



Citrus pectin modified by microfluidization and ultrasonication: Improved emulsifying and encapsulation properties

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ARTICLE INFO

Keywords:

Pectin
Ultrasound
Interfacial property
Microencapsulation
Emulsion

ABSTRACT

In this study, modified citrus pectin treated with a combination of microfluidization and ultrasonication was compared to the original and ultrasonication treated pectin on hydrodynamic diameter, molecular weight, polydispersity, zeta potential, apparent viscosity, Fourier-transform infrared spectroscopy (FTIR), 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging capacity, scanning electron microscope (SEM), atomic force microscopy (AFM), their emulsifying properties and encapsulation properties. Modified pectin treated with a combination of microfluidization and moderate ultrasonication (MUB) was found to have lowest hydrodynamic diameter (418 nm), molecular weight (237.69 kDa) and polydispersity (0.12), and relatively low apparent viscosity among all pectin samples. Furthermore, it showed significantly higher DPPH radical scavenging capacity than the original pectin although only slightly higher than that of ultrasonication treated one (UB). MUB showed a thin fibrous morphology and decreased degree of branching from SEM and AFM. Emulsion stabilized by MUB had highest centrifugal and thermal stability compared to emulsions stabilized by UB and the original pectin. This could be attributed to higher interfacial loading of MUB (17.90 mg/m²) forming more compact interfacial layer observed by confocal laser scanning microscopy (CLSM). Moreover, both MUB and UB exhibited improved encapsulation functionality to protect cholecalciferol (vitamin D₃) from UV degradation compared to the original pectin.

1. Introduction

As a biological processing byproduct, pectin has been widely used as a thickening agent, gelling agent and stabilizer in bakery, dairy products, jams, and confectionary. Meanwhile it also can be utilized as dietary fiber, prebiotics and fat replacer [1]. Recently, the role of pectin as an emulsifier and encapsulation wall material was gaining more attention [2–4]. In general, the emulsifying capacity of pectin is mainly attributed to the hydrophobic groups in pectin molecules such as methoxyl group, acetyl group, etc. depending on the species and chemical structure [5]. In terms of emulsion stability, pectin is capable of stabilizing emulsions, largely owing to its ability to elevate the apparent viscosity of the continuous phase in emulsions [6] and its unique amphiphilic character which assists in reducing the interfacial tension between oil and aqueous phases [7–9]. However, natural pectin has limited emulsifying properties therefore modification of pectin is under consideration.

Ultrasound is an environmentally friendly physical technique to extract, degrade and breakdown biopolymers with relatively low cost [10]. It has been manifested effectively to increase antioxidant capacity and solubility, reduce viscosity and molecular size for natural pectin [11–13], soy protein [14] and pea protein [15]. In addition to the size-reduction effect, ultrasonication also showed profound effects on the chemical motifs, such as decreasing the degree of esterification and branching [16]. These chemical modifications may further alter surface properties and mobility of pectin molecules, which were highly associated with its emulsifying properties [17]. Recently, Mungure, et al. [2] summarized potential application of pectin for the stabilization of nanoemulsions indicating that power ultrasound would improve the encapsulation load of pectin nanoparticles. Our previous study has also demonstrated that ultrasound treatment enabled the improved emulsification stability of pectin and the encapsulation feasibility when applied to coffee-like aroma compound [18].

As an alternative to homogenizations, microfluidization is gaining

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renewed interest owing to its superb capacity of producing nanoemulsions [19]. A typical microfluidizer applies an extremely high pressure to guide the flow stream through microchannels toward the impingement area, meanwhile creating a very high shearing force and cavitation effects which is able to reduce emulsion droplet size as well as break down biopolymers [19,20]. Chen, et al. [21] found a substantial size reduction of pectin from 627 nm to 274 nm after microfluidization at 200 MPa, which demonstrated the possibility of achieving better emulsifying properties of pectin. Therefore, we hypothesized a new approach coupling microfluidization and ultrasonication, can have a synergistic effect in breaking down pectin structure and resulting in modified pectin that persist enhanced antioxidant capacity, emulsifying and encapsulation properties.

The specific objectives of this study were to 1) investigate the impact of microfluidization and ultrasonication on the physical properties and antioxidant capacity of citrus pectin, and 2) compare the stability, interfacial properties, and encapsulation properties of emulsions stabilized by pectin treated differently. It is anticipated that this study could provide insights on how microfluidization and ultrasonication benefits the emulsifying and encapsulation properties of pectin and the underlying mechanism.

2. Materials and methods

2.1. Material and chemical reagents

Citrus pectin (P9135) was purchased from Sigma-Aldrich. It had 485 kDa of the weight average molecular weight and 57.86% of degree of methoxylation (Table 2). Canola oil (Crisco, Orrville, OH, USA) was purchased from a local store. All other chemical reagents were analytical grade from Fisher Scientific Co.

2.2. Treatment of pectin

2.2.1. Microfluidization of pectin

The experiment was divided into eight groups (see Table 1). Among them, three groups of pectin (0.5 g/100 mL) were treated by microfluidizer (Microfluidics™ M-110P, Westwood, MA, USA) under 138 MPa (20000 psi) for twice. PH of pectin samples were tested around 3.7 before and after treatments.

2.2.2. Ultrasonication of pectin

Ultrasound (20 kHz, 750 W) was transmitted through a probe with a tip of 13 mm diameter (Sonics, VC750, USA). Duty cycle of ultrasound pulse was set at 50% (2 s on : 2 s off). Each time, 60 mL of pectin solution at the concentration of 0.5 g/100 mL was added in a 100 mL beaker placed in an ice bath to avoid overheating under sonication for 20 min and the probe was immersed into the solution with 10 mm height. The amplitude percentage (%) set on the device could be transferred into power density (W/mL) by the calorimetric method. In this study, the amplitude of 40%, 70%, 100% were equivalent to power density of 0.40, 0.76, 1.12 W/mL, respectively. Treated pectin solution was held at room temperature for 1 h before further analysis. PH of pectin samples were tested around 3.7 before and after treatments.

Table 1
Experimental design.

	Microfluidization	Ultrasonid
Control	N/A	N/A
UA	N/A	0.40 W/mL
UB	N/A	0.76 W/mL
UC	N/A	1.12 W/mL
MUA	138 MPa, twice	0.40 W/mL
MUB	138 MPa, twice	0.76 W/mL
MUC	138 MPa, twice	1.12 W/mL

2.3. Characterization of pectin

2.3.1. Hydrodynamic diameter, polydispersity index (PDI) and zeta-potential

The pectin solutions were diluted 20 times prior to hydrodynamic diameter analysis. The hydrodynamic diameters of the pectin nanoparticles were determined using dynamic light scattering (DLS) by ZetaPALS (Brookhaven Instruments Corporation, Brookhaven, NY, USA) and analyzed by multimodal size distribution (MSD) mode. Zeta-potential was analyzed by the same instrument after 200 times of dilution.

2.3.2. Molecular weight (Mw)

The Mw of pectin samples were determined using previous HPLC method [11].

2.3.3. Degree of methylation (DM) and degree of acetylation (DA)

DM and DA were expressed as the molar percent of methanol or acetic acid to the galacturonic acid content using HPLC methods according to our previous study [11].

2.3.4. Apparent viscosity

The apparent viscosity of the pectin solution samples was determined using a rotational rheometer (ARES G2, TA Instruments, New Castle, DE, USA) with a parallel steel plate (50 mm diameter, 1.0 mm gap). Flow curves with increasing shear rate from 0.1 to 100 s⁻¹ were measured at 25 °C.

2.3.5. Scanning electron microscope (SEM)

The morphological properties of pectin with or without treatments were obtained using a scanning electron microscope (SEM) equipped with a field emission electron gun (S-4700 High Resolution SEM, Hitachi, Tokyo, Japan). Samples were subjected to freeze-drying (Labconco Freezone 6, Labconco Corp., Kansas City, MO, USA) for 48 h, followed by adhering to a conductive carbon tape and sputter coating (model Dest-1 TSC, Denton Vacuum LLC., Moorestown, NJ, USA) with a gold layer before imaging.

