Study on the emulsification and oxidative stability of ovalbumin-pectin-pumpkin seed oil emulsions using ovalbumin solution prepared by ultrasound

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ABSTRACT

Pumpkin seed oil (PSO), which is a valuable compound with high nutritional value used for the prevention of various chronic diseases, is prone to oxidation. In this work, small and uniform (su) ovalbumin (OVA) and pectin (PEC) were used to stabilize PSO in the form of an emulsion. The results showed that suOVA-PEC-PSO emulsion with a droplet size of 9.82 ± 0.05 μm was successfully self-assembled from PSO, PEC, and suOVA solution (with a droplet size of 230.13 ± 14.10 nm) treated with 300 W ultrasound, owing to the formation of a more stable interfacial film on the surface of droplets. The interfacial, rheological, emulsifying, and antioxidant properties of the suOVA-PES-PSO emulsions were excellent, owing to the synergistic effects between PEC and suOVA solution. Moreover, the physical stability of the suOVA-PEC-PSO emulsions to salt stress, a freeze-thaw cycle, and heat treatment was also increased and the oxidation of linolenic acid was notably delayed. These results have extended the food-related applications of OVA and PSO, and provide a promising foundation for further exploration of the self-assembly of composite emulsions by small and uniform proteins.

1. Introduction

Pumpkin seed oil (PSO), obtained from the seeds of Cucurbita moschata Duch., has a high nutritional value (unsaturated fatty acids > 80%), intense aroma, and contains various active components, including tocopherols, phytosterols, and phenolic acid [1,2]. PSO has the potential effect to prevent diseases such as prostate, hypertension and kidney stones; this activity may be related to its antioxidant properties [3]. However, the exposure of PSO to oxygen and heat treatment during storage and processing have been reported to degrade its nutritional and sensory qualities and even cause the formation of harmful compounds [4]. Therefore, it is important to prevent or inhibit the oxidation and degradation of PSO.

Various emulsifiers and/or technologies have been tested to meet this aim and to improve the stability (another crucial parameter) of emulsions [5–7]. For example, proteins have been used extensively to stabilize food emulsions owing to their amphiphilic nature [8]. However, the impact of ionic strength and storage conditions on the stability of protein-containing emulsions were well studied [9–11].

Polysaccharides, including gum arabic, gelatin, and pectin, have also been used to enhance the stability of food emulsions [9,11]. Coating the emulsion droplets with polysaccharides (and/or proteins) leads to greater resistance to environmental stress, and the effect of protein-polysaccharide mixtures was better than that of proteins or polysaccharides alone [9]. This may be attributable to the formation of a thick-compact interfacial layer on the surface of the oil droplets to
In recent years, ultrasound, as a safe and “green” technology, has been used to improve the physicochemical and/or functional characteristics, solubility, and foaming and gelling properties of protein emulsions primarily owing to its cavitation and microstreaming effects [13–15]. It has also been reported that the emulsifying properties of whey proteins and bovine myofibrillar proteins were enhanced by high-intensity ultrasonic treatment as a result of the structural disruption, exposure of hydrophobic groups, and particle size reduction [16,17]. In addition, the stability of ovalbumin (OVA)-stabilized emulsions was greatly improved by an OVA-gum arabic (w/w) complex [9]. However, to the best of our knowledge, the combined effects of OVA and PEC on the properties of emulsions have not been reported previously. Therefore, the objective of the present study was to investigate the impact of ultrasound-generated small particles of OVA and PEC on the rheology, emulsifying properties (emulsifying activity index (EAI) and emulsifying stability index (ESI)), and physical (NaCl treatment, thermal treatment, and cold storage) and oxidative stability of emulsions. The knowledge obtained from this study will help to elucidate the underlying mechanisms for the stability of self-assembled by PSO, PEC, and OVA emulsions with different particle sizes prepared by ultrasound.

2. Materials and methods

2.1. Materials and reagents

PSO (98%) was purchased from Tongze Biotech Co., Ltd. (Xi’an, Shaxi, China), pectin (galacturonic acid ≥ 74%, dry basis) was purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China), and ovalbumin (A-5253, purity: 62–88%) was purchased from Sigma-Aldrich (USA); all other chemicals and reagents (analytical grade) were purchased from E-Merck (Germany).

