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Effect of ultrasonic power on the stability of low-molecular-weight oyster peptides functional-nutrition $W_1/O/W_2$ double emulsion

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ABSTRACT

Ultrasonic-assisted treatment is an eco-friendly and cost-effective emulsification method, and the acoustic cavitation effect produced by ultrasonic equipment is conducive to the formation of stable emulsion. However, its effect on the underlying stability of low-molecular-weight oyster peptides (LOPs) functional-nutrition $W_1/O/W_2$ double emulsion has not been reported. The effects of different ultrasonic power (50, 75, 100, 125, and 150 W) on the stability of double emulsions and the ability to mask the fishy odor of LOPs were investigated. Low ultrasonic power (50 W and 75 W) treatment failed to form a well-stabilized double emulsion, and excessive ultrasound treatment (150 W) destroyed its structure. At an ultrasonic power of 125 W, smaller particle-sized double emulsion was formed with more uniform distribution, more whiteness, and a lower viscosity coefficient. Meanwhile, the cavitation effect generated by 125 W ultrasonic power improved storage, and oxidative stabilities, emulsifying properties of double emulsion by reducing the droplet size and improved sensorial acceptability by masking the undesirable flavor of LOPs. The structure of the double emulsion was further confirmed by optical microscopy and confocal laser scanning microscopy. The ultrasonic-assisted treatment is of potential value for the industrial application of double emulsion in functional-nutrition foods.

1. Introduction

Marine natural products, particularly food-derived peptides, have recently attracted increasing attention for their potential benefits in promoting health and preventing or delaying the onset of disease [1]. Compared with proteins, protein peptides have better bioavailability due to their low molecular weight and ease of digestion and absorption [2], which benefits populations with special nutritional needs, such as postoperative malnourished patients. Oysters are the globally most cultured economic shellfish, with a protein content of 39.1–53.1 % (based on dry flesh weight), making them a good source of marine peptides [3]. Low-molecular-weight oyster peptides (LOPs) have potential health benefits and carry out several biological activities, such as anti-fatigue, anti-inflammatory, anti-hypertensive, antioxidant, and immune modulation [4]. In our previous study, we reported that LOPs can effectively improve the nutritional and immune status by ameliorating intestinal flora dysbiosis and immunosuppression in chemotherapy-treated Lewis lung cancer mice treated [5]. Hence, as a functional ingredient, food-derived LOPs hold promise in the development of functional-nutrition supplements.

However, the water-soluble peptides of marine origin possess undesirable flavor and taste profiles (e.g., fishy smell and bitterness) that further limit their storage as novel ingredients and their application in functional nutrition supplements [6,7]. Thus, it is crucial to effectively protect marine peptides from external environmental stresses and mask their negative flavor. The $W_1/O/W_2$ double emulsion is an effective delivery system for the encapsulation of hydrophilic actives and has promising applications in masking odors and controlling the release of active ingredients from the food during digestion [8,9]. Therefore, $W_1/O/W_2$ double emulsion could be an important strategy to preserve LOPs. Unfortunately, the double emulsions are unstable, leading to aggregation of oil droplets, migration of internal water phases, and flocculation or phase separation during processing or storage, thus limiting the functional-nutrition applications of this emulsion system [10]. With the

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popularization of the concept of nutritious food in recent years, one of the most favored approaches has been the addition of natural or food-derived proteins and polysaccharides to the external aqueous phase, owing to their edibility, high nutritional value, and ability to improve the stability of emulsions [11,12]. The demand is increasing for nutritional foods with functional ingredients, such as dietary fats rich in polyunsaturated fatty acids, as it has equally high nutritional and health values. In this regard, one of the promising approaches to regulating their presence in foods is to have a smaller proportion of saturated fatty acids and a larger proportion of polyunsaturated fatty acids as suitable lipid phases in double emulsions, leading to functional-nutrition emulsion products that are more aligned to health advice [11,13].

An appropriate emulsification method significantly impacts the performance and stability of the emulsion; the commonly used emulsification methods include ultrasound, high-pressure homogenization, high-speed homogenization, and the use of a microfluidizer [14]. Among these technologies, ultrasound has attracted more attention due to its green, safe, and energy-efficient features [15]. Unlike other high-energy methods that use high shear or pressure to reduce droplet size, ultrasound has a special cavitation effect [16]. Acoustic cavitation from ultrasonic devices, defined by Ashokkumar as "the growth and collapse of pre-existing microbubbles in a liquid by the action of an ultrasonic field", significantly affects the stability of emulsions [17]. The cavitation effect has a number of potential benefits for emulsions, such as small droplet size and narrow size distribution of emulsions, which can enhance the long-term stability of emulsions [16]. Leong et al. [18] successfully used 20 kHz ultrasound to prepare $W_1/O/W_2$ double emulsion in skim milk and found that ultrasound treatment could produce more stable double emulsions with fewer surfactants. In a subsequent study, the double emulsion was prepared by ultrasonication and high-pressure homogenization, and the particle size of the ultrasonically treated double emulsion was slightly smaller, with significantly increased stability than that produced by high-pressure homogenization [19].

Most studies until now have focused more on the design, formation, structure, and performance of double emulsion to overcome the problems associated with the production of stable double emulsion [20] and less on the application in the development of healthy functional foods. In this study, the primary double emulsion was prepared by a two-step high-speed homogenization process using the whey protein-maltodextrin-fructooligosaccharides complex solution as the external aqueous (W_2) phase and sunflower oil-medium chain triglyceride-fish oil dietary fat as the oil (O) phase. Then, the $W_1/O/W_2$ double emulsion with encapsulated LOPs was prepared by ultrasound treatment. The effects of different ultrasonic power on the physicochemical properties, microstructure, stability, and odor of double emulsion were investigated. The effect of ultrasonic treatment on the stability of the double emulsion against environmental stress was also explored. Thus, this study investigated the effect of ultrasonic power on double emulsion to increase its potential application in functional-nutrition supplements.

2. Material and methods

2.1. Materials

The LOPs were provided by Beijing Shengmeinuo Biotechnology Co., ltd (Beijing, China). The amino acid composition and the main peptide sequence of LOPs are reported in our previous publication [5]. Sunflower oil was procured from China Oil and Foodstuffs Corporation (Beijing, China). Medium-chain triglycerides (MCT) were obtained from the Musim Mas Group (Sumatera Utara, Indonesia). The fish oil (with 18.23 % eicosapentaenoic acid and 12.06 % docosahexaenoic acid content) was kindly provided by Zhoushan Sinomega Biotech Engineering Co., ltd (Zhoushan, China). Whey protein, fructooligosaccharides, and maltodextrins were purchased from Yuanye Biotechnology Co., ltd (Shanghai, China). Nile red was procured from Sigma-Aldrich (St. Louis, MO, USA). Other reagents were of analytical grade, and deionized water was used for all experiments.

