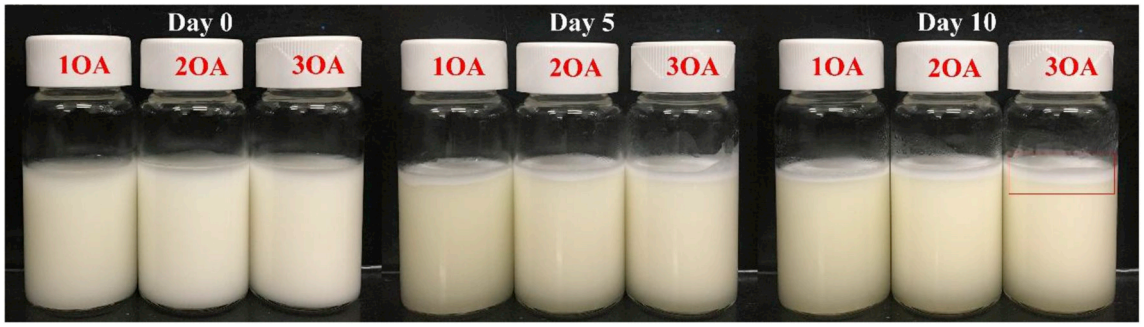


Fig. 1. Stability of chitosan coating formulations with cellulose nanocrystals Pickering emulsions comprised of 1 g/kg cellulose nanocrystals, 20 g/kg chitosan and oleic acid at 10, 20, and 30 g/kg (1OA, 2OA, 3OA). Day 0, 5, and 10 pictured here in ambient conditions (20 °C).

design was applied, and a one-way ANOVA was used to determine any significant differences between the 5 treatments at each sampling time. For both datasets, *post-hoc* least significant difference (LSD) was performed at a significance level of $P \leq 0.05$ with SAS statistical software (SAS v 9.2, The SAS Institute, Cary, NC, USA). Pooled standard deviation values were retrieved from SAS ANOVA output and reported to represent reproducibility of data.

3. Results and discussion

3.1. Properties of emulsions and derived films

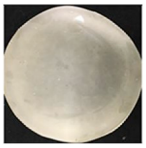
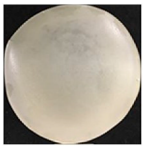
When monitored over 10 days, emulsion cream layer height of 1OA, 2OA and 3OA increased with time and with increased OA concentration (Fig. 1). Jung et al. (2019) also reported significant increases in emulsion cream layer height with increasing OA concentration, indicating decreased emulsion stability (Berton-Carabin & Schroën, 2015). Minimal unincorporated oil was observed on the surface of the 1OA and 2OA films, while there were easily observable large droplets on the 3OA film (Table 1). This was likely due to an excess of oil which could not be stabilized by the concentration of CNC Pickering agent, contributing to the instability. Film thickness significantly increased ($P < 0.05$) with increased OA concentration from 0.143 mm (1OA) to 0.231 mm (3OA) (Table 1), as the water component available to evaporation decreased with additional oil. Regarding hydrophobicity of the films, WVP decreased as OA increased, although not significantly, which is in agreement with the previous study as well as other investigations of the effect of OA on chitosan films (Jung et al., 2019; Vargas, Albors, & Chiralt, 2009). A hydrophobic component of the emulsions (OA) may therefore strengthen the water barrier property of the coating, but is not linearly effective. Increased OA concentration was positively correlated with L* and c* values, indicating higher purity of color, which could be attributed to the increased film thickness. Considering the higher emulsion stability, oil incorporation, and findings from previous studies, the 1OA and 2OA CH–PCNC emulsion formulations were selected as treatments to coat pears for long-term storage and analysis.

3.2. Validation of CH–PCNC coatings during simulated commercial long-term storage

3.2.1. Weight loss

The weight loss of pears from all treatments increased steadily over time (Fig. 2). Pears stored in CAS had the lowest ($P < 0.05$) weight loss until 5 months. At 7 months, the weight loss of pears from all treatments was below an average of 4.5%, and 2OA coated pears had the

Table 1
Appearance and properties of films derived from chitosan coating (20 g/kg) with cellulose nanocrystals (1 g/kg) Pickering emulsion and oleic acid at 10, 20, and 30 g/kg (1OA, 2OA, 3OA). Film color was recorded as L* = lightness, h° (hue angle) = $\tan^{-1}(\frac{b^*}{a^*})$, and c* (chroma) = $\sqrt{(a^*)^2 + (b^*)^2}$ from 2 films. Thickness and WVP (water vapor permeability) data represent the average of 3 films.