2.3.6. Atomic force microscopy (AFM)

The original pectin and pectin after treatments were imaged using a Cypher ES Environmental AFM (Cypher ES, Oxford Instruments Asylum Research, Santa Barbara, CA, USA). Pectin samples were diluted to the concentration of 10 µg/mL, dehydrated on the mica surface, and then scanned by AFM with the BS-Tap300Al (Budget Sensors, USA) tip using the Acoustical Alternating Current (AAC) tapping mode.

2.3.7. Fourier-transform infrared (FTIR) spectroscopy

Prior to FTIR analysis, freeze dried pectin sample was dehydrated at 45 °C for at least 2 h to minimize the impact of the residual moisture. After that, the pectin sample (5 mg) was mixed with KBr (1:100 w/w) and pressed into a semitransparent pellet for FTIR analysis. The spectrum was recorded using an IR spectrometer (Thermo Nicolet Nexus 670, Thermo Scientific Inc., Waltham, MA, USA) within the frequency range of 4000 to 400 cm⁻¹, and the sample was scanned 32 times at the resolution of 4 cm⁻¹.

2.3.8. Antioxidant capacity

Total antioxidant capacity was determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, according to our previous published methods with minor modifications [11]. The results of DPPH assay were expressed as percentage of DPPH radical inhibition (%).

2.4. Emulsion preparation

All emulsions were prepared using canola oil and pectin solutions from different treatment conditions. Six milliliter canola oil containing

vitamin D₃ (0.5 mg/mL) was added dropwise into the 24 mL of pectin solution (0.5 g /100 mL) while mixing with a high-speed homogenizer at 12,000 rpm for 40 s (IKA-ULTRA-TURRAX® T25 basic, IKA® Works, Inc., Wilmington, NC, USA). The mixture was then homogenized for another 1 min at 12,000 rpm after all the oil was added, prior to the following analysis. PH of emulsion samples were tested around 3.7, which were the same as pectin samples.

2.5. Emulsion characterization

2.5.1. Droplet size analysis

The size distributions of emulsion were analyzed using a laser diffraction particle sizer (SALD-2300, Shimadzu, Kyoto, Japan). The volume average diameter ($d_{4,3}$) was reported and surface average diameter ($d_{3,2}$) was used for interfacial loading calculation in section 2.5.5. The refractive indices were taken to be 1.47 for the oil phase and 1.33 for the dispersant.

2.5.2. Centrifugal stability of emulsion

The centrifugal stability was evaluated by accelerated centrifugal test. 1.8 mL of emulsion was placed into 2 mL micro centrifuge tubes. The samples were then centrifuged at 14,000 g for 20 min (model MiniSpin® plus, Eppendorf, Westbury, NY, USA). The weight (mg) of released oil in upper layer was transferred and weighed.

2.5.3. Thermal stability of emulsion

The thermal stability was evaluated under 45, 55, and 65 °C. One milliliter freshly made emulsion was placed in the oven at specific temperature. After 90 min, droplet sizes of emulsion were measured using the same method in section 2.5.1.

2.5.4. Interfacial structure

The interfacial structure of the emulsion droplets was observed using a Zeiss LSM 700 confocal microscope (Zeiss, Germany) with two excitation lines (488 nm for the FITC dye and 639 nm for the Nile red dye). The emulsion samples were first diluted 20 times using deionized water and then 1 mL of diluted samples were stained with a mixture (40 µL) of 0.5 mg/mL FITC and 1 mg/mL Nile red in 1:1 ethanol and water (v:v) solution for 60 min. Images were taken at a magnification of 20x, 40x, and further processed using the software ZEN 2.3 (Carl Zeiss Microscopy GmbH, Oberkochen, Germany).

2.5.5. Interfacial loading

The interfacial loading of pectin (τ) was quantified following a protocol from a previous study [17]. Briefly, the fresh emulsion was centrifuged at 100 g (model IEC CENTRA CL2, Thermo Scientific Inc., Waltham, MA, USA) for 20 min. The pectin concentration in the serum phase was determined using the weighing method. The interfacial loading Γ (mg/m²) can be calculated by the following equation (1):

$$\Gamma(\text{mg}/\text{m}^2) = (C_0 - C_f) \times d_{3,2}/6\phi \quad (1)$$

where C_0 is the initial pectin concentration in the aqueous phase, C_f is the final concentration of pectin in the serum phase, $d_{3,2}$ is the surface average diameter of emulsion droplets, and ϕ is the volume fraction of oil.

2.6. UV stability of vitamin D₃

The stability of vitamin D₃ was measured following a previous work [14]. Briefly, emulsion samples were divided into three replicates for each group and 4 mL of each were placed into 15 mL poly-styrene tubes and exposed to UV light (Transilluminator FBTIV-614, Fisher Scientific, Pittsburgh, PA, USA). After 0, 20, 40, and 60 min, 0.1 mL was sampled from each tube. Then 0.9 mL of pure methanol was added, vortexed, and centrifuged for vitamin D₃ extraction. After filtration (0.22 µm pore size, 13 mm diameter, PTFE syringe filter, Whatman, Piscataway, NJ,

USA), 10 µL of extracted sample was injected into reverse phase HPLC (Waters e2695 Separations Module, Waters, Milford, MA, USA) with a C18 column (ODS-2 Hypersil, 5 µm, 250 × 46 mm, Thermo Scientific Inc., Waltham, MA, USA) at 40 °C and a photo diode array detector (Waters PDA 2996) at 265.3 nm for accurate quantification of vitamin D₃. The mobile phase was 100% methanol at 1 mL/min.

2.7. Statistical analysis

All experiments were performed in three replicates. The data was analyzed using one-way ANOVA with Duncan test by SPSS (16.0) at a significant level of 0.05.

3. Results and discussion

3.1. The impact of microfluidization and ultrasonication on pectin

3.1.1. Hydrodynamic diameter, polydispersity, zeta potential and Mw of pectin

In this study, it was found that ultrasonication was an effective approach in reducing hydrodynamic diameter and M_w , decreasing polydispersity for pectin (Table 2). Pectin treated by ultrasound with higher power density (UB and UC) showed lower hydrodynamic diameter than the lower power density treatment (UA) ($P > 0.05$), which could be due to enhanced degradation effect resulted from stronger cavitation effect. Besides, the combination of microfluidization and ultrasonication (MUA, MUB, MUC) slightly decreased the hydrodynamic diameter of pectin when compared to UA, UB and UC, respectively. This slight decrease was consistent with the change of molecular weight leading to a small sized mesoscopic structure [22]. However, there was no significant difference of hydrodynamic diameter between MUA, MUB, and MUC, indicating pectin with hydrodynamic diameter of 400–500 nm could not be further severely degraded under the condition used in this study. Moreover, there was no significant difference for DM and DA among those pectin samples although DM of MUA, MUB, and MUC were slightly lower than UA, UB and UC, respectively. With respect to polydispersity, all modified pectin solution showed significantly ($P < 0.05$) lower values than the natural pectin, indicating narrower hydrodynamic diameter distribution of pectin. Among all treated groups, MUB (pectin treated by the combination of microfluidization and ultrasound with power density of 0.76 W/mL) showed the lowest polydispersity, which suggested pectin after MUB was the most homogenous sample. These results about reduction of hydrodynamic diameter and polydispersity could attribute to degradation of pectin by ultrasound and microfluidization. Previous studies also found that the hydrodynamic diameter in the solution was highly and positively correlated with the molecular weight of pectin [21,23]. Wang, et al. [10] reviewed effect of ultrasound on the molecular weight and polydispersity of two types of most common pectin products including citrus pectin and apple pectin. Both molecular weight and polydispersity decreased significantly due to the breakdown of glycosidic bond after intensive mechanical forces and sonochemical reaction provided by sonication. Chen, et al. [21] found that high pressure microfluidization degraded pectin by decreasing molecular weight and hydrodynamic diameter. However, no significant reduction was found in the zeta-potential after all the treatments or their combinations ($P > 0.05$), indicating the free carboxyl group of pectin that directly related to degree of methoxylation did not change dramatically after treatment, which accord with the results of DM in Table 2.