2.2. Preparation of double emulsions of low-molecular-weight oyster peptides

The double emulsions of LOPs were prepared via a two-step process. The inner aqueous (W_1) phase contained 0.9 % (w/v) NaCl, which suppressed Ostwald ripening in the double emulsion by balancing the differences in Laplace pressure [10,21]. The LOPs were added at 40 % (w/w) to the W_1-phase. The O-phase comprised dietary oils (sunflower oil, MCT, fish oil, in a 2:2:1 ratio, w/w) with 8 % (w/w) of the hydrophobic emulsifier polyglycerol polyricinoleate (PGPR) and kept for magnetic agitation for 30 min to ensure complete dissolution. The total coverage of the interface by PGPR at this concentration produced the highest concentration of dispersed phase water droplets [6]. The W_1/O emulsion was prepared as described by Yang et al. [7]. Briefly, the W_1-phase was slowly added to the O-phase to a final ratio of 4:6 (w/w) and homogenized at 13000 rpm for 2 min using a high-speed homogenizer (IKA T18 digital, Ultra-Turrax, Staufen, Germany).

A 2:2:1 (w/w) mixture of whey protein, maltodextrin, and fructooligosaccharides was dissolved in distilled water, along with 0.25 % (w/w) Tween 80 as the hydrophilic emulsifier, and 0.1 % (w/v) sodium azide to inhibit microbial growth. The mixture was stirred at room temperature for 2 h and then left overnight (12 h) at 4 °C until fully hydrated. The prepared mixture was used as the external aqueous (W_2) phase, and the content of solids in the W_2-phase was maintained at 28 % (w/w). The W_1/O was added to the W_2-phase at a mass ratio of 15:85 and homogenized at 7000 rpm for 2 min using a high-speed homogenizer. The coarse emulsion was placed a circulating temperature controller (CYDC-2010, Hangzhou Chuanyi Experimental Instruments Co., ltd., Hangzhou, China) maintain the temperature below 20 ℃, and then emulsified using an ultrasound generator (Branson Sonifier 250D, Branson Ultrasonics Co., ltd, Shanghai, China) at 50, 75, 100, 125, and 150 W input power for 5 min. The ultrasound probe of 6 mm diameter was placed 1 cm from the bottom of the emulsion. The ultrasound parameters were set to a frequency of 20 kHz and a pulse period of 6 s (4 s on and 2 s off), and the amplitude was adjusted in range of 10 %-70 %, and the total input sonication power was 250 W. All freshly prepared emulsions were stored at 4 °C until further analysis.

2.3. Determination of turbidity and whiteness

The turbidity of all freshly prepared emulsions was determined based on the method of Wang et al. [22] with some minor modifications. Briefly, the double emulsions were diluted 100 times in 10 mmol/L phosphate buffer solution (pH 7.0) and the absorbance of the sample was measured at 600 nm. The whiteness values of the emulsion were measured following a previously described method [23] using a hand-held colorimeter (CR-400, Konica Minolta Sensory, Tokyo, Japan). The turbidity and whiteness were calculated using the following formula:

$$\text{Turbidity} = (2.302 \times A_{600} \times 100) / 0.01$$

$$\text{Whiteness} = 100 - \sqrt{(100 - L^*)^2 + a^{*2} + b^{*2}}$$

Here, A_{600} is the absorbance at 600 nm, 100 is the dilution factor, 0.01 is the optical path difference, L* is the lightness, a* is the redness/greenness, and b* is the yellowness/blueness.

2.4. Determination of emulsifying properties

The emulsifying activity index (EAI) and emulsifying stability index (ESI) of emulsion were measured as described by Han et al. [24] with some modifications. Twenty microliters of the emulsion sample were mixed with 4 mL of 0.1 % (w/v) sodium dodecyl sulfate solution and

immediately vortexed for 30 s. The absorbance of the mixture was determined at 500 nm, and the EAI and ESI were calculated as follows:

$$EAI\ (m^2/g) = (2 \times 2.303 \times A_0 \times N) / (10000 \times C \times \theta)$$

$$ESI\ (\%) = (A_{60} / A_0) \times 100$$

Here, A_0 is the absorbances at 0 min, N is the dilution factor (200), C is the concentration of protein (g/mL), θ is the oil volume fraction of emulsion, and A_{60} is the absorbances at 60 min.

2.5. Determination of particle size and zeta potential

The size distribution and average particle size of double emulsions loaded with LOPs were measured by dynamic light scattering on a Zetasizer Nano ZS90 (Malvern Instruments ltd, Malvern, Worcestershire, UK) following the method of Zhang et al. [25]. The Z-average diameter represents the particle size of the emulsion, and the polydispersity index (PDI) represents the width of the particle size distribution. The zeta potential of the emulsion was also measured. All measurements were made in triplicate.

2.6. Microstructure observation

2.6.1. Optical microscopy

The microstructure of the double emulsion was visualized by optical microscopy (CX43, OLYMPUS, Japan). For this, 5 μL of the sample was placed on a microscope slide and covered with a coverslip. The images were captured using an objective lens (100 x) with oil immersion.

2.6.2. Confocal laser scanning microscopy (CLSM)

The multiple structures of the double emulsions were observed by CLSM (SpinSR, OLYMPUS, Japan) [26]. Briefly, 1 mL of the emulsion was mixed with 40 μL of 0.01 % (w/w) Nile Red for 1 min, and the excitation wavelength was analyzed at 488 nm. Then, 5 μL of the sample was added to a microscopic slide, covered with a coverslip, and observed under a 100 × objective with oil immersion.

2.7. Apparent viscosity

The rheological properties of double emulsions at different ultrasonic powers were recorded using a rheometer (HAAKE MARS III, Thermo Fisher Scientific, USA). The apparent viscosity of the emulsion was derived from the flow curve using a 60 mm parallel plate with shear rates in the range of 0.001 to 100 s^{-1}. The equation used herein was obtained by running the Herschel-Bulkley model using OriginPro 2022 (OriginLab Corporation Inc., USA) as previously described [16].

$$\sigma = \sigma_0 + k \times \gamma^n$$

Here, σ is shear stress (Pa), σ_0 is yield stress (Pa), γ is the shear rate (s^{-1}), k is the viscosity coefficient (Pa·s^n), and n is the flow behavior index.