Formulation	Appearance	Thickness (mm)	Film Color			WVP (g mm/m^2 day kPa)
			L*	h°	c*	
1OA		0.143^{a}*	47.52^{a}	−1.15^{a}	11.68^{a}	61.7^{a}
2OA		0.194^{b}	53.22^{b}	−1.18^{ab}	13.14^{ab}	55.3^{a}
3OA		0.231^{c}	58.65^{c}	−1.24^{b}	14.90^{b}	48.6^{a}
Pooled standard deviation		*0.007*	*3.94*	*0.09*	*3.21*	*8.6*

*Means sharing superscripts in the same column were not significantly different ($P > 0.05$).

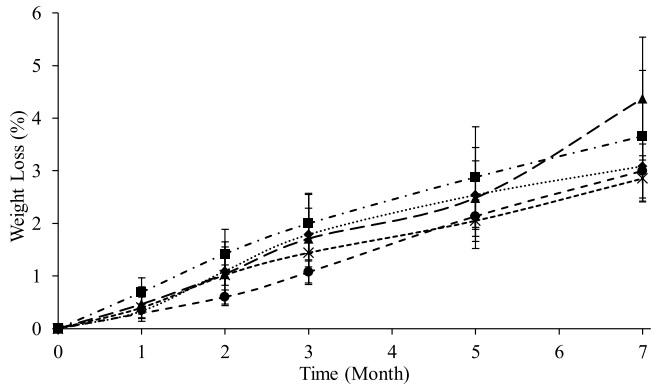


Fig. 2. Weight loss of 'Bartlett' pears in cold storage over time (−1.1 °C, n = 21). ···◆···: uncoated control; – ● –: controlled atmosphere storage; --✱--: carnauba wax; —▲—: Pickering emulsion, 20 g oleic acid/kg; – ■–: Pickering emulsion, 10 g oleic acid/kg.

highest value (Fig. 2). The rapid increase in weight loss in 2OA coated pears between 5 and 7 months might be explained by an error in storage conditions, during which time the polyethylene liner was not replaced around them, but its performance was improved from 1OA coating for at least 5 months. Necessity of the polyethylene liners was explored by Kaur, Dhillon, and Mahajan (2013), who confirmed the consistent benefit of a liner in minimizing weight loss of pears over 75 days.

Weight loss of fruit occurs due to water loss from the plant cells and tissues as transpiration and respiration, causing dehydration, shriveling, textural changes, and loss of marketability (Yahia & Carrillo-Lopez, 2018). Therefore, it was imperative to minimize weight loss to maintain fruit quality. Because the CTRL, CARNB, and CAS pears all had equivalent ultimate weight loss values, the low temperature and high RH storage conditions (>90%) might have had a greater impact on controlling weight loss than the different treatments by minimizing cellular processes and maintaining near equilibrium of moisture between the fruit and the surrounding environment. Lidster (1990) found similar increased weight loss values of 'McIntosh' apples between chambers of different gas compositions as RH increased from 75% to 96%, showing the impact of humidity control in sustaining equilibrium with pome fruits. Increased hydrophobicity and lower WVP of the 2OA coating compared to 1OA might have also contributed to the decreased weight loss for the first 5 months of storage as shown in Table 1.

