Influence of milk fat globule membrane and milk protein concentrate treated by ultrasound on the structural and emulsifying stability of mimicking human fat emulsions

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Abstract

The primary objective of the present study was to investigate the effectiveness of ultrasonic treatment time on the particle size, molecular weight, microstructure and solubility of milk fat globule membrane (rich in phospholipid, MPL) and milk protein concentrate (MPC). The mimicking human fat emulsions were prepared using modified proteins and compound vegetable oil and the structural, emulsifying properties and encapsulation efficiency of emulsions were evaluated. After ultrasonic treatment, the cavitation caused particle size decreased and structure change of both MPL and MPC, resulting in the enhancement of protein solubility. While, there was no significant change in molecular weight. Modified proteins by ultrasonic may cause a reduction in particle size and an improvement in emulsifying stability and encapsulation efficiency of emulsions. The optimal ultrasonic time to improve functional properties of MPL emulsion and MPC emulsion were 3 min and 6 min, respectively. The emulsifying stability of MPL emulsion was superior to MPC emulsion, which indicated that MPL is more suitable as membrane material to simulate human fat. Therefore, the obtained results can provide basis for quality control of infant formula.

1. Introduction

Although human milk is the ideal food for the newborn infants feeding, some reasons for not breastfeed and infant formula is the best substitute. Protein and fat are important components of infant formula, which provides energy for neonates. Proteins are widely used as emulsifier in infant formula to promote the stability of emulsions because of their special emulsifying properties in the food system, which enable them to adsorb on the oil–water interface and to form interface films.

The milk fat is present in the shape of milk fat globules, composing of triglycerides (TGs) surrounded by the milk fat globule membrane (MFGM). MFGM is an important functional component in human milk, which is rich in membrane proteins, polar lipids, cholesterol [1] and other active substances to protect the fat globule from enzymatic degradation and polymerization [2]. Furthermore, these complex lipids play critical roles on neonates health and linked with nourishment of brain development and cognitive function [3,4]. Previous research has found that the main compositions of bovine MFGM are similar to that of human MFGM [5]. Hence, in terms of nutrition, the addition of bovine MFGM in infant formula is beneficial to narrow the gap with human milk.

Milk protein concentrate (MPC), also known as aggregated proteins, is a high milk protein product, which contains casein and whey protein in the same proportions as whole milk. MPC is increasingly being used as an ingredient in food due to its high protein (40%-90%), low fat and low sugar properties. Unfortunately, MPC has poor functional properties in comparison with other milk protein powders such as whey protein concentrate and sodium caseinates [6]. It is widely known that MPC powder from commercial source has extremely low solubility, which limits the application of MPC in milk powder [7]. Moreover, previous
report also indicated the emulsifying ability of MPC is significantly lower than that of whey protein in simple O/W emulsions [8], so that it is necessary to improve functional characteristics of MPC.

Milk protein or soy protein is often used as membrane material to envelop milk fat globules in infant formula. Presently, commercial milk fat globule membrane and milk protein concentrate have been paid much more attention due to their high nutritional value. Some studies [9,10] have added commercial milk fat globule membrane and milk protein concentrate as emulsifiers to wrap milk fat globules to mimic breast milk fat. It is worth noting that the physical, structural and stability characteristics of mimicking human fat emulsion are of great significance for milk product processing. In recent years, sonication is a promising emerging technology and widely used in the food industry [11,12]. By some studies, ultrasonic treatment has potential to modify the physical, structural and functional properties of milk proteins and dairy products [13]. For instance, Ultrasonic vibration causes a dispersion of lipids into the aqueous part of the milk, and then cavitation stimulates the rupture of the lipid droplets, resulting in the reduction of particle size [14]. The studies also reported that the solubility of whey proteins increased [15] and emulsifying properties improved by high-intensity ultrasound [12].

