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Effect of microwave-assisted phosphorylation modification on the structural and foaming properties of egg white powder

Peishan Li, Zhuo Sun, Meihu Ma, Yongguo Jin*, Long Sheng**

National Research and Development Center for Egg Processing, College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei, PR China

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ABSTRACT

Egg white protein was phosphorylated with microwave assistance (200 W, 300 W, 400 W, 500 W and 600 W) in the presence of sodium tripolyphosphate at pH 8.0, and the structural and foaming properties of the protein were investigated in this work. The degree of phosphorylation increased at first and then remained unchanged. Fourier Transform infrared spectroscopy showed that egg white had new characteristic absorption bands by phosphorylation. Circular dichroism spectroscopy and surface hydrophobicity analysis revealed that the phosphorylated proteins had a more unfolded and flexible structure. The phosphorylated proteins had higher absolute values of zeta potential and a lower content of free -SH. After treatment with microwave at the 500 W level, phosphorylated protein had a 0.44 times ($P < 0.05$) greater foaming ability and 1.1 times ($P < 0.05$) greater foaming stability compared with the untreated sample. The results suggested that microwave-assisted phosphorylation modification is a practical method to improve egg white protein foaming properties.

1. Introduction

Egg white protein is not only a nutritious food but also an excellent functional material, with applications such as foaming, gelling, etc (Amagliani & Schmitt, 2017). Egg white protein is used to provide structure and texture in aerated food, such as meringues, cakes, cookies and chocolate mousses due to its high foaming properties (Huang et al., 2017). To improve the quality of aerated food products based on egg white protein, it is necessary to enhance the foaming properties of the protein.

When air is introduced into a protein solution, soluble globular proteins diffuse to the air–water interface, concentrate and reduce the interfacial tension, then denature to initially orient the hydrophilic regions toward the water and the hydrophobic regions toward air. Egg white protein undergoes a rapid conformational change and rearrangement as a final step, forming cohesive viscoelastic films. The adsorption ability of a protein at the liquid surface is of vital importance in determining the protein conformation (Dickinson, 2011; Sadahira, Rodrigues, Akhtar, Murray, & Netto, 2018). Thus, protein structural changes might influence their foaming ability and stability.

Deliberate modification of food protein is a popular method for improving their properties (Li et al., 2010). It has been proven that phosphorylated proteins can exhibit many enhanced functional properties, including foaming, solubility, emulsifying, calcium absorption, gelling, etc (Li, Ibrahim, Sugimoto, Hatta, & Aoki, 2004; Li, Salvador, Ibrahim, Sugimoto, & Aoki, 2003). Proteins bound to phosphate groups have a higher electronegativity and a lower pI, which can change the functional properties of the base protein (Zhang, Li, & Ren, 2007). Huang et al. (2017) studied fish gelatin phosphorylated with sodium trimetaphosphate (STMP) at 50 °C for 0, 0.5, 1 or 2 h and found that fish gelatin phosphorylation could improve the gel properties in the short term, while the long-term effects of phosphorylation improved emulsion stability. Miedzianka and Pęksa (2013) succeeded in potato protein isolate phosphorylation modification with STMP using a chemical phosphorylation method at ambient temperature and the results showed that potato protein isolate phosphorylated under slightly alkaline pH (8.0) could exhibit some improved functional properties, such as water and oil absorption capacity, emulsifying activity and foaming capacity.

Phosphorylation is mainly achieved by chemical phosphorylation and enzymatic phosphorylation. Compared to chemical phosphorylation, enzymatic phosphorylation introduces too few phosphate groups, thus it is difficult to use this technique for improving protein functional properties (Campbell, Shih, & Marshall, 1991). As a controlled method, chemical phosphorylation is an economical and effective modification method which has been widely performed (Wang & Chi, 2012).

Microwave-assisted chemical techniques have been used in the performance of chemical reactions for a few years. Compared with the

* Corresponding author.
** Corresponding author.
E-mail addresses: jinyongguo@mail.hzau.edu.cn (Y. Jin), stheaven16@126.com (L. Sheng).

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use of conventional heating methods, chemical synthesis with microwave assistance can significantly shortened reaction times, along with improved yield and purity of the product (Guan et al., 2011). Some researchers have already employed microwave-assisted techniques in the chemical modification of food protein. Guan, Qiu, Liu, Hua, and Ma (2006) used a microwave heating method to assist soy protein isolate–saccharide graft reactions and found that microwave irradiation was able to speed up the graft reactions of the SPI–saccharides. Wang and Chi (2012) phosphorylated soy protein isolate assisted with microwave at 600 W for 3 min and the result showed that the phosphorylated protein had a 2 times greater emulsifying activity and 1.4 times greater emulsifying stability compared with the untreated sample.

Although phosphorylated egg white protein has been studied for several decades, the phosphorylation method mainly used is dry-heating (Hayashi et al., 2009; Li et al., 2004, 2003; Yin, Yang, Zhao, & Li, 2014). Phosphorylation modification with microwave assistance has not been applied for egg white protein. In this paper, egg white protein was phosphorylated by a chemical phosphorylation with microwave assistance, and the structural and foaming properties of the phosphorylated egg white protein in the presence of sodium tripolyphosphate were investigated. The information obtained in this study will provide theoretical and practical references and expand the applications of egg white protein.