2.8. Electronic nose (e-nose)

The flavor profile of the double emulsions was analyzed using a portable e-nose system (PEN3; Win MUster AirSense Analytics Inc., Germany) to assess the odor masking of LOPs. The e-nose contains 10 sensors with varying sensitivity to different compounds, including W1C (aromatic compounds), W5S (oxynitride), W3C (ammonia and aromatic compounds), W6S (hydrogen), W5C (alkanes and aromatic compounds), W1S (methane), W1W (sulfur compounds), W2S (ethanol), W2W (aromatic and organic sulfur compounds), and W3S (alkanes)[27]. The sensor was flushed for 2 min before each test to restore to its initial state, and each sample was tested for 100 s at a flow rate of 300 mL/min.

2.9. Stability analyses of the emulsion

2.9.1. Creaming index (CI)

The freshly prepared double emulsions were stored at 4 °C for 30 days, and every 5 days, the emulsification separation height (H_s) and a total height of the emulsion (H_t) were recorded [28]. The CI was calculated using the following equation:

$$CI\ (\%) = (H_s / H_t) \times 100$$

2.9.2. Conjugated dienes (CD)

The lipid oxidation in the emulsion was evaluated by assessing the production of CD, as described by Vallath et al. [29]. Fifty microliters of double emulsion were added to 10 mL of isooctance/2-propanol (2:1, v/v) solution and immediately mixed thoroughly for 1 min. The mixture was then centrifuged at 5000 g for 5 min and passed through a 0.22 μm filter to eliminate the interfering proteins. The absorbance of the filtrate was measured at 232 nm. The double emulsions were stored at 4 °C for 30 days, and the CD value was measured every 5 days.

2.10. Physical stability of emulsions to environmental stresses.

To investigate the stability of the emulsions under thermal processing, the emulsions were placed in a water bath and heated (30, 50, 70, or 90 °C) for 30 min and then cooled to 25 °C. To evaluate the stability of the emulsions against ionic stress, different quantities of NaCl were added to the W_2 phase to obtain final concentrations of 0, 50, 100, 200, and 300 mM. The stability of the emulsions against pH changes was examined after adjusting the pH of fresh emulsions to 3.0–9.0 using different concentrations of HCl or NaOH. All emulsions were prepared and kept at 25 °C for 30 min; then, the average particle size and zeta potential of the samples were determined [24].

2.11. Statistical analysis

Each experiment was performed in triplicate, and the results were expressed as the mean ± standard deviation. Data analyses and processing were performed by SPSS version 26 (IBM, USA). Data were analyzed using a one-way analysis of variance, followed by Duncan's multiple range test. A value of $P < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Turbidity and whiteness analysis

The turbidity and whiteness are two important indicators of a functional-nutrition emulsion product that reflect the color sensory and affect consumer acceptance [30,31]. Consumers generally find it difficult to accept the original color of the LOPs solution; thus, the acceptability of the appearance of double emulsion with encapsulated LOPs was assessed by measuring turbidity and whiteness (Fig. 1). Higher turbidity and whiteness values imply a more milky appearance of the emulsions. A comparison of five emulsion samples obtained by ultrasonic treatments, showed that the double emulsions prepared at 125 W and 150 W had higher turbidity (30773 ± 246.19 and 30523.55 ± 290.20) and whiteness (77.99 ± 0.18 and 77.66 ± 0.26). Particle size may be the most significant factor that influences the turbidity and whiteness of the emulsion, and higher turbidity may be the result of smaller emulsion droplet sizes [31]. The double emulsion prepared at 125 W ultrasonic power had higher turbidity and whiteness, probably due to the smaller droplet size and consistent with the findings of Zhou et al. [23].

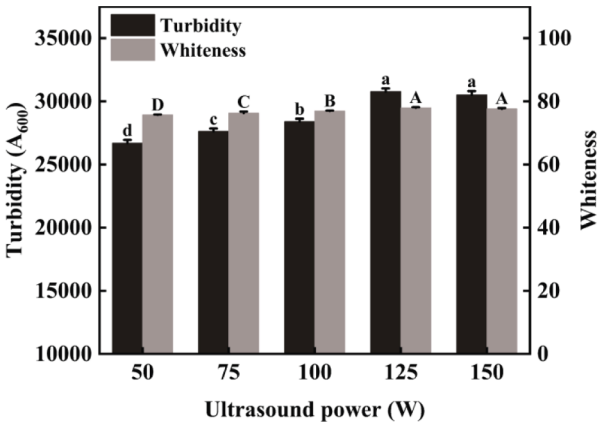

Fig. 1. Turbidity and whiteness of LOPs double emulsion at different ultrasonic power. Different letters represent significant differences ($P < 0.05$).

3.2. Analysis of emulsifying properties

Emulsifying property of an emulsifier is its capacity to form and stabilize an emulsion by adsorbing to the oil–water interface and reducing interfacial tension [32]. The EAI and ESI values of the double emulsion treated with different ultrasonic powers are mentioned in Fig. 2. With the increase in the ultrasonic power, the EAI and ESI of the double emulsion first increased and then tended to level off. Better emulsifying performance of the double emulsion was observed at 125 W ultrasonic power, with the corresponding EAI and ESI values being 1.61 ± 0.38 m^2/g and 98.37 ± 0.21 %, respectively. The cavitation effect and physical shear stress of ultrasound treatment effectively reduce the large sized nutrient particles (proteins, polysaccharides, and other edible components) in the outer aqueous phase, improving their dispersibility and solubility; in addition, the powerful micro-jet generated by ultrasound treatment renders greater uniformity to the emulsion system with better emulsifying properties [33,34]. Interestingly, the emulsifying properties of the double emulsion did not improve further after ultrasonic treatment at 150 W. This may be because a high ultrasonic power may disrupt the spatial structure of the protein to thus form aggregates, leading to a decrease in the emulsifying properties of the emulsion system, consistent with that reported earlier [35].

3.3. Particle distribution, size, and polydispersity index analysis

The particle size and polydispersity index reflect the stability and other functional properties of emulsion, as smaller particle size and a more uniform distribution can effectively improve emulsion stability [36]. The particle distribution, size, and PDI of the double emulsion tended to vary with the increase in ultrasonic treatment power (Fig. 3A and B). As the ultrasonic power increased, there was a concomitant enhancement in the uniformity and concentration of particle distribution of the double emulsion, showing a shift from a multi-peak to a single-peak distribution and from a broad-peak to a narrow-peak distribution. The single-peak distribution was more uniform at 125 W ultrasonic power treatment. When the ultrasonic power was<100 W, the droplet size and PDI value of the double emulsion increased, suggesting that the double emulsion prepared under this condition had poor uniformity and could not maintain structural stability. The situation improved significantly with the increase in ultrasonic power and showed the smallest average particle size and PDI at 125 W treatment ($P < 0.05$). The smaller average particle size and PDI facilitated the adsorption of large particle-sized nutrients at the oil–water interface and promoted the stability of the emulsion [28]. As the cavitation yield increased with the increase in ultrasonic power, the number of bubbles generated increased, that thus improved intermolecular collisions and effectively reducing particle size; hence the ultrasound treatment leads to a more uniform and stable emulsion [22]. Nonetheless, when the ultrasonic power was increased to 150 W, the average particle size and PDI values of the double emulsion instead increased only slightly. These observations suggest that excessive ultrasonic treatment may lead to forceful squeezing and agglomeration of smaller droplets. This agglomeration causes the expansion of the surface area, and the gradual formation of larger particles by inhomogeneous aggregation leads to a poorly dispersed system [21].

