Structure and stability characterization of pea protein isolate-xylan conjugate-stabilized nanoemulsions prepared using ultrasound homogenization

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ABSTRACT
Preparation of pea protein isolate-xylan (PPI-X) conjugate-stabilized nanoemulsions using ultrasonic homogenization and the corresponding structure and environmental stability were investigated in this study. Conditions used to prepare nanoemulsions were optimized using a response surface methodology as follows: protein concentration 8.86 mg/mL, ultrasound amplitudes 57 % (370.5 W), and ultrasound time 16 min. PPI-X conjugate-stabilized nanoemulsions formed under these conditions exhibited less mean droplet size (189.4 ± 0.45 nm), more uniform droplet distribution, greater absolute value of zeta-potential (44.8 ± 0.22 mV), and higher protein adsorption content compared with PPI-stabilized nanoemulsions. PPI-X conjugate-stabilized nanoemulsions also exhibited even particle distribution and dense network structure, which might be reasons for the observed high interfacial protein adsorption content of conjugate-stabilized nanoemulsions. Moreover, better stability against environmental stresses, such as thermal treatment, freeze–thaw treatment, ionic strength and type, and storage time was also observed for the conjugate-stabilized nanoemulsions, indicating that this type of nanoemulsions possess a potential to endure harsh food processing conditions. Therefore, results provide a novel approach for the preparation of protein-polysaccharide conjugate-stabilized nanoemulsions to be applied as novel ingredients to meet special requirements of processed foods.

1. Introduction

Emulsions are defined as a colloidal dispersion of one immiscible fluids/phases/droplets in the other liquid, which commonly consist of an oil phase and an aqueous phase. The formation of emulsions usually needs energy or pressure provided by an external force, such as high-speed shearing, homogenization, ultrasound, to form available physicochemical properties [1]. Nanoemulsions are one of the most promising type of emulsions applied in the functional food, drug, and cosmetic industries with a diameter <500 nm [2]. Compared with traditional emulsions, nanoemulsions have a number of potential advantages due to the small size of oil droplets and high loading capacity for the encapsulation and delivery of hydrophobic bioactive compounds, and improving the shelf life of fruits and vegetables as edible films or coatings [3,4]. However, for the preparation of nanoemulsions, a feasible stabilizer is needed to overcome the instability of this thermodynamic colloids.

A number of stabilizer used to stabilize nanoemulsions derives from native proteins, such as soybean protein isolate, whey protein concentrate or isolate, sodium caseinate, and pea protein isolate (PPI). Thereinto, PPI extracted from dry peas has been confirmed to be a suitable alternative to animal proteins and be used as an stabilizer in food emulsions due to its excellence in nutrients with gluten free, low cost, free cholesterol, and great environmental sustainability [5,6]. However, compared with wildly commercialized soy protein isolate, PPI exhibits weaker functionality, such as lower solubility in neutral aqueous solution [7]. Therefore, it is necessary to modify the molecular structure or surface charge distribution of PPI to improve functional properties.

As a promising approach, glycosylation occurred by means of the condensation between protein and reducing polysaccharide molecules could modify the structural and functional properties of native protein via the Maillard reaction [8]. Compared with nanoemulsions stabilized

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by sole proteins, nanoemulsions stabilized by protein-poly saccharide complexes or conjugates possess better emulsifying ability and resistance against various environmental stresses [9,10]. After glycosylation, polysaccharide chains are covalently attached to protein molecules, and the resulting amphiphilic conjugates provide a more flexible protein structure for the quick move and adsorption to the oil/water interface [11]. Furthermore, polysaccharide chains also provide a strong steric barrier at the interface to stabilize nanoemulsions effectively for preventing their flocculation and coalescence [12,13]. Recently, many studies have focused on the modification of PPI by glycosylation to stabilize nanoemulsions. Caballero and Davidov-Pardo [14] demonstrated that nanoemulsions stabilized by PPI-dextran conjugates exhibited higher physical stability, such as the storage and pH stability than that stabilized by native PPI. In addition, PPI-gum Arabic conjugates formed through the Maillard reaction endowed nanoemulsions with small particle size, high surface charge, and strong steric hindrance effectively against environmental stresses (specially at acidic pH, high temperature treatment, and high salt concentration) and lipid oxidation [15]. Therefore, it is a promising way to stabilize nanoemulsions by protein-poly saccharide conjugates.

Ultrasound emulsification concentrates on the production of excellent nanoemulsions with small droplet sizes for a long-term stability by acoustic cavitation [16,17]. Compared with other methods, ultrasound emulsification exhibits more advantages due to the high energy efficiency, easy clean and operation [18], and strong nanoemulsions stability [19,20]. For the ultrasonic-assisted preparation of nanoemulsions, two steps are generally involved: 1) coarse emulsions are formed by high-speed shearing mixers; 2) the acoustic cavitation generated during emulsification process can gradually breakdown the coarse emulsions to form uniform, fine, and stable nanoemulsions [20]. In general, varying ultrasound time, amplitudes and concentrations of stabilizer have different effects on the mean droplet size, polydispersity index, and zeta potential of nanoemulsions. For instance, Qamar, Bhandari, Sangeeta, and Prakash [21] prepared pea protein concentrate-stabilized nanoemulsions which showed a significant decrease in their $D_{4,3}$ with increasing sonication time. Additionally, McCarthy et al. [22] also comparatively studied the effectiveness of the formation of nanoemulsions using ultrasound emulsification, homogenization, and microfluidization and results showed that sonication could form emulsions with more uniform and smaller droplets. Therefore, as a high energy emulsification method, ultrasound emulsification is increasingly applied to prepare nanoemulsions.

Previous studies have mainly focused on the application of glycosylation in the improvement of functional properties of pea protein and the physical stability evaluation of nanoemulsions prepared using high pressure homogenization [14,23]. However, PPI-X conjugate-stabilized nanoemulsions prepared using ultrasound emulsification has not been reported. In this study, glycosylation combined with ultrasound emulsification using PPI and xylan as raw materials was performed to construct nanoemulsions in order to obtain desirable characteristics. PPI-X conjugate-stabilized nanoemulsions were prepared using ultrasound emulsification under the optimized protein concentration, ultrasound amplitude, and ultrasound time using a response surface methodology (RSM). Then, the turbidity, percentage of adsorbed proteins, micromorphology, and environmental stability (i.e., thermal, ionic strength, freeze–thaw, and storage stability) of the nanoemulsions were investigated, aiming to preparing stable nanoemulsions to meet physical requirements with processed foods.