3.1.2. Flow behavior

Apparent viscosity was measured to characterize the rheological properties of pectin solutions with or without treatments (Fig. 1) and the flow curves were fitted to the Power-law equation as follows,

$$\sigma = K \cdot \dot{\gamma}^n(2)$$

Table 2
Physicochemical properties of pectin.

	Hydrodynamic diameter (nm)	Polydispersity	Zeta potential (mV)	Mw (kDa)	DM (%)	DA (%)
Control	1288.97 ± 47.17 ^a	0.27 ± 0.03 ^a	-17.98 ± 1.95 ^a	485.00 ± 8.11 ^a	57.86 ± 0.19 ^a	1.57 ± 0.10 ^a
UA	480.45 ± 26.52 ^b	0.15 ± 0.00 ^b	-16.96 ± 0.67 ^a	286.37 ± 25.66 ^b	56.75 ± 2.83 ^a	1.67 ± 1.13 ^a
UB	436.15 ± 5.16 ^{bc}	0.18 ± 0.00 ^b	-15.10 ± 2.54 ^a	252.13 ± 2.97 ^{cd}	54.87 ± 3.99 ^a	1.92 ± 0.03 ^a
UC	429.95 ± 7.42 ^{bc}	0.16 ± 0.01 ^b	-15.70 ± 1.83 ^a	247.10 ± 4.38 ^{cd}	56.60 ± 4.13 ^a	1.31 ± 0.51 ^a
MUA	440.17 ± 12.61 ^{bc}	0.15 ± 0.01 ^b	-14.81 ± 0.76 ^a	255.29 ± 10.09 ^c	55.78 ± 1.17 ^a	1.36 ± 0.42 ^a
MUB	418.60 ± 16.62 ^c	0.12 ± 0.02 ^c	-15.13 ± 2.16 ^a	237.69 ± 3.78 ^d	53.57 ± 1.99 ^a	1.89 ± 0.08 ^a
MUC	422.20 ± 21.03 ^c	0.16 ± 0.01 ^b	-15.22 ± 1.12 ^a	240.59 ± 8.01 ^{cd}	54.95 ± 4.53 ^a	1.65 ± 0.47 ^a

Control: untreated pectin; UA: ultrasound modified pectin using power density of 0.40 W/L; UB: ultrasound modified pectin using power density of 0.76 W/L; UC: ultrasound modified pectin using power density of 1.12 W/L; MUA: pectin treated by microfluidization and 0.40 W/L of ultrasound; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound; MUC: pectin treated by microfluidization and 1.12 W/L of ultrasound; Mw: Molecular weight; DM: Degree of methoxylation; DA: Degree of acetylation.

Different lower letters in the superscript within the column indicated significant difference ($P < 0.05$).

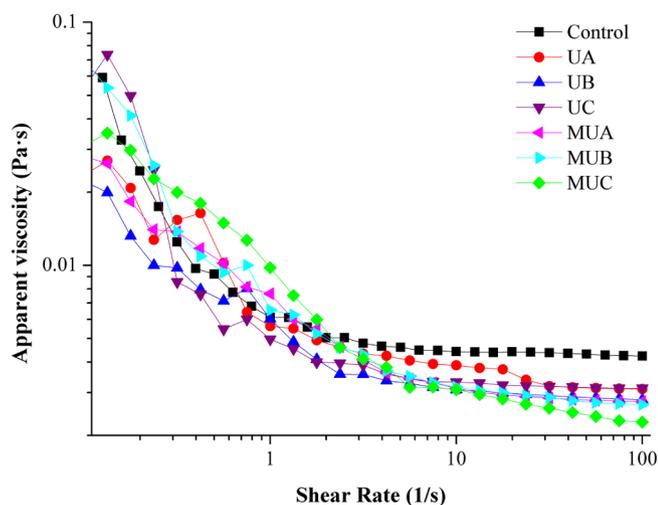


Fig. 1. Flow behavior of pectin solution (0.5%) with different treatments. Control: untreated pectin; UA: ultrasound modified pectin using power density of 0.40 W/L; UB: ultrasound modified pectin using power density of 0.76 W/L; UC: ultrasound modified pectin using power density of 1.12 W/L; MUA: pectin treated by microfluidization and 0.40 W/L of ultrasound; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound; MUC: pectin treated by microfluidization and 1.12 W/L of ultrasound.

Table 3
Power-law parameters of apparent viscosity of pectin.

	K	n	R^2
Control	0.005 ± 0.000	0.973 ± 0.007	1.000
UA	0.004 ± 0.000	0.924 ± 0.012	0.999
UB	0.003 ± 0.000	0.956 ± 0.007	1.000
UC	0.003 ± 0.000	0.974 ± 0.013	0.999
MUA	0.003 ± 0.000	0.948 ± 0.012	0.999
MUB	0.004 ± 0.000	0.922 ± 0.016	0.998
MUC	0.004 ± 0.000	0.856 ± 0.017	0.997

Control: untreated pectin; UA: ultrasound modified pectin using power density of 0.40 W/L; UB: ultrasound modified pectin using power density of 0.76 W/L; UC: ultrasound modified pectin using power density of 1.12 W/L; MUA: pectin treated by microfluidization and 0.40 W/L of ultrasound; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound; MUC: pectin treated by microfluidization and 1.12 W/L of ultrasound.

where σ is the shear stress (Pa), γ is shear rate (s^{-1}), K is consistency index ($Pa \cdot s^n$), and n is the flow behavior index (dimensionless). The values of γ and K were shown in Table 3. The flow behavior indices of the all pectin solutions were < 1.0 , indicating all the pectin solutions shared a shear thinning behavior, which are consistent with previous works [6,11]. Consistency index (K) of all modified pectin solutions

showed lower values than the control (Table 3), contributing to lower viscosity. At low shear rates, the apparent viscosity of all samples extensively fluctuated, without a significant difference among each other. As the shear rate increased to $> 2 s^{-1}$, the original pectin without treatment became more viscous compared to other treated samples. MUB and MUC, which processed by microfluidization at 138 MPa twice and 0.76 W/mL or 1.12 W/mL power density of ultrasound for 20 min respectively, resulted in lower viscosity at high shear rate which was correlated with their reduced molecular weight in Table 2. The decrease of viscosity after microfluidization and ultrasonication was attributed to the molecular size reduction which was closely related to the decrease of molecular weight and polydispersity [24,25]. According to the Mark-Houwink-Sakurada equation (3), the viscosity of a solution is positively correlated with the molecular weight [26].

$$\eta = kM^\alpha \quad (3)$$

where η was the intrinsic viscosity (L/g); k and α were constants depending on the solution and temperature; M was the viscosity-average molecular weight. As the molecular weight was reduced by microfluidization and ultrasonication, the viscosity of pectin solution decreased.

3.1.3. FTIR

The FTIR spectra of pectin are shown in Fig. 2(a). For the original pectin without treatment, a major absorption at around $3411 cm^{-1}$ was found due to the stretching of hydroxyl groups, while the absorption at $2933 cm^{-1}$ was attributed to C-H stretching of CH_2 groups. An absorption around $1750 cm^{-1}$ was attributed to C=O stretching vibration of ester carbonyl, while the absorption around $1630 cm^{-1}$ was attributed to C=O stretching vibration of carboxyl group. The ratio of the peak area around $1750 cm^{-1}$ (COO-R) over the sum of areas under the bands at $1750 cm^{-1}$ (COO-R) and $1630 cm^{-1}$ (COOH) is typically denoted as the degree of methoxylation (DM). In this study, those two peaks were almost the same among all three pectin samples, indicating similar degree of methoxylation of pectin were found among the control, ultrasound-treated one, and the one treated by the combination of microfluidization and ultrasonication [11]. Although Zhang, et al. [16] found citrus pectin treated by ultrasound for 40 min obtained relatively lower DM than the control, DM of treated pectin in this study did not alter significantly, possibly due to short treatment time (20 min). These results also supported close values of zeta-potential from pectin solutions and similar level of DM of pectins shown in Table 2.

