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Improvement of the emulsifying and oxidative stability of myofibrillar protein prepared oil-in-water emulsions by addition of zein hydrolysates

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ABSTRACT

This study investigated the effects of zein hydrolysate (ZH) on the improvement of the emulsifying and oxidative stability of oil-in-water (O/W) emulsions prepared by myofibrillar protein (MP). The emulsifying activity index (EAI) and emulsion stability index (ESI) increased with the increase of ZH concentrations. Emulsions with 5 mg/mL ZH had the highest ESI and ζ-potential, the smallest mean particle size, and the lowest creaming index ($p < 0.05$). Confocal laser scanning microscopy (CLSM) observation proved that emulsions with ZH possessed relatively small oil droplets, especially emulsions with 5 mg/mL ZH, which were coincident with the results of droplet diameters and particle size distributions. The micrographs demonstrating the adsorption of ZH on the interfacial membrane of oil droplets in emulsions by CLSM also revealed that the interfacial membrane in emulsions with 5 mg/mL ZH were more compact and more massive than that those without ZH. The addition of ZH significantly reduced the peroxide values and thiobarbituric acid-reactive substances values ($p < 0.05$) of emulsions during 10 days of storage. In general, our results revealed that the ZH promoted the adsorption of protein on the O/W interface and improved the emulsifying and oxidative stability of the MP O/W emulsion.

1. Introduction

Oil-in-water (O/W) emulsions are associated with many food ingredients and commercial foods, including beverages, ice cream, coffee, sauces, soups, desserts, dips, dressings, and delivery systems [1]. However, from the view of thermodynamics, food emulsions are unstable systems that lean toward separation into an aqueous and oil phase by some mechanisms, including creaming, coalescence, aggregation, sedimentation, and flocculation of the dispersed droplets [2]. Stability against coalescence and creaming relates to the shelf life of the emulsion products. Food manufacturers are therefore challenged to seek desirable ingredients in order to stabilize food emulsion systems. Emulsifiers are amphiphilic molecules that are commonly incorporated into O/W emulsions in order to improve the physical and chemical stability of emulsions [3]. Proteins and surfactants are the two most important types of emulsifiers [4], and O/W emulsions are often emulsified and stabilized by a variety of proteins. For example, the salt-soluble myofibrillar protein is currently considered as an endogenous emulsifier in meat products [5]. It contributes to the composition and stability of emulsions by forming a protective interfacial coating with a high degree of rigidity and continuity around the droplets and lowering the interfacial tension [6,7].

Oxidation is a dominating factor influencing the quality characteristics of O/W emulsions during preparation and storage [8]. Many antioxidants, including protein hydrolysates [7,9], polysaccharides [8,10] and polyphonic compounds [7,11], have been added to emulsions to inhibit oxidation and improve the emulsifying stability. As potential alternatives to traditional antioxidants, protein hydrolysates have brought constant attention. Unlike synthetic antioxidants, protein hydrolysates and peptides tend to be multifunctional and can modify the physical properties of foods aside from the inhibition of lipid oxidation [12]. The antioxidant mechanisms of peptides and protein hydrolysates in emulsion systems include both physical and chemical pathways. Protein hydrolysates can act as electron or hydrogen donors, metal ion chelators, or radical stabilizers [13], and they can also form physical barriers around lipid droplets in emulsion to reduce the diffusion and penetration of lipid oxidation initiators [9]. Due to increased charge and reduced size, protein hydrolysates are distributed easily in the

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aqueous phase compared to native protein and can absorb at the oil-water interface, thus inhibiting the initiation of lipid oxidation [13]. Once protein hydrolysates are absorbed onto the surface of the lipid droplets, they can form a protective coating that helps to prevent the droplets from aggregating in O/W emulsions [9].

Emulsifying stability can be influenced by many factors: average droplet size and droplet size distribution [1]; the type of oil, oil volume fraction, protein concentration and protein adsorbed at the interface [14]; and droplet aggregation, viscosity of the continuous phase, storage temperature and storage time [15]. Different protein concentrations and protein adsorbed at the interface are among the major factors that can influence both the physiochemical properties and oxidative stability of emulsions [8]. Nevertheless, there is little information regarding the improvement of the emulsifying and oxidative stability by protein hydrolysates in O/W emulsions; stability mechanisms also remain unknown.