However, there are few studies on whether proteins treated by ultrasound have the ability to improve the physical and emulsifying stability of mimicking human fat emulsions. Therefore, the current study was designed to investigate the changes of pH, solubility, particle size and microstructure in milk fat globule membrane and milk protein concentrate under different ultrasonic treatment time. Subsequently, the emulsions of mimicking human fat were prepared using modified proteins and compound vegetable oil and their microstructure, emulsifiability and encapsulation efficiency were evaluated. The study increased new knowledge on improvement in emulsifying stability of mimicking human fat emulsions by ultrasonic treatment and provided a new idea for the application of ultrasound in infant formula.

2. Materials and methods

2.1. Materials

Milk fat globule membrane protein (rich in phospholipid, MPL) was purchased from Hilmar (USA). Milk protein concentrate (MPC) was obtained from the Fuxin Industrial Co., LTD (Shanghai, China). All the vegetable oil (rapeseed oil, sunflower seed oil, soybean oil, palm oil, and coconut oil) used in our study were commercially available (Harbin, China). The MPL comprised about 18% lipids with 1.96% phosphatidylcholine (PC), 1.26% phosphatidylethanolamine (PE), 1.42% sphingomyelin (SM), and it also comprised 70% protein, 8% MFGM protein and small amount of lactose. The composition of MPC contained 85% protein, 4% lipids, 5% lactose and 6% ash. Nile Red and Fast Green were provided by Sigma-Aldrich (Beijing, China).

2.2. MPL and MPC solutions and ultrasound treatments

MPL and MPC powders were dissolved in distilled water at room temperature to obtain solutions at concentrations with 2% (w/w). The 0.02% (w/w) of sodium azide was added to the solution to inhibit microbial growth. All proteins solutions were constantly stirred at 20 ± 2°C for 1 h by using a magnetic stirrer.

An ultrasonic processor (DY-1200Y, Yibang instruments Co. Ltd, Zhejiang, China) with a 2-cm diameter titanium probe was applied to sonicate MPL and MPC solutions at concentrations of 2% (w/w). The protein solutions were sonicated at a frequency of 20 kHz with a maximum power of 600 W. The ultrasonic intensity was calculated as 43 ± 3.4 W/cm² according to reported literature [16]. The ultrasound treatment times were 0, 3, 6, 9 and 12 min in 6 s : 4 s work/rest cycles. The ultrasonic process was carried out in an ice bath to reduce heat gain.

2.3. pH determination

The pH of MPL and MPC solutions before and after ultrasonic treatment were determined by using a pH meter (FE-28, Metter Toledo, Shanghai, China). The instrument was calibrated with standard solution of pH before using.

2.4. Solubility

The solubility of protein samples was measured using a modified method based on Martinez et al [17]. The samples (2%, w/w) treated by ultrasound were diluted to 0.5% w/w, and 0.5% (w/w) protein solution was prepared directly as control solution. The protein solutions were centrifuged at 12,000 × g for 30 min at 25°C. The obtained supernatant was freeze-dried for 48 h using a vacuum freezing dryer (LGJ-10 N, Xaying Co., LTD, China). The calculation formula of solubility was as follows:

\[
\text{Solubility} = \frac{\text{total soluble solids of supernatant}}{\text{total solids}} \times 100
\]

2.5. Particle size determination

The particle size of MPL and MPC after ultrasound treatment was measured through a light scattering technique by a particle size analyzer (S3500, Microtrak, USA). Particle size is reported as D₄₃, which stands for the average particle size of the samples.

2.6. SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) was performed to observe the changes of protein molecular structure before and after ultrasonic, using DYY-6C electrophoresis apparatus and mini electrophoresis tank (Beijing Liuyi Biological Technology Co., LTD, China). The sparting gel and staking gel were formed by a 12% polyacrylamide resolving gel and a 5% acrylamide stacking gel, respectively. The protein samples at 1% (w/w) concentration were diluted with native sample buffer in a 3:1 vol ratio. Afterwards, the samples were heated in boiling water for 5 min to cause the protein denaturation. 10 μL samples were loaded into the polyacrylamide wells in a stacking gel and run at 60 V. Then the samples were within the sparting gel, gel electrophoresis was carried out at 120 V. Finally, the gels were stained with coomassie allocation stain fluid for 45 min and de-stained with decolored solution overnight until a clear background emerged.

