Food Chemistry 309 (2020) 125693

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Mechanisms and performance of cellulose nanocrystals Pickering emulsion chitosan coatings for reducing ethylene production and physiological disorders in postharvest 'Bartlett' pears (*Pyrus communis* L.) during cold storage

Jooyeoun Jung[1], Zilong Deng[2], Yanyun Zhao*

Department of Food Science & Technology, Oregon State University, Corvallis, OR 97331-6602, USA

ARTICLE INFO

Keywords:
Pickering emulsion
Cellulose nanocrystal
Chitosan coating
'Bartlett' pears
Long-term cold storage

ABSTRACT

Pickering emulsion coating (CNCP-CH) composed of oleic acid (OA, 1, 2, and 3%, w/w), cellulose nanocrystal (CNC, 0.1, 0.3, and 0.5%, w/w), and 2% chitosan (CH) was optimized for high emulsion stability. It was found that OA concentration played significant role on emulsion stability. Increasing OA from 1 to 3% reduced emulsion stability ~43%, indicated by the thickness of separated cream layer in the emulsion. 'Bartlett' pears (*Pyrus communis* L.) coated by CNCP-CH containing 1% OA showed significantly reduced ethylene production than that coated with 2% and 3% OA at 1-month of accelerated cold storage at 1.7 °C. The superficial scald on pear peels was only observed on fruit coated by CNCP-CH with 3% OA, but not that with 1% or 2% OA. Therefore, CNCP-CH coating with 1% OA, 0.1% CNC, and 2% CH was suggested for delaying ripening and superficial scald of 'Bartlett' pears during the long-term cold storage.

1. Introduction

'Bartlett' (*Pyrus communis* L.) pear is one of the predominant pear cultivars produced in the US Pacific Northwest. In 2014, Washington led the United States in pear production with 832 million pounds valued at $233.8 million; Oregon produced 432 million pounds valued at $127.4 million, and California produced 378 million pounds valued at $88.6 million. From these three states, 776 million pounds were Bartlett pears valued at $180.7 million (NASS, 2015). The pears are normally held for 1–2 months in cold storage (−1.1 °C and 90–94% relative humidity (RH)) or 3–5 months in controlled atmosphere (CA) storage (Drake, Elfving, Drake, & Visser, 2004; Kupferman, 2003; Wang & Sugar, 2013). Retaining the green status and reducing physiological disorders, especially superficial and senescent scald, during the prolonged cold storage are essential for increasing the values of postharvest pears in retail and export markets (Wang & Sugar, 2015; Whitaker, Villalobos-Acuña, Mitcham, & Mattheis, 2009). Edible coatings, such as shellac and carnauba wax or Semperfresh™ (a mixture of sucrose fatty acid esters, sodium carboxymethyl cellulose, and mono- and diglycerides), have been commercially used for providing gloss and reducing water loss and shrinkage of citrus and pome fruit for short-term storage (Arnon, Zaitsev, Porat, & Poverenov, 2014; Dhall, 2013). Controlled atmosphere (CA) system is applied for retaining quality of fruit for the long-term cold storage, but the system is expensive (de Chiara, Pal, Licciulli, Amodio, & Colelli, 2015; East, Smale, & Trujillo, 2013; Vijayan, Arjunan, & Kumar, 2016). The pear industry has been seeking competitive coating technique comparable to or better than above mentioned postharvest technologies.

Our previous study developed cellulose nanocrystal (CNC) Pickering emulsion chitosan (CH) coating (CNCP-CH) with high moisture barrier under high relative humidity (RH) cold storage and better adhesion onto pear surface (Deng, Jung, Simonsen, & Zhao, 2018). The emulsion coating used CNC as Pickering emulsion agent and oleic acid (OA) and CH as oil and water phases, respectively. The coating showed superior performance in reducing senescent scald of pears for up to 3-months of cold storage (at > 90% RH and 1.7 °C). This study thus simulated the commercial long-term cold storage (at −1.1 °C for 6-months) and validated the effectiveness of the developed CNCP-CH coating for

* Corresponding author.
E-mail addresses: jjung9@unl.edu (J. Jung), zilongdeng@tongji.edu.cn (Z. Deng), yanyun.zhao@oregonstate.edu (Y. Zhao).
[1] Current affiliation: Department of Food Science & Technology, University of Nebraska-Lincoln, Lincoln, NE 68588-6205, USA.
[2] Current affiliation: State Key Laboratory of Pollution Control and Resource Reuse, School of Environmental Science and Engineering, Tongji University, Shanghai 200092, China.

https://doi.org/10.1016/j.foodchem.2019.125693
Received 4 April 2019; Received in revised form 8 October 2019; Accepted 9 October 2019
Available online 19 October 2019
0308-8146/

delaying ripening of fresh 'Bartlett' pears in comparison with controlled atmosphere (CA) storage, 1-methylcyclopropene (1-MCP) treatment, and Semperfresh™ coating. Based upon the results, however, it led to the need of further stabilization of CNCP-CH formulation since the emulsion stability directly affects its hydrophobicity and barrier property, related to the capability for prolonging its effect on delaying ripening of postharvest pears (Chiumarelli & Hubinger, 2012; Darder, Colilla, & Ruiz-Hitzky, 2003; Gómez-Estaca, Montero, Giménez, & Gómez-Guillén, 2007).

In an emulsion coating system, the ratio between oil phase and emulsifier is critical for its stability, i.e., preventing separation of oil phases (Paraskevopoulou, Boskou, & Kiosseoglou, 2005; Taherian, Britten, Sabik, & Fustier, 2011). It was thus hypothesized that the stability and hydrophobicity of CNCP-CH emulsion coating is impacted by OA and CNC concentrations, and an optimal value should be identified in order to achieving sufficient coating performance for reducing ethylene production and delaying ripening of coated fruit.