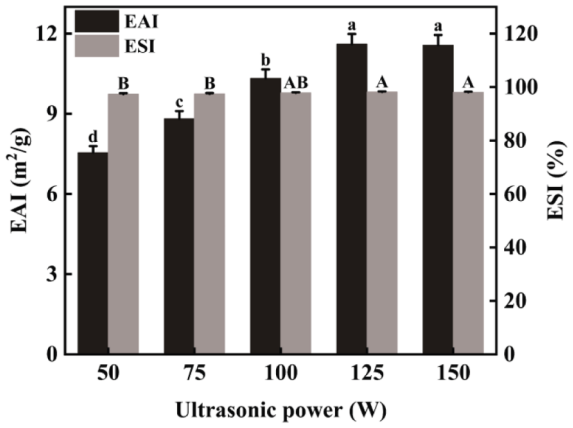

Fig. 2. Emulsifying activity index (EAI) and emulsifying stability index (ESI) of LOPs double emulsion at different ultrasonic power. Different letters for the same index represent significant differences ($P < 0.05$).

3.4. Microstructure observation

The success of double emulsion preparation can be effectively proved by microstructure analysis [37]. The microstructure of the double emulsions was analyzed herein using optical microscopy and CLSM. The double emulsion exhibited a well-defined morphology under the microscope, and the CLSM results showed a darker aqueous phase and brighter oil phase (when no fluorescence was detected, it was marked black; when a strong fluorescence was detected, it was marked red) (Fig. 4 and Fig. 5), confirming the double emulsion morphology of water-in-oil-in-water. The freshly prepared emulsion was macroscopically stratified at 50 W, and its microstructure showed a non-uniform morphology with larger particles and aggregated droplets. The cavitation effect produced by ultrasound or its force directly breaks up oil droplets or emulsion droplets into smaller particles, thereby forming fine and stable emulsions [38]. Therefore, with the increase in ultrasonic power, the droplet size of the double emulsion decreased, and the droplet distribution was more uniform. This structure improved the stability of double emulsion against aggregation and flocculation [24]. Notably, a higher ultrasonic power led to the disruption of the double emulsion structure, which may be caused by the local thermal effect of the excessive ultrasound treatment [16]. The thermal effect thus disrupted the W_1/O interfacial layer and changed the emulsion architecture from a double to a single layer, and the number of droplets with a double emulsion structure was significantly reduced when treated at 150 W.

3.5. Analysis of rheological properties

The rheological properties are the quantitative relationship between the strain and stress of an object under the action of an external force and are one of the key parameters to describe the characteristics of an emulsion [39]. Fig. 6 exhibits the variation in apparent viscosity with the shear rate for the double emulsion prepared with different ultrasonic powers. In all the double emulsions, a decrease in apparent viscosity was observed with the increase in ultrasonic power. Recent studies have demonstrated that ultrasound treatment reduces the apparent viscosity of emulsions [40,41]. The apparent viscosity, the consistency coefficient

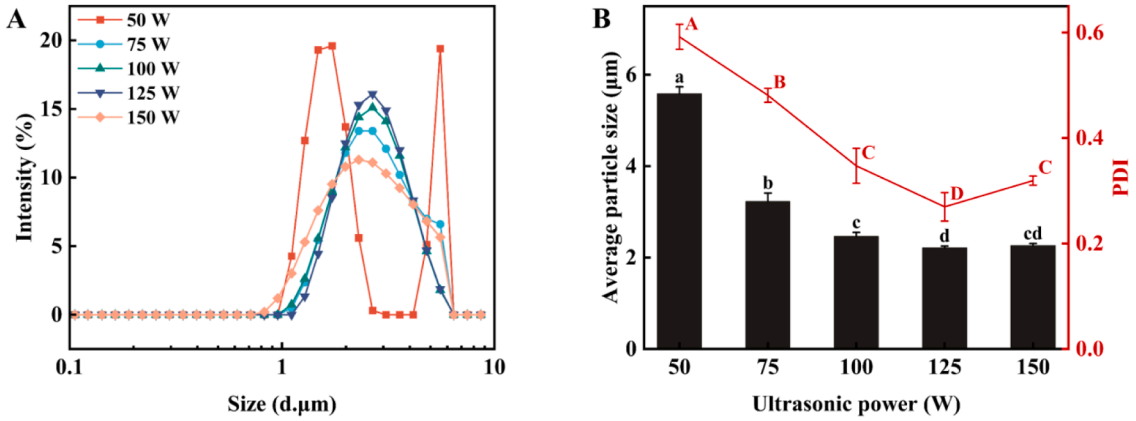

Fig. 3. Effect of different ultrasonic power on the droplet size of LOPs double emulsion. (A) Particle distribution. (B) Average particle size and polydispersity index (PDI). Different letters for the same index represent significant differences ($P < 0.05$).