2.2. Preparation of suOVA-PEC-PSO emulsion

450 mg OVA powder was dispersed into 30 mL deionized water in a beaker and stirred for 2 h until it was completely dissolved, and was then subjected to ultrasonic irradiation (Scientz-IID, Ningbo Scientz Biotechnology Co., Ltd. Ningbo, China) at different powers (0 W, 100 W, 200 W, 300 W, 400 W, and 500 W, 4.9 cm²) for 30 min (duty ratio = 1:1, probe diameter: 6 mm) in an ice-water bath [18]. Then, to prepare the suOVA-PEC solution, pectin (to give a final concentration was 10 mg/mL) was immediately added into the OVA solution and stirred for 6 h, and then stored overnight at 4°C. Subsequently, the suOVA-PEC solution was mixed with PSO (80% suOVA-PEC solution; 20% PSO) and sheared at 20,000 rpm for 2 min with a high-speed homogenizer (T18, IKA, Germany) to prepare oil-in-water emulsion [5]; finally, 0.02% (w/w) sodium azide was added as an antimicrobial agent. After preparation, the emulsion was sealed and stored at 4°C until further analysis.

2.3. Particle size measurements of suOVA solution

The mean size of particles in the suOVA solution after a 100-fold dilution in deionized water was determined using a commercial dynamic light scattering instrument (Zetasizer Nano-ZS, Malvern Instruments, Worcestershire, UK) [19].

2.4. FTIR and fluorescence spectroscopy

Freeze-dried powder of the suOVA-PEC mixtures or OVA solution
were mixed with dried KBr (1:250 w/w) in a mortar, pressed into tablets, and then analyzed using an FTIR spectrometer (Perkin Elmer Instruments, Massachusetts, USA). The scanning range was 400–4000 cm\(^{-1}\) and the resolution was 4 cm\(^{-1}\) [20]. The intrinsic fluorescence of the solutions at 0.1 mg/mL was measured using a spectrophotometer (F-7000, Hitachi, Japan) equipped with a 1.0 cm quartz cell. The emission wavelength range used was 300–400 nm and the excitation wavelength was 280 nm, and the excitation slit and emission slit widths were 5 nm [21].

2.5. Interfacial rheology measurements

The suOVA-PEC solutions were diluted 100-fold in deionized water and the interfacial adsorption and/or dilatational properties (including pressure (\(\pi\)), interfacial pressure, viscoelasticity, and adsorption kinetics) of these complexes at the oil/water interface were measured over time using an automated drop tensiometer (Tracker-H, Teclis, France) at 25 °C [22,23].

2.6. Measurement of emulsification properties

The EAI and ESI were determined according to a previously described method [15] with minor modifications. In brief, 100 μL of the suOVA-PEC-PSO emulsion was prepared, left to stand for 10 min, and then mixed well with 10 mL of 0.1% sodium dodecyl sulfate (SDS) solution. The absorbance of the dilute emulsion was recorded at 500 nm and the EAI and ESI were calculated from the following equations:

\[
EAI = \frac{2 \times A_0 \times DF}{C \times Q \times \theta \times 10000}
\]

Fig. 3. Change in interface properties of suOVA-PEC solutions induced by suOVA solution refined by ultrasound with different power. (0 W: OVA-PEC solution without ultrasound; 100 ~ 500 W: suOVA-PEC solutions). A: oil drop shape diagram; B: interfacial tension; C: \(\pi-t^{1/2}\) curve; D: E-time curve; E: \(E_d\)-time curve.
ESI = A₀ × 10⁻¹⁻°⁻¹

DF: dilution factor (100); C: protein concentration (g/mL); Φ: optical path (0.01 m); θ: volume fraction of oil phase in the emulsion (0.20); A₀ and A₁₀: the absorbance value of emulsion at 0 min and 10 min, respectively.