The specific objectives of this study were 1) to validate the previously developed CNCP-CH emulsion coating on postharvest pears through a simulated long-term commercial cold storage trial, 2) to identify the optimal OA and CNC concentrations in CNCP-CH formulation for enhancing oil incorporation efficiency, emulsion stability, and hydrophobicity of derived films, and 3) to understand the correlation of CNCP-CH formulations derived from different concentrations of OA and CNC with emulsion stability, film hydrophobicity, and ethylene production of coated fruit through the Principal Component Analysis (PCA) and Pearson's correlation matrix. It is anticipated that the optimized CNCP-CH coating formulation could be a potential alternative for other postharvest technologies, such as CA or 1-MCP treatment.

2. Materials and methods

2.1. Materials

The CNCP-CH emulsion coating was composed of CH (149 kDa, 97% degree of deacetylation, Premix, Iceland), CNC (11.8% slurry, Process Development Center of the University of Maine, Orono, ME, USA), OA (Alfa Aesar, Ward Hill, MA, USA), and acetic acid (J. T. Baker, Phillipsburg, NJ, USA). All chemicals were reagent grade. Green 'Bartlett' pears (*Pyrus communis* L.) utilized for the simulated commercial cold storage trial were harvested from the Oregon State University Research Extension farm in Hood River County on August 6th, 2018 and stored at −1.1 °C for 3-weeks prior to the experiments (in order to coordinate the schedule of using the facility for coating studies). Firmness (76 N), soluble solid content (12 °Brix), and acidity (5.4%) of freshly harvested pears were measured. For the optimization of emulsion coating formulation, 'Bartlett' pears originated from Argentina were purchased from a local supermarket (Corvallis, OR, USA) right after they arrived in the store, washed, and subjected to coating treatments on the same day. Uncoated and coated pears were stored at 1.7 °C and 90% RH, a higher temperature than the commercial storage to accelerate the ripening and quality deterioration of pears during 1-month. This temperature was lower than the intermediate temperature of 2.5–10 °C for pears, thus without leading to any potential harm to the fruit (BMT, 2012).

2.2. Simulated commercial long-term storage trial

Four treatments, including CNCP-CH and Semperfresh™ coatings, 1-MCP treatment, and CA storage, were applied along with a control (uncoated fruit). CNCP-CH Pickering emulsion coating showed superior performance in reducing senescent scald of pears for up to 3-months of cold storage in comparison with CNC reinforced CH coating without oil phase from our previous bench-scale study (Deng et al., 2018), thus evaluating for its performance during a simulated commercial long-term storage in this study. Controlled atmosphere (CA) storage, 1-methylcyclopropene (1-MCP) treatment, and Semperfresh™ coating are among the commercially applied postharvest technologies, therefore, were selected for the purpose of comparing with CNCP-CH coating. For each treatment and control, 420 pears were used for 6-months of cold storage. Previous study reported that CA storage generally delayed ripening of 'Bartlett' pears up to 6 months (Villalobos-Acuna & Mitcham, 2008). For comparing coating performance with CA storage, 6-month storage was thus selected. For preparing CNCP-CH coating developed from our previous study (Deng et al., 2018), 3% OA was slowly added into 0.1% CNC aqueous suspensions and homogenized for 3 min (PT10-35, Polytron, Luzernerstrasse, Switzerland) to get CNC Pickering emulsion. A 2% CH (dissolved in 1% acetic acid (w/w)) was then incorporated into the CNC Pickering emulsion and homogenized for another 1 min (Deng et al., 2018). The 2% CH was selected because it could form homogenous matrix and have good compatibility with CNC Pickering emulsion). A commercial coating product Semperfresh™ (Pace International, Wapato, WA) was prepared at a diluted concentration of 0.5% (w/v, wet basis). For 1-MCP treatment, fruit was exposed to 0.15 mg kg^{-1} 1-MCP (SmartFresh®, AgroFresh, Spring House, PA, USA) in an airtight room (39.75 m^3) with a circulation fan at −1.1 °C for 24 h (Xie, Song, Wang, & Sugar, 2014). For controlled atmosphere (CA) storage, fruit were stored in a tightly sealed CA cabinet at −1.1 °C with a semi-static concentration of 1.6% O_2 established within 3 d after fruit were moved in. A semi-static O_2 concentration was established via purified N_2 generated from a membrane gas generator (CPA-5, Permea, St. Louis, MO, USA). CO_2 < 1.0 kPa was maintained by adding 0.5 kg per box of hydrated lime. All of 1-MCP treated, CA stored, and coated pears were stored at −1.1 °C for 6-month. Pears were collected monthly and evaluated visually for appearances and senescent core breakdown. Ethylene production was also measured monthly. Briefly, pears were weighed, sealed in glass jar, and stored in the air-tight jar (3.8 L) with lid holding a 10 mm rubber septa for 1 h. The collected headspace gas was measured by a gas chromatograph (GC-8A, Shimadzu, Tokyo, Japan) with a flame ionization detector (FID) and Porapak Q column (80/100 mesh, 3 mm in diameter, 2 m long) (Sigma-Aldrich, Saint Louis, MO, USA). The carrier gas was nitrogen with a flow rate of 0.8 mL s^{-1}, the oven temperature was 90 °C, and the injector and detector temperatures were both set at 140 °C.

2.3. Performance enhancement of CNCP-CH emulsion coating formulation

Based upon the results from the simulated commercial storage trial, the previously developed CNCP-CH emulsion coating formulation was further improved regarding its stability for prolonging its effect on reducing ethylene production of pears in comparison with other treatments (more details below). For improving emulsion stability and coating performance on pears, the concentration of OA (1, 2, and 3%, w/w) and CNC (0.1, 0.3, and 0.5%, w/w) were considered as two critical factors in the formulation and the significant effect of each factor and their interactions were investigated by using a 3^2 completely randomized factorial design.