2. Materials and methods

2.1. Materials and reagents

Dry pea (Fengyou No. 1) was purchased from local grocery store. Medium chain triglyceride-oil (MCT-oil, food grade) was bought from Tropicana Food Products, Inc. (San Pablo City, Laguna, Philippines). Xylan with an average molecular weight of 1025 Da (confirmed by HPSEC analysis) and Lowry Protein Assay Kit were purchased from Solarbio Science & Technology Co., Ltd. (Beijing, China). All other chemicals used were of analytical grade and obtained from Chengdu Chron Chemical Co., Ltd. (Chengdu, China).

2.2. Preparation of pea protein isolate

PPI was extracted using an isoelectric precipitation method described by Gao, Shen, Lan, Cui, and Rao [24] with a slight modification. Dry peas were first ground into powder to pass through an 80 mesh screen and the sifted powder was defatted twice using ethyl ether with a ratio of 1:4 (w/v) at room temperature. The defatted powder was dispersed in 10-fold distilled water and the suspension was adjusted to pH 9.0 using 2 M NaOH. After stirring at 1000 rpm for 1 h, the suspension was centrifuged at 8000 g for 30 min at 4 °C. The collected supernatant was adjusted to pH 4.5 using 2 M HCl, stored at 4 °C for 2 h, and then centrifuged at 8000 g and 4 °C for 20 min. The protein precipitate was washed twice using distilled water and re-adjusted to pH7.0 using 2 M NaOH. Finally, the solution was freeze-dried and stored at 4°C for further study. The protein content of PPI determined using an automatic kieldahl apparatus (KDN-1, Shanghai Yidian Scientific Instrument Co., Ltd, Shanghai, China) was 89.70 % (w/w, dry basis).

2.3. Preparation of PPI-X conjugate

The PPI-X conjugate was prepared using a dry-heating method [25]. PPI was dissolved in ultrapure water to reach a protein concentration of 20 mg/mL and the suspension was mildly stirred at room temperature for 2 h. After adding xylan with a xylan/PPI mass ratio of 2:1, the mixture was further mildly stirred at room temperature for 2 h and stored at 4 °C overnight. Then, the solution was adjusted to pH 8.15 using 2 M NaOH and was freeze-dried (FD-1-50, Beijing Biocool Experimental Instrument Co., ltd, Beijing, China), ground, and placed in a petri dish. The solid samples were then put in an incubator (LHS-250, Shengyuan Instrument Co., ltd., Zhengzhou, China) to conduct the glycosylation for a certain time (160 min). The temperature and relative humidity were kept at 60 °C and 79 %, respectively. The degree of grafting of the obtained conjugate was 60.92 % according to our previous study [26].

2.4. Preparation of PPI-X conjugate-stabilized nanoemulsions

The PPI-X conjugates were dissolved in ultrapure water and the pH of the suspension was adjusted to pH 7.0 using 2 M HCl or NaOH solutions, followed by continuously stirring for 2 h at room temperature. The supernatant solution was obtained after centrifuging at 12,364 g for 15 min. The protein concentrations (1, 4, 7, 10, 13, and 16 mg/mL) of the final PPI-X conjugate were determined according to the Lowry’s method. MCT-oil was added to the PPI-X conjugate with a volumetric ratio of 1:4 (w/v) at room temperature. The mixture was further mildly stirred at room temperature for 2 h and then centrifuged at 8000 g for 4 °C overnight. The resulting coarse emulsions were subjected to the ultrasound processor (JY92-IIN, Shengyuan Instrument Co., ltd, Shanghai, China) to conduct the glycosylation for a certain time (160 min). The temperature and relative humidity were kept at 60 °C and 79 %, respectively. The degree of grafting of the obtained conjugate was 60.92 % according to our previous study [26].

The PPI-X conjugates were subsequently subjected to the ultrasound processor (JY92-IIN, Ningbo Xinzhi Biotechnology Co., ltd. Ningbo, Zhejiang, China) at 20 kHz in an ice bath. The samples were treated at ultrasound amplitudes of 0 (0 W), 30 % (195 W), 40 % (260 W), 50 % (325 W), 60 % (390 W), 70 % (455 W), and 80 % (520 W), ultrasound treatment time of 5, 10, 15, 20, and 25 min (pulse duration of 5 s on-time and 5 s off-time). The PPI coarse emulsions without ultrasound homogenization (NUPPI-X), and PPI-stabilized nanoemulsions with ultrasound homogenization were set as control groups. PC-300 (0.1 %) was used as an antimicrobial agent. The protein concentration, ultrasound amplitude, and ultrasound time were optimized by combining single factor experiment and RSM.
The droplet size and zeta potential were selected to study the appropriate numerical ranges of factors [27]. Thereafter, a Box-Benhken design was used to optimize the conditions for the preparation of nanoemulsions using the droplet size as a response value.

2.5. Characterization of PPI-X conjugate-stabilized nanoemulsions

2.5.1. Measurement of particle size distribution

Droplet size, zeta potential, and polydispersity index (PDI) of nanoemulsions were determined using a dynamic laser droplet size analyzer (Zetasizer Nano ZS, Malvern Instruments, Ltd., Great Malvern, UK) according to a previous method [28]. The droplet size and zeta potential of nanoemulsions diluted in 50-fold ultrapure water were measured. Each measurement was performed in triplicate at 25 °C.

2.5.2. Turbidity measurement

The turbidity was determined according to our modified method [28]. Nanoemulsions were diluted 50-fold in ultrapure water and the turbidity was recorded at 600 nm using a spectrophotometer (Varioskan Flash, Thermo Fisher Scientific Co., Ltd., Vantaa, Finland). The obtained optical density (OD$_{600}$) value was used to indicate the turbidity. Ultrapure water was used as a blank. Each test was performed in triplicate.

2.5.3. Determination of percentage of adsorbed proteins

Determination of percentage of adsorbed proteins (AP%) of nanoemulsions was measured according to a previous method with a slight modification [29]. Briefly, 3 mL of each sample was centrifuged at 7040 g and 25 °C for 30 min, and the lower aqueous phase was sucked by a syringe. The process was repeated three times. Then the lower aqueous phase was filtered through a 0.22 μm filtration membrane. After the sample was diluted 50 times, the Lowry’s method was used to determine the protein concentration of the lower aqueous phase. The formulas of AP (%) is listed below.

\[
\text{AP} \% = \frac{A_0 - A_1}{A_0} \times 100\%
\]

where $A_0$ was the protein concentration in the initial protein solution, $A_1$ was the protein concentration of the unadsorbed layer.