3.2.2. Fruit appearance

The I_{AD} of CTRL, CARNB, 2OA, and 1OA pears in cold storage decreased over 7 months from 2.0 to 1.2 while CAS pears remained at 1.8 after 7 months (data not shown). After ripening 5 days in ambient conditions to mimic consumer experience, CTRL peel chlorophyll degradation began after 2 months cold storage, averaging an I_{AD} of 0.35 for the remainder of storage compared to the initial 2.0, significantly ($P < 0.05$) lower than all other treatments (Fig. 3a and b; Table 2). At 5 months of cold storage, 2OA maintained the highest post-ripening I_{AD} of 0.85, statistically equivalent to CAS (0.76), but significantly ($P < 0.05$) outperforming 1OA (0.63), CARNB (0.51), and CTRL (0.23) (Fig. 3b), equivalent to a decrease of 58%, 62%, 69%, 75%, and 89% of peel chlorophyll, respectively. Comparison of the I_{AD} at month 5 provided the best accuracy due to the increase in I_{AD} of CTRL at month 7, which likely occurred due to the development of brown areas of senescent scald on the peel surface, causing interference with the chlorophyll measurement (Fig. 3b). Because of the minimization of physiological processes in CAS low oxygen conditions, chlorophyll was well maintained in cold storage. After 5 days in ambient conditions without such limitation on metabolic activity, however, chlorophyll of CAS pears was equivalent to other treatments, suggesting discontinuation of its effects (Sugar, 2002).

The internal flesh of the pears when ripened after 7 months of cold storage is illustrated in Fig. 3a, showing the presence of senescent core breakdown (SCB) as brown areas of internal flesh in the CTRL, CARNB, and 1OA pears. This result corresponded to the degree of external chlorophyll degradation in the CTRL, CARNB, and 1OA pears, while the 2OA and CAS pears never showed signs of SCB within the testing period. Development of SCB might be due to increased respiration, furthering metabolic processes of the fruit (Meheriuk, Prange, Lidster, & Porritt, 1994; Zhi et al., 2019). The stronger emulsion coating (2OA) therefore prevented internal browning and chlorophyll degradation by decreasing metabolic activity. A similar result was seen in 'Ya Li' and 'Laiyang Chili' pears, as a higher oil proportion in an emulsion coating lowered incidence of internal browning (Ju, Duan, & Ju, 2000). The I_{AD} data and external and internal appearance of pears suggested that the 2OA CH–PCNC coating created the most similar microenvironment to CAS, attuned to the 'Bartlett' pears for maintaining peel chlorophyll and preventing senescence disorders.

3.2.3. Firmness

After 7 months of cold storage, there was no significant difference in firmness between CTRL, CARNB, 1OA, and 2OA pears ($P > 0.05$), all of which experienced a decrease of 17–21% from the initial 79.9 ± 1.2 N (Fig. 4a; Table 2). The firmness of CAS fruit remained significantly ($P < 0.05$) higher than other treatments, with a decrease of only 13%. When the pears ripened for 5 days in ambient conditions, they experienced an accelerated drop in firmness at each sampling time, with pears from all treatments reaching <15 N (>82% decrease) by month 2, and no significant differences between treatments by month 3 (Fig. 4b).

Fruit flesh firmness decreases due to metabolic processes fostered by depolymerization of the cell wall components over time, exacerbated by increased respiration of the fruit (Hussain, Meena, Dar, & Wani, 2010; Yahia & Carrillo-Lopez, 2018). In our previous study, the 3OA CH–PCNC coating was found to maintain pear flesh firmness significantly higher than the uncoated pears at 3 months of storage (Deng et al., 2018). However, neither 2OA or 1OA coatings in this study produced this significant difference from the CTRL, nor did CAS or CARNB, indicating that the decreased firmness trend might correspond with harvest maturity, handling, or storage of the pears more than the postharvest preservation treatment. Notably, if 'Bartlett' pears were stored too long, they would never soften to the ideal firmness for consumption (14–24 N) (Zhi et al., 2019). All treatments remained in this ideal window at 7 months of cold storage (Fig. 4b), indicating good eating quality throughout the storage period.

3.2.4. Respiration and ethylene production

When measured 1 day after removal from cold storage, a consistent increase across the 7 month period was observed in the respiration and ethylene production rates of CARNB and 2OA pears (Fig. 5a and c). The respiration rates of CTRL and 1OA pears and ethylene production of 1OA pears also followed an increasing trend but reached the climacteric peak at 5 months. Pears in CAS did not show evidence of ripening in cold storage, maintaining consistently low, stable production of both CO_2 and ethylene between 3 and 7 months, ranging from 17.55 to 18.90 mL CO_2/kg h and 17.72–20.72 μL/Lgh, respectively (Fig. 5a and c). At each sampling period, the day 1 ethylene production rate of the CTRL pears was significantly ($P < 0.05$) higher than other treatments, with a maximum of 51.46 μL/Lgh compared to 37.81 (CARNB), 34.11 (1OA), 31.84 (2OA), and 20.72 (CAS) μL/Lgh.