3.1.4. DPPH scavenging capacity

Pectin is basically a polymer of α -(1-4)-linked D-galacturonic acid with plenty of hydroxyl groups at the 2' and 3' carbon atom positions. Those hydroxyl groups have hydrogen- or electron-donating properties meanwhile DPPH having an unpaired electron at nitrogen atom is a stable free radical. Pectin from different sources including apple pectin,

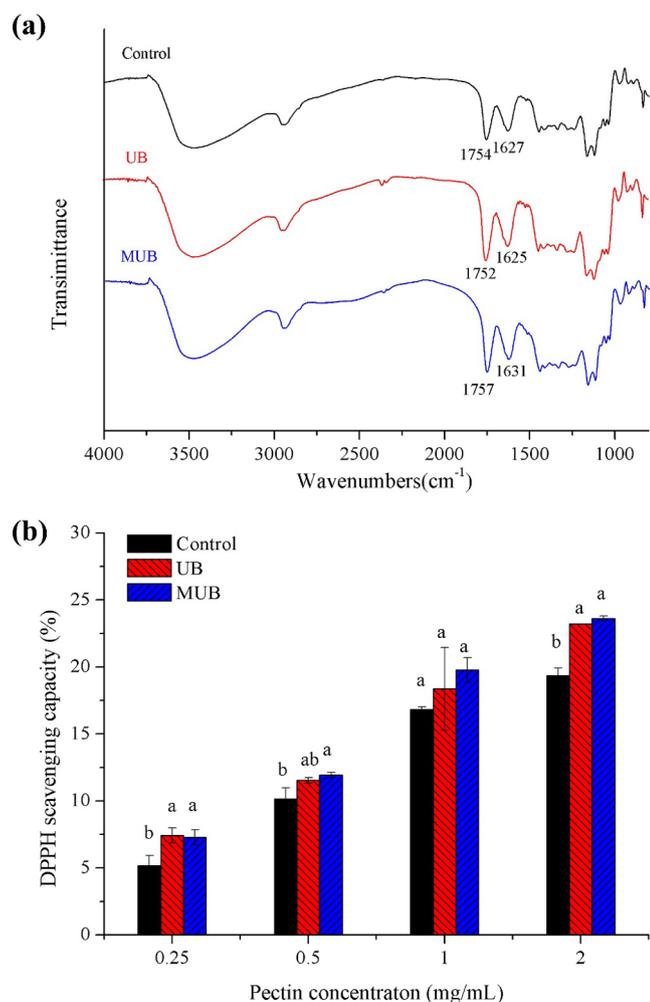


Fig. 2. FTIR and DPPH scavenging capacity of treated pectin. (a) FTIR; (b) DPPH scavenging capacity. Control: untreated pectin; UB: ultrasound modified pectin using power density of 0.76 W/L; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound.

citrus peel pectin, and hawthorn pectin have been reported to have great antioxidant activity by DPPH free radical scavenging assay [27,28]. As shown in the Fig. 2(b), DPPH scavenging capacity of all pectin samples were positively correlated with the concentration. Although there was no significant difference ($P > 0.05$) between UB and MUB, the DPPH scavenging capacity of MUB was slightly higher than that of UB at the concentration of 0.5 mg/mL, 1 mg/mL and 2 mg/mL, indicating that the combination of microfluidization and ultrasonication made some contributions to the antioxidant capacity of pectin. Besides, the DPPH scavenging capacity of MUB was significantly higher than that of the original pectin at the concentration of 0.25 mg/mL, 0.5 mg/mL, and 2 mg/mL ($P < 0.05$), which proved their capability of inhibiting oxidative degradation for active compounds when being used as the wall material for encapsulation. The enhanced antioxidant capacity could be explained from two aspects. On one hand, lower viscosity of MUB and UB promoted the interaction between hydroxyl groups existing in pectin with free radicals [29]. Ogotu and Mu [30] found that antioxidant capacity of pectin treated by 200 W and 400 W sonication (led to lower viscosity) was much higher than those of the original and pectin treated by 100 W sonication, which showed insignificant structural changes with improved antioxidant activity. Our previous work also demonstrated that ultrasound treatment lowering viscosity of pectin was beneficial to the antioxidant capacity of pectin [11]. Regarding microfluidization, DPPH radical scavenging capacity

was found to be significantly improved in treated lentinan and polysaccharides from *Mesona chinensis* Benth [31,32]. On the other hand, ultrasound was found to increase percentage of side chains attached to rhamnogalacturonan-I, possessing considerable bioactivity potentially [33]. Moreover, sonication could expose the hidden functional groups and create more functional groups (e.g. carbonyl groups) at the scission sites by cavitation effect, acting as hydrogen or electron atom donors in order to quench the radical species [30].

3.1.5. AFM and SEM

To visualize and confirm the structural change after treatments, AFM and SEM images are presented in the Fig. 3. AFM has been successfully applied for imaging the heterogenous pectin structural properties at nanolevel [16]. It qualitatively and quantitatively illustrated the conformational and structural evolution of pectin before and after treatments in this study. According to Fig. 3a, several forms of structures were observed in the original pectin including sphere (s), linear strands of varying lengths (ls), single branched (br), and multiply branched (mbr) structures, indicating the original pectin was highly branched long-chain polymer. Regarding UB, “br” structure reduced significantly from 17.44% to 10.97% but the number of “s” structure increased from 42.85% to 51.51%, indicating that the mechanical and chemical effect from ultrasound already degraded pectin molecules [34]. With respect to MUB, significantly less “mbr” (7.04%) could be found compared to control (20.08%) and UB (16.65%) while the number of “s” structure increased to 64.28%, indicating degree of branching decreased with further degradation happened when treated by combining microfluidization with ultrasonication, which agreed with the result of lower molecular weight and hydrodynamic diameter of MUB pectin mentioned in 3.1.1. Also, more similar chain lengths of MUB pectin shown in Fig. 3a correlated with the lower polydispersity meaning narrower distribution. Fig. 3b are the SEM images of freeze-dried pectin with or without treatments. The pectin without treatments showed a smooth and continuous morphology, indicating the structural integrity. Such a smooth morphology was not found in the ultrasound-modified pectin (UB), which instead presented as ripped-apart structure. With microfluidization pretreatment, the pectin showed a thin fibrous morphology. Similar findings were reported by Wang, et al. [12] that pectin extracted by using ultrasound assisted heating method showed wrinkled surface compared with pectin extracted by using conventional heating method. Chen, et al. [21] reported that after being microfluidization-treated, smaller chips were found in pectin compared to the original flake-like structure, which was likely due to the molecule breakdown by microfluidization. Meanwhile, pectin samples showed increased diameters of the pores as microfluidization pressure increased. AFM and SEM images in this study indicated that microfluidization and ultrasonication might have worked synergistically in breaking down the pectin structure.

3.2. Characterization of modified pectin emulsion

3.2.1. Centrifugal stability and interfacial pectin loading

The emulsion stability was characterized by centrifugal stability and interfacial pectin loading (Table 4). Centrifugal stability characterized by use of the amount of oil phase leaked after the centrifugation process, and a high value refers to decreased stability. According to Table 4, centrifugal stability value of the control group showed the highest among all groups, which indicated the emulsion formed by original pectin were most unstable. With the increasing power density of sonication from 0.40 W/mL to 1.12 W/mL, the centrifugal stability gradually improved from 35.43 to 24.80 mg. Emulsion stabilized by pectin treated by combination of microfluidization and ultrasonication (MUA, MUB, and MUC) further improved the centrifugal stability compared to emulsion stabilized by pectin samples treated by corresponding sonication power alone (UA, UB, UC), respectively. The differences in centrifugal stability among different samples could be