2.7. Microstructure observation

After freeze-drying, the microstructure of MPL and MPC powders were observed by using a Cryo Scanned Electron Microscopy (SU8010, HITACHI, Japan). The conductive tape was attached to a sample stage of scanning electron microscopy. Then the metal film with thickness of 100–150 Å was coated on the surface of the samples by ion beam sputtering deposition. Coated samples were observed at a magnification of 8.0 K, operating with an accelerating voltage of 5 kV.

2.8. Emulsion preparation

The oil-in-water emulsions were made by mixing of protein (MPL/MP) treated by different ultrasonic time (2%, w/w) with compound oil by a ratio of 4:1. The compound oil phase consisted of rapeseed oil (10%), sunflower seed oil (10%), soybean oil (15%), palm oil (40%), and coconut oil (25%). The mixture was prepared and emulsified by a high shear mixer (JRJ-300-L, SHBBMX, China) at a speed of 10000 rpm for 2 min at room temperature to obtain an oil-in-water pre-emulsion. Then, the pre-emulsion was homogenized by Microfluidizer (AFM-3, ATS, Canada) at 30 MPa for 2 times to form oil-in-water emulsion.
2.9. Droplet size of emulsions

A laser particle analyzer (Microtrac S3500U, US) was used to determine the size distribution of the fat globules using three laser sources [18]. The 0.2 mL emulsion and 1 mL EDTA/NaOH (35 mM, pH 7.0) were mixed for 1 min to dissociate the casein micelles in milk. The mixed sample was diluted with Ultrapure water to 100 mL and then the volume-weighted diameters \( D_{4,3} \) of the fat globules in milk were measured with 10% obscuration. The volume-weighted diameter \( D_{4,3} \) was measured. The refractive indices used were 1.458 and 1.460 for milk fat at 633 and 466 nm, respectively, and 1.33 for water.

2.10. Emulsifying properties of emulsions determination

The emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to a previously published method [19]. 50 \( \mu \)L emulsion was added to 5 mL SDS solution (0.1 g/L) and the absorbance of mixture \( (A_0) \) was measured at 500 nm. Let the emulsion stand for 30 min, 50 \( \mu \)L emulsion was also taken to measure the absorbance \( (A_{30}) \). EAI and ESI were calculated as follows:

\[
\text{EAI (m}^2/\text{g)} = 2 \times 2.303 \frac{A_0 \times N}{\rho(q \times 10000)} \tag{2}
\]

\[
\text{ESI/min} = \frac{A_0}{(A_0 - A_{30}) \times 30} \tag{3}
\]

Where \( N \) is the dilution multiple (250), \( \rho \) is the emulsion concentration (g/mL), \( q \) is the fractional volume of oil phase.

2.11. Encapsulation efficiency of emulsions determination

5 mL sample was loaded into dialysis bag, and then the dialysis bag was placed in the PBS buffer (500 mL, 0.005 mol/L, pH 7.0) for 16 h of dialysis. After dialysis, 1 mL sample was added into 5 mL \( \text{C}_2\text{H}_5\text{OH} \) for 30 min of ultrasonic treatment. The ultrasonic sample was mixed with 50 mL petroleum ether, and the absorbance of mixture was measured at 212 nm. The formula as follows:

\[
\text{Encapsulation efficiency(\%)} = \frac{A_0}{A_0 - A_{30}} \times 100 \tag{4}
\]

Where \( A \) is the absorbance of sample after dialysis, \( A_0 \) is the absorbance of without dialysis sample.

The encapsulation efficiency of MPL treated for 3 min and MPC treated for 6 min was further determined for 30 d storage at 4 \( ^\circ \)C to investigate the storage stability of emulsions.

2.12. Confocal laser scanning microscopy of emulsions

A super-resolution microscope (Deltavision OMX SR, US) was used to observe the proteins and lipids of emulsions. Prior to analysis, emulsion samples were labled with two fluorescent dyes. Nile Red was used at a concentration of 42 g/mL in ethanol to dye neutral lipid. Fast Green was used at a concentration of 1 mg/mL in deionized water to mark protein. 500 \( \mu \)L of the sample was mixed with Nile red (100 \( \mu \)L) and Fast green (250 \( \mu \)L). The prepared sample (5 \( \mu \)L) was further taken to observe on the microscope.