2.5.4. Fourier transform infrared (FTIR) analysis

Structural characteristics of PPI-X conjugate-stabilized nanoemulsions were analyzed using FTIR spectroscopy (Nicolet iS10, Thermo Fisher Scientific Corp., Madison, WI, USA). The freeze-dried samples (2 mg) were thoroughly mixed with dried potassium bromide (200 mg) and were then pressed into a thin salt tablet. The spectra were acquired using a 32 scan in a range of 500–4000 cm$^{-1}$ at a resolution of 4 cm$^{-1}$. A pure potassium bromide tablet was used to remove the background. The FTIR spectral data were analyzed using an OMNIC software (Thermo Electric Corporation, Chicago, IL, USA).

2.5.5. Microstructure observation

The morphological properties of nanoemulsions was observed using a field emission scanning electron microscope (SEM, Quanta 25, FEI company, Hillsboro, OR, USA) and a field emission transmission electron microscope (TEM, Tecnai G2 F20, FEI company, USA) from different perspectives. The freeze-dried nanoemulsions were fixed on the objective table, and coated with a conductive layer. Samples were observed at an accelerating voltage of 5 kV. In addition, a certain amount freeze-dried nanoemulsions was dissolved in appropriate ethanol, ultrasonically dispersed for 5–10 min, and then placed onto a copper wire for observing the surface morphology and size of droplet particles using TEM. The handle voltage was 200 kV and the camera constant was 300.

2.6. Stability analysis

The stability of nanoemulsions was characterized against thermal treatment, freeze–thaw cycles, ionic strength and type, and storage by analyzing the changes in droplet size and zeta potential.

Specifically, nanoemulsions heated at 60, 70, 80, and 90 °C for 30 min was conducted to evaluate the thermal stability. The ionic strength stability of nanoemulsions were investigated by adding NaCl into the nanoemulsions with a final ionic strength of 50, 100, 200, and 400 mM, respectively. As for different salt ions, Na$^+$ (NaCl), K$^+$ (KCl), Mg$^{2+}$ (MgCl$_2$), and Fe$^{2+}$ (FeSO$_4$) were respectively added to fresh samples with a final ionic strength of 50 mM. 4 mL nanoemulsions was stored at −18 °C for 24 h and then thawed at 25 °C for 3 h to determine the droplet size and zeta potential at room temperature. The rest samples were re-frozen, and repeated in seven cycles. The freeze–thaw stability of nanoemulsions was determined after each cycle. The storage stability of nanoemulsions was observed during storage at 4 °C (0, 2, 4, 6, 10, 14, 21, and 28 days) and at 25 °C (0, 2, 4, 6, 10, and 14 days), respectively.

2.7. Statistical analysis

Each group of experiments was repeated three times and the data were expressed as a form of mean ± standard deviation. The RSM was performed using Design Expert 8.0.6 software. All tests were subjected to one-way analysis of variance (one-way ANOVA) and Duncan test using SPSS 21.0 software (IBM®, Chicago, IL, USA). All tests were set at a significance level of $p < 0.05$. All figures were drawn using Origin 9 software (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Optimization of PPI-X conjugate-stabilized nanoemulsions preparation

Ultrasound emulsification is usually used to produce nanoemulsions with small droplet size and thus long-term stability [17]. Different preparation conditions, such as concentration of stabilizer, ultrasound power, and time, have different effects on the ultrasound-assisted emulsification. For instance, Qamar et al. [21] revealed that the particle size and creaming of nanoemulsions decreased with increasing ultrasoundation time. Therefore, it is important to optimize the effects of critical conditions on the particle size of the nanoemulsions stabilized by PPI-X conjugate.

An appropriate particle size can inhibit the aggregation of oil droplets to form stable nanoemulsions [30]. The zeta potential, as an indicator of the potential stability of nanoemulsions, reflects the surface charge density of proteins [31]. The changes in droplet size and zeta potential of the obtained nanoemulsions at various protein concentrations are shown in Fig. 1A. It is generally believed that protein concentration plays an important role in the stabilization of nanoemulsions. When being enough protein molecules can sufficiently cover the oil droplets, thus promoting the formation of a stable interfacial film [32]. When the protein concentration increased from 1 to 10 mg/mL, the droplet size decreased gradually (p < 0.05). However, when the concentration further increased, there was a slight increase in droplets size due to an excess of non-adsorbing protein, consequently accelerating droplets flocculation by depletion phenomenon [33]. Amine, Dreher, Helgason, and Tadros [34] also reported the similar result when ultrasound-assisted prepared emulsions stabilized by pea proteins with a series of concentration from 0.1 % to 7.5 %. Moreover, when protein concentration reached 10 mg/mL, the zeta potential absolute value was 40.7 ± 1.38 mV, indicating a stronger interparticle electrostatic repulsion existed between PPI-X conjugate molecules. It is generally believed that the increased absolute value of zeta-potential of nanoemulsions provided a high energy barrier between emulsion droplets to stabilize nanoemulsions [31]. Therefore, under the selected conditions, an
appropriate protein concentration should be considered for ultrasound emulsification.

As shown in Fig. 1B, when the ultrasound amplitude changed from 30 % to 80 %, the droplet size first significantly decreased ($p < 0.05$), and then slowly decreased at an ultrasound amplitude of 50 %. More importantly, all droplet size was less than 200 nm within the applied ultrasound amplitude (50 %–80 %). Belgheisi, Motamedzadegan, Milani, Rashidi, and Rafe [35] also showed a significant reduction in droplet size of maltodextrin/pectin-stabilized nanoemulsions with an increasing sonication amplitude. However, no significant difference in zeta potential was observed among the nanoemulsions prepared at different ultrasound amplitude ($p > 0.05$). This result disagreed with a previous study which revealed a first decrease and then increase in zeta potential for ovalbumin/pectin complexes-stabilized nanoemulsions prepared using an ultrasound method [36]. Thus, it is important to select greater ultrasound amplitude via considering energy cost and purpose of the experiment.