2.3.1. Oil incorporation efficiency in derived films

Coating formulations were derived into films using the method described in the study of Deng, Jung, Simonsen, Wang, and Zhao (2017) for investigating oil incorporation efficiency (OIE). Briefly, 60 mL of coating suspension was uniformly cast onto a 150 mm diameter polystyrene petri dish (VWR, Radnor, PA, USA), and dried at room temperature (~23 °C) 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, USA) before evaluation.

For measuring OIE, 3 × 3 cm^2 film specimen was cut and weighted precisely. Films were immersed in 20 g of 95% ethanol for 30 s to solubilize the non-incorporated OA from the film to ethanol. The absorption of ethanol suspensions was measured using the UV160U spectrophotometer (Shimadzu Co., Kyoto, Japan) at 292 nm wavelength. For the control, the absorbance of each coating formulation (20 g) before casting into films was also measured. The OIE was calculated by dividing incorporated amount of OA over total amount of initially incorporated OA (control) for each individual film specimen.

2.3.2. Hydrophobicity of derived films

Water vapor permeability (WVP) of derived films was measured using a cup method (Jung, Deng, Simonsen, Bastías, & Zhao, 2016). The conditioned film sample was sealed using vacuum grease between the lid and the Plexiglas test cup containing 11 mL of distilled water. The seal ring was tightly closed with rubber bands. Test cups were stored at 25 °C and 50% RH in a controlled environment chamber (T10RS 1.5, Hyland Scientific, Stanwood, WA, USA) and weighed hourly for 6 h.

Contact angle (CoA) of water onto the derived films was determined using a video contact angle system (FTA 32, First Ten Angstroms, Inc., Portsmouth, VA, USA) equipped with a face contact angle meter. A 10 μL of water was dropped from 10 mm height to a horizontal surface of prepared films. CoA was recorded after 30 s for excluding the influence of dispersing time on spreadability of water onto film surface. A lower CoA indicated a decreased hydrophobicity of film.

2.3.3. Emulsion stability

CNCP-CH emulsion coating suspensions were transferred to test tubes (internal diameter = 11.5 mm, height = 33.6 mm) for observing phase behavior for 7-day. The percentage of cream layer (CL) height over the whole suspensions was calculated at the end of 7-day of storage.

2.3.4. Validation of CNCP-CH coatings on pears

For validating CNCP-CH coatings on pears, three formulations with different concentrations (1, 2, and 3%) of OA were selected since OA concentration played significant role on emulsion stability ($P < 0.05$). The selected CNCP-CH coating formulations were applied on 'Bartlett' pears using the dipping method and stored at 1.7 °C and 90% RH for 1-month. 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.

Fruit weight loss (WL, %) was calculated as weight change after 2-week of storage, divided by the initial weight, and multiplied by 100% (3 pears per replicate and 9 pears in total for triplicates for each treatment). Chlorophyll content of pear peels was measured on the opposite sides of the equator of each individual fruit using a delta absorbance (DA) meter (Sinteleia, Bolonga, Italy) (Xie et al., 2014). Chlorophyll degradation (CD, %) was calculated as the reduced amount of chlorophyll content after 2-week, divided by the initial value, and multiplied by 100%.

Fruit ethylene production was determined using a gas chromatograph (GC-2014, Shimadzu, Kyoto, Japan) with a flame ionization detector (FID). The self-assembled RH (83%) controlled glass jars (3.5 L) with Vaseline sealed lid holding a 10 mm rubber septa were prepared (Deng et al., 2018). Uncoated and coated pears were precisely weighed, placed inside the jar and stored at the ambient temperature for 3 h. A 1 mL of headspace gas was collected using an air tight syringe (Series A, Valco Instrument Co., Poulsbo, WA, USA) 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, USA). 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™, Plumsteadville, PA, USA), and GC solution software (Shimadzu, Kyoto, Japan) was used for calculating the amount of ethylene production for each treatment. The obtained data were further processed to express the decreased percentage of ethylene production in coated pears in comparison with that in uncoated pears at 2- and 4-week cold storage (1.7 °C).

2.4. Experimental design and statistical analysis

All experiments were conducted in triplicates except for ethylene production (n = 2). In the CNCP-CH formulation optimization study, the analysis of variance (ANOVA) was conducted to evaluate significant effect of the individual factor and their interactions through the completely randomized two factorial design. 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 $P < 0.05$. To further understand the correlation of various CNCP-CH formulations with the measured quality properties of coating suspensions, derive films, and coated fruit, Principal Component Analysis (PCA, XLSTAT, New York, NY, USA) and Pearson's correlation matrix using SPSS Version 20 (IBM, Chicago, IL, USA) were applied.