After 5 days ripening in ambient conditions, CARNB and CTRL pears peaked in both ethylene production and respiration by month 3 (Fig. 5b and d). The respiration rates of CAS, 1OA, and 2OA pears reached a maximum 2 months later with no significant ($P > 0.05$) difference between them, however, ethylene production differentiated the

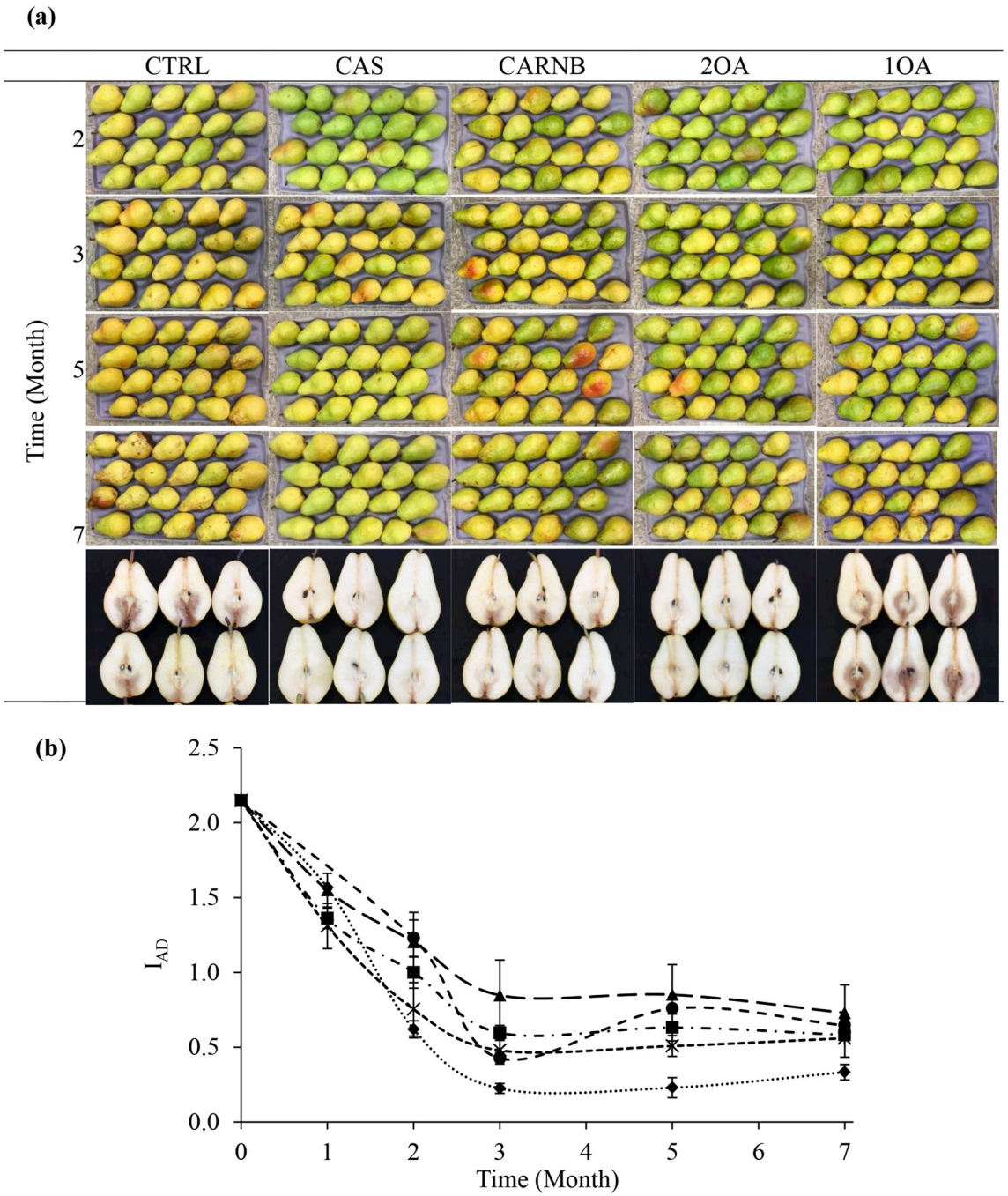


Fig. 3. Appearance (a) and index of absorbance difference (I_{AD}; n = 21) (b) of 'Bartlett' pears over time stored at −1.1 °C followed by 5 days of ambient ripening. Cut pears (a) show internal appearance after 7 months of cold storage. Error bars indicate standard deviation of I_{AD} (b). ···◆···: uncoated control (CTRL); – ● –: controlled atmosphere storage (CAS); - -✱- -: carnauba wax (CARNB); —▲—: Pickering emulsion, 20 g oleic acid/kg (2OA); – ■–: Pickering emulsion, 10 g oleic acid/kg (1OA).

Table 2
Initial qualities of uncoated 'Bartlett' pears immediately following harvest. I_{AD}: index of absorbance difference (n = 21). Firmness, total soluble solids (TSS) and titratable acidity (TA) data were from 3 replicates of 10 pears. Ethylene production and respiration rate represent averages of 3 replicates of 5 pears.

Parameter	Mean	*Standard Deviation*
Color (I_{AD})	2.03	*0.02*
Firmness (N)	79.9	*1.2*
TSS (g/100 g)	12.0	*0.2*
TA (g/100 mL)	0.38	*0.0*