2.7. Analysis of particle size and zeta potential

The average particle size of emulsion was measured using a Mastersizer 2000 (Malvern Instruments Ltd., UK) in accordance with the method of Hu et al., with some modifications [24]. The refractive index of PSO and water was 1.330 and 1.47 with an absorption coefficient of 0.01 and the pump speed was 2,000 rpm. The zeta potential of emulsion was measured using a Zetasizer Nano-ZS (Malvern Instruments, U.K.) after 50-fold dilution in deionized water at 25 °C [25].

2.8. Rheological analysis

The rheological behavior of the suOVA-PEC-PSO emulsions were measured using a rotational rheometer (HAAKE RS 6000, Thermo Fisher, USA). The amplitude oscillatory strain applied ranged from 0.1% to 100% at a frequency of 1 Hz. Under steady-state shear, the shear rate was increased from 0.01 to 100 s⁻¹ and all data were collected in logarithmic mode [23].

2.9. Optical microscopy and confocal laser microscopy analysis

The morphology of the suOVA-PEC-PSO emulsions diluted 3-fold in deionized water was observed using an optical microscopy (Leica DM2500, Leica Microsystems GmbH, Wetzlar, Germany). The images were captured with an M shot MD130 digital camera (at 40× magnification) and analyzed using Micro-Shot Basic version 1.0 [26]. The microstructure of the suOVA-PEC-PSO emulsions were characterized using a laser scanning confocal microscope (Leica Microsystems, Mannheim, Germany). For analysis, 1 mL of the emulsion was stained with 10 µL Nile red and Nile blue solution (1 mg/mL in isopropanol) and observed at 488 nm for Nile red and 638 nm for Nile blue (excitation spectra) [27].

2.10. Assessment of storage stability

The change in particle size was used to determine the stability of the suOVA-PEC-PSO emulsions to various storage conditions. To determine the thermal stability of the emulsions, the change in particle size was measured after the suOVA-PEC-PSO emulsions were heated at 90 °C for 30 min in a water bath, cooled to room temperature, and stored at 25 °C for 24 h. To determine the freeze-thaw stability of the emulsions over a single cycle, the suOVA-PEC-PSO emulsions prepared at −18 °C for 24 h were thawed at 25 °C for 24 h. To study the effect of storage in salt concentration of the emulsion, 5 mL of the suOVA-PEC-PSO emulsion was mixed with 0, 50, 100, 250 mmol/L aqueous NaCl solution and then...
stored at 25 °C for 24 h [9].

2.11. Assessment of the oxidative stability

2.11.1. Determination of peroxide value (PV)

The PV of the suOVA-PEC-PSO emulsions were evaluated using the method of (Nasrabadi et al. 2020) with some modifications [6]. In brief, 2 g emulsion was dissolved in 20 mL of a glacial acetic acid and isooctane mixture (3:2, v/v) and stirred for 30 s. Immediately, 500 μL of saturated KI solution and 30 mL of deionized water were added, shaken (or mixed) for 1 min, and then titrated against 0.01 mol/L Na$_2$S$_2$O$_3$ solution containing 500 μL of 1% starch until the blue/purple color disappeared. The PV was calculated from the following equations:

\[
PV = \frac{(S - B) \times N \times 1000}{W}
\]

S: the volume of Na$_2$S$_2$O$_3$ used in sample; B: the volume of Na$_2$S$_2$O$_3$ used in blank; N: the normality (or concentration) of Na$_2$S$_2$O$_3$; W: the sample weight (g).

2.11.2. Determination of free radical scavenging ability

The DPPH radical scavenging activity of the suOVA-PEC-PSO emulsions were evaluated using the method of Hu et al. [28] with minor modifications. First, 50 μL of emulsion was diluted to a volume of 3 mL and then was mixed with 3 mL of DPPH solution (0.1 mmol/L in ethanol). The mixture was incubated in the dark for 30 min at room temperature and then centrifuged at 6,000 rpm for 5 min. The absorbance of the supernatant was measured at 517 nm (A) and the absorbance of 3 mL of H$_2$O containing 3 mL of DPPH solution as a control group (A$_0$). The DPPH radical scavenging rate was calculated from the following formula:

\[
\text{DPPH radical scavenging rate} (%) = \frac{A_0 - A}{A_0} \times 100\%
\]

2.12. Analysis of fatty acids

The fatty acids were extracted from suOVA-PEC-PSO, converted to fatty acids methyl esters (FAMES), and then analyzed by Gas chromatography-mass spectrometry detector (GC-MSD) (Agilent 7890B/5977A) equipped with a DB-WAX capillary column (30 mm × 0.25 mm × 0.25 mm) in accordance with the method described in Wang et al [29].