3. Results and discussion

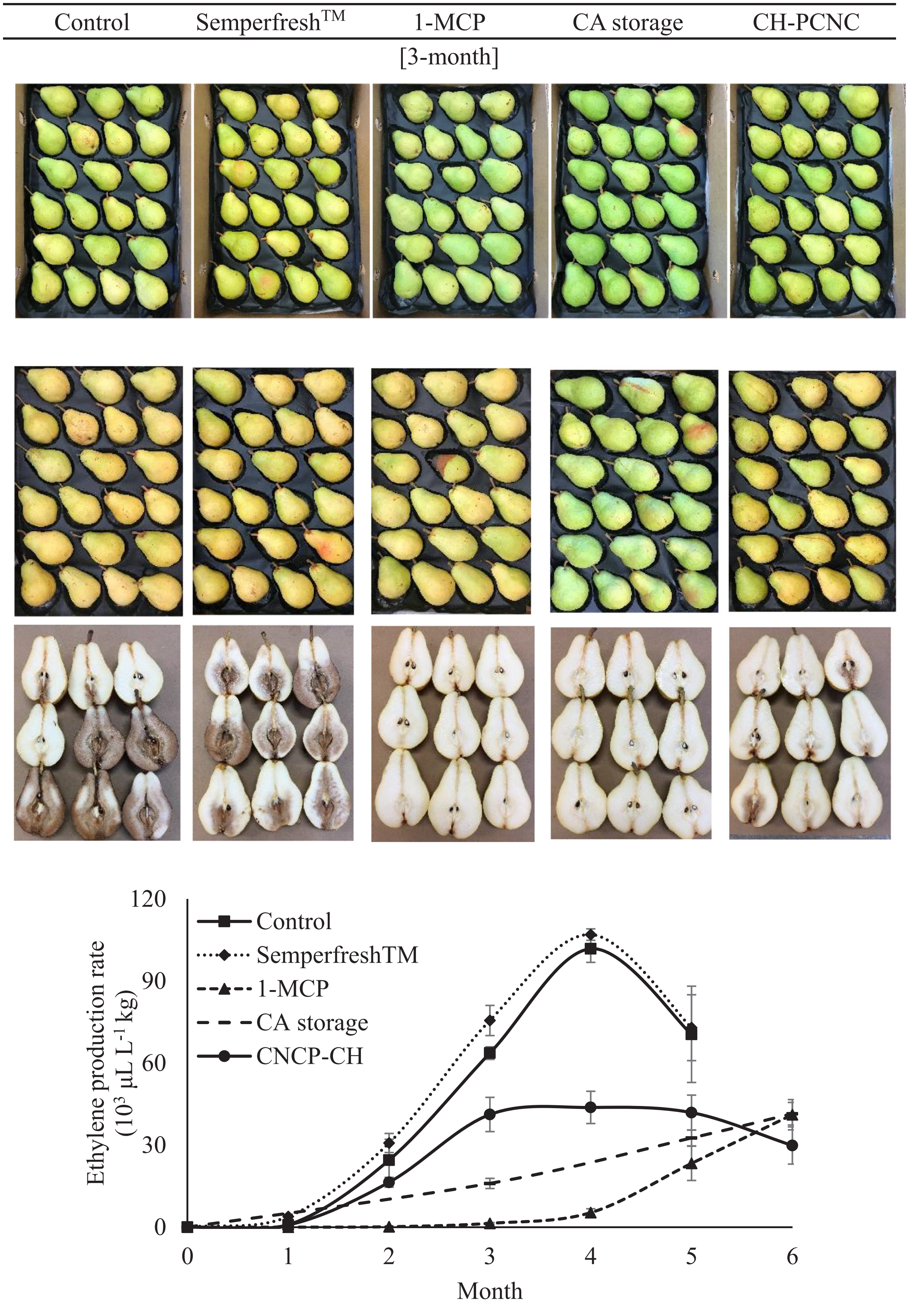
3.1. Simulated commercial long-term storage trial

The appearance, senescent core breakdown, and ethylene production of CNCP-CH coated pears during the simulated commercial long-term cold storage (−1.1 °C and > 90% RH) were compared with control (uncoated), Semperfresh™ coated, 1-MCP treated, and CA stored fruit (Fig. 1). At the end of 3-month storage, CNCP-CH, 1-MCP, and CA treated fruit retained higher chlorophyll pigment than those from control and Semperfresh™ coating. At the end of 5-month storage, CA pears retained the highest chlorophyll pigment and showed mostly delayed senescence scald, followed by CNCP-CH coated ones (Fig. 1). Meanwhile, CNCP-CH, 1-MCP, and CA samples experienced significantly lower senescent core breakdown, which could also reduce the incidence of senescent flesh browning (or breakdown). CNCP-CH, 1-MCP, and CA samples also exhibited significantly lower ethylene production than control and Semperfresh™ fruit. While the ethylene production of CNCP-CH fruit was gradually increased during the first 3-month storage, it remained significantly lower than that of control and Semperfresh™ fruit, and showed no difference from CA and 1-MCP samples from the 5th month of storage. These results indicated that the CNCP-CH coating was able to delay pear ripening, senescence scald, and quality deterioration, but its performance was not as competitive as CA and 1-MCP treatments for storage beyond 5 months. This might be because of the lack of CNCP-CH emulsion stability, which decreased its hydrophobicity and barrier property during the cold storage. Considering the cost and other side effects of CA and 1-MCP treatments on pears and the benefit of coating (Deng et al., 2017), it was necessary to optimize CNCP-CH formulation for enhancing emulsion stability and coating performance on pears. Fig. 2

3.2. Performance enhancement of CNCP-CH Pickering emulsion coating

3.2.1. Quantitative descriptive analysis

The CNCP-CH coating was further improved to satisfy the following criteria: 1) higher CoA and lower CL of coating suspension, 2) higher OIE and lower WVP of derived film, and 3) lower WL, CD, and ethylene production of coated fruit. Pearson's correlation matrices were performed in order to identify the correlations among these quality parameters of coating suspensions, derived films, and coated fruit (Table 1). The significantly positive correlations were found between quality parameters as follows: CL vs. OIE, CL vs. ethylene at 4-week, WVP vs. WL, and ethylene production at 2-week vs. that at 4-week (Table 1). Although it was aimed to obtain a negative correlation between OIE and CL for ensuring emulsion stability, they were found positively correlated, probably owning to the prolonged storage causing the separation of oil from the emulsion. While CL was measured at 7-day of



(caption on next page)

Fig. 1. Fruit appearance and senescent core breakdown at 5-month and ethylene production during 6-month of cold storage (−1.1 °C) (mean ± S.D.); Control: uncoated fruit; 1-MCP: 1-methylcyclopropene treatment; CA: controlled atmosphere storage; 3OA/0.1CNC: CNCP-CH coating with 3% oleic acid (OA), 0.1% cellulose nanocrystal (CNC), and 2% chitosan (CH); 420 pears were used for 6 months of cold storage for each treatment and control; Ethylene production of control and Semperfresh™ samples couldn't be measured as those are rotten.

storage, OIE was measured on films derived from freshly prepared coating suspension without storage. The significantly negative correlation between the quality parameters were found as follows: CL vs WVP and WL, CoA vs. WVP and WL, WVP vs. OIE, and OIE vs. WL (Table 1). Although CL, WVP, and WL were all expected to have linear relationship, they were negatively correlated. As stated above, storage time might affect CL, but not on WVP of derived films and WL of coated fruit since freshly prepared coating suspensions were used for making films and coating fruit.