Ethylene (uL/L g h)	Not detectable	*N/A*
Respiration Rate (mL CO_2/kg h)	11.1	*1.0*

treatments: 1OA pears peaked at 35.72 μL/Lgh by month 3, but CAS and 2OA pears increased through month 5–49.34 and 31.91 μL/Lgh, respectively. While not simultaneous, the day 5 ethylene peak intensities of the CTRL, CAS and CARNB pears were statistically equivalent, at 47.14 (CTRL), 49.34 (CAS) and 40.59 μL/Lgh (CARNB) (Fig. 5d). Meanwhile, 1OA and 2OA coatings delayed the timing of the ethylene production peak compared to the CTRL while decreasing its intensity significantly ($P < 0.05$) from CTRL and CAS.

The observed peaks and subsequent declines in respiration and ethylene were characteristic of climacteric fruit, which experience a period of increased metabolic activity during ripening (Hansen, 1942; Yahia & Carrillo-Lopez, 2018). Reducing ethylene synthesis and respiration were essential in preventing degradation of pear firmness and

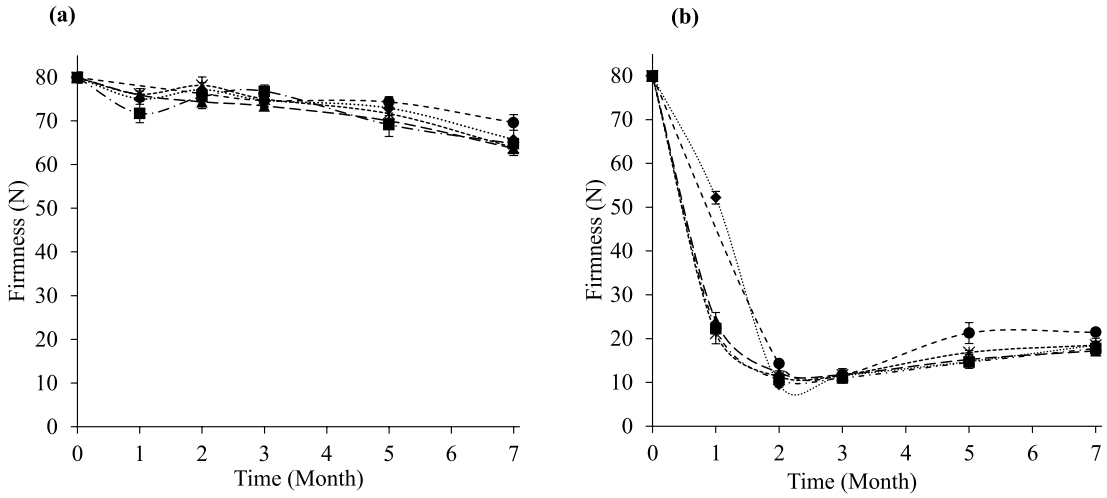


Fig. 4. Firmness of 'Bartlett' pears at 1 day (a) and 5 days (b) after removal from cold storage (−1.1 °C) at each sampling period. ···◆···: uncoated control; – ● –: controlled atmosphere storage; --✱--: carnauba wax; —▲—: Pickering emulsion, 20 g oleic acid/kg; – ■–: Pickering emulsion, 10 g oleic acid/kg. Error bars indicate standard deviation of 3 replicates of 10 pears.