Effects of ultrasound time on the droplet size and zeta potential in PPI-X conjugate-stabilized nanoemulsions are shown in Fig. 1C. The droplet size tended to decrease significantly with the increasing time and the smallest droplet size ($199.7 \pm 2.28$ nm) was observed when treated for 15 min. This might be related to that the energy generated by cavitation caused the droplets to be violently agitated resulting in a reduction of the particle size [37]. However, when ultrasound time was 20 min, the droplet size of nanoemulsions stabilized by PPI-X conjugate increased again. Similar results were reported by Zou et al. [29]. This phenomenon may be due to the “over-processing” to result in droplet aggregation [38]. Besides, when ultrasound time changed from 0 to 25 min, the zeta potential first decreased and then increased, demonstrating that ultrasound treatment affected the charge distribution on the surface of nanoemulsions droplets. The reason for these results was that excess ultrasound reduced electrostatic repulsion between nanoemulsions droplets at a longer duration [36]. Therefore, for the preparation of PPI-X conjugate-stabilized nanoemulsions, protein concentration of 7, 10, and 13 mg/mL, ultrasound amplitudes of 40 %, 50 %, and 60 %, and ultrasound time of 10, 15, and 20 min, were selected to perform optimization test. The RSM results were described in the Supplementary material. The regression model constructed based on the protein concentration, ultrasound amplitudes, and ultrasound treatment time, showed an excellent ability to predict the droplet size value. As exhibited in Table S1, the optimized preparation conditions were protein concentration of 8.86 mg/mL, ultrasound amplitudes of 57 % (370.5 W), and ultrasound time of 16 min. Under these optimal conditions, the actual droplet size was $189.4 \pm 0.45$ nm which was 97.25 % of the predicted value, indicating that the experimental result was well in agreement with the predicted values. Therefore, the PPI-X conjugate-stabilized nanoemulsions used to perform the following analysis were obtained under above optimized conditions.

### 3.2. Characterization of PPI-X conjugate-stabilized nanoemulsions

#### 3.2.1. Particle size distribution analysis

As listed in Table 1, compared with the NUPPI (droplet size $> 1 \mu m$), both PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions...
Table 1  
The droplet size, zeta potential and PDI of NUPPI, NUPPI-X, and the nanoemulsions stabilized by PPI and PPI-X conjugate with ultrasound homogenization.

| Sample               | Droplet size (nm) | Zeta potential (mV) | PDI       |
|----------------------|-------------------|---------------------|-----------|
| NUPPI                | >1000             | -36.8 ± 0.82a       | 1.000 ± 0.00a |
| NUPPI-X              | >1000             | -38.1 ± 0.12b       | 0.894 ± 0.10b |
| PPI                  | 287.4 ± 3.65      | -38.7 ± 0.73b       | 0.427 ± 0.00b |
| PPI-X                | 189.4 ± 0.45      | -44.8 ± 0.22c       | 0.147 ± 0.04d |

NUPPI, the PPI coarse emulsions without ultrasound homogenization; NUPPI-X, PPI-X conjugate coarse emulsions without ultrasound homogenization; PPI, pea protein isolate; X, xylan; Different letters following the data in the same column mean significant differences, p < 0.05.

...exhibited a significant smaller droplet size. In addition, the absolute value of zeta potential (-44.8 ± 0.22 mV) and PDI (0.147 ± 0.04) of PPI-X conjugate-stabilized nanoemulsions were greater than those of PPI-stabilized nanoemulsions under the same preparation conditions, which might be related to the increase in net negative charges surrounding the protein molecules and the thicker layer of conjugates [31]. In comparison, NUPPI and NUPPI-X exhibited lower absolute value of zeta potential, demonstrating the ability to form droplets for PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions in the nannometric range under ultrasound homogenization. Wang et al. [39] indicated that the increased charge can be related with the originally dense structure of proteins unfolded in the ultrasonic power and thus the polar groups inside exposed. PPI-X conjugate-stabilized nanoemulsions displayed a monomodal particle size distribution and NUPPI exhibited trimodal particle size distribution. All other nanoemulsions (PPI-stabilized nanoemulsions and NUPPI-X) exhibited two smooth peaks (Fig. 2S). These findings suggest that a stable and uniform nanoemulsion can be formed by ultrasound homogenization.

3.2.2. Turbidity analysis

The turbidity of an nanoemulsions negatively correlated with its stability is not only related to droplet size, but also closely related to the droplet shape and distribution [40]. The turbidity (OD\textsubscript{600}) of nanoemulsions prepared under different conditions was shown in Fig. 2A. The OD\textsubscript{600} value of both PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions were lower than that of the NUPPI and NUPPI-X (without ultrasound homogenization), indicating that the turbidity of nanoemulsions changed significantly after ultrasound homogenization. It can be seen that the change trend of turbidity was consistent with the droplet size, zeta potential, and PDI results mentioned above. Wang et al. [40] explained that the strong physical force of ultrasound waves crushed the droplets by ultrasound, resulting in the reduction of particle size, more uniform droplet distribution, and decreased turbidity. In addition, the PPI-X conjugate-stabilized nanoemulsions showed a significantly reduced OD\textsubscript{600} value compared with the PPI-stabilized nanoemulsions (p < 0.05), which might be related to the repulsive steric interaction among the Maillard conjugates [41].

3.2.3. Percentage of absorbed proteins

The AP% is an index for analyzing the interfacial protein adsorption to better understand the protein content adsorbed on droplet surface in emulsions. Generally, the larger proportion of absorbed proteins is, the stronger the emulsification ability obtains [29]. As exhibited in Fig. 2A, the nanoemulsions prepared by PPI-xylan conjugate exhibited much high AP% values. This might be related to the formation of a stable interfacial protein film or specifically a denser and more stable interfacial layer on the surface of oil droplets because of the covalent and non-covalent cross-linking among protein molecules induced by the Maillard reaction [42]. This phenomenon was consistent with the result of micromorphological observation. Moreover, PPI-X conjugate-stabilized nanoemulsions exhibited higher protein adsorption at the oil-water interface compared with NUPPI-X, demonstrating that ultrasound emulsification increased the AP%. Zou et al. [29] revealed that ultrasonication decreased the particle size of oil droplets to improve the adsorption of proteins on the oil-water interface. Thus, the combination of the preparation of PPI-X conjugate and ultrasound treatment can be used to stabilize food nanoemulsions.

3.2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra (shown in Fig. 2B) can provide structural properties of nanoemulsions. The bands in the regions of 3700–3200 cm\textsuperscript{-1}, 1700–1600 cm\textsuperscript{-1} (Amide I), and 1600–1500 cm\textsuperscript{-1} (Amide II) reflect the characteristics of proteins on the oil-water interface. Thus, the combination of the preparation of PPI-X conjugate and ultrasound treatment can be used to stabilize food nanoemulsions.