Supplement Table 1 shows ANOVA results demonstrating the effect of individual factor and their interactions on measured quality parameters of coating suspensions, derived films, and coated fruit during 1-month of cold storage (1.7 °C). The concentration of OA had significant ($P < 0.05$) effect on measured parameters except on color degradation (CD) of fruit. On the other hand, the concentration of CNC only significantly ($P < 0.05$) affected the CL, and significant interaction of OA and CNC was only observed on CoA.

The properties of CNCP-CH coating suspensions and derived films with different OA and CNC concentrations are reported in Table 2. Note that only the factor showing the significant ($P < 0.05$) effect based upon ANOVA results were reported. With increased OA concentration, the CL was significantly ($P < 0.05$) enlarged, indicating less emulsion stability. The oil-phase volume fraction and storage time played a dominant role in creaming of emulsions (Sun & Gunasekaran, 2009). It was found that the phase behavior of stabilized emulsions changes along with storage time. For examples, the CNCP-CH with 2% OA showed the increased CL with extended storage time (Table 2). The OIE of CNCP-CH significantly ($P < 0.05$) increased with increased OA. For interpreting the OIE result, however, the actual encapsulation efficiency should be considered. The CNCP-CH with 1% OA had a 53% OIE, but those with higher OA (2 and 3%) were not linearly increased, having 66% and 74% OIE, respectively (Table 2), indicating that the encapsulation efficiency was higher in CNCP-CH with 1% OA than that with 2 or 3% OA. On the other hand, WVP of derived films was significantly ($P < 0.05$) lower in the CNCP-CH with higher OA concentration (2 or 3%) than that with 1% OA. These results might attribute to the non-emulsified free oil within coating formulations enhancing hydrophobicity of coating, which in turn resulted in the superficial scald of fruit due to the free oil accumulation on fruit surface. The increased concentration of CNC could significantly ($P < 0.05$) decrease the CL due to the sufficient emulsification by higher concentration of CNC Pickering agent (Kargar, Fayazmanesh, Alavi, Spyropoulos, & Norton, 2012). The significant ($P < 0.05$) interaction effect was only observed on the CoA of water on the derived films (Fig. 3). For the CNCP-CH with 1% and 2% OA, CNC didn't show significant ($P > 0.05$) effect on the CoA. At 3% OA, however, the CoA significantly ($P < 0.05$) decreased with the increased CNC concentration, indicating the increased hydrophilicity of derived films. For this reason, although the higher concentration of CNC could enhance the emulsion stability, this study selected 0.1% CNC for the modified CNCP-CH coating formulation by considering the coating hydrophobicity and cost. Hence, the CNCP-CH with 1% OA, 0.1% CNC, and 2% CH was selected as an optimal formulation to provide good emulsion stability and hydrophobicity for fruit coating, and validated on pears as reported below.

3.2.2. Quality characteristics of coating suspensions, derived films, and coated fruit

Fig. 2 illustrates the influence of OA concentration on the reduced ratio of ethylene production in coated fruit compared to control during 1-month cold storage. At 2-week, the reduced ratio of ethylene production on fruit coated by CNCP-CH with 1% OA was significantly ($P < 0.05$) higher than that of fruit coated by CNCP-CH with 2 or 3% OA. The CNCP-CH with 1 and 3% OA delayed ripening and senescence scald of fruit as shown by better retained chlorophyll pigment than that with 2% OA (Fig. 2). However, the CNCP-CH with 3% OA coating resulted in superficial scald on the surface of pears (Fig. 2), probably due to the accumulation of free oil on cells resulting in the cell damage. The oil phase in the emulsion can be divided into two parts depending on the success of emulsification: emulsified oil by surfactant molecules as spherical droplets and non-emulsified oil liquid (Zhao et al., 2018). The excessive amount of OA which was unable to be emulsified by CNC could be accumulated onto fruit surface. Successfully emulsified films/coatings with good emulsion stability and homogeneity could exhibit high tensile strength, elongation at break, and water barrier properties (Galus & Kadzińska, 2015; Quezada Gallo, Debeaufort, Callegarin, & Voilley, 2000). Hence, the modified CNCP-CH (1% OA, 0.1% CNC, and 2% CH) with good emulsion stability and less degradation of emulsion

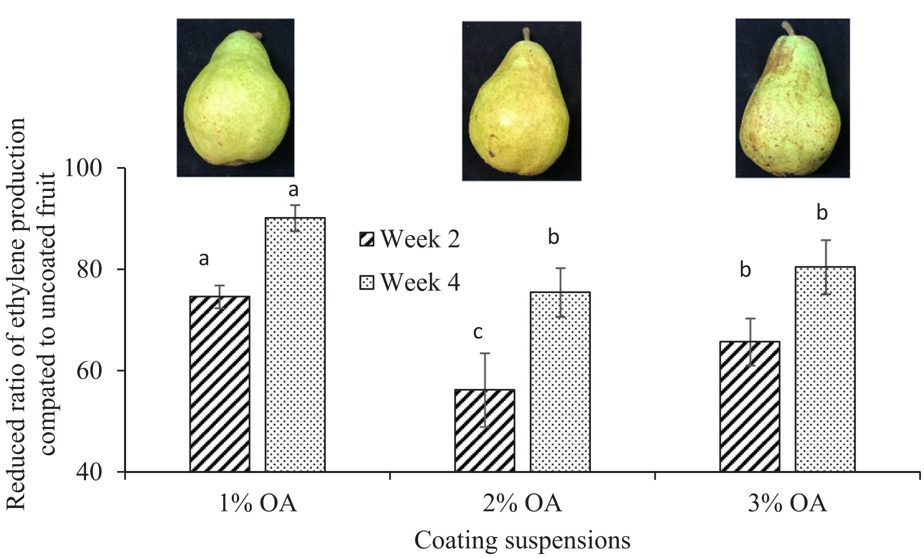


Fig. 2. Reduced ratio of ethylene production in coated fruit, compared to uncoated one at 2- and 4-week of cold storage (1.7 °C) and appearance of brown spots on fruit surface; Coatings (CNCP-CH) included different levels of OA, 0.1% cellulose nanocrystal (CNC), and 2% chitosan (CH). Five pears were used in each individual 3.5 L tightly sealed jar.