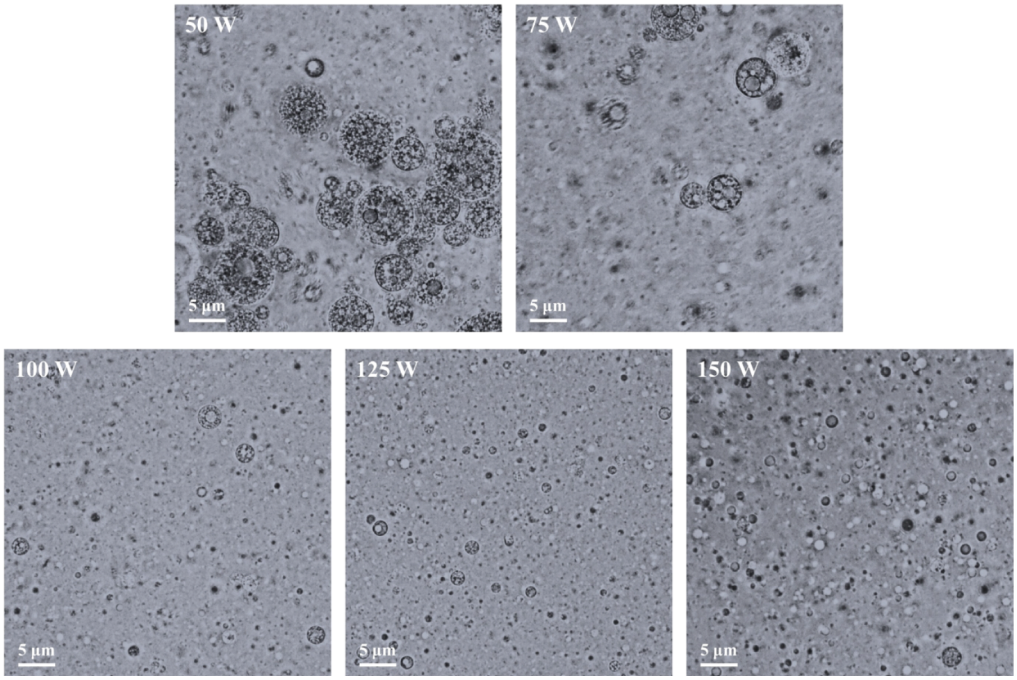

Fig. 4. The Optical microscopy images (100 × magnification) of LOPs double emulsion at different ultrasonic power.

(k), and flow index (n) also reflect the significant rheological properties of the emulsion, and the Herschel-Bulkley model is practically relevant for calculating the k and n values of food emulsions [39]. When $\sigma_0 = 0$, the fluid can be categorized as a shear-thinning (pseudoplastic) fluid ($0 < n < 1$) and a shear-thickening (dilatant) fluid (n greater than 1). With the increase in shear rate, the emulsion droplets begin to elongate, leading to a decrease in the viscosity of the system, which is characteristic of non-Newtonian fluids [42]. In this study, the apparent viscosity of the double emulsion decreased with increasing shear rate; the n value was in the range of 0 and 1, indicating a shear-thinning behavior. Generally, smaller emulsion particle sizes result in lower viscosity and a higher n value, with strong shear thinning properties of the emulsions [16]. This might be the reason for the lowest k and highest n values of the double emulsion prepared at 125 W ultrasonic power.

3.6. Electronic nose analysis

LOPs, as a marine origin peptide, have the characteristic unpleasant odor of aquatic products and are difficult to be accepted by consumers. Therefore, an e-nose analysis was performed using principal component analysis (PCA) to characterize the odor-masking effect of double emulsions prepared with different ultrasound powers on LOPs. All samples were distributed in different regions (Fig. 7A), indicating that the e-nose combined with PCA effectively distinguished the odors of emulsions, and the total contribution of the PC1 and PC2 was far greater than 90 %. In our previous study, the odor of LOPs was observed to mainly originate from W1W (indicating sensitivity to organic sulfur), which is the primary source of the unpleasant marine fishy odor [7]. With the increase in ultrasonic treatment power, the difference in PC1 gradually increased between each treatment group and LOPs. The odor of samples treated with 50, 75, and 150 W ultrasonic power was closer to that of the LOPs, indicating that the odor of LOPs was not well masked by ultrasonic treatment at low power, while a too-high ultrasonic power would lead to the destruction of the inner W_1/O emulsion, thus causing the leakage of LOPs to the W_2-phase, in accordance with the microstructural observations described above. Variations in droplet size and distribution can affect the stability, optical properties, rheology and sensory properties (e.g., odor) of an emulsion. Smaller droplet sizes and narrower droplet

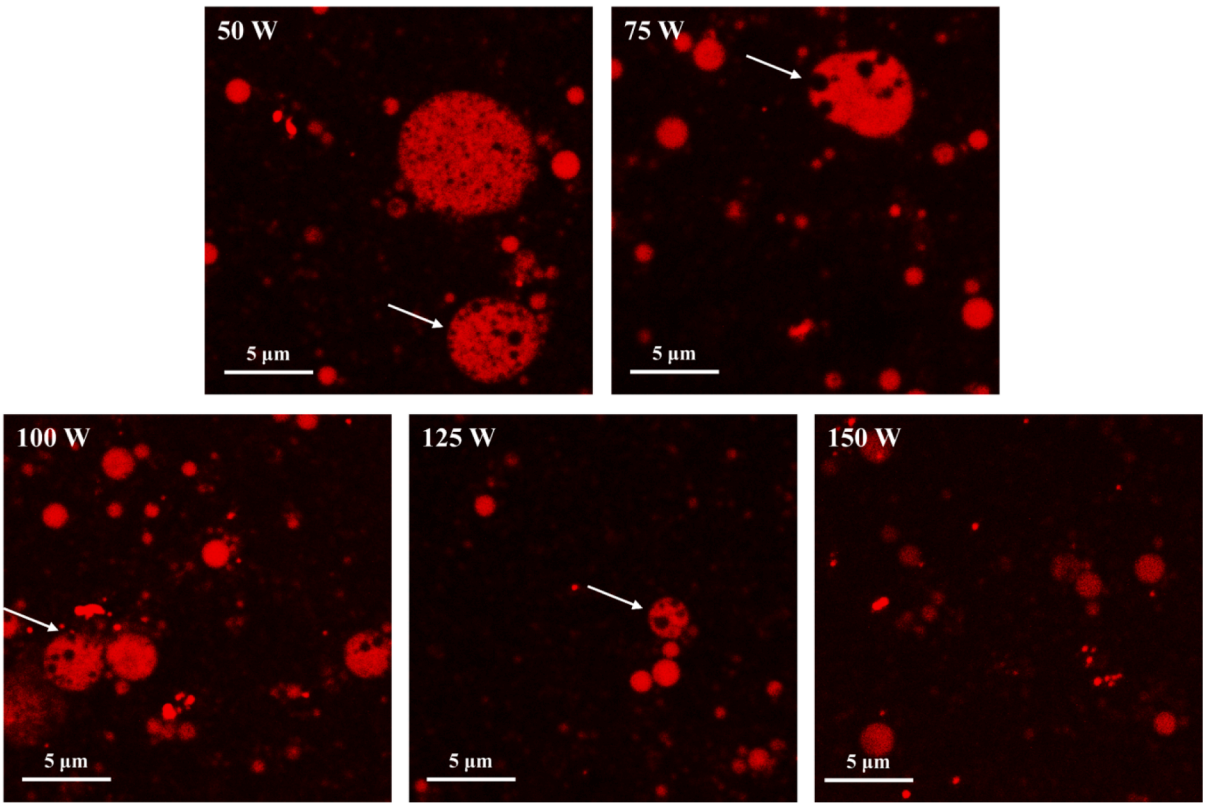

Fig. 5. The confocal laser scanning microscopy (CLSM) images (100 × magnification) of LOPs double emulsion at different ultrasonic power. Arrows represent W_1/O/W_2 double emulsion.