2. Materials and methods

2.1. Materials

Fresh eggs were bought from a local farm. Tris, citric acid monohydrate, 5,5′-dithiobis-(2-nitrobenzoicacid) (DTNB), glycine (Gly), sodium dodecyl sulfate (SDS), and 1-anilino-8naphthalenesulphonate (ANS) were of analytical grade. Sodium tripolyphosphate (STP) was purchased from Aladdin Industrial Co. Ltd. (Shanghai, China). All other reagents were of analytical grade.

2.2. Preparation of the egg white protein solution

The egg white protein solution was prepared as follows. Egg white protein was separated from infertile eggs and equilibrated using a magnetic stirrer for 12 h at 4 °C. Afterward, the egg white protein was added together with double its volume of deionized water and equilibrated with a magne tic stirrer for 6 h at 4 °C, then insoluble protein was removed. This solution was used for the preparation of egg white protein powder.

2.3. Preparation of the phosphorylated egg white protein powder

STP was dissolved to the egg white protein solution to form a 30 g/L mixed solution at pH 8.0 by adjusting the pH with citric acid monohydrate (pH 2.0). Then, the solutions were put into a microwave reactor at 200 W, 300 W, 400 W, 500 W, and 600 W power levels for 2 min without stirring. When the reaction was finished, the solutions were placed into ice water immediately to prevent further reaction. The solutions were dialyzed for 24 h at 4 °C against deionized water to remove free phosphate. Finally, the powders were obtained using a spray dryer, in which the outlet temperature was 170 °C and the speed of fluid flow was 1200 mL/h. These samples are referred to as 200W-PP-EWP, 300W-PP-EWP, 400W-PP-EWP, 500W-PP-EWP and 600W-PP-EWP. The blank control group was called native egg protein (N-EWP).

2.4. Determination of phosphorylation degree of N-and PP-EWP

The total content of phosphorus was determined as previously reported (Chen, Toribara, & Warner, 1956). A 0.5 g portion of each sample was digested in nitric acid, sulfuric acid perchloric acid and perchloric acid at 120 °C for 45 min, at 180 °C for 3 h and then at 220 °C for 30 min. The amount of phosphorus in the digest was regarded as the total phosphorus of the protein. For the determination of inorganic phosphorus (Pi), 5 mL of 0.1 g/mL trichloroacetic acid was added with the same volume of the 10 mg/mL sample solution, and the solution was centrifuged at 3000 g for 20 min. The phosphorus content of the supernatant was regarded as Pi. The amount of phosphorus connected to proteins was estimated by the difference between the total phosphorus and the Pi content.

2.5. Determination of the content of free -SH groups

Sulfhydryl thiol groups were determined using DTNB according to the method of Sheng et al. (2018b). The 1 mg/mL egg white dispersion was dissolved in 0.1 moL/L Tris–glycine buffer (pH 8.0) and 50 g/L SDS, and then the solution was incubated at 40 °C for 30 min. After incubation, 40 μL of DTNB solution (4 mg in 1 mL 0.1 moL/L Tris–glycine buffer, pH 8.0) was added to 4 mL of the sample and then incubated in the dark for 30 min. The absorbance was then measured at 412 nm.

2.6. Measurement of the surface hydrophobicity (Ho) of N- and PP-EWP

The surface hydrophobicity of the protein was determined by following the method of Xiong, Zhang, and Ma (2016) with slight modification. The 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL egg white dispersions were dissolved in 0.1 moL/L phosphate buffer (pH 7.2). Then, 4 mL of each of the different dilutions was added with 20 μL of 8 mmoL/L ANS solution (0.1 moL/L phosphate buffer solution, pH 7.2). The fluorescence intensity was then determined using a fluorescence spectrophotometer at an excitation wavelength of 390 nm and a 470 nm emission wavelength. The ANS solution lacking protein was used to correct for background fluorescence. Ho was determined according to the slope method used in a previous paper (Alizadeh & Lichan, 2000).

2.7. Fourier Transform infrared (FTIR) spectroscopy analysis

Each sample was mixed with KBr and triturated and then analyzed over the wavenumber range of 4000–400 cm^{-1} at 25 °C with an FTIR spectrometer (Nicolet Nexus 470). The resolution of the instrument was 4 cm^{-1}, and air was scanned as the background for each sample. Each measurement was a superposition of 32 scans.

2.8. Circular dichroism (CD) spectroscopy analysis

The secondary structures of N- and PP-EWP were measured by CD spectroscopy from 190 to 250 nm, performed with a spectropolarimeter (Jasco Co J-810., Tokyo, Japan). Each sample (0.1 mg/mL) was dissolved in 0.1 moL/L phosphate buffer solution (pH 7.2). CD spectra are presented in terms of mean residue ellipticity (mdeg). CD spectra were measured with a resolution of 0.2 nm, 100 nm/min scan speed, response time of 0.25 s, bandwidth of 1.0 nm, and sensitivity of 20 mdeg and measurements were performed using a circular quartz cell of 0.1 cm path length at 25 °C (Sheng et al., 2017). The results of CD spectroscopy were analyzed using Spectra Manager software and the contents of secondary structure was calculated by the Yang principle.

2.9. Zeta potential analysis

Each sample (0.1 mg/mL) was dissolved in 0.1 moL/L phosphate buffer solution (pH 7.2). After filtration through a 0.45-mm microporous membrane, the diluted samples were injected directly into the chamber of a Zetasizer Nano-ZS instrument (Malvern Instruments, Worcester-shire, UK) prior to zeta potential analysis at 25 °C (Gouda, Zu, Ma, Sheng, & Ma, 2018).