Table 1
Pearson's correlation matrix of eight measured quality parameters for coating suspension, derived film and coated fruit. All experiments were conducted in triplicates except for ethylene production ($n = 2$).

	Coating suspension		Derived film		Coated fruit			
	CL**	CoA	WVP	OIE	WL	CD	Ethylene (Week 2)	Ethylene (Week 4)
CL	–							
CoA	0.648	–						
WVP	−0.918*	−0.725*	–					
OIE	0.826*	0.665	−0.921*	–				
WL	−0.824*	−0.719*	0.891*	−0.788*	–			
CD	−0.028	−0.231	0.128	−0.166	0.351	–		
Ethylene (Week 2)	0.511	−0.117	−0.470	0.550	−0.308	0.125	–	
Ethylene (Week 4)	0.682*	0.019	−0.605	0.587	−0.523	0.128	0.879*	–

*Correlation is significant at the level of 0.05 (2-tailed).
**CL: percentage of cream layer height over the whole suspension after being stored at ambient conditions for 7-day; CoA: contact angle of water on derived film surface; WVP: water vapor permeability; OIE: oil incorporation efficiency; WL: weight loss; CD: color degradation; Ethylene: reduced ratio of ethylene production in coated fruit, compared to uncoated one, at 2- and 4-week of storage.

Table 2
Effects of oleic acid (OA; 1%, 2% and 3%, w/w) and cellulose nanocrystal (CNC; 0.1%, 0.3% and 0.5%, w/w) concentrations on percentage of cream layer (CL) formed in emulsion, oil incorporation efficiency (OIE), water vapor permeability of derived film, fruit weight loss at 7-day of storage, microscopic pictures at initial stage and phase behavior of stabilized emulsions as a function of time.

Levels of factors	Parameters				Levels of factors	Parameters	Phase behavior of stabilized emulsions as a function of time		
OA (%)*	CL (%)**	OIE (%)	WVP ($g \cdot mm/m^2 \cdot d \cdot kPa$)	WL (%)	CNC (%)	CL (%)	Day 0***	Day 3	Day 7
1	8.2[c]	52.8[c]	2.99[a]	1.21 [a]	0.5	10.1[c]			
2	12.6[b]	65.5[b]	2.40[b]	1.02[b]	0.3	11.7[b]			
3	14.4[a]	74.3[a]	2.03[b]	0.88[b]	0.1	13.4[a]			

*All emulsion formulations were incorporated with 2% chitosan (CH, w/w, wet basis).
**The CL was calculated as the percentage of cream layer height over the whole suspension after being stored at ambient conditions for 7-day. OIE: oil incorporation efficiency; WVP: water vapor permeability; WL: weight loss.
***The representative sample was modified CNC Pickering emulsion formulation (CNCP-CH) with 2% oleic acid (OA), 0.3% cellulose nanocrystal (CNC), and 2% CH. All experiments were conducted in triplicates ($n = 3$).

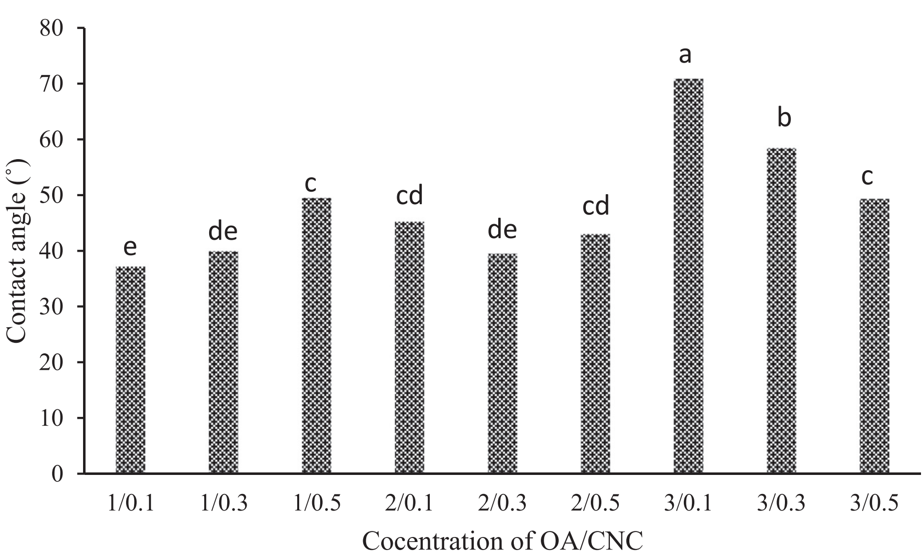


Fig. 3. Effects of oleic acid (OA) and cellulose nanocrystal (CNC) concentrations on contact angle (CoA) of water on films derived from various CNC Pickering emulsion (CNCP-CH); 1/0.1, 1/0.3, 1/0.5…3/0.5 indicated the concentration of OA/CNC; All CNCP-CH contained 2% chitosan; The same letter placed above bar chart were not significantly different ($P > 0.05$) using LSD.