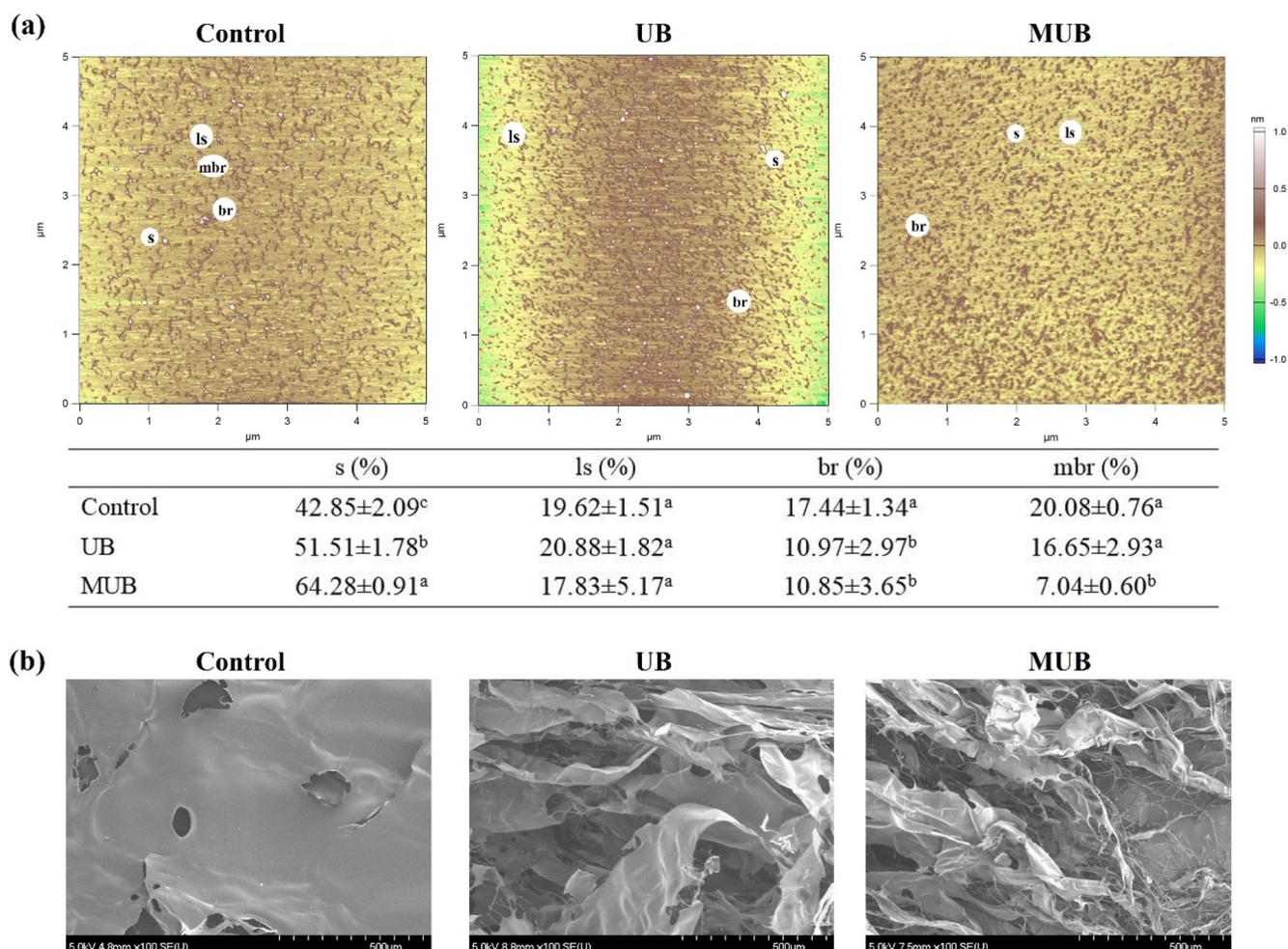


Fig. 3. AFM and SEM of treated pectin. (a) AFM; (b) SEM. Control: untreated pectin; UB: ultrasound modified pectin using power density of 0.76 W/L; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound.

Table 4
Centrifugal stability and surface loading.

	Centrifugal stability (mg)	Interfacial loading Γ (mg/m ²)
Control	55.16 ± 5.15 ^a	14.21 ± 2.87 ^c
UA	35.43 ± 7.42 ^b	14.96 ± 0.25 ^{bc}
UB	31.45 ± 2.93 ^{bc}	15.46 ± 2.22 ^{bc}
UC	24.80 ± 3.83 ^{cd}	17.52 ± 1.04 ^b
MUA	30.55 ± 2.47 ^{bc}	17.95 ± 0.68 ^{ab}
MUB	27.23 ± 0.55 ^{cd}	17.90 ± 1.72 ^{ab}
MUC	22.05 ± 2.68 ^d	20.87 ± 0.34 ^a

Control: untreated pectin; UA: ultrasound modified pectin using power density of 0.40 W/L; UB: ultrasound modified pectin using power density of 0.76 W/L; UC: ultrasound modified pectin using power density of 1.12 W/L; MUA: pectin treated by microfluidization and 0.40 W/L of ultrasound; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound; MUC: pectin treated by microfluidization and 1.12 W/L of ultrasound.

Different lower letters in the superscript within the column indicated significant difference ($P < 0.05$).

highly associated with the amount of pectin being adsorbed at the oil–water interface. Typically, a threshold interfacial coverage had to be achieved in order to ensure the stability of emulsion droplets against coalescence [35]. In this study, it was found that the interfacial loading of the original pectin was only 14.21 mg/m², the lowest among all groups. With mild sonication (UA and UB), the interfacial loading of pectin increased (14.96 and 15.46 mg/m², respectively) but there was no significant difference. Further increase of ultrasonic power density

was able to enhance the interfacial loading (UC, 17.52 mg/m²), which in turn improved the centrifugal stability of emulsion (24.80 mg). These results agreed with our previous work [18], demonstrating ultrasound treatment significantly improved emulsifying capacity of pectin such as centrifugal stability by enhancing interfacial loading. When stabilized by pectin treated by the combination of microfluidization and ultrasonication, MUA, MUB and MUC emulsions resulted in higher pectin interfacial loading compared to UA, UB, and UC, respectively, thus leading to desirable centrifugal stability. This could be due to the reduced molecular weight of pectin producing a reduction in aggregate size (mesoscopic structure), which improved the interfacial capacity and emulsifying ability by promoting their distribution at the interface against flocculation or coalescence [36]. The formation of thicker interfacial layers could give better accessibility of surface-active groups or increase adsorption kinetics [5]. Zhao, et al. [22] found decreased size of hydrolyzed citrus pectin resulted in a greater distribution at the O/W interface, and hence more hydrophobic groups could adsorb on the interface, improving the interfacial capacity. It was also reported that reducing the molecular weight of polysaccharide emulsifiers enhanced their interfacial properties [37]. However, the excessive depolymerization of pectin could lead to a partial loss of the polymer emulsifying ability to produce very thin adsorbed layers around oil droplets which influenced the steric stabilization of emulsions [38,39]. Overall, microfluidization coupled with ultrasonication to effectively improve emulsion stability has been proved to be a synergistic approach in our study.

Table 5
Droplet size of emulsions and their thermal stability.

	No thermal treatment	45 °C		55 °C		65 °C	
		Droplet size after storage	Size increase (%)	Droplet size after storage	Size increase (%)	Droplet size after storage	Size increase (%)
Control	29.04 ± 1.27 ^{d,C}	32.35 ± 2.10 ^{c,BC}	11.40	34.31 ± 0.92 ^{c,B}	18.15	38.51 ± 0.20 ^{d,A}	32.61
UA	34.82 ± 0.10 ^{bc,B}	37.28 ± 2.90 ^{bc,B}	7.06	40.32 ± 4.20 ^{bc,B}	15.80	50.21 ± 3.26 ^{ab,A}	44.20
UB	31.88 ± 4.74 ^{cd,B}	32.73 ± 0.42 ^{c,B}	2.67	44.39 ± 5.55 ^{ab,A}	39.24	44.78 ± 5.82 ^{bcd,A}	40.46
UC	46.30 ± 0.46 ^{a,A}	47.99 ± 6.43 ^{a,A}	3.65	48.95 ± 5.32 ^{a,A}	5.72	53.80 ± 1.64 ^{a,A}	16.20
MUA	38.97 ± 1.57 ^{b,B}	40.54 ± 5.29 ^{abc,AB}	4.03	42.20 ± 0.14 ^{ab,AB}	8.29	46.15 ± 2.62 ^{bc,A}	18.42
MUB	36.99 ± 0.93 ^{b,A}	37.50 ± 5.63 ^{bc,A}	1.38	35.11 ± 2.41 ^{c,A}	-5.08	40.57 ± 1.14 ^{cd,A}	9.68
MUC	44.22 ± 2.19 ^{a,A}	44.92 ± 6.79 ^{ab,A}	1.58	45.93 ± 4.39 ^{ab,A}	3.87	43.97 ± 5.39 ^{bcd,A}	-0.57

Control: untreated pectin; UA: ultrasound modified pectin using power density of 0.40 W/L; UB: ultrasound modified pectin using power density of 0.76 W/L; UC: ultrasound modified pectin using power density of 1.12 W/L; MUA: pectin treated by microfluidization and 0.40 W/L of ultrasound; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound; MUC: pectin treated by microfluidization and 1.12 W/L of ultrasound.

