IMPROVE THE EMULSIFYING STABILITY OF MYOFIBRILLAR PROTEIN PREPARED OIL-IN-WATER EMULSIONS BY ZEIN HYDROLYSATES

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Abstract

This study investigated the effects of zein hydrolysate (ZH) on the improvement of the emulsifying stability of oil-in-water (O/W) emulsions prepared by myofibrillar protein (MP). Emulsions with 5 mg/mL ZH had the highest ESI and ζ-potential, the smallest mean particle size (P<0.05). Confocal laser scanning microscopy observation proved that emulsions with ZH possessed relatively small oil droplets, and the interfacial membrane in emulsions with 5 mg/mL ZH were more compact and more massive than that those without ZH. In general, our results revealed that the ZH promoted the adsorption of protein on the O/W interface and improved the emulsifying stability of the MP O/W emulsion.

Keywords: Zein hydrolysate, Myofibrillar protein, Emulsifying stability, Oil-in-water emulsion

I. INTRODUCTION

From the view of thermodynamics, oil-in-water (O/W) emulsions are unstable systems that lean toward separation into an aqueous and oil phase [1].

Proteins are protein hydrolysates that are commonly incorporated into O/W emulsions in order to improve the physical and chemical stability of emulsions [2]. For example, the salt-soluble myofibrillar protein is currently considered as an endogenous emulsifier in meat products [3]. Due to increased charge and reduced size, protein hydrolysates can absorb at the oil-water interface. Once protein hydrolysates are absorbed onto the surface of the lipid droplets, they can form a protective network structure that helps to prevent the droplets from aggregating in O/W emulsions [4].

The objectives of this study were to investigate the effect of ZH on the improvement of emulsifying stability in O/W emulsions emulsified by MP. Moreover, the possible stability mechanisms of emulsions were investigated.

II. MATERIALS AND METHODS

1. Preparation of zein hydrolysates

Zein hydrolysates (ZH) were prepared according to the method of Kong and Xiong with some modifications [5]. In our related paper, the antioxidant activity and emulsifying properties of ZH as influenced by hydrolysis time were evaluated. The results revealed that ZH obtained by 1 h hydrolysis had better antioxidant and emulsion activity, and the degree of hydrolysis for 1 h ZH was 8.7%. ZH obtained by 1 h hydrolysis was therefore used in the following study.

2. Emulsion preparation

MP was prepared by the procedure of Xia et al. [6] and then the MP was diluted with phosphate buffer (0.6 M NaCl, 50 mM, pH 6.2) to obtain aqueous phase solution. MP emulsions were first prepared by mixing 24 mL of 10 mg/mL MP solution with 6 mL of soybean oil. ZH were then added to the emulsions to obtain final ZH concentrations of 0, 1.25, 2.5, 5, and 10 mg/mL. Emulsions were homogenised at 17,500 rpm for 2 min each. The emulsions were transported immediately to glass weighing bottles 70 mm high and 30 mm in diameter. Then, sodium azide (3 mM in final emulsion) was added into each emulsion to prevent microbial growth. The emulsions were then stored both at room temperature to evaluate the emulsion stability in the dark for 0, 1, 3, 5, 7 and 10 d.

3. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to the method of Ramírez-Suárez and Xiong.[1].

4. ζ-potential measurements

The ζ-potentials of the emulsions were measured by static light scattering using a Nano ZS series Particle Analyzer after the emulsions were stored at approximately 22 °C for 10 d.

5. Particle size determination

The droplet mean diameters and particle size distributions of different emulsions were analyzed by the laser light scattering method using a Mastersizer 2000 instrument when emulsion were stored at approximately 22 °C for 10 d.

6. Creaming profile

Some of the emulsions separated into a top cream layer and a bottom serum layer during storage. The movement of any creaming boundary was tracked after 0, 1, 3, 5, 7 and 10 d.

7. Confocal laser scanning microscopy measurement

The distributions of protein and oil droplets in emulsions were assessed by CLSM. Nile Red solution and Nile Blue solution were used to dye the oil phase and the aqueous protein phase, respectively. After dyed, the emulsions were placed on a microscope slide and observed under the microscope to collect the CLSM images using a 40 × oil immersion objective lens at 22 °C. Excitation wavelengths of 488 nm and 633 nm were used to observe the distributions of oil droplets and proteins, respectively.

8. Statistical analysis

All data were presented as the mean ± standard deviations (SD) and analysed statistically using the General Linear Models procedure of the Statistix 8.1 software package (Analytical Software, St Paul, MN, USA). One-way analysis of variance (ANOVA) with Tukey's multiple comparison was performed to measure the significance of the main effects (P<0.05).