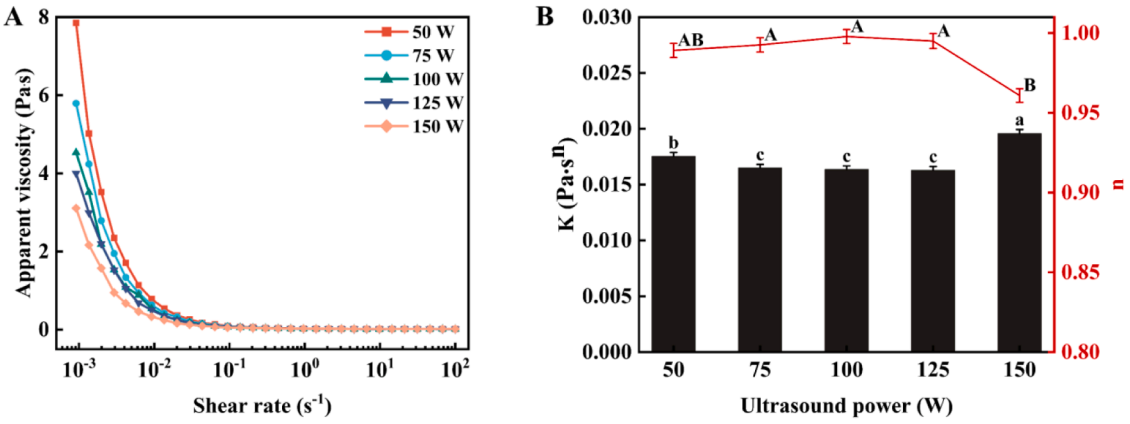

Fig. 6. (A) Apparent viscosity of LOPs double emulsion at different ultrasonic power. (B) The viscosity coefficient (k) and the flow behavior index (n) from the Herschel-Bulkley model.

size distributions often mean better stability and emulsification properties of the emulsions [16]. Interestingly, the odor of the sample treated with 125 W ultrasonic power was far away from that of the LOPs, probably owing to the smaller droplets and the structural integrity of the double emulsion prepared under this condition, which was able to mask the peculiar odor of the LOPs. A well-stabilized double emulsion is a potentially useful strategy for masking undesirable odors of nutrients and bioactive compounds and improving the sensory properties of food products [11]. Consequently, double emulsions prepared using ultrasound treatment have potential applications in the functional-nutrition emulsions industry. Further correlation heat map analysis showed a positive correlation of W1W with W5S (sensitivity to hydrogen compounds) and a negative correlation with W1C, W3C, and W5C, showing that the unpleasant odor of LOPs was also due to some hydrogen compounds, and that this unpleasant odor could be effectively ameliorated by the aromatic compounds in the double emulsion after the ultrasound (Fig. 7B).

3.7. Storage stability analysis

The CI of an emulsion can be described as the intensity with which an emulsion resists the separation and remains in the dispersion and directly reflects the stability of the emulsion with time [43]. A higher CI value represents significant delamination of the emulsion, indicating the extreme instability of the emulsion. Fig. 8A and B show the changes in appearance and CI of the double emulsion treated with different

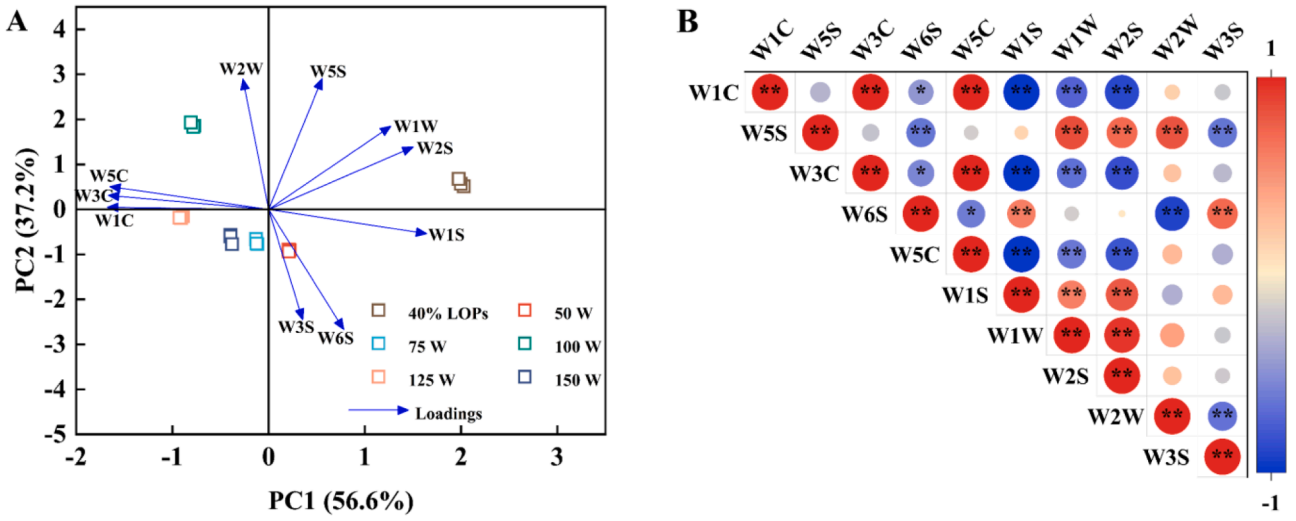

Fig. 7. The e-nose results of double emulsion on the fishy odor of LOPs at different ultrasonic power. (A) Principal component analysis (PCA). (B) Pearson correlations heatmap. Different colors of squares represent different R values of Pearson correlations. $^{*}P < 0.05$, $^{**}P < 0.01$.