![Fig. 2. The turbidity, AP (A), and FTIR (B) of NUPPI, NUPPI-X, and the nanoemulsions stabilized by PPI and PPI-X conjugate with ultrasound homogenization (NUPPI, PPI coarse emulsions without ultrasound homogenization; NUPPI-X, PPI-X conjugate coarse emulsions without ultrasound homogenization; PPI-X, pea protein isolate-xylan; PPI, pea protein isolate; X, xylan; Different letters represent significant difference, p < 0.05).](image-url)
glycosylation with xylan, a strong peak at 1042 cm\(^{-1}\) was also observed in NUPPI-X and PPI-X conjugate-stabilized nanoemulsions, suggesting that the glycosylation greatly changed the protein secondary structures [44]. It was observed that the peak in the Amide I region (1700–1600 cm\(^{-1}\)) was slightly shifted from 1658 cm\(^{-1}\) for the PPI-stabilized nanoemulsions to 1647 cm\(^{-1}\) for the NUPPI. Likewise, the peak in Amide II region (1600–1500 cm\(^{-1}\)) shifted from 1543 cm\(^{-1}\) for the PPI-X conjugate-stabilized nanoemulsions to 1548 cm\(^{-1}\) for the NUPPI. These results indicated that ultrasound treatment has a substantial impact on the protein secondary structure, which might related to the unfolding of protein molecules due to the acoustic cavitation during sonication [45,46]. Wang et al. [47] also found that the cavitation effect of ultrasound could change the protein secondary structure to increase the protein flexibility.

3.2.5. Micromorphological properties

The micromorphology observed using SEM was shown in Fig. 3I. As exhibited in Fig. 3Ia, big microsized pores or cavities were clearly observed for the NUPPI, which was also in connection with the results of flocculation and coalescence of oil droplets. In comparison, the PPI-stabilized nanoemulsions (Fig. 3Ib) exhibited a more smooth structure with a relatively smooth surface. More importantly, compared with the NUPPI-X (Fig. 3Ic), the PPI-X conjugate-stabilized nanoemulsions (Fig. 3Id) showed an even particle distribution and dense network structure due to the stronger steric hindrance. Moreover, from the appearance of freeze-dried powder of NUPPI (Fig. 3IIA) and NUPPI-X (Fig. 3IIC), the leaking of oil was observed, further indicating that ultrasound homogenization could improve the stability of nanoemulsions. The TEM images of the PPI-stabilized nanoemulsions (Fig. 3E) and PPI-X conjugate-stabilized nanoemulsions (Fig. 3F) exhibited regular spherical shape with completely intact surface and were in good agreement with the average droplet size results shown in Table 1. Similar spherical shapes were also reported by Chang et al. [48] who studied the micromorphology of soybean protein isolate or whey protein isolate-stabilized nanoemulsions. Particularly, PPI-X conjugate-stabilized nanoemulsions were homogeneously dispersed with smaller droplet size, indicating that the grafting of polysaccharide chains to protein molecule minimize the aggregation of oil droplets. This might be due to the formation of massive dense layer surrounding to oil droplets formed by protein-polysaccharide interaction in an aqueous phase [49]. Therefore, the Maillard modification combined with ultrasound homogenization can effectively improve the emulsifying properties.

3.3. Stability of nanoemulsions

3.3.1. Thermal stability

Commercial emulsion products may undergo a variety of heat treatments during production, storage, and usage, such as the pasteurization process. Therefore, it is of practical significance to study the thermal stability of nanoemulsions. As shown in Fig. 4A, compared with PPI-stabilized nanoemulsions, PPI-X conjugate-stabilized nanoemulsions exhibited smaller particle size and higher zeta potential at 60–90 \(^\circ\)C for 30 min. It is noteworthy that PPI-X conjugate-stabilized nanoemulsions exhibited a slight increase in droplet size at 70 \(^\circ\)C, indicating that the protein-polysaccharide conjugates applied as a stabilizer was quite stable and showed greater thermal stability. This change was similar to that reported by Gumus, Davidov-Pardo, and McClements [50] who investigated the thermal stability of sodium...
caseinate-dextran conjugate-stabilized lutein-loaded nanoemulsions. From the appearance of nanoemulsions (Fig. S4), creaming and phase separation were not observed, and no noticeable appearance changes were observed for both PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions. These results indicate that the grafted polysaccharide chains weakened the absorption of peptide chains caused by heat due to the strong steric hindrance of polysaccharide moieties, thus increasing the thermal stability of the prepared nanoemulsions [51].

3.3.2. Ionic strength stability

The influence of NaCl concentration (0–400 mmol/L) on the droplet size and zeta potential of nanoemulsions was shown in Fig. 4B. Compared with the nanoemulsions without NaCl, no significant increase in droplet size was observed for PPI-X conjugate-stabilized nanoemulsions within the entire ionic strength range. Moreover, even when 400 mmol/L salt was added, the droplet size was still small. In addition, PPI-X conjugate-stabilized nanoemulsions showed greater zeta potential value (-30.5 ± 0.78 mV) at a higher NaCl concentration (200 mmol/L), suggesting that the particle size distribution of PPI-X conjugate-stabilized nanoemulsions was uniform. In comparison, when the NaCl concentration was greater than 200 mmol/L, the droplet size of PPI-stabilized nanoemulsions was greater than 1 μm and the zeta potential significantly decreased (p < 0.05). Chen, Wang, Guo, Li, Meng, and Liu [52] reported that whey protein isolate-gum Acacia conjugate-stabilized nanoemulsions also exhibited better ionic strength stability compared with whey protein isolate-stabilized nanoemulsions due to the
conjugate-formed thicker interfacial adsorbed layer. Therefore, it is an effective way to improve salt tolerance of nanoemulsions by applying PPI-xylan conjugate as a stabilizer. This finding extends the usage of PPI as a stabilizer in high-salty foods, such as ham sausage and luncheon meat.

3.3.3. Salt ions stability

Nanoemulsions-based delivery systems can be used for manufacturing functional food and beverage products which generally contain various types of salt ion. Therefore, the stability of nanoemulsions against different types of salt was also investigated. As for the addition of NaCl and KCl (monovalent salt ions), the least droplet size (about 200 nm) and PDI (<0.2) were observed in PPI-X conjugate-stabilized nanoemulsions, and its zeta potential also exhibited a high absolute value (shown in Table 2). Furthermore, the absolute value of zeta potential of PPI-X conjugate-stabilized nanoemulsions was greater than that of PPI-stabilized nanoemulsions, suggesting that monovalent salt ion at the applied concentration has little effect on the stability of PPI-X conjugate-stabilized nanoemulsions. This might be related to the increase in the interfacial layer thickness of PPI-X conjugate-stabilized nanoemulsions resulting in the improvement of nanoemulsions stability for the addition of NaCl and KCl [53]. In comparison, with the addition of MgCl₂ and FeSO₄ at the same concentration, the droplet size significantly increased (>1 μm) in both PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions, indicating the occurrence of droplet flocculation in nanoemulsions. Moreover, for the addition of FeSO₄, the absolute zeta potential of PPI-stabilized and PPI-X conjugate-stabilized nanoemulsions decreased to 2.4 ± 0.13 mV and 2.1 ± 0.12 mV, respectively. A clear water layer was observed in all samples when MgCl₂ was added (Fig. S4). It can be concluded that the prepared nanoemulsions are prone to coalescence, creaming, and phase separation triggered by MgCl₂ and FeSO₄, which could be attributed to ion binding or ion bridging effects induced by these bivalent salt ions [54].