2.13. Statistical analysis

Each experiment was performed in triplicate and the results were expressed as the mean ± standard deviation. The statistical significance of the difference between means was evaluated by Duncan’s test using SPSS 23.0. Values of p < 0.05 were considered statistically significant.

3. Results and discussion

3.1. Effect of ultrasonic power on the particle size in OVA solution

The average particle size (ranging from 230.13 ± 14.10 nm to 342.73 ± 18.50 nm) and PDI (ranging from 0.32 ± 0.02 to 0.50 ± 0.01) of the suOVA solutions were significantly smaller than those of the OVA solution (particle size = 536.53 ± 4.22 nm; PDI = 0.50 ± 0.01),
respectively (Fig. 1 & Table 1). This suggests that the small and uniform OVA solution was successfully prepared by the ultrasonic treatment, likely because of the vigorous collisions of protein particles breaking the noncovalent bonds between them [30]. Moreover, the size of particles in the suOVA solutions decreased gradually as the ultrasonic power (0–300 W) and then increased (300–500 W); hence, the optimum suOVA solution was obtained when the ultrasonic power was 300 W (Table 1). A similar observation was reported in protein isolate extracted from dephenolized sunflower meal [31], and may be due to the protein aggregation that occurred during treatment [15].

3.2. Characterization of suOVA-PEC complexes

3.2.1. FTIR and endogenous fluorescence spectra

As shown in Fig. 2A, the characteristic absorption peaks of OVA and PEC were presented at 3300 cm⁻¹ (O–H), 1654 cm⁻¹ (C=O in amide I), 1544 cm⁻¹ (N–H in amide II), and 3439 cm⁻¹ (O–H), 1752 cm⁻¹ (C=O), 1016 cm⁻¹, respectively [32]. Compared with OVA, the amide I and amide II peaks in OVA-PEC were shifted from 1654 cm⁻¹ to 1656 cm⁻¹ and from 1544 cm⁻¹ to 1546 cm⁻¹, respectively. This indicates that the electrostatic interactions between suOVA and PEC were formed via the carboxyl (COO⁻) and amino (NH⁺) groups [33]. Moreover, the intensity of the characteristic peak (at 1656 cm⁻¹) of suOVA-PEC prepared by ultrasound at/or less than 300 W was enhanced, indicating that the combination of OVA and PEC could be promoted by suOVA treated by
Change in fatty acids of PSO and OVA, PEC, OVA-PEC and suOVA-PEC emulsions. More chromophores [36]. Ultrasound irradiation expands the spatial structure of OVA and exposes more chromophores [36].

3.2.2. Interfacial properties of suOVA-PEC complexes

It was apparent that the surface of oil droplets became wrinkled when PEC was mixed with OVA, as a small amount of the oil phase was removed from the emulsion and a similar result was obtained for the suOVA treated with ultrasound, especially at 300 W (Fig. 3A). This suggested that covalently crosslinked interfacial membranes between OVA with PEC were formed [37] and more stable interfacial membranes between them could be achieved and maintained by small and uniform proteins, such as suOVA.

The interfacial tension of suOVA-PEC complexes was significantly lower than that of the OVA solution and first decreased and then increased as the ultrasonic power was increased; the lowest value for interfacial tension was obtained with 300 W (Fig. 3B). This may be attributable to the increased fluidity of small particles (suOVA) that could be rapidly adsorbed at the oil-water interface [38]. Similarly, the diffusion coefficient (or slope) for suOVA was bigger than OVA and the largest value was obtained at 300 W (Fig. 3C). This indicates that the adsorption rate of suOVA was enhanced by appropriate ultrasound irradiation, which could be attributed to the small and uniform proteins bind more tightly with pectin through increased electrostatic and hydrophobic interactions [39].