2.13. Statistical analysis

All determinations were made in duplicate, and each value represents the mean of at least two measurements from two independent ultrasound treatments. One-way ANOVA was performed using IBM SPSS Statistics 23. Graphs were drawn using Origin 2018.

### Table 1

| Sonication time (min) | pH MPL | pH MPC |
|-----------------------|--------|--------|
| 0                     | 6.54 ± 0.008 | 7.21 ± 0.005 |
| 3                     | 6.48 ± 0.000 | 7.23 ± 0.004 |
| 6                     | 6.45 ± 0.012 | 7.25 ± 0.000 |
| 9                     | 6.41 ± 0.012 | 7.25 ± 0.004 |
| 12                    | 6.48 ± 0.008 | 7.22 ± 0.017 |

Fig. 1. The solubility of MPL and MPC after ultrasonic treatment.

3. Results and discussion

3.1. Effect of ultrasonic time on physical properties of MPL and MPC

3.1.1. pH

The pH of MPL and MPC solution treated with different ultrasonic time were shown in Table 1. It can be seen that the pH of MPL solution decreased significantly \((P < 0.05)\) with the increase of ultrasonic time except for 12 min of ultrasonic treatment. Bermudez et al. [20] reported that the pH of milk reduced after ultrasonic treatment, which is similar to the pH change of MPL solution in our study. Sakurai et al. [21] mentioned that a reduction in pH of protein can be attributed to the exposure of acidic amino acid residues which were contained within the aggregated structure of the protein micelles without ultrasonic treatment. The pH of MPC solution after ultrasound increased slightly compared with that without ultrasound, but the overall change was not significant (Table 1). Some researchers found that different ultrasonic time had no effect on the pH of milk protein [22].

3.1.2. Solubility

Solubility is one of the intuitive indicator to measure protein aggregation. Hence, it is a critical index of infant formula. Any improvement in solubility of commercial protein isolates is likely to lead to improvement in their industrial application. Compared to untreated, the solubility of MPL and MPC was greatly increased after sonication, as illustrated in Fig. 1. The result obtained is supported by Jambrak [23] and Jiang [24], which indicated that ultrasonic treatment caused an increase in solubility of soy proteins and black beans protein isolates. On the other hand, an investigation indicated that the solubility of whey protein concentrate had no significant difference after high intensity ultrasound [25]. This is because of the large amount of lactose in whey protein concentrate, which exhibited protective effect during ultrasonic treatment. The solubility of MPL increased significantly from 36% to 85.88% after 6 min of ultrasonic treatment, and the solubility of MPC...
increased significantly from 32% to 97.5% after 3 min of ultrasonic treatment (Fig. 1). When proteins are treated more than 6 min, the solubility decreased slightly, but not significantly. Furthermore, the solubility of MPC after sonication was higher than that of MPL. The conclusions may be due to the unfolding of protein molecular structure is beneficial to the improvement of protein solubility after ultrasonic treatment. Similarly, Previous study [26] also found a substantial enhancement in the solubility of millet protein concentrates after sonication.

### 3.2. Effect of ultrasonic time on structural properties of MPL and MPC

#### 3.2.1. Particle size distribution

The effect of ultrasonic time on the size of MPL and MPC was investigated. The evolution of volume-weighted diameter (D_{4,3}) of each protein are shown in Table 2. The volume-weighted diameter (D_{4,3}) of MPL and MPC after ultrasonic treatment were significantly smaller than those of untreated samples (P < 0.05). The decrease in protein size is observed to be due to the high shear forces caused by ultrasonic cavitation, as well as micro-streaming and turbulent motion [27]. The particle size of MPC without ultrasonic treatment was seriously aggregated, and with prolonging the time of ultrasonic treatment, the size of MPC samples gradually reduced. While, ultrasonic treatment also affected the size of MPL though not enough to be noticeable. Similarly, Jonathan et al [28] reported that ultrasound treatment reduced the size of whey protein isolate and milk protein isolate. Furthermore, the average volume-weighted diameter of untreated MPC solution (2.63 ± 0.02 μm) was significantly larger than that of MPL solution (42.55 ± 0.02 μm).