3.3.4. Freeze-thaw stability

A number of physicochemical processes, including fat crystallization, ice crystallization, and phase separation, occur during freeze-thawing of emulsion-based products, such as cheese and cream sauces [55]. Effects of freeze–thaw cycles on the droplet size and zeta potential of nanoemulsions were shown in Fig. 4C. With an increasing number of freeze–thaw cycle, the droplet size of PPI-X conjugate-stabilized nanoemulsions slightly increased and a slow decrease in zeta potential were observed. Furthermore, at the seventh freeze–thaw cycles oil droplets layering was not observed in PPI-X conjugate-stabilized nanoemulsions (Fig. S3), which was in accordance with the changes in droplet size and zeta potential. On the contrary, the particle size of PPI-stabilized nanoemulsions increased significantly (p < 0.05). After the first freeze–thaw cycle, the droplet size of PPI-stabilized nanoemulsions reached 461.1 ± 0.70 nm, which is mainly due to the occurrence of flocculation or coalescence between oil droplets during freeze–thaw cycle [56]. Moreover, at the first cycle, there was certain emulsion floatation with a clear oiling off phenomenon in PPI-stabilized nanoemulsions (Fig. S3). Therefore, PPI-X conjugate-stabilized nanoemulsions possessed better freeze–thaw stability than PPI-stabilized nanoemulsions. A similar finding was reported by O’Regan and Mulvihill [57] who observed that the maltodextran-grafted sodium caseinate at the droplet surface significantly improved freeze–thaw stability of nanoemulsions due to hydrodynamic and steric stabilization effects.

3.3.5. Storage stability

The storage stability of emulsions was evaluated by investigating the changes in droplet size and zeta potential at 4 °C for 4 weeks and at 25 °C for 2 weeks, respectively. As shown in Fig. 4D, PPI-X conjugate-stabilized nanoemulsions exhibited no distinct change in droplet size (<206 nm) and were stable during the duration at 4 °C, but the droplet size and zeta potential of PPI-stabilized nanoemulsions showed a decreased trend in overall with an irregular change during storage, which might be in connection with the protein aggregation. In comparison, the droplet size, with 197.7 ± 1.06 nm after a 14-day storage at 25 °C (shown in Fig. 4E), seems to be stable in PPI-X conjugate-stabilized nanoemulsions during storage, and no noticeable aggregation and subsequent precipitation were observed after two weeks (Fig. S4). In addition, for the same storage time, the zeta potential of PPI-X conjugate-stabilized nanoemulsions was higher than that of PPI-stabilized nanoemulsions. The mean size of PPI-stabilized emulsion sharply increased to 705.7 ± 5.75 nm for 10 days of storage. This finding is consistent with that reported by Li et al. [58] who revealed that the galactose-grafted whey protein isolate-stabilized emulsion showed a greater storage stability compared with the whey protein isolate-stabilized nanoemulsions due to the relatively easily attachment of glycosylated protein onto the surface of oil droplets. Thus, these results suggest that the glycosylation of PPI with xylan could extend the storage time of protein-based nanoemulsions against flocculation and coalescence at 4 °C or room temperature.

3.4. Possible mechanism for the stabilization of nanoemulsions by PPI-xylan conjugate

Fig. 5 depicts the possible mechanisms of the stabilization of nanoemulsions by PPI-xylan conjugate assisted with ultrasound homogenization. As revealed above, PPI-X conjugate-stabilized nanoemulsions exhibited a significantly small droplet size, indicating that the cavitation effect and physical force through ultrasound waves crushed the droplets by ultrasoundondation [40]. Moreover, the increasing charge can be related with the originally dense structure of proteins unfolded by the ultrasonic power and thus the polar groups inside exposed [39]. Concurrently, the grafting of xylan onto PPI molecules via the Maillard reaction minimizes the aggregation of oil droplets and then the adsorption of proteins on the oil–water interface increased. Furthermore, the formation of a stable interfacial PPI-xylan conjugate layer and dense network structure of nanoemulsions correlated to the strong steric hindrance provided by xylan moieties, which are sufficient to inhibit nanoemulsions flocculation and coalescence. Therefore, protein glycosylation combined with ultrasound homogenization can appropriately change the structure of native protein and thus effectively construct nanoemulsions with desirable physicochemical properties.

4. Conclusions

In this study, PPI-X conjugate-stabilized nanoemulsions were prepared using a ultrasound emulsification method and characterized centering on the structural properties and environmental stability. The

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Table 2

| Salt type | PPI | PPI-X |
|----------|-----|-------|
| Droplet size (nm) | Zeta potential (mV) | PDI | Droplet size (nm) | Zeta potential (mV) | PDI |
| Control | 287.4 ± 39.3 | 0.427 | 189.4 ± 44.8 | 0.147 |
| NaCl | 3.65 | 0.73 ± 0.00 | 0.45 | 0.22 ± 0.00 |
| KCl | 11.84 | 0.05 ± 0.09 | 4.46 | 0.56 ± 0.01 |
| MgCl₂ | 2.83 ± 1.00 | 5.15 | 199.2 ± 35.3 | 0.178 |
| FeSO₄ | 15.00 | 0.34 ± 0.00 | 5.15 | 1.00 ± 0.00 |

* - indicates the PDI is unstable; PPI-X, pea protein isolate-xylan conjugate; PPI, pea protein isolate; X, xylan; Different letters following the data in the same column mean significant differences (p < 0.05).
conditions for the preparation of PPI-xylan conjugate-stabilized nanoemulsions were optimized as follows: protein concentration of 8.86 mg/mL, ultrasound amplitudes of 57%, and ultrasound time of 16 min. The PPI-X conjugate-stabilized nanoemulsions prepared under these optimal conditions exhibited decreased droplet size, more uniform droplet distribution, higher absolute zeta potential, decreased turbidity, and larger proportion of adsorbed proteins compared with the PPI-stabilized nanoemulsions. Besides, an even droplet distribution and dense network structure could be clearly observed in the PPI-X conjugate-stabilized nanoemulsions. The nanoemulsions also exhibited excellent stability against thermal treatment (60–90 °C, holding time 30 min), ionic strength (0–400 mM), freeze–thaw treatment (seven cycles), and storage. These findings demonstrated that the combination of formation of protein conjugate and ultrasound homogenization is a promising method to prepare food nanoemulsions with enhanced physicochemical properties, which provides an innovative way to broaden the development and utilization of pea proteins.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Dan Zhao: Conceptualization, Methodology, Investigation, Writing – original draft. Yuhong Ge: Investigation. Xianrong Xiang: Validation. Hongmin Dong: Writing – review & editing. Wen Qin: Writing – review & editing. Qing Zhang: Supervision, Funding acquisition, Project administration, 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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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References