The interfacial dilational viscoelastic properties (E and E′) are usually used to evaluate the interfacial structure and intermolecular interactions at the oil-water interface [39]. Both the E and E′ values of the suOVA-PEC complexes were significantly higher than those of the OVA solution, and the largest value was also obtained at 300 W (Fig. 3D & E). This demonstrated that the adsorption of the suOVA molecules at the oil-water interface was increased and that elastic interfacial films were formed through stronger hydrophobic or electrostatic interactions [39].

3.3. Characterization of suOVA-PEC-PSO emulsions

3.3.1. Emulsifying properties

EAI and ESI of suOVA-PEC-PSO emulsions were significantly better than those of control groups (OVA & PEC). The emulsifying properties of suOVA-PEC-PSO emulsions were increased as the ultrasonic power increased to 300 W (EAI = 2100 m²/g; ESI = 1135 min) and then decreased slightly (Fig. 4A). This finding was similar to the changes in emulsifying properties of muscle sarcoplasmic proteins treated with ultrasound for up to 30 min [30]. Therefore, it was concluded that the best emulsifying properties for the suOVA-PEC-PSO emulsions were obtained from an ultrasonic treatment of 300 W. It is generally accepted that PEC adsorbed at the oil-water interface can enhance the electrostatic interaction and steric hindrance between emulsion droplets to improve emulsification [40]. In addition, the better emulsifying properties of the suOVA-PEC-PSO emulsions can be attributed to the size and

| Table 2 | Change in fatty acids of PSO and OVA, PEC, OVA-PEC and suOVA-PEC emulsions. |
|---------|---------------------------------------------------------------------------|
|          | Fatty acids (%) | 0 day Initial oil | Oil | OVA | PEC |
|          | Palmitic | 16.44 ± 0.03 | 20.33 ± 0.11 | 19.65 ± 0.02 | 18.68 ± 0.03 |
|          | (C16:0) | 0.03± | 0.01± | 0.01± | 0.03± |
|          | Stearic (C18:0) | 0.09 ± 0.01± | 2.85 ± 0.16 | 2.55 ± 0.01 | 2.18 ± 0.02 |
|          | Oleic (C18:1) | 0.60± | 0.06± | 0.07± | 0.17± |
|          | Linoleic (C18:2) | 0.34± | 0.60± | 0.84± | 0.07± |
|          | Linolenic (C18:3) | 0.03± | 0.10± | 0.42± | 0.05± |
|          | Arachidic (C20:0) | 0.87 ± 0.03± | 0.99 ± 0.92± | 0.92 ± 0.11± |
|          | SFA | 0.14± | 0.24± | 0.13± | 0.01± |
|          | MUFA | 0.43± | 0.31± | 0.13± | 0.12± |
|          | PUFA | 0.06± | 0.07± | 0.17± | 0.07± |
|          | UFA | 0.05± | 0.53± | 0.39± | 0.13± | 0.60± | 0.19± | 0.07± | 0.26± | 0.06± |
|          | 7 days | 0 W | 100 W | 200 W | 300 W | 400 W | 500 W | 17.87 ± 0.06 | 18.31 ± 0.07 | 17.74 ± 0.08 | 17.22 ± 0.02 | 17.98 ± 0.05 | 19.27 ± 0.03 |
|          | 100 W | 21.09 ± 0.03 | 20.79 ± 0.03 | 20.37 ± 0.03 | 19.78 ± 0.03 | 20.56 ± 0.03 |
|          | 200 W | 21.09 ± 0.03 | 20.79 ± 0.03 | 20.37 ± 0.03 | 19.78 ± 0.03 | 20.56 ± 0.03 |
|          | 300 W | 21.09 ± 0.03 | 20.79 ± 0.03 | 20.37 ± 0.03 | 19.78 ± 0.03 | 20.56 ± 0.03 |
|          | 400 W | 21.09 ± 0.03 | 20.79 ± 0.03 | 20.37 ± 0.03 | 19.78 ± 0.03 | 20.56 ± 0.03 |
|          | 500 W | 21.09 ± 0.03 | 20.79 ± 0.03 | 20.37 ± 0.03 | 19.78 ± 0.03 | 20.56 ± 0.03 |
molecular flexibility of OVA modified by ultrasound at the appropriate power. The poorer emulsifying properties of the suOVA-PEC-PSO emulsions (>300 W) may be attributed to the aggregation of denatured OVA molecules [31].