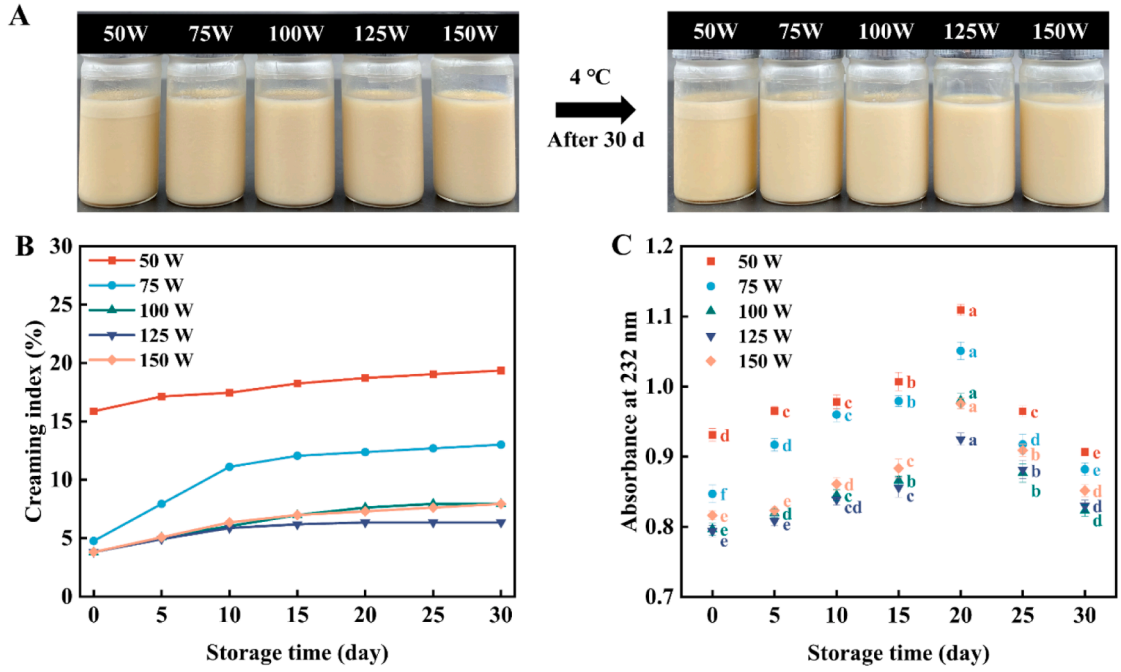

Fig. 8. Storage stability of LOPs double emulsion at different ultrasonic power. (A) Visual appearance. (B) Creaming index. (C) Conjugated dienes (CD) value. Different letters for the same ultrasonic power group represent significant differences.

ultrasonic powers during storage at 4 °C. The freshly prepared double emulsion presented a uniform milky yellow color except for that prepared by 50 W treatment. After 30 days of storage, the double emulsions prepared at 50 W and 75 W ultrasonic treatment exhibited obvious separation of the inner W_1/O emulsion and outer W_2-phases; the highest CI value was obtained at 50 W, indicating poor stability of the double emulsion prepared at low power ultrasound, while this phenomenon clearly improved with the increase in ultrasonic power. The emulsion prepared by ultrasound treatment exhibited a lower CI value, which may be due to the cavitation effect that can increase the strength of repulsion between droplets and effectively inhibit the aggregation and phase separation of the emulsion [28]. In this study, the double emulsion exhibited excellent storage stability after treatment with more than 100 W ultrasonic power. This is mainly because ultrasound contributes to the close association between the large particle-sized nutrients with oil droplets, forming emulsions with smaller droplet sizes and thus enhancing stability [44].

CD is typically formed during the oxidative deterioration of food samples due to double bond rearrangements, leading to the synthesis of hydrogen peroxide from unsaturated fatty acids, facilitating CD measurement as an effective tool for the analysis of lipid oxidation [29]. The high content of unsaturated fatty acids in the oil phase is the functional characteristic of the functional-nutrition double emulsion; thus, it was essential to assess the oxidation quality of the double emulsion in this study. The CD values of the double emulsion treated with different ultrasonic powers during the storage of 30 days are shown in Fig. 8C. As the storage time increased, the CD values of all double emulsions first increased and then decreased, and the highest level was observed on day

20. The decrease in observable CD values could be attributed to the formation and detection of secondary oxidation products when the primary oxidation products were not enough to be measured [45,46]. The CD values of the double emulsion treated with 50 W and 75 W ultrasonic power remained high during storage. This was because the cavitation effect produced by low power was not capable of effectively reducing the droplet size of the double emulsion, which resulted in an unstable system, while the inner W_1/O emulsion could not be completely coated, which aggravated the oxidation of the O-phase, which is rich in polyunsaturated fatty acids. The double emulsion treated by ultrasonic power higher than 100 W showed relatively low CD levels during storage. Besides the improvement in the stability of the double emulsion due to ultrasonic treatment, possibly the proteins in the double emulsion also inhibited lipid oxidation [24].

3.8. Analysis of physical stability

Heat sterilization is a common processing method in the food industry, as it can effectively inhibit the growth and reproduction of microorganisms [33]. Therefore, it was necessary to study the effect of temperature on the stability of LOPs double emulsion under 125 W ultrasonic treatment. As shown in Fig. 9A and B, the particle size and PDI of the double emulsion increased with the increase in temperature. The particle size and PDI did not differ significantly at 30 °C and 50 °C, indicating that the double emulsions remained relatively stable within this temperature range. This may be because nutrients with large particles size are well-dispersed at the oil–water interface during the ultrasound, forming a thicker interfacial layer, leading to an orderly arrangement of the complexes at this interface and decreasing the exposure of the hydrophobic end of the proteins [47]. The double