![Particle Size Distribution](image)

**Table 2**

| Ultrasonic time (min) | 0          | 3          | 6          | 9          | 12         |
|-----------------------|------------|------------|------------|------------|------------|
| MPL (μm)              | 2.63 ± 0.02 | 0.43 ± 0.01 | 0.39 ± 0.01 | 2.46 ± 0.03 | 0.99 ± 0.02 |
| MPC (μm)              | 42.55 ± 0.02 | 0.21 ± 0.01 | 0.19 ± 0.01 | 0.18 ± 0.02 | 0.17 ± 0.02 |

*Significantly not differences vs. untreated sample at p > 0.05.

*Significantly differences vs. untreated sample at p < 0.05.

Fig. 2. The effect of ultrasonic time on particle size distribution of MPL solution (A) and MPC solution (B).

![SDS-PAGE Profile](image)

**Fig. 3.** SDS-PAGE profile of ultrasound-treated MPL and MPC solutions. A: Milk fat globule membrane (MPL); B: Milk protein concentrate (MPC).

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Protein particle size distribution in MPL and MPC solution are illustrated in Fig. 2. Compared with untreated MPL and MPC, the size distribution of all proteins was changed after all ultrasonic treatments. The particle size of all the MPL solutions became multi-peak distribution, and the size distribution range of MPL by 3 min and 6 min of sonication was narrower than that of 0, 9, 12 min of sonication (Fig. 2A). The particle size of MPC solution always maintained unimodal distribution. Meanwhile, after ultrasonic treatment, the particle size distribution of MPC solution was in the range of 0.05–1 μm, which was relatively consistent (Fig. 2B). Previous research result [22] revealed that the particle size of reconstituted milk protein concentrate decreased from 28.45 μm to 0.13 μm after 0.5 min of ultrasonic treatment. Overall, ultrasonic treatment significantly reduced the particle size (D_{4,3}, volume-weighted diameter).
of MPL and MPC solutions. However, ultrasound had a more significant effect on the particle size of MPC, which may be due to the lower solubility of MPC solution itself.

3.2.2. Molecular structure

SDS-PAGE was used to analysis whether the ultrasonic treatment can change the protein molecules weight and lead to the aggregation or degradation of molecules weight. The compositions of untreated and ultrasound treated proteins MPL and MPC are displayed in Fig. 2. It was evident from the figure (Fig. 3A) that the major proteins in MPL are MUC1, PAS III/IV, CD 36, BNT, ADRP, and PAS 6/7 with molecular weight of 200 kDa, 95 kDa, 77 kDa, 67 kDa, 52 kDa and 48 kDa.

Fig. 4. Microstructure of MPL (A) and MPC (B) after ultrasonic treatment. 1–5 represents the ultrasound time of 0, 3, 6, 9 and 12 min, respectively.
respectively. This is relatively consistent with the composition of bovine milk fat globule membrane reported in the previous literature [29]. The MPC was separated to obtain BSA (68 kDa), α-CN (35 kDa), β-CN (30 kDa), κ-CN (28 kDa), β-Lg (18 kDa) and α-La (14 kDa) by using SDS-PAGE (Fig. 3B). The SDS-PAGE profile results of MPL and MPC also showed that there is no significant difference between the untreated and ultrasound treated samples in molecular weight. These results are in agreement with those studied by Abdul et al. [30], who observed no changes in molecular weight of Lac-US-α-lactalbumin and US-α-lactalbumin emulsion with ultrasonic time prolonged. Sun et al. [22] also found that ultrasonic treatment had no effect on molecular weight of reconstituted milk protein concentrate. They used an ultrasonic treatment of 600 W output power at a frequency of 20 kHz and 50% amplitude, which the ultrasonic intensity is differ from our study. These results indicate that shear stress and turbulence produced by different ultrasonic intensity can not cause the splitting of protein molecular structure.