III. RESULTS AND DISCUSSION

1. Emulsifying properties

EAI represents the ability of proteins or protein hydrolysates to be adsorbed at the interface of fat globules. MP emulsions with ZH showed a tendency to become steady with the increase of ZH concentrations as shown in Table 1. So, in O/W emulsions of MP, ZH could be considered as an emulsifying stabilizer.

The ESI of the MP emulsion was 29.0%, which increased as the ZH concentration increased from 1.25 mg/mL to 5 mg/mL and then decreased as the ZH concentration became 10 mg/mL. The highest ESI (85.0%) was observed in the MP emulsion with 5 mg/mL ZH.

2. ζ-potential

As shown in Table 1, the ζ-potential of the O/W emulsions ranged from -5.2 mV to -20.5 mV. The significant difference confirmed that ZH concentrations had a notable influence on the ζ-potential values (P < 0.05), and the emulsion with 5 mg/mL ZH had the highest negative charge among the samples. Emulsions with a low absolute ζ-potential value tended to coagulate or flocculate, whereas emulsions with a high absolute ζ-potential value were electrically stabilized [2]. Therefore, a relatively strong electrostatic repulsion in the emulsion with 5 mg/mL ZH was adequate to overcome the attractive hydrophobic interactions acting in emulsions, thereby preventing flocculation and aggregation and improving the emulsifying stability.

Table 1. Emulsifying activity and ζ-potential of the myofibrillar protein oil-in-water emulsions with different concentrations of zein hydrolysates (ZH).

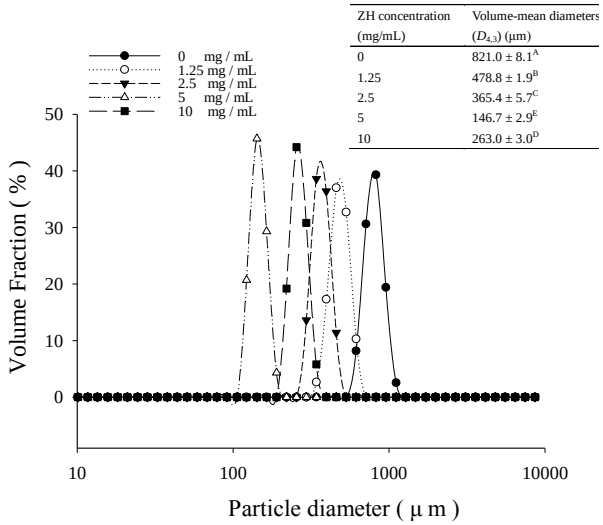
ZH concentration (mg/mL)	EAI (m^2/g)	ESI (%)	ζ-potential (mV)
0	21.32 ± 0.86^{D}	28.95 ± 0.43^{E}	-5.18 ± 0.17^{D}
1.25	25.37 ± 0.49^{C}	65.62 ± 0.74^{D}	-13.78 ± 0.94^{C}
2.5	29.27 ± 1.19^{B}	70.51 ± 0.55^{C}	-14.97 ± 0.24^{C}
5	36.65 ± 0.52^{A}	85.00 ± 1.72^{A}	-20.46 ± 1.20^{A}
10	36.54 ± 0.23^{A}	74.01 ± 1.03^{B}	-17.34 ± 0.57^{B}

3. Droplet diameters and particle size distributions

The mean particle size of the O/W emulsions was shown in Figure 1. For the MP emulsions, the mean droplet diameter ($D_{4,3}$) was

821 μm; the addition of ZH from 1.25 to 5 mg/mL led to a gradual reduction in $D_{4,3}$ from 478.8 μm to 146.7 μm ($P < 0.05$), respectively. The volume-based droplet size distributions of the samples verified the consistent results (Figure 1.), which showed a leftward shift of the distribution peak of the MP emulsions with ZH toward smaller sizes when compared to the emulsion without ZH; additionally, the emulsion with 5 mg/mL ZH had the most leftward shift. The results confirmed that the MP emulsion with 5 mg/mL ZH was enough to cover the totality of the oil droplets and, in other words, adsorb at the interface and form a protective film on the droplet surface; proof will be shown below by CLSM and partition of protein analysis.

Figure 1. Volume-mean diameters and particle size distributions (% volume) in the myofibrillar protein oil-in-water emulsions with different concentrations of zein hydrolysates after storing for 10 d.