Different lower letters in the superscript within the column indicated significant difference ($P < 0.05$).

Different upper letters in the superscript within the row for emulsion droplet size indicated significant difference ($P < 0.05$).

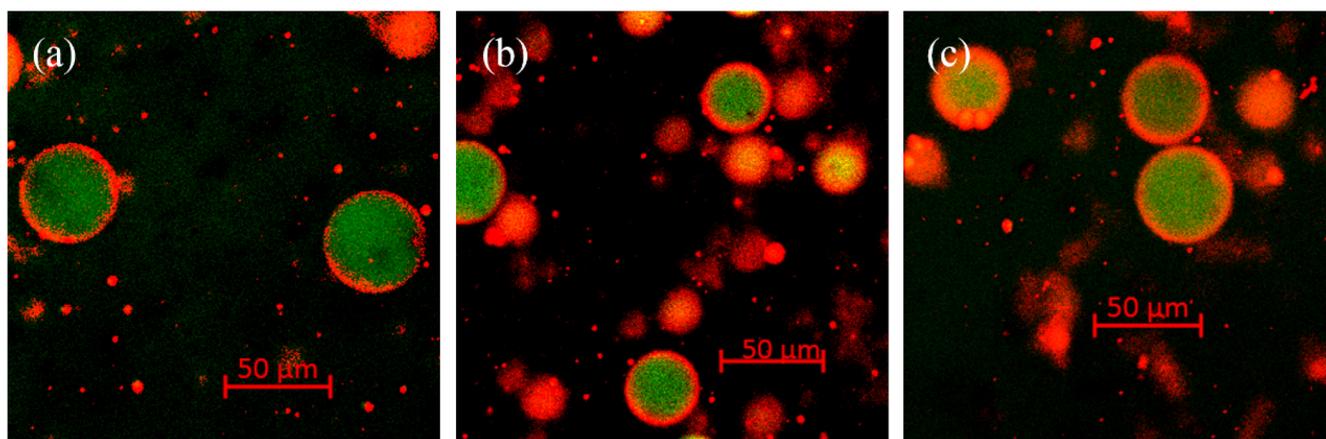


Fig. 4. CLSM image of pectin emulsion. (a) Control: untreated pectin; (b) UB: ultrasound modified pectin using power density of 0.76 W/L; (c) MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound.

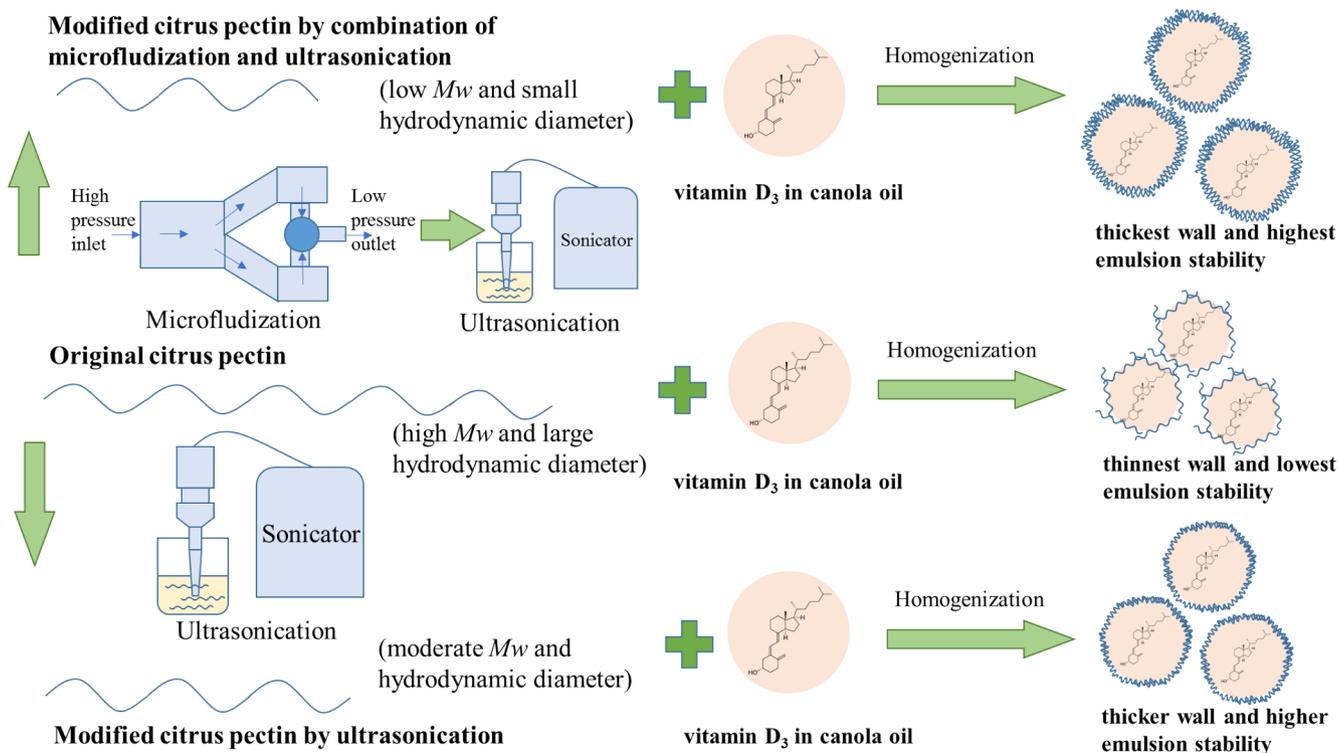


Fig. 5. Possible mechanism of modified pectin improving emulsion stability.

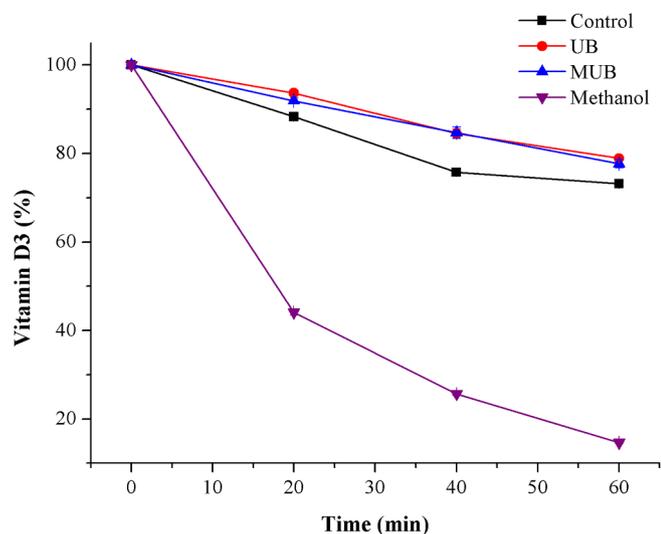


Fig. 6. Vitamin D3 stability after UV exposure. Methanol: Vitamin D3 dissolved in methanol. Control, UB, and MUB: Vitamin D3 emulsion using different pectin as the wall material. Control: untreated pectin; UB: ultrasound modified pectin using power density of 0.76 W/L; MUB: pectin treated by microfluidization and 0.76 W/L of ultrasound.