[1] K. Sneha, A. Kumar, Nanoemulsions: Techniques for the preparation and the recent advances in their food applications, Innov. Food Sci. Emerg. Technol. 76 (2022), 102994, https://doi.org/10.1016/j.ifset.2021.102994.
[2] M. Marhamati, G. Ranjbar, M. Rezaie, Effects of emulsifiers on the physicochemical stability of Oil-in-water Nanoemulsions: a critical review, J. Mol. Liquids 340 (2021), 117218, https://doi.org/10.1016/j.molliq.2021.117218.
[3] D.J. Mc Clements, Advances in edible nanoemulsions: digestion, bioavailability, and potential toxicity, Prog. Lipid Res. 81 (2021), 101081, https://doi.org/10.1016/j.plipres.2020.101081.
[4] A. Naseema, L. Kovoorn, A.K. Behera, K.P.P. Kumar, P. Srivastava, A critical review of synthesis procedures, applications and future potential of nanoemulsions, Adv. Colloid Interface Sci. 287 (2021), 102318, https://doi.org/10.1016/j.cis.2020.102318.
[5] Z.L. Gao, P.Y. Shen, Y. Lan, L.Q. Cai, J.B. Ohm, B.C. Chen, J.J. Rao, Effect of alkaline extraction pH on structure properties, solubility, and benny flavor of yellow pea protein isolate, Food Res. Int. 131 (2020), 109045, https://doi.org/10.1016/j.foodres.2019.109045.
[6] B.R. Zhang, X.M. Kang, Y.H. Cheng, B. Cai, A.M. Abd El-Aty, Impact of high moisture contents on the structure and functional properties of pea protein isolate during extrusion, Food Hydrocolloid. 127 (2022), 107508, https://doi.org/10.1016/j.foodhyd.2022.107508.
[7] Z.J. Zhi, L. Yan, H. Li, K. Desvetting, P.V.D. Meeren, R. Liu, F.V. Bockstaele, A combined approach for modifying pea protein isolate to greatly improve its solubility and emulsifying stability, Food Chem. 380 (2022), 131832, https://doi.org/10.1016/j.foodchem.2021.131832.
[8] L. Li, C.Z. Wang, K.X. Li, D.T. Wu, B. Hu, W.Y. Yang, H.M. Dong, Q. Zhang, Influence of soybean protein isolate-dextran conjugates on the characteristics of glucono-δ-lactone-induced tofu, IWT 139 (2021), 110588, https://doi.org/10.1016/j.iwt.2020.110588.
[9] J.L. Feng, C.C. Berton-Carabin, V. Fogliano, K. Schroes, Maillard reaction products as functional components in oil-in-water emulsions: a review highlighting interfacial and antioxidant properties, Trends Food Sci. Technol. 121 (2022) 129–141, https://doi.org/10.1016/j.tifs.2021.02.008.
[10] M. Evans, L. Ratcliffe, P.A. Williams, Emulsion stabilisation using polyaccharide-protein complexes, Car. Opin. Colloid Interface Sci. 18 (2013) 272–282, https://doi.org/10.1016/j.jocs.2013.04.004.
[11] J.A. O' Maloney, K.P. Drapala, E.M. Mulcathy, D.M. Mulvihill, Controlled glycation of milk proteins and peptides: Functional properties, Int. Dairy J. 77 (2017) 16–34, https://doi.org/10.1016/j.idairyj.2016.09.012.
[12] X.H. Kan, G.J. Chen, W.T. Zhou, X.X. Zeng, Application of protein-poly saccharide Maillard conjugates as emulsifiers: Source, preparation and functional properties, Food Res. Int. 150 (2021), 110740, https://doi.org/10.1016/j.foodres.2021.110740.
[13] Q. Zhang, Y.Y. Zhou, W. Qin, H.M. Dong, T. Vasanthan, Nanostructures of protein-poly saccharide complexes or conjugate for encapsulation of bioactive compounds.
C. Amine, J. Dreher, T. Helgason, T. Tadros, Investigation of emulsifying properties of Maillard conjugates improves physical and oxidative stability of oil-in-water emulsions, Food Chem. 285 (2022) 112607, https://doi.org/10.1016/j.foodchem.2021.112607.

A. Taha, E. Ahme, T. Hua, X. Xu, S. Pan, H. Hu, Effects of different ionic strengths on the physicochemical properties of plant and animal proteins-stabilized emulsions fabricated using ultrasonic emulsification, Ultrason. Sonochem. 58 (2020) 106195, https://doi.org/10.1016/j.ultsonch.2018.12.012.

M. Nooshkam, M. Varidi, Physicochemical stability and gastrointestinal fate of biotechnology-derived functional proteins: impact of GaLA conjugation on the stability, intestinal and solubility properties of wheat gluten proteins in the presence of bile salts and carbohydrates, Compr. Rev. Food Sci. Food Saf. 13 (2014) 1-16, https://doi.org/10.1111/1541-4337.12190.

W. Huang, X.L. Xiang, X.Y. Luo, X.T. Li, X.W. Yu, S.G. Li, Study on the emulsification and oxidative stability of ovalbumin-pectin-pumpkin seed oil emulsions using ultrasonic emulsification, Ultrason. Sonochem. 78 (2022) 105717, https://doi.org/10.1016/j.ultsonch.2021.105717.

T. Wang, N. Wang, N. Li, X.R. Ji, H.W. Zhang, D.Y. Yu, L.Q. Wang, Effect of high-intensity ultrasound on the physicochemical properties, microstructure, and stability of soy protein isolate-stabilized emulsions, Ultrason. Sonochem. 82 (2022) 105871, https://doi.org/10.1016/j.ultsonch.2021.105871.

S. Kentsh, T.J. Wooster, M. Ashokkumar, S. Balachandran, R. Mawson, L. Simons, The use of ultrasound for nanoemulsion preparation, Innov. Food Sci. Emerg. Technol. 9 (2007) 176-175, https://doi.org/10.1016/j.ifset.2007.07.005.

T. Wang, X. Chen, W.N. Wang, L.Q. Wang, L.Z. Jiang, D.Y. Yu, F.Y. Xie, Effect of ultrasound on the properties of rice bran protein and its chlorogenic acid complex, Ultrason. Sonochem. 71 (2021), 105758, https://doi.org/10.1016/j.ultsonch.2021.105758.