peel chlorophyll, as well as minimizing senescence disorders (Zhi et al., 2019). The consistency measured over time in the CAS pears was expected and indicative of minimal fruit ripening due to controlled levels of CO_2 and O_2 in the storage environment, reducing respiration and ethylene synthesis through inhibition of metabolic processes (Özden & Bayindirli, 2002; (Thompson, Prange, Bancroft, & Puttongsiri, 2018). Once the pears were moved from CAS to ambient conditions, however, the effects of CAS were discontinued, and ethylene production increased to the highest values recorded of any treatment in months 3–7 (Fig. 5d). The most comparable coating to CAS was 2OA, which delayed

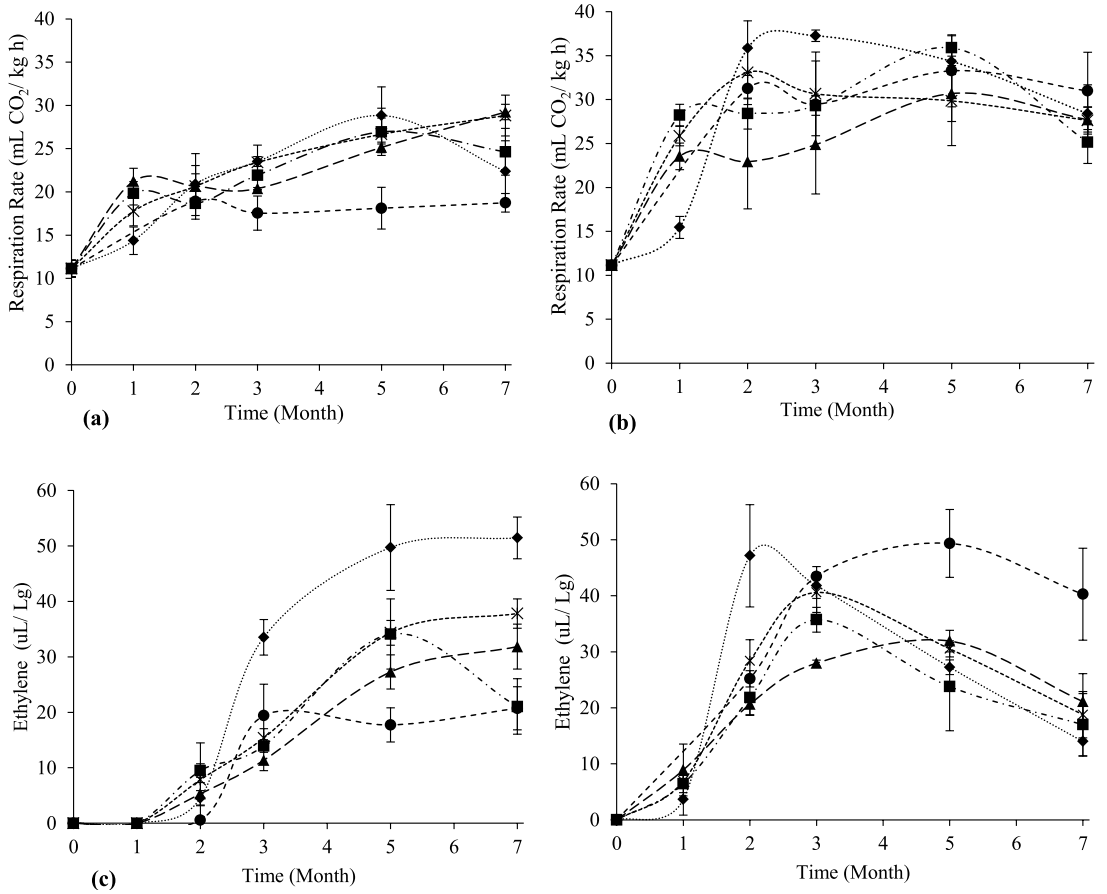


Fig. 5. Respiration rate (a, b) and ethylene production rate (c, d) of 'Bartlett' pears after 1 day of removal from cold storage at −1.1 °C (a, c) and 5 days of ripening in ambient conditions (b, d). ···◆···: uncoated control; – ● –: controlled atmosphere storage; --✱--: carnauba wax; —▲—: Pickering emulsion, 20 g oleic acid/kg; – ■–: Pickering emulsion, 10 g oleic acid/kg. Error bars indicate standard deviation from 3 replicates of 5 pears.

and decreased respiration and ethylene production, controlled respiration better than CARNB and 1OA coatings until month 5 and month 7, respectively, and maintained lower day 5 respiration and ethylene production than CAS fruit for the entirety of storage. Deng et al. (2017) found that the stability and barrier properties of chitosan films with cellulose nanocrystals reinforcement reduced oxygen permeation and lowering gas transmission, thereby slowing down ethylene synthesis and delaying ripening of pears, as observed here. In addition, emulsion coatings with more oil were shown by Ju et al. (2000) to inhibit respiration over 6 months compared to lower oil concentrations and uncoated control, corroborating the observed effects of 2OA over 1OA. The increased monthly respiration rate of 1OA for the first 5 months of cold storage might also be a contributing factor to the severe SCB observed (Fig. 3), indicating insufficient barrier property of 1OA coating and increased rate of cellular processes, observable as the expected correlated yellowing of peel color (Meheriuk et al., 1994).

3.2.5. TSS and TA