3.2.2. Thermal stability

The thermal stability of emulsions was evaluated by storage at elevated temperatures (45 °C, 55 °C, and 65 °C), and the result is shown in Table 5. The thermal stability of emulsion was characterized by comparing the variation of droplet size before and after thermal storage, and the average size increase (%) was calculated [40]. At 45 °C, all the emulsions were relatively stable with minor droplet size evolution. The greatest size increase was found with the emulsion stabilized by original pectin, with an average droplet size increase of 11.4%. All the other groups, including using pectin treated by sonication alone and combination of microfluidization and ultrasonication resulted in less droplet size increase. Among them, MUB and MUC emulsions showed the least droplet size increase (1.38% and 1.58%, respectively), indicating the synergistic effect between microfluidization and ultrasonication in improving the emulsifying capacity of pectin. When the storage temperature increased to 55 °C and 65 °C, similar results were found that MUB and MUC emulsions had relatively low droplet size increase against temperature elevation followed by MUA and UC emulsions. However, pectin treated by mild sonication (UA and UB) did not result in improving thermal stability of their emulsions. These results could be related to the hydrodynamic diameter (Table 2) and interfacial loading of pectin (Table 4). Lower hydrodynamic diameter of pectin mostly contributed to obtaining higher interfacial loading and forming a more compact interfacial layer, providing efficient steric stabilization that impedes emulsion coarsening under high temperature environment [17].

3.2.3. Interfacial properties

The emulsifying properties of pectin samples with different treatments were compared by confocal laser scanning microscopy (CLSM). The CLSM images illustrated the interface structure of emulsion oil droplets stabilized by pectin. Red in the figure indicates pectin whereas green represents oil. According to the Fig. 4a, the emulsion interface stabilized by original pectin was not smoothly covered, leaving a relatively thin interfacial layer which meant weak adsorption of original pectin. However, the UB and MUB presented a much more complete interfacial coverage with thicker layer, suggesting that the interfacial activity of pectin was improved after modification. Similar finding was reported in recent research [18], the difference could be attributed to the structural evolution that exposed the hydrophobic portion such as

methoxyl groups of the pectin. This result confirmed our hypothesis that the modified pectin treated by sonication or combination of microfluidization and ultrasonication, due to the smaller molecular weight and hydrodynamic diameter, and thus smaller sized mesoscopic structure and better mobility in water, was able to form a thicker layer at the oil/water interface, potentially provide more effective protection for the core material in the oil phase, which is also illustrated in Fig. 5.

3.3. UV stability of cholecalciferol (vitamin D₃) microcapsules

In this study, pectin with or without treatments were used to encapsulate cholecalciferol (vitamin D₃) in order to evaluate their feasibility as encapsulation wall materials. Compared to the vitamin D₃ stability in methanol, which lost 85% of vitamin D₃ after 60 min, the encapsulated vitamin D₃ using groups of pectin as protection layer exhibited acceptable capability in stabilizing vitamin D₃ (Fig. 6). This result verified that pectin could be used as the wall material through emulsification to form a protective layer at the oil–water interface which may act as a UV light and oxygen barrier, protecting vitamin D₃ from the oxidation. Moreover, there was still a prominent difference between the original pectin and modified pectin, in terms of their encapsulated vitamin D₃ stability. After 60 min of UV exposure, the original pectin was able to retain 73% of vitamin D₃, while the modified pectin samples (UB and MUB) were able to retain significantly ($P < 0.05$) higher vitamin D₃ (78%). Similar results were found in our previous work that the ultrasound-modified pectin significantly enhanced performance on flavor retention when encapsulating oil phase containing the flavor compounds compared with the original one [18]. Herein, the improved stability could be attributed to three perspectives: 1) the enhanced emulsion stability by UB and MUB simultaneously improved vitamin D₃ stability by preventing the leakage of the oil phase containing vitamin D₃; 2) UB & MUB formed a more intact and thicker protective layer providing complete coverage at the oil–water interface, to prevent vitamin D₃ from UV light and oxygen exposure; 3) the antioxidative capacity of UB and MUB inhibited the oxidation of vitamin D₃.

4. Conclusions

In this work, the efficacy of microfluidization-ultrasonication combined treatment was used to improve the physiochemical properties and emulsion-stabilizing properties of pectin. Microfluidization and ultrasonication worked synergistically to break down pectin molecules by decreasing hydrodynamic diameter, molecular weight and polydispersity, and was confirmed from SEM and AFM images. Meanwhile, antioxidant capacity of modified pectin was improved. Further emulsifying tests suggested that the combination of microfluidization and ultrasonication substantially improved the emulsifying stability of pectin, in both centrifugal and thermal stability assay. CLSM and interfacial loading results indicated that the improved emulsion-stabilizing properties could be attributed to better interfacial coverage which meant thicker wall of pectin in the interfacial layer. Moreover, both pectin treated by ultrasonication alone and combination of microfluidization and ultrasonication exhibited excellent encapsulation feasibility to protect vitamin D₃ from UV degradation. Future study will focused on developing more effective microencapsulation system when applying modified pectin from this study in prebiotics, packaging and other related research domains.

CRedit authorship contribution statement

Wenjun Wang: Conceptualization, Methodology, Investigation, Formal analysis, Writing - original draft. **Yiming Feng:** Methodology, Investigation, Formal analysis, Writing - original draft. **Weijun Chen:** Investigation, Formal analysis. **Kyle Adie:** Formal analysis, Writing - review & editing. **Donghong Liu:** Conceptualization, Supervision,

Writing - review & editing. **Yun Yin:** Supervision, Funding acquisition, Writing - review & editing.

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.

Acknowledgement

The authors acknowledge the funding support from Virginia Agriculture Experiment Station.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ultsonch.2020.105322>.