W.N. Wang, R.Y. Wang, J. Yao, S.N. Luo, X. Wang, N. Zhang, L.Q. Wang, X.Q. Zhu, Effect of ultrasound power on the emulsion stability of rice bran protein - cholic acid emulsion, Ultrason. Sonochem. 84 (2022), 105959, https://doi.org/10.1016/j.ultsonch.2022.105959.

P.G. Nagaraju, P. Sindhu, T. Dubey, S. Chinnathambi, C.G.P. Priyadarshini, J. Rao, Influence of sodium caseinate, malto dextri n, pectin, and their Maillard conjugate on the stability, in vitro release, antioxidant property and cell viability of Eugenol oil in nanoemulsions, Int. J. Biol. Macromol. 183 (2021) 158-170, https://doi.org/10.1016/j.ijbiomac.2021.04.122.

J.J. Yu, Y.F. Zhang, J. Yan, S.H. Li, Y. Chen, A novel glycoprotein emulsion using high-denatured peanut protein and sesbania gum via cold plasma for encapsulation of β-carotene, Innov. Food Sci. Emerg. Technol. 74 (2021), 102840, https://doi.org/10.1016/j.ifset.2021.102840.

U. Etruglu, S. Namli, O. Tas, K. Oktacagly, V. Gokmen, S.G. Sunmu, M.H. Ortop, Physicochemical properties of acetylated fennel oil nanoparticles prepared by microfluidization, LWT (2015), 111939, https://doi.org/10.1016/j.lwt.2015.11.039.

Y.T. Shen, Y.H. Li, Acylation modification and/or gum guar conjugation enhanced functional properties of pea protein isolate, Food Hydrocolloid. 117 (2021), 105442, https://doi.org/10.1016/j.foodhyd.2021.105442.

J.J. Cheng, L.Q. Cui, Effects of high-intensity ultrasound on the structural, optical, mechanical and physicochemical properties of pea protein isolate-based edible film, Ultrason. Sonochem. 80 (2021), 105890, https://doi.org/10.1016/j.ultsonch.2021.105890.

A.B. Khatkar, A. Kaur, S.K. Khatak, N. Mehta, Characterization of heat-stable whey protein isolate: Impact of ultrasound on rheological, thermal, structural and morphological properties, Ultrason. Sonochem. 49 (2018) 333-342, https://doi.org/10.1016/j.ultsonch.2018.08.026.

M. Wang, X.N. Zhou, W.N. Wang, L.Q. Wang, L.Z. Jiang, T.Y. Liu, D.Y. Yu, Effect of high-intensity ultrasound on the structure and solubility of soy protein isolate–pectin complex, Ultrason. Sonochem. 80 (2021), 105808, https://doi.org/10.1016/j.ultsonch.2021.105808.

M. Chang, Y.W. Guo, Z.R. Jiang, L.K. Shi, T. Zhang, Y.D. Wang, M.G. Cen, T. Wang, R.X. Lin, R.J. Liu, Y. Wang, Q.Z. Jin, X.G. Wang, Sea buckthorn pulp oil nanoemulsions fabricated by ultra-high pressure homogenization process: A promising carrier for nutraceutical, J. Food Eng. 287 (2020), 110129, https://doi.org/10.1016/j.jfoodeng.2020.110129.

S. Potdar, U. Bagale, I. Potoroko, V.S. Hakke, Y. Maralla, M. Sivakumar, S. Sonawane, Sonication-assisted synthesis of softfurf oil based low fat emulsion: Effect of ultrasonic parameters, Mater. Today: Proc. 57 (2022) 1619–1625, https://doi.org/10.1016/j.mtprod.2021.123.223.

C.E. Gumus, G. Davidov-Pardo, D.J. McClements, Lutein-enriched emulsion-based delivery systems: Impact of Maillard conjugation on physicochemical stability and gastrointestinal fate, Food Hydrocolloid. 60 (2016) 38–49, https://doi.org/10.1016/j.foodhyd.2016.03.021.

W.H. Zhu, W.T. Xu, M.L. Han, Y. Bu, X.P. Li, J.R. Li, Preparation, characterization, and gel characteristics of nanoemulsions stabilized with dextrin-conjugated clam Meretrix meretrix lineatus protein isolate, Food Chem. 375 (2022), 131664, https://doi.org/10.1016/j.foodchem.2021.131664.

W.J. Chen, W.J. Wang, M.M. Gao, Y.C. Li, F.B. Meng, D.H. Liu, Whey protein isolate-gum O. Maxilla conjugates as emulsions: Impact of glycation methods on physicochemical stability and in vitro bioavailability of β-carotene emulsions, Food Chem. 351 (2022), 131706, https://doi.org/10.1016/j.foodchem.2021.131706.

M. Nooshkam, M. Varidi, Physicochemical stability and gastrointestinal fate of β-carotene-loaded oil-in-water emulsions stabilized by whey protein isolate-low acyl gellan gum conjugates, Food Chem. 347 (2021), 129079, https://doi.org/10.1016/j.foodchem.2021.129079.

H.F. Cui, Q.H. Liu, D.J. McElory, L.L. Li, S.L. Liu, Y. Li, Development of salt- and gastric-resistant whey protein isolate stabilized emulsions in the presence of cinnamaldehyde and application in salad dressing, Foods 10 (2021) 1868, https://doi.org/10.3390/foods10051868.

B.M. Degener, C. Chung, V. Schlegel, R. Hutkins, D.J. McClements, Factors influencing the freeze-thaw stability of emulsion-based foods, Compr. Rev. Food Sci. Food Saf. 13 (2014) 98–113, https://doi.org/10.1111/1541-4337.12050.
[56] X.D. Zang, C.H. Yue, M.J. Liu, H.Y. Zheng, X.F. Xia, G.P. Yu, Improvement of freeze-thaw stability of oil-in-water emulsions prepared with modified soy protein isolates, LWT 102 (2019) 122–130, https://doi.org/10.1016/j.lwt.2018.09.004.

[57] J. O'Regan, D.M. Mulvihill, Heat stability and freeze-thaw stability of oil-in-water emulsions stabilised by sodium caseinate-maltodextrin conjugates, Food Chem. 119 (2010) 182–190, https://doi.org/10.1016/j.foodchem.2009.06.019.

[58] M. Li, Y. Liu, J.L. Zhao, R. Yu, M.A. Hussain, A. Qayum, Z.M. Jiang, B. Qu, Glycosylated whey protein isolate enhances digestion behaviors and stabilities of conjugated linoleic acid oil in water emulsions, Food Chem. 383 (2022), 132402.