3.2.3. Microstructures

The microstructures of the ultrasound-treated proteins were observed after lyophilized, and the microstructure images of MPL and MPC were collected by scanning electron microscope at 5000 × magnification and 1000 × magnification, respectively. As depicted in Fig. 4. MPL without ultrasonic treatment were in the form of continuous lamellar structure. Meanwhile, a small number of protein particles, varying in size and uneven distribution, were exposed on the surface (Fig. 4 A1). After sonication, the morphologies of MPL powder were changed in different ultrasonic treatment time, and more protein particles were exposed to the surface. With the increase of ultrasonic time, the protein particle sizes were significantly reduced in the microscopic image, which was consistent with the results of the aforementioned particle size determination of protein solution in Fig. 4 A2-5 and Table 1. MPL powder treated for 6 min remained continuous flake, and protein particles were relatively uniformly distributed (Fig. 4 A3). Moreover, the protein fragments treated for 9 min were cracked in Fig. 4 A4. The protein structure was seriously broken and the holes could be observed, especially after 12 min of ultrasonic treatment (Fig. 4 A5), indicating time prolongs for sonication had a significant impact on protein structure.

It also can be seen that MPC particles are relatively large compared with MPL. Visually, the surface of MPC treated for 3 min (Fig. 4 B2), 6 min (Fig. 4 B3), 9 min (Fig. 4 B4) and 12 min (Fig. 4 B5) appeared homogeneous with the presence of various degrees of crack. With the extension of ultrasonic time, the morphology of MPC were gradually broken from continuous flake (Fig. 4 B1) to protein micell (Fig. 4 B2-5).

3.3. Effect of ultrasonic time on structural properties and emulsifying stability of mimicking human fat emulsions

3.3.1. Particle size distribution

The particle size of emulsion is a major indicator to evaluate the emulsion stability, which can reflect the physicochemical characteristics and functional properties of emulsion. The volume-mean diameter D₄,₃ and particle size distribution of MPL and MPC emulsions sonicated for 0, 3, 6, 9 and 12 min are shown in Figs. 5-6. Compared to untreated emulsions, the particle size of all treated samples had a significant decrease. When the ultrasonic time prolongs, the volume-mean diameter D₄,₃ gradually reduced in both MPL and MPC emulsions. The average particle size in MPL emulsion was 148 ± 3.25 μm, and 12 min of sonication decreased the average particle size to 1.62 ± 0.36 μm. Meanwhile, the particle size of MPC reduced from 209.3 ± 3.68 to 20.04 ±
2.32 μm by the 12 min of sonication. The obtained results in our study are in agreement with reported literature [33]. The particle size distribution curve of MPL emulsion was multimodal during 0 and 9 min of sonication (Fig. 6A) suggesting that droplet accumulate severely. While, except for 3 min of ultrasonic treatment, the particle size distribution of all MPC emulsion showed unimodal distribution after ultrasonic treatment (Fig. 6B), indicating that there had a dramatic improvement on MPC emulsion by ultrasonic treatment. The particle size distribution of MPL and MPC emulsions were narrower for 12 and 6 min of sonication, respectively. The particle size change observed here for MPL and MPC
could be originated shear forces, turbulent and cavitational forces of ultrasound effect [34].

3.3.2. Confocal laser scanning microscopy

The microstructure of MPL and MPC emulsions are presented in Fig. 7A-B. Proteins and lipids were observed in red and green by using confocal laser scanning microscopy. The lipid droplets of all emulsion samples were enveloped by proteins and evenly distributed in the emulsion system except for MPC emulsion without ultrasonic treatment. Significantly, the lipid droplets of untreated MPC emulsion was seriously aggregated (Fig. 7B1), which indicated that the original MPC had a poor stability. This also confirmed the previous result that MPC solubility was relatively low (Fig. 1). Confocal laser imaging also showed that the fat globules of all emulsions became smaller with the extension of ultrasonic time, which was consistent with the particle size results (Fig. 5). In general, the fat globules of MPC emulsion were larger than that of MPL emulsion, and there was a significant improvement in fat globules aggregation of MPC emulsion by ultrasound treatment. Moreover, a large number of proteins can be easily attach to the surface of fat globules after ultrasonic treatment. The explanation is that, with the increase of ultrasonic treatment time, the size of fat globules gradually decreased and the interface of the droplets were covered by more proteins.