References

- [1] F. Naqash, F.A. Masoodi, S.A. Rather, S.M. Wani, A. Gani, Emerging concepts in the nutraceutical and functional properties of pectin-A Review, *Carbohydr. Polym.* 168 (2017) 227–239.
- [2] T.E. Mungure, S. Roohinejad, A.-E.-D. Bekhit, R. Greiner, K. Mallikarjunan, Potential application of pectin for the stabilization of nanoemulsions, *Curr. Opin. Food Sci.* 19 (2018) 72–76.
- [3] L. Aberkane, G. Roudaut, R. Saurel, Encapsulation and oxidative stability of PUFA-rich oil microencapsulated by spray drying using pea protein and pectin, *Food Bioprocess Technol.* 7 (2014) 1505–1517.
- [4] F.J. Rossier-Miranda, K. Schroën, R. Boom, Microcapsule production by an hybrid colloidosome-layer-by-layer technique, *Food Hydrocolloids* 27 (2012) 119–125.
- [5] K. Alba, V. Kontogiorgos, Pectin at the oil-water interface: Relationship of molecular composition and structure to functionality, *Food Hydrocolloids* 68 (2017) 211–218.
- [6] X. Yang, T. Nisar, Y. Hou, X. Gou, L. Sun, Y. Guo, Pomegranate peel pectin can be used as an effective emulsifier, *Food Hydrocolloids* 85 (2018) 30–38.
- [7] M.I. Guerra-Rosas, J. Morales-Castro, L.A. Ochoa-Martínez, L. Salvia-Trujillo, O. Martín-Belloso, Long-term stability of food-grade nanoemulsions from high methoxyl pectin containing essential oils, *Food Hydrocolloids* 52 (2016) 438–446.
- [8] T.A. Verrijssen, L.G. Balduyck, S. Christiaens, A.M. Van Loey, S. Van Buggenhout, M.E. Hendrickx, The effect of pectin concentration and degree of methyl-esterification on the in vitro bioaccessibility of β -carotene-enriched emulsions, *Food Res. Int.* 57 (2014) 71–78.
- [9] A. Gharsallaoui, G. Roudaut, L. Beney, O. Chambin, A. Voilley, R. Saurel, Properties of spray-dried food flavours microencapsulated with two-layered membranes: Roles of interfacial interactions and water, *Food Chem.* 132 (2012) 1713–1720.
- [10] W. Wang, W. Chen, M. Zou, R. Lv, D. Wang, F. Hou, H. Feng, X. Ma, J. Zhong, T. Ding, Applications of power ultrasound in oriented modification and degradation of pectin: a review, *J. Food Eng.* 234 (2018) 98–107.
- [11] W.J. Wang, X.B. Ma, P. Jiang, L.L. Hu, Z.J. Zhi, J.L. Chen, T. Ding, X.Q. Ye, D.H. Liu, Characterization of pectin from grapefruit peel: a comparison of ultrasound-assisted and conventional heating extractions, *Food Hydrocolloids* 61 (2016) 730–739.
- [12] W. Wang, X. Ma, Y. Xu, Y. Cao, Z. Jiang, T. Ding, X. Ye, D. Liu, Ultrasound-assisted heating extraction of pectin from grapefruit peel: optimization and comparison with the conventional method, *Food Chem.* 178 (2015) 106–114.
- [13] W. Wang, X. Wu, T. Chantapakul, D. Wang, S. Zhang, X. Ma, T. Ding, X. Ye, D. Liu, Acoustic cavitation assisted extraction of pectin from waste grapefruit peels: a green two-stage approach and its general mechanism, *Food Res. Int.* 102 (2017) 101–110.
- [14] H. Lee, G. Yildiz, L. Dos Santos, S. Jiang, J. Andrade, N.J. Engeseth, H. Feng, Soy protein nano-aggregates with improved functional properties prepared by sequential pH treatment and ultrasonication, *Food Hydrocolloids* 55 (2016) 200–209.
- [15] S. Jiang, J. Ding, J. Andrade, T.M. Rababah, A. Almajwal, M.M. Abulmeaty, H. Feng, Modifying the physicochemical properties of pea protein by pH-shifting and ultrasound combined treatments, *Ultrason. Sonochem.* 38 (2017) 835–842.
- [16] L.F. Zhang, X.Q. Ye, S.J. Xue, X.Z. Zhang, D.H. Liu, R.F. Meng, S.G. Chen, Effect of high-intensity ultrasound on the physicochemical properties and nanostructure of citrus pectin, *J. Sci. Food Agric.* 93 (2013) 2028–2036.
- [17] Y. Feng, Y. Lee, Surface modification of zein colloidal particles with sodium caseinate to stabilize oil-in-water pickering emulsion, *Food Hydrocolloids* 56 (2016) 292–302.
- [18] W. Wang, Y. Feng, W. Chen, Y. Wang, G. Wilder, D. Liu, Y. Yin, Ultrasonic modification of pectin for enhanced 2-furfurylthiol encapsulation: process optimization and mechanisms, *J. Sci. Food Agric.* 100 (2020) 110–118.
- [19] S. Mahdi Jafari, Y. He, B. Bhandari, Nano-emulsion production by sonication and microfluidization—a comparison, *Int. J. Food Prop.* 9 (2006) 475–485.
- [20] J. Chen, D. Gao, L. Yang, Y. Gao, Effect of microfluidization process on the functional properties of insoluble dietary fiber, *Food Res. Int.* 54 (2013) 1821–1827.
- [21] J. Chen, R.-H. Liang, W. Liu, C.-M. Liu, T. Li, Z.-C. Tu, J. Wan, Degradation of high-methoxyl pectin by dynamic high pressure microfluidization and its mechanism, *Food Hydrocolloids* 28 (2012) 121–129.
- [22] S. Zhao, W. Ren, W. Gao, G. Tian, C. Zhao, Y. Bao, J. Cui, Y. Lian, J. Zheng, Effect of mesoscopic structure of citrus pectin on its emulsifying properties: compactness is more important than size, *J. Colloid Interface Sci.* (2020).
- [23] C.-M. Liu, L. Liang, X.-X. Shuai, R.-H. Liang, J. Chen, Dynamic high-pressure microfluidization-treated pectin under different ethanol concentrations, *Polymers* 10 (2018) 1410.
- [24] H.M. Chen, X. Fu, Z.G. Luo, Properties and extraction of pectin-enriched materials from sugar beet pulp by ultrasonic-assisted treatment combined with subcritical water, *Food Chem.* 168 (2015) 302–310.
- [25] L.F. Zhang, X.Q. Ye, T. Ding, X.Y. Sun, Y.T. Xu, D.H. Liu, Ultrasound effects on the degradation kinetics, structure and rheological properties of apple pectin, *Ultrason. Sonochem.* 20 (2013) 222–231.
- [26] Y. Feng, Y. Lee, Microfluidic assembly of food-grade delivery systems: Toward functional delivery structure design, *Trends Food Sci. Technol.* (2019).
- [27] X. Chen, Y. Qi, C. Zhu, Q. Wang, Effect of ultrasound on the properties and antioxidant activity of hawthorn pectin, *Int. J. Biol. Macromol.* 131 (2019) 273–281.
- [28] X. Wang, X. Lu, Characterization of pectic polysaccharides extracted from apple pomace by hot-compressed water, *Carbohydr. Polym.* 102 (2014) 174–184.
- [29] J. Ro, Y. Kim, H. Kim, S.B. Jang, H.J. Lee, S. Chakma, J.H. Jeong, J. Lee, Antioxidative activity of pectin and its stabilizing effect on retinyl palmitate, *Korean J. Physiol. Pharmacol.* 17 (2013) 197–201.
- [30] F.O. Ogutu, T.H. Mu, Ultrasonic degradation of sweet potato pectin and its antioxidant activity, *Ultrason. Sonochem.* 38 (2017) 726–734.
- [31] L. Huang, M. Shen, X. Zhang, L. Jiang, Q. Song, J. Xie, Effect of high-pressure microfluidization treatment on the physicochemical properties and antioxidant activities of polysaccharide from *Mesona chinensis* Benth, *Carbohydr. Polym.* 200 (2018) 191–199.
- [32] X. Huang, Z. Tu, Y. Jiang, H. Xiao, Q. Zhang, H. Wang, Dynamic high pressure microfluidization-assisted extraction and antioxidant activities of lentinan, *Int. J. Biol. Macromol.* 51 (2012) 926–932.
- [33] E.G. Maxwell, N.J. Belshaw, K.W. Waldron, V.J. Morris, Pectin - An emerging new bioactive food polysaccharide, *Trends Food Sci. Technol.* 24 (2012) 64–73.
- [34] W. Wang, W. Chen, O. Kahraman, T. Chantapakul, T. Ding, D. Liu, H. Feng, Manothermosonication (MTS) treatment by a continuous-flow system: effects on the degradation kinetics and microstructural characteristics of citrus pectin, *Ultrason. Sonochem.* 63 (2020) 104973.
- [35] S. Tcholakova, N. Denkov, A. Lips, Comparison of solid particles, globular proteins and surfactants as emulsifiers, *PCCP* 10 (2008) 1608–1627.
- [36] S. Zhao, W. Gao, G. Tian, C. Zhao, C. DiMarco-Crook, B. Fan, C. Li, H. Xiao, Y. Lian, J. Zheng, Citrus oil emulsions stabilized by citrus pectin: The influence mechanism of citrus variety and acid treatment, *J. Agric. Food Chem.* 66 (2018) 12978–12988.
- [37] J. Li, X. Hu, X. Yan, X. Li, Z. Ma, L. Liu, Effects of hydrolysis by xylanase on the emulsifying properties of *Artemisia sphaerocephala* Krasch. polysaccharide, *Food Hydrocolloids* 76 (2018) 158–163.
- [38] M. Akhtar, E. Dickinson, J. Mazoyer, V. Langendorff, Emulsion stabilizing properties of depolymerized pectin, *Food Hydrocolloids* 16 (2002) 249–256.
- [39] E.D. Ngouémazong, S. Christiaens, A. Shpigelman, A. Van Loey, M. Hendrickx, The emulsifying and emulsion-stabilizing properties of pectin: a review, *Compr. Rev. Food Sci. Food Saf.* 14 (2015) 705–718.
- [40] X. Li, Y. Feng, S. Ting, J. Jiang, Y. Liu, Correlating emulsion properties to microencapsulation efficacy and nutrients retention in mixed proteins system, *Food Res. Int.* 115 (2019) 44–53.