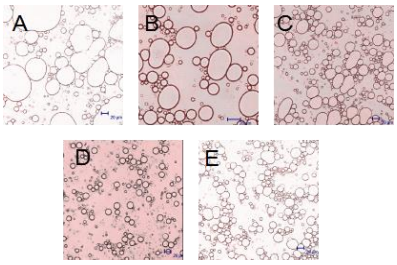


ZH concentration (mg/mL)	Volume-mean diameters ($D_{4,3}$) (μm)
0	821.0 ± 8.1^{A}
1.25	478.8 ± 1.9^{B}
2.5	365.4 ± 5.7^{C}
5	146.7 ± 2.9^{E}
10	263.0 ± 3.0^{D}

4. Confocal laser scanning microscopy

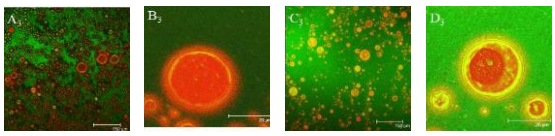
The emulsions were composed of differently sized oil droplets and aqueous phase protein. As expected, the microscopy of emulsions without ZH showed relatively large oil droplets in the MP emulsion (Figure 2A). With the increase of ZH concentrations from 0 and 5 mg/mL, the size of oil droplets obviously decreased; the smallest oil droplets were observed with the 5 mg/mL ZH (Figure 2D). The results were coincident with droplet diameters and particle size distributions.

Figure 2. Confocal laser scanning microscopy micrographs of the myofibrillar protein oil-in-water emulsions with different concentrations of zein hydrolysates (ZH) to observe the size and distribution of oil droplets. ZH concentrations were 0 mg/mL (A), 1.25 mg/mL (B), 2.5 mg/mL (C), 5 mg/mL (D), and 10 mg/mL (E) at 10 days. The bar length represents 20 μm.



CLSM was also used to evaluate the distribution of ZH on the interfacial membrane of oil droplets in emulsions (Figure 3). The MP emulsions with or without 5 mg/mL ZH were dyed by Nile Red solution and Nile Blue to visualize the ZH coating at the fat-protein interfacial layer. Oil droplets and protein exhibited less flocculation and aggregation in the MP emulsion with 5 mg/mL ZH than the emulsion without ZH (Figure 3A and 3C). To more clearly observe the distribution of ZH on the interfacial membrane, a typical fat droplet in the emulsion was selected for observation under high magnification (Figure 3B and 3D). When 5 mg/mL ZH was used, it could be observed that more protein (MP and ZH) surrounded the interface of the oil droplets in the MP emulsion, and the interfacial membrane was more compact and massive. The CLSM images further endorsed the assumption that the distribution and size of droplets were largely affected by ZH concentrations.

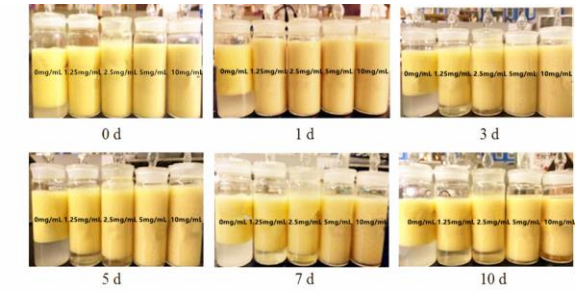
Figure 3. Confocal laser scanning microscopy micrographs of the myofibrillar protein oil-in-water emulsions to observe the adsorption of ZH on the interfacial membrane. (A) and (C) represent the emulsion without ZH, (B) and (D) represent the emulsion 5 mg/mL ZH.



5. Creaming profile

Changes of the creaming profile photos of emulsions at different storage times were presented in Figure 4. The creaming profile photo observation proved that the increase in ZH concentrations from 0 to 5 mg/mL significantly decreased the phase separation and enhanced the stability of the O/W emulsion; the highest separation occurred in the MP emulsion without ZH and the MP emulsion with 5 mg/mL ZH also had almost no visible phase separation until 10 d of storage. The creaming profile photos demonstrated that the maximal physical stability was observed in the emulsion with 5 mg/mL ZH. The MP emulsion with 5 mg/mL ZH had the smallest mean particle size and the highest ζ-potential, so it had the ability to minimize creaming formation. This may result from the formation of a compact and rigid interfacial membrane on oil droplets in the emulsion system at an optimal ZH concentration that inhibited phase separation by immobilization of dispersed oil droplets On the other hand, the creaming profile was lessened on account of the increased viscosity of the emulsion by addition of ZH .

Figure 4. Creaming profile during storage of the myofibrillar protein oil-in-water emulsions with different concentrations of zein hydrolysates (ZH).



IV. CONCLUSION

The emulsifying stability of the MP emulsions were effectively improved by adding ZH. The emulsion with different concentrations of ZH significantly increased the EAI and ESI, which should have a relationship with increased ζ-potential, decreased mean particle size, reduced creaming index and strengthened interfacial membrane. CLSM observation revealed that the interfacial membrane in the emulsion with 5 mg/mL ZH was more compact and massive than that without ZH. ZH can absorb to the surfaces of the lipid droplets and form a protective coating that helps prevent the droplets from aggregation and reduces the oxidation in O/W emulsions. This study suggests that ZH has good application potential in the muscle-contained emulsification of foods.

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