3.3.3. EAI and ESI

The emulsifying properties of proteins are indicated by emulsion activity index (EAI) and emulsion stability index (ESI). EAI represents that protein per unit mass can stabilize the oil-in-water interface area, and ESI represents the ability of protein to maintain emulsion stability at a pre-determined time [35]. The effects of ultrasonic time on EAI and ESI for MPL and MPC emulsions were illustrated in Fig. 8. The lowest EAI values were calculated in untreated emulsions with 32.2 m$^2$/g of MPL emulsion and 28.1 m$^2$/g of MPC emulsion. After ultrasonic treatment, EAI values of both MPL and MPC emulsions were increased obviously. The maximum EAI values were 55.3 m$^2$/g and 53.4 m$^2$/g for MPL emulsion and MPC emulsion at 6 min and 9 min of ultrasonic treatment. The obtained results also revealed that there was no significant difference in EAI values of both MPL and MPC emulsions treated for 6–12 min. For ESI, the longer the ultrasonic time, the higher the ESI value of both MPL and MPC emulsions. The maximum values of treated MPL and MPC emulsion in ESI were 61.3 min and 54.1 min, respectively. After sonication, the bigger improvement of the physical and structure characteristics of protein, the smaller emulsion particle size and the lesser of particle size aggregation lead to a faster adsorption of the proteins to the oil–water interface, thus the emulsifying properties of emulsions can be improved.
3.3.4. Encapsulation efficiency

The encapsulation efficiency reflected the stability of the oil-in-water emulsion. The encapsulation efficiency of MPL and MPC emulsions after ultrasonic treatment was significantly increased in comparison with untreated emulsions. The encapsulation efficiency of MPL emulsion was the highest at 92.19% after 3 min of ultrasonic treatment (Fig. 9 A1), and the encapsulation of MPC emulsion was the highest at 93.83% after 6 min of ultrasonic treatment (Fig. 9 B1). Hence, in order to investigate the storage stability of emulsions, the encapsulation efficiency of MPL treated for 3 min and MPC treated for 6 min was further determined for 30 d storage at 4°C. As shown in Fig. 9 A-B, the encapsulation efficiency of MPL emulsion and MPC emulsion reduced as the storage time prolonged, which is consistent with the research by Mayumi [36]. After the 10 days of storage at 4°C, although the encapsulation efficiency of MPL and MPC emulsions treated with ultrasound and without ultrasound decreased significantly, the encapsulation efficiency of both MPL and MPC emulsions by ultrasound treatment was still above 80%. This is most likely due to the large amounts of droplets aggregation resulting in poor emulsion stability, and the cavitation produced by ultrasound makes the lipid droplets more dispersed and smaller. Therefore, ultrasound treatment improved the physical properties of the protein which enhanced the stability of the emulsion.

4. Conclusions

The obtained results showed that ultrasound treatment (20 kHz, 43 ± 3.4 W/cm²) can significantly decrease the particle size of proteins and cause the unfolding of protein structure, thus the solubility of MPL and MPC increased from 36% and 32% to 85.88% and 97.5%. However, ultrasound time had no effect on the molecular weight of both MPL and MPC. Moreover, the functional properties of emulsion prepared by modified protein and compound vegetable oil were distinctly improved. Interestingly, after ultrasound treatment, EAI and ESI values of MPL and MPC emulsions were increased obviously. Although the encapsulation efficiency decreased with the prolonging storage time, the encapsulation efficiency of emulsions stored for 10 days at 4°C was still above 80%. Therefore, ultrasound reduces particle size, improves emulsification stability, and increases the encapsulation efficiency of mimicking human fat emulsions, resulting in better application foreground in infant formula.

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