Our previous study proved that alcalase-hydrolyzed zein expressed a strong antioxidant activity in the liposome system. The antioxidant activity was attributed to the increased protein solubility and the composition of specific amino acids/peptides present in the zein hydrolysates (ZH) [12]. The SDS-PAGE analysis of the hydrolyzed zein showed that zein proteins were susceptible to alcalase, showing a steady degradation over time. Within 0.5 h, the band intensity (peak area) had decreased obviously, and after 5 h, only trace amounts of the proteins were left [12]. The antioxidant activity and emulsifying properties of ZH as influenced by the different degree of hydrolysis (DH) were also evaluated in previous study [16]. The results demonstrated that hydrolysis increased antioxidant activity of zein, but it caused the decrease of emulsifying activities at higher DH. When the DH was 8.7% (1 h hydrolysis), the emulsifying activity and ζ- potential reached maximum and the stability of emulsion was best. The objectives of this study were to investigate the effect of ZH on the improvement of oxidative and emulsifying stability in O/W emulsions emulsified by MP. Moreover, the possible stability mechanisms of emulsions were investigated by analysis with confocal laser scanning microscopy (CLSM) and of properties such as ζ-potential, droplet size, creaming index and partition of protein in emulsions.

2. Materials and methods

2.1. Samples and materials

Porcine longissimus muscle was obtained within 24 h after slaughter (pH was approximately 5.6–5.8) from the Beidahuang Meat Corporation (Harbin, Heilongjiang, China). The samples kept on ice were transported to the laboratory to prepare MP on the same day. The dry zein powder containing 92% protein on a dry weight basis was purchased from Freeman Industries LLC (Tuckahoe, NY). Alcalase 2.4 L (6×10^4 U/g) was provided by Novozymes (Bagsvaerd, Denmark). All chemicals and reagents were analytical grade. Distilled deionised water was used for the preparation of all solutions.

2.2. Preparation of zein hydrolysates

Zein hydrolysates (ZH) were prepared according to the method of Kong and Xiong [12] with some modifications. The dry zein powder (5% w/v aqueous solution) was hydrolyzed with alcalase at 50 °C for 1 h. The enzyme to substrate ratio (E/S) was 2.5:100 (g/g). The pH of the zein solution was adjusted to the optimal values for alcalase (pH 9.0) before hydrolysis was initiated, and it was readjusted to the optimal value every 10 min during hydrolysis with 1 M NaOH. After hydrolysis, the pH of the solution was brought to 7.0 with 1 M HCl, and the solution was then heated at 95 °C for 10 min to inactivate the enzyme. After that, the solution was centrifuged at 6000*g* for 20 min at 4 °C to remove the insoluble material; the supernatant then was freeze-dried (LGJ-1 Freeze-Dryer, Shanghai, China) and stored at 4 °C until use. 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 DH for 1 h ZH was 8.7% [16]. ZH obtained by 1 h hydrolysis was therefore used in the following study.

2.3. Preparation of myofibrillar protein and aqueous phase solution

MP was prepared by the procedure of Xia et al. [5] and protein content was determined by the Biuret method using bovine serum albumin as a standard [17]. The MP was diluted with phosphate buffer (0.6 M NaCl, 50 mM, pH 6.2) to obtain aqueous phase solution.

2.4. Emulsion preparation

MP emulsions were produced according to the procedure of Wu et al. [18] with some modifications. 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 subsequently homogenised with an Ultra-Turrax homogenizer (IKA T18 basic, IKA-Werke GmbH and Co., Staufen, Germany) 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 MP emulsions with or without ZH were then stored both at room temperature (approximately 22 °C) to evaluate the emulsion stability and at 37 °C in an incubator to evaluate oxidative stability in the dark for 0, 1, 3, 5, 7 and 10 days.

2.5. Emulsifying properties

Emulsifying activity index (EAI) and emulsion stability index (ESI) were determined according to the method of Ramírez-Suárez and Xiong [19] with slight modifications. Emulsions (20 μL) with or without ZH were carefully pipetted from the bottom of bottles immediately after homogenisation at time 0 and 180 min and were diluted with 7 mL of sodium dodecyl sulphate (SDS) solution (0.1% W/V). Absorbance at 500 nm was determined using SDS solution as the blank. EAI was calculated by the absorbance at 0 min. ESI (%) was calculated using the absorbance at time 0 min divided by absorbance at 180 min and then multiplying by 100.

2.6. ζ-Potential measurements

The ζ-potentials of the emulsions were measured by static light scattering using a Nano ZS series Particle Analyzer (Malvern Instruments Ltd, Worcestershire, UK) after the emulsions were stored at room temperature (approximately 22 °C) for 10 days. Samples were diluted with 10 mM phosphate buffer (pH 7.0) to attain an oil-phase volume fraction of approximately 0.001%. One millilitre of each diluted sample was put in a visibly clear disposable zeta cell (Model DTS 1060C, Malvern Instruments Co. Ltd., Worcestershire, UK) without any air bubbles. The equilibrium time was 1 min.

2.7. 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 (Malvern instruments

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