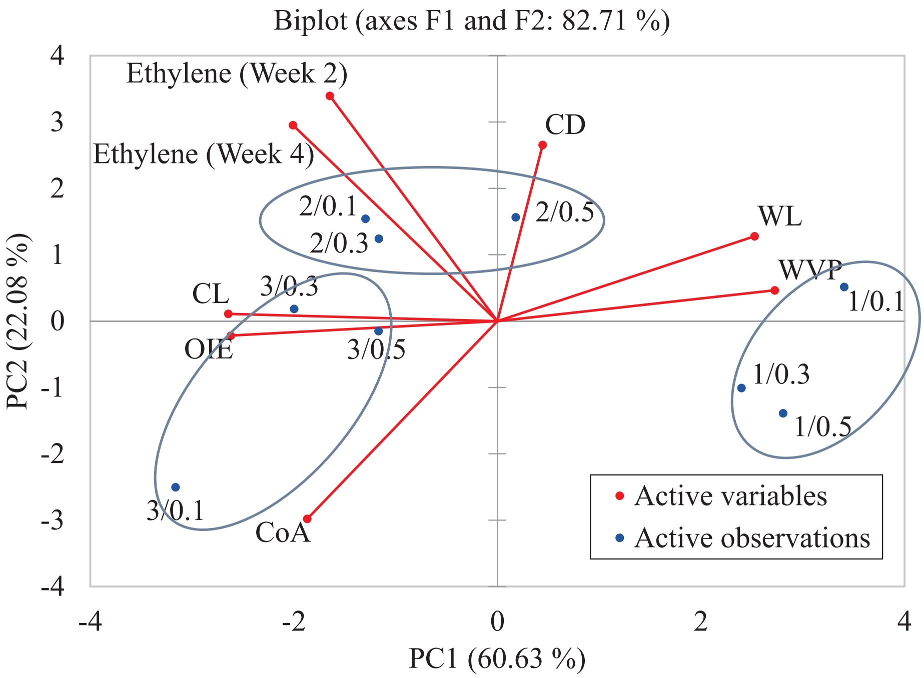


Fig. 4. Principal component analysis (PCA) bi-plots for different Pickering emulsion (CNCP-CH) in relation to fruit quality; 3/0.1, 3/0.3, 3/0.5, 2/0.1… 1/0.5 indicated the concentration of oleic acid (OA)/cellulose nanocrystals (CNC); CL: percentage of cream layer height over the whole suspension at 7-days of storage; CoA: contact angle of water on derived film surface; WVP: water vapor permeability; OIE: oil incorporation efficiency; WL: weight loss; CD: color degradation; Ethylene: reduced ratio of ethylene production in coated fruit, compared to uncoated one, at 2- and 4-week of storage.

during prolonged storage could provide stable barrier onto pear surface for delaying fruit ripening and superficial scald.

3.3. Correlation between CNCP-CH formulations and measured quality parameters of coating formulation, derived films, and coated fruit

The principal component analysis (PCA) was applied to understand the correlation between CNCP-CH formulations with different concentrations of OA and CNC and measured quality parameters of coating formulations, derive films, and coated fruit (Fig. 4). PCA can profile data in a smaller number of dimensions than the total attributes in the profile (principal component) (Moussaoui & Varela, 2010). The first and the second principal components described 82.71% of the variability (60.63 and 22.08%, respectively). The positive relation was found between CL and OIE, ethylene production at 2-week and 4-week, and WL and WVP, respectively, corresponding to the Pearson's correlation matrices. The first principal component (PC1) separated 9 different types of CNCP-CH mainly depending on concentration of OA, grouping CNCP-CH with 1% OA from those with 2 and 3% OA. This result could corroborate the ANOVA results where the concentration of OA was the significant ($P < 0.05$) factor on the measured quality parameters (Supplement Table 1). It was also observed that the CNCP-CH with 1% OA was negatively correlated to the ethylene production regardless of the concentration of CNC, indicating the least ethylene production of fruit. Hence, this result demonstrated that the modified CNCP-CH Pickering emulsion coating could delay ripening and reduced superficial scald of coated pears.

4. Conclusions

The emulsion stability highly depends on the ratio between oil phase and emulsifier, which could directly affect emulsion coating performance on postharvest pears. The CNCP-CH Pickering emulsion coating were modified by enhancing emulsion stability during the prolonged cold storage, which was anticipated to provide stable barrier for green 'Bartlett' pears. Through a systematic experimental approach using a completely randomized two factorial design and Principal Component Analysis (PCA), it was identified that the concentration of oil phase (oleic acid in this study) was the most critical factor affecting emulsion stability and hydrophobicity, and 1% OA was the optimal concentration for 0.1% CNC Pickering agent. The CNCP-CH emulsion coating was thus enhanced with 1% OA, 0.1% CNC, and 2% CH. Consistently, the ethylene production and superficial scald of coated fruit with the modified CNCP-CH coating was significantly reduced in comparison with the CNCP-CH with other OA and CNC concentrations. The simulated commercial long-term storage trial for the developed CNCP-CH in comparison with 1-MCP and CA storage is under the way. The effect of developed Pickering emulsion coating will be also validated for other climacteric fruit, such as apple, mango, or avocado, for delaying ripening and retaining quality during the prolonged storage time.

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.

Acknowledgements

The authors express their gratitude for the financial support of Oregon Department of Agricultural Specialty Crop Block Grant and Ms. Caoxia Li and Dr. Yu Dong from the Mid-Columbia Agricultural Research and Extension Center, Oregon State University for their help in the long-term storage of fruit.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.125693.