### 3.3.2. Particle size and zeta-potential

The particle size of the suOVA-PEC-PSO emulsion was significantly smaller than that of the OVA and PEC emulsions/solutions (Fig. 4B). The smallest droplet size (9.82 ± 10.33 μm) for the emulsions was obtained using suOVA treated with ultrasound between 200 and 400 W (Fig. 4B) and this phenomenon was mainly attributed to the size of suOVA prepared by ultrasound. Moreover, compared with the control groups (OVA: -32.8 mV; PEC: -33.8 mV), the zeta-potential of the suOVA-PEC-PSO emulsions became slightly more negatively charged as the ultrasound power was increased, with the lowest value (-40.2 to -39.6 mV) obtained at 300–400 W (Fig. 4B). This indicated that the stability of OVA-PEC-PSO emulsion was promoted by PEC and suOVA by the enhanced electrostatic and spatial repulsion between them [41]. Meanwhile, the expanded suOVA treated with moderate ultrasound in combination with PEC also increased the negative charge on the surface of droplet, and the reassembly of suOVA treated with excess ultrasound reduced this effect [30].

### 3.3.3. Rheological characteristics

The storage modulus (G’) was higher than loss modulus (G”) for all emulsions (Fig. 5A & B), which was attributed to their gel-like structure [42]. The viscoelasticity (G’ and G”) followed the order: suOVA-PEC > OVA-PEC > OVA > PEC (Fig. 5A & B), indicating that the viscoelasticity of emulsion was synergistically enhanced by PEC and OVA, especially for suOVA treated with ultrasound at 300 W. This was consistent with fish myofibrillar protein emulsions stabilized with xanthan gum and treated with ultrasound [43], which was mainly due to the smaller sizes of protein or emulsion droplets [44]. Moreover, similar values for creep were obtained, as demonstrated by the opposite trend for strain observed with these emulsions (Fig. 5C).

### 3.3.4. Microstructure

Optical and confocal laser scanning microscopy (CLSM) were used to characterize the microstructure, including the particle size and interface, of the suOVA-PEC-PSO emulsions. The trends in the changes in the mean droplet diameter and the size distribution of the suOVA-PEC-PSO emulsions determined using optical microscopy and CLSM were consistent with the results measured by the Mastersizer (Fig. 6 & Fig. 4B). Smaller and uniform droplets were obtained in the emulsion prepared by suOVA treated with ultrasound at 300 W (Fig. 6A–F, 6B–F), and the flocculation or coalescence may occurred in these emulsions prepared with larger OVA treated with inappropriate ultrasound (100–200 W & 400–500 W) (Fig. 6A).

Furthermore, as shown in CLSM images, the drops of PSO (green) were encapsulated by OVA and/or PEC emulsifiers (red) (Fig. 6B), showing that all these emulsions were oil-in-water (O/W) systems [43]. Although the particle size of the OVA-PEC complexes was smaller than that of the OVA and PEC solutions, the droplet diameter in the suOVA-PEC-PSO emulsion (300 W) was significantly larger than that of the OVA and PEC solutions and the larger-sized OVA-PEC emulsions (Fig. 6B). This may be explained by the tight and stable network, which is more likely to be formed by small and uniform proteins, improving the stability of the emulsion [30,45].

### 3.3.5. Physical and storage stability

Commercial foods or beverages emulsions are often subjected to salt stress, thermal treatment, or cold storage (freezing); as such, it is necessary to investigate the physical stability of the suOVA-PEC-PSO emulsions under different conditions by assessing particle size or zeta-potential [9]. The mean particle diameter and zeta-potential of the OVA and PEC emulsions appreciably increased when the NaCl concentration was increased from 50 to 250 mM, and those of the OVA-PEC and suOVA-PEC emulsions increased slowly, notably for the emulsion prepared by OVA treated with 300 W ultrasound (Fig. 7A & B). These observations may be attributed to the coalescence of emulsion droplets in the presence of a high concentration of salt, which decreases the electrostatic repulsion between emulsion droplets and then caused the desorption of PEC from the emulsion droplets [9,46]. More importantly, it could be concluded that the stability of the suOVA-PEC-PSO emulsions was improved by the OVA-PEC mixture, likely owing to the stable interfacial layer formed on the surface of oil droplet through electrostatic interaction between OVA and PEC [43].