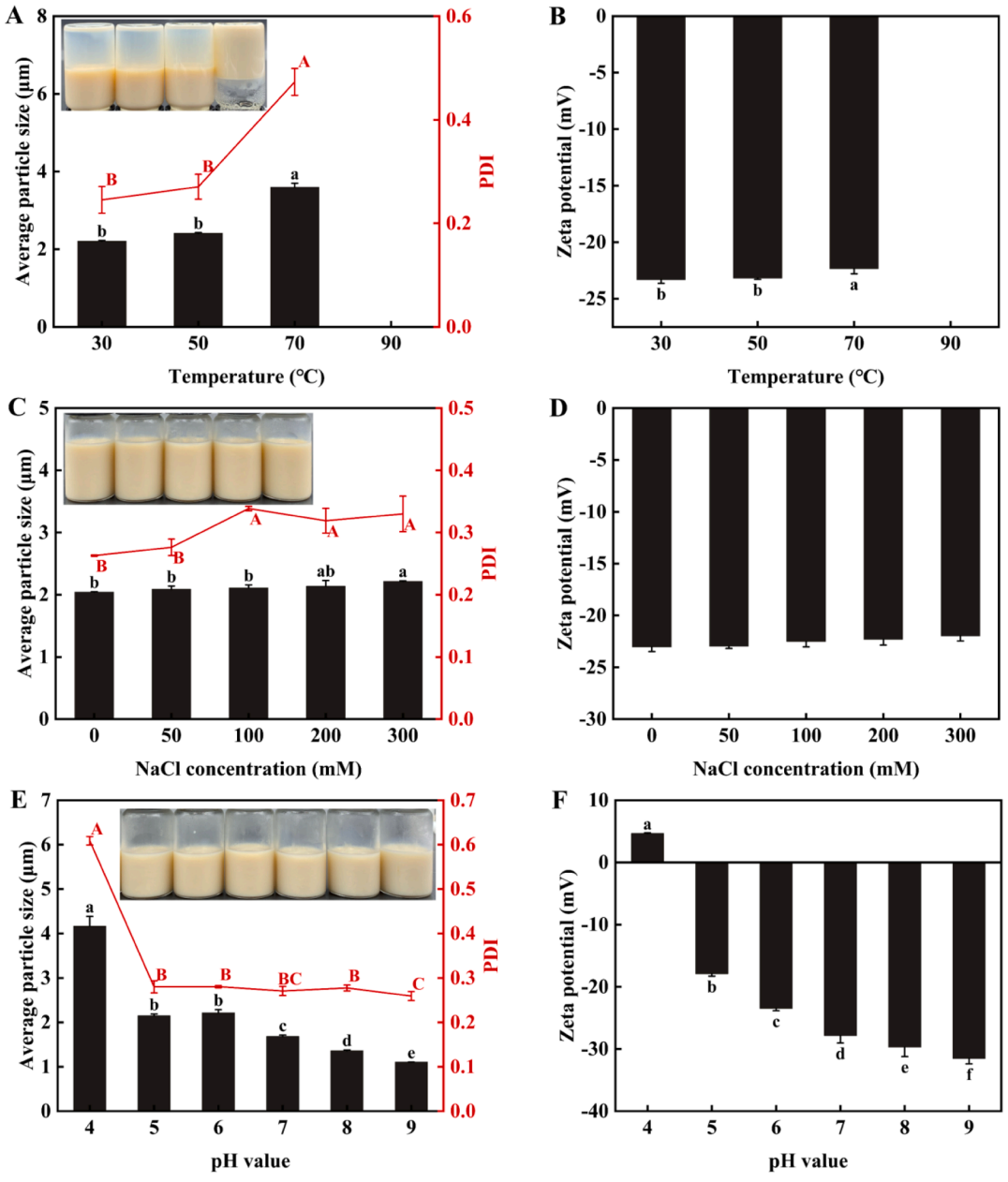

Fig. 9. Physical stability of LOPs double emulsion at 125 W ultrasonic power to environment stresses. (A) Average particle size and (B) zeta potential at temperatures of 30, 50, 70, and 90 ℃ (left to right). (C) Average particle size and (D) zeta potential under different ionic strengths, using NaCl at different concentrations of 0, 50, 100, 200, and 300 mM (left to right). (E) Average particle size and (F) zeta potential under different pH conditions from 4.0 to 9.0 (left to right). Different letters for the same index represent significant differences ($P < 0.05$).

emulsion particle size and PDI increased significantly at 70 °C and formed a gel at 90 °C. This may allow the whey proteins to form a stable gel network through aggregation and exchange under thermal induction, which disrupts the interfacial layer, thus allowing gradual gelling of the emulsion [48]. With the increase in temperature, the absolute zeta potential value of the double emulsion decreased significantly from 23.30 ± 0.35 mV to 22.33 ± 0.46 mV, indicating that the heat treatment had a similar effect on the zeta potential of the double emulsion. Thermal treatment disrupts the interfacial layer of the double emulsion and reduces the electrostatic interactions between molecules by causing the clumping of the large particle-sized nutrients, resulting in unstable or even gelatinized emulsions.

Fig. 9C and D present the stability of the double emulsions post-sonication at different salt ionic strengths. With the increase in ionic concentration, the droplet size of the double emulsion stabilized by ultrasound increased gradually, and the absolute value of zeta potential decreased slightly, suggesting that the double emulsion without salt addition was more stable with the smallest droplet size (2.04 ± 0.09 μm), PDI (0.263 ± 0.001), and the largest absolute zeta potential value (23.03 ± 0.45 mV). One of the reasons for these changes might be the addition of sodium ions reduces the amount of charge around the droplet, thus weakening the electrostatic interaction [49]. Another possible reason is that Tween 80 is a nonionic surfactant, and the dehydration effect of the hydrophilic head is enhanced as the concentration of sodium ions increases, suggesting that Tween 80 prevents double emulsion droplet aggregation mainly through resistance instead of electrostatic repulsion at the spatial site [24].

The particle size, PDI, and zeta potential of the double emulsions at different pH are mentioned in Fig. 9E and F. At pH 4, the particle size and PDI of the double emulsion were the highest, at 4.17 ± 0.22 μm and 0.609 ± 0.010, respectively. The particle size of the double emulsion decreased gradually to 1.10 ± 0.01 μm with an increase to pH 9, and there was no significant difference at pH 5 and 6. A strongly acidic environment may lead to a decrease in electrostatic repulsions to be less than hydrophobic interactions and van der Waals forces, thus destabilizing the emulsion [50]. The absolute value of the double emulsion zeta potential increased with increasing pH, consistent with the results of Wang et al. [33].

4. Conclusions

In this study, the ultrasonic treatment was used to successfully prepare $W_1/O/W_2$ double emulsion as an emulsion-based functional-nutrition supplement rich in LOPs. The ultrasonic treatment significantly improved the stability and sensory acceptability of the double emulsion. The 125 W ultrasonic power was effective in improving emulsification properties, reducing the droplet size of double emulsions, alleviating fat oxidation, and improving sensory acceptability, such as improved whiteness, and reduced viscosity. The treatment at 125 W could also masked the special flavor of LOPs, thus improving the long-term stability of the double emulsion. However, sonication at a high power (150 W) caused over-squeezing of the emulsion droplets, leading to flocculation and aggregation, resulting in phase separation. The successful preparation of double emulsions with good stability and sensory acceptability was attributed to the cavitation effect produced by ultrasound which resulted in the reduction of particle size and uniform dispersion of all components in the matrix. In the future, the nutrition support mechanism of double emulsion-type functional-nutrition supplements based on LOPS will be studied to provide a theoretical basis for the development and clinical application of special foods to treat malnutrition.

CRediT authorship contribution statement

Jinzhen Li: Methodology, Formal analysis, Investigation, Writing – original draft, Data curation, Visualization. **Shuo Wang:** Formal analysis, Investigation. **Hua Wang:** Investigation, Software. **Wenhong Cao:** Investigation, Validation. **Haisheng Lin:** Investigation, Validation. **Xiaoming Qin:** Investigation, Validation. **Zhongqin Chen:** Investigation, Validation. **Jialong Gao:** Investigation, Validation. **Leiyan Wu:** Project administration, Writing – review & editing. **Huina Zheng:** Funding acquisition, Project administration, Supervision, 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.

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