References

Arnon, H., Zaitsev, Y., Porat, R., & Poverenov, E. (2014). Effects of carboxymethyl cellulose and chitosan bilayer edible coating on postharvest quality of citrus fruit. *Postharvest Biology and Technology, 87*, 21–26.
BMT. (2012). Pears. from http://www.cargohandbook.com/index.php/Pears.
Chiumarelli, M., & Hubinger, M. D. (2012). Stability, solubility, mechanical and barrier properties of cassava starch – Carnauba wax edible coatings to preserve fresh-cut apples. *Food Hydrocolloids, 28*(1), 59–67.
Darder, M., Colilla, M., & Ruiz-Hitzky, E. (2003). Biopolymer–Clay nanocomposites based on chitosan intercalated in montmorillonite. *Chemistry of Materials, 15*(20),

3774–3780.
de Chiara, M. L. V., Pal, S., Licciulli, A., Amodio, M. L., & Colelli, G. (2015). Photocatalytic degradation of ethylene on mesoporous TiO_2/SiO_2 nanocomposites: Effects on the ripening of mature green tomatoes. *Biosystems Engineering, 132*, 61–70.
Deng, Z., Jung, J., Simonsen, J., Wang, Y., & Zhao, Y. (2017). Cellulose nanocrystal reinforced chitosan coatings for improving the storability of postharvest pears under both ambient and cold storages. *Journal of Food Science, 82*(2), 453–462.
Deng, Z., Jung, J., Simonsen, J., & Zhao, Y. (2018). Cellulose nanocrystals Pickering emulsion incorporated chitosan coatings for improving storability of postharvest Bartlett pears (*Pyrus communis*) during long-term cold storage. *Food Hydrocolloids, 84*, 229–237.
Dhall, R. K. (2013). Advances in edible coatings for fresh fruits and vegetables: A review. *Critical Reviews in Food Science and Nutrition, 53*(5), 435–450.
Drake, S. R., Elfving, D. C., Drake, S. L., & Visser, D. B. (2004). Quality of modified atmosphere packaged "Bartlett" pears as influenced by time and type of storage. *Journal of Food Processing and Preservation, 28*(5), 348–358.
East, A. R., Smale, N. J., & Trujillo, F. J. (2013). Potential for energy cost savings by utilising alternative temperature control strategies for controlled atmosphere stored apples. *International Journal of Refrigeration, 36*(3), 1109–1117.
Galus, S., & Kadzińska, J. (2015). Food applications of emulsion-based edible films and coatings. *Trends in Food Science & Technology, 45*(2), 273–283.
Gómez-Estaca, J., Montero, P., Giménez, B., & Gómez-Guillén, M. C. (2007). Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*). *Food Chemistry, 105*(2), 511–520.
Jung, J., Deng, Z., Simonsen, J., Bastías, R. M., & Zhao, Y. (2016). Development and preliminary field validation of water-resistant cellulose nanofiber based coatings with high surface adhesion and elasticity for reducing cherry rain-cracking. *Scientia Horticulturae, 200*, 161–169.
Kargar, M., Fayazmanesh, K., Alavi, M., Spyropoulos, F., & Norton, I. T. (2012). Investigation into the potential ability of Pickering emulsions (food-grade particles) to enhance the oxidative stability of oil-in-water emulsions. *Journal of Colloid and Interface Science, 366*(1), 209–215.
Kupferman, E. (2003). Controlled atmosphere storage of apples and pears. *Acta Horticulturae, 600*, 729–735.
Moussaoui, K. A., & Varela, P. (2010). Exploring consumer product profiling techniques and their linkage to a quantitative descriptive analysis. *Food Quality and Preference, 21*(8), 1088–1099.
National Agricultural Statistics Service (NASS). United States Department of Agriculture (USDA). (2015). Noncitrus Fruits & Nuts. 78.
Paraskevopoulou, A., Boskou, D., & Kiosseoglou, V. (2005). Stabilization of olive oil – Lemon juice emulsion with polysaccharides. *Food Chemistry, 90*(4), 627–634.
Quezada Gallo, J.-A., Debeaufort, F., Callegarin, F., & Voilley, A. (2000). Lipid hydrophobicity, physical state and distribution effects on the properties of emulsion-based edible films. *Journal of Membrane Science, 180*(1), 37–46.
Sun, C., & Gunasekaran, S. (2009). Effects of protein concentration and oil-phase volume fraction on the stability and rheology of menhaden oil-in-water emulsions stabilized by whey protein isolate with xanthan gum. *Food Hydrocolloids, 23*(1), 165–174.
Taherian, A. R., Britten, M., Sabik, H., & Fustier, P. (2011). Ability of whey protein isolate and/or fish gelatin to inhibit physical separation and lipid oxidation in fish oil-in-water beverage emulsion. *Food Hydrocolloids, 25*(5), 868–878.
Vijayan, S., Arjunan, T. V., & Kumar, A. (2016). Mathematical modeling and performance analysis of thin layer drying of bitter gourd in sensible storage based indirect solar dryer. *Innovative Food Science & Emerging Technologies, 36*, 59–67.
Villalobos-Acuna, M., & Mitcham, E. J. (2008). Ripening of European pears: The chilling dilemma. *Postharvest Biology and Technology, 49*, 187–200.
Wang, Y., & Sugar, D. (2013). Internal browning disorder and fruit quality in modified atmosphere packaged 'Bartlett' pears during storage and transit. *Postharvest Biology and Technology, 83*, 72–82.
Wang, Y., & Sugar, D. (2015). 1-MCP efficacy in extending storage life of 'Bartlett' pears is affected by harvest maturity, production elevation, and holding temperature during treatment delay. *Postharvest Biology and Technology, 103*, 1–8.
Whitaker, B. D., Villalobos-Acuña, M., Mitcham, E. J., & Mattheis, J. P. (2009). Superficial scald susceptibility and α-farnesene metabolism in 'Bartlett' pears grown in California and Washington. *Postharvest Biology and Technology, 53*(1), 43–50.
Xie, X., Song, J., Wang, Y., & Sugar, D. (2014). Ethylene synthesis, ripening capacity, and superficial scald inhibition in 1-MCP treated 'd'Anjou' pears are affected by storage temperature. *Postharvest Biology and Technology, 97*, 1–10.
Zhao, S., Tian, G., Zhao, C., Li, C., Bao, Y., DiMarco-Crook, C., ... Zheng, J. (2018). The stability of three different citrus oil-in-water emulsions fabricated by spontaneous emulsification. *Food Chemistry, 269*, 577–587.