The mean particle diameter of these emulsions after heat treatment and a single freeze-thaw cycling were significantly higher (P < 0.05) than that of the freshly prepared emulsion (Fig. 7C & Fig. 7D). This suggests that the flocculation of emulsions may occur, owing to reactive groups and/or hydrophobic regions exposed from the interior of denatured (folded) proteins [11]. Moreover, the mean particle diameter of the OVA (76.90 μm), PEC (23.89 μm), and OVA-PEC (14.57 μm) emulsions after heat treatment were 7.56, 2.26, and 1.43 times higher, respectively, than that of suOVA treated with ultrasound at 300 W (Fig. 7C) and similar results were also observed in the that were subjected to a freeze-thaw cycle (Fig. 7D). This suggested that the stability of emulsions subjected to heat treatment or a freeze-thaw cycle was enhanced by PEC and suOVA. The results agreed with the adsorption of OVA/GA (ovalbumin/gum arabic) complexes at the emulsion interface, which was shown to improve the thermal stability of emulsions [47].

### 3.3.6. Oxidation stability and resistance

#### 3.3.6.1. DPPH scavenging ability

The DPPH scavenging rate of OVA-PEC (emulsion: 40.40%; solution: 57.36%) was significantly higher than that of OVA (emulsion: 17.09%; solution: 51.26%) and PEC (emulsion: 28.21%; solution: 11.33%) and significantly lower than that of the suOVA-PEC emulsions (52.86%–59.80%) and solutions (61.15%–68.22%) (Fig. 8). This confirmed synergistic effect of PEC and OVA combination on the DPPH radical scavenging, and that was further enhanced by suOVA, with the highest value of 68.22 ± 1.35% obtained (at an ultrasonic power of 300 W). Similar results were reported in rice peptide nanoparticles and proteins from Chlorella pyrenoidosa [48,49]. This was mainly due to the caviation effect (shearing action) of moderate ultrasound, which increased the contact area for suOVA, exposing more hydrophobic groups, and providing more free radical reaction sites or hydrogen donors [50,51].

#### 3.3.6.2. Changes in PV and fatty acids during storage

The PV of the suOVA-PEC-PSO emulsions (33.91–40.50 meq/kg) was lower than that of the OVA (63.53 meq/kg), PEC (53.78 meq/kg), and OVA-PEC (42.78 meq/kg) emulsions over 5–14 days of storage at 25 °C, although the PV of all emulsions gradually increased over time (Fig. 9). This showed that the oxidation of lipids has been delayed by the combination of OVA and PEC, and this effect was further enhanced by suOVA. This could be explained by the much more stable and thicker interface formed on the suOVA-PEC-PSO emulsion droplets, which provided a physical barrier to prevent the contact between oil, oxygen, and pro-oxidants, and/or the exposure of more antioxidant groups owing to the decreased particle size and increased number of groups [48,52].

Changes in unsaturated fatty acids also have been used to characterize the oxidative stability and quality of oils [53]. The polyunsaturated (e.g., linoleic and linolenic) lipids were gradually oxidized to form saturated, monounsaturated (oleic) lipids and free fatty acids over 7 days of storage at 60 °C (Fig. 9, Table 2) [28]. The oxidation of linolenic acid in suOVA (300 W)-PEC-PSO emulsion was notably delayed (46.58%) compared with the OVA (8.37%), PEC (17.81%) and OVA-PEC (18.87%) emulsions. Thus, it could be inferred that the oxidation of (polyunsaturated) lipids in these emulsions were suppressed.
by suOVA owing to the denser films that were formed at the interface of emulsion droplets.

4. Conclusion

suOVA solution with a particle size of 230.13 ± 14.10 nm was prepared by treatment with 300 W ultrasound, and the average particle size by suOVA owing to the denser films that were formed at the interface of -

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