Sugar and acid content are highly important flavor components of pears, and as these parameters change with ripening, sensory studies showed a decrease in consumer liking of taste (Zhou et al., 2008). Therefore, it is important that the postharvest preservation method minimizes cellular processes and respiration to maintain total soluble solids and titratable acidity as long as possible. Tables 2 and 3 show that the TSS of each treatment increased from the initial 12.0 g/100 g to a peak due to the breakdown of starch and pectin to soluble sugars, then subsequently decreased (Hussain et al., 2010; Yahia & Carrillo-Lopez, 2018). At the end of 7 months of cold storage, there were no significant differences in TSS values between the treatments ($P > 0.05$), which was consistent with the results of Zhi et al. (2019). After ripening in ambient conditions for 5 days, however, 1OA and 2OA pears had significantly ($P < 0.05$) higher TSS (12.8 g/100 g) compared to CTRL (12.1 g/100 g), CAS (12.2 g/100 g), and CARNB (12.1 g/100 g). Due to the long-term storage condition of the fruit, optimum ripeness in terms of sugar content might have been achieved and then surpassed as soluble sugars were further broken down. Similar trends have been observed between coated and uncoated 'Huanghua' pears and 'Yali' pears in studies where TSS eventually decreased in long-term cold storage due to continued respiration and metabolism of the fruit (Lin et al., 2008; Zhou et al., 2008).

The TA of pears in cold storage decreased steadily over time from the initial 0.38 g/100 mL to 0.15 g/100 mL (CTRL), 0.19 g/100 mL (CAS), 0.18 g/100 mL (CARNB), 0.21 g/100 mL (2OA), and 0.20 g/100 mL (1OA) after 7 months of cold storage (Table 2; Table 3). The ultimate values for 1OA and 2OA coated pears were significantly ($P < 0.05$) higher than all other treatments upon removal from cold storage as well as after 5 days ripening in ambient conditions. Pear TA followed a generally decreasing trend throughout storage, which was corroborated by Zhou et al. (2008), Lin et al. (2008) and Hussain et al. (2010) in their storage studies on pears. Both TSS and TA data indicate that 1OA and 2OA fruits were earlier in the ripening process than other treatments, controlling metabolic processes while in cold storage and upon ripening in ambient conditions.

4. Conclusion

The cellulose nanocrystal Pickering emulsion incorporated chitosan coating with 20 g oleic acid/kg demonstrated its effectiveness on improving postharvest quality of 'Bartlett' pears throughout simulated commercial long-term cold storage. The 2OA coating and controlled atmosphere storage retained peel chlorophyll, reduced senescent core browning, and decreased and delayed the natural, climacteric ethylene production and respiration peaks of 'Bartlett' pears compared to control, 1OA and carnauba wax coatings. The results of this study directly validated the potential use of optimized CH–PCNC coatings to replace CAS, which could increase accessibility of extending long-term commercial 'Bartlett' pear storage across the industry and supply chain. However, evaluation of the developed CH–PCNC emulsion coating application on a commercial fruit coating line is necessary to provide more insight into the feasibility of this process. Expansion of CH–PCNC beyond 'Bartlett' pears to other pear varieties such as 'D'Anjou' or 'Comice' is also worth studying.

Author contribution statement

Rachel Rosenbloom: Investigation, formal analysis, original draft preparation, review & editing.

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Yanyun Zhao: Supervision, funding acquisition, reviewing and editing.

Table 3
Total soluble solids (TSS) and titratable acidity (TA) of triplicate groups of 'Bartlett' pears (n = 10) 1 day after removal from cold storage (−1.1 °C), and again after 5 days in ambient conditions (20 °C). CTRL (uncoated control), CAS (controlled atmosphere storage), CARNB (carnauba wax coating), 2OA and 1OA emulsion coatings comprised of 20 g chitosan/kg, 1 g cellulose nanocrystals/kg, and 20 or 10 g oleic acid/kg, respectively.

Day 1						Day 5					
Month	1	2	3	5	7	Month	1	2	3	5	7
TSS (g/100 g)											
CTRL	13.1^{a}*	11.9^{a}	12.2^{bc}	12.1^{a}	11.9^{a}	CTRL	13.5^{a}	12.6^{bc}	13.3^{ab}	12.6^{b}	12.1^{b}
CAS	–	12.4^{a}	12.8^{a}	12.1^{a}	12.1^{a}	CAS	–	12.8^{b}	13.3^{a}	11.6^{e}	12.2^{b}
CARNB	12.3^{b}	12.0^{a}	11.8^{c}	11.5^{b}	11.9^{a}	CARNB	12.8^{c}	12.1^{c}	12.4^{c}	11.9^{d}	12.1^{b}
2OA	12.6^{ab}	12.4^{a}	12.4^{ab}	12.3^{a}	12.1^{a}	2OA	12.9^{bc}	13.2^{ab}	13.2^{ab}	13.0^{a}	12.8^{a}
1OA	12.6^{ab}	12.3^{a}	12.4^{ab}	12.0^{ab}	11.7^{a}	1OA	13.4^{ab}	13.4^{a}	12.8^{b}	12.2^{c}	12.8^{a}
Pooled SD	*0.29*	*0.29*	*0.31*	*0.27*	*0.31*	*Pooled SD*	*0.30*	*0.34*	*0.24*	*0.14*	*0.27*
TA (g/100 mL)											
CTRL	0.35^{a}	0.29^{a}	0.23^{b}	0.17^{d}	0.15^{c}	CTRL	0.34^{b}	0.31^{a}	0.22^{c}	0.20^{c}	0.19^{c}
CAS	–	0.28^{a}	0.29^{a}	0.22^{b}	0.19^{b}	CAS	–	0.32^{a}	0.29^{b}	0.21^{c}	0.19^{b}
CARNB	0.31^{b}	0.29^{a}	0.23^{b}	0.19^{c}	0.18^{b}	CARNB	0.31^{b}	0.33^{a}	0.25^{c}	0.22^{c}	0.19^{b}
2OA	0.32^{b}	0.28^{a}	0.28^{a}	0.25^{a}	0.21^{a}	2OA	0.33^{b}	0.34^{a}	0.29^{b}	0.28^{a}	0.23^{a}
1OA	0.35^{a}	0.30^{a}	0.28^{a}	0.23^{b}	0.20^{a}	1OA	0.38^{a}	0.34^{a}	0.33^{a}	0.25^{b}	0.24^{a}
Pooled SD	*0.01*	*0.02*	*0.02*	*0.01*	*0.01*	*Pooled SD*	*0.02*	*0.02*	*0.02*	*0.01*	*0.01*

*Means sharing superscripts in the same column were not significantly different ($P > 0.05$). Pooled SD: pooled standard deviation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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