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Effect of structure changes on hydrolysis degree, moisture state, and thermal denaturation of egg white protein treated by electron beam irradiation

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ABSTRACT

This research focused on electron beam irradiation (EBI) effects on quality characteristics of egg white protein (EWP). The EWP was treated by 0, 1.08, 3.24, and 5.40 kGy doses of EBI. Quality characteristics were measured including degree of hydrolysis (DH), moisture state, and denaturation temperature. Low-field nuclear magnetic resonance (LF-NMR) was used to evaluate the change on moisture state and thermal denaturation. Scanning electron microscopy (SEM) and mid-infrared spectroscopy (MIR) were used to investigate the microcosmic surface and secondary structure of EWP. The DH of EWP treated by EBI at dose of 5.40 kGy was significantly ($P < 0.05$) increased to 23.79% ± 0.14%. Besides, LF-NMR results showed EBI treatment had effects on reducing bound water content and could reduce denaturation temperature of EWP to 25 °C. Through SEM and MIR analysis, EBI treatment would not damage the secondary structure of EWP but lead to puncture pores on microcosmic surface of EWP granules, which resulted in the improvement of EWP hydrolysis with the EBI doses increasing. These results provided data support for irradiation applying on foodborne protein in the future and solving the problem of the low production efficiency of peptides.

1. Introduction

In recent years, because the food were easily contaminated by kinds of pathogenic bacteria in preserving process, the food safety problems have been emerged in endlessly and caused national attention (Hagiwara et al., 2005). The traditional food processing technology such as adding antiseptic and heat sterilizing not only destroy the food original structure, influence the unique food flavor and taste, but also easy to cause food chemical pollution and harm to human health. Electron beam irradiation (EBI) was one kind of irradiation technology widely used in food process (Kim et al., 2014). The irradiated food by electron beam from electron accelerator can generate physics, chemistry, and biological effects to kill microorganisms, delay mature, sprout inhibition, and promote materials conversion, then, achieving the purpose of food preservation. Compared with the traditional food processing, EBI is conform to the trend of the current environmental protection with unique characteristics such as low temperature, high efficiency, and less energy consumption (Farkasa & Mohácsi-Farkas, 2011). As a powerful cold pasteurization technology in food process EBI treatment can cause very small temperature changes inside foods and has been paid much attention on it.

Some researches on the application of irradiation technology in the starch and protein were gradually increasing, which were focused on the physical and chemical properties (Josimovic, Radojcic, & Milosavljevic, 1996; Soliman & Furuta, 2009; Wang, Sun, Zeng, & Lu, 2000). It was reported that gamma irradiation had great influence on the physical and chemical properties of gluten protein and soy protein isolate. When the irradiation doses was less than 16 kGy, it could induce the molecular structure change, the disruption of the gluten molecules and the cracking of the polypeptide chains significantly (Myoungsuk, Sehee, & Kyung,

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2005). In addition, the EBI tecnology was also applied in the enzymatic hydrolysis in bio-resource utilization, protein digestibility and gel properties of protein and so on (Bak et al., 2009; Duan, Xing, Shao, & Zhao, 2010; Karthika et al., 2013). These researches showed the possible reason for EBI effects on protein was the destruction of the hydrogen bond or hydrophobic group which lead to protein denaturation. Besides, EBI irradiation could also improve the smell of food (Lopez–gonzalez, Murano, Brennan, & Murano, 1999). However, whether EBI irradiation has effect on egg white protein (EWP) has not been reported and the proper doses for treatment were not clear as well. Therefore, research on the effect of EBI treatment on the quality characteristics of EWP should be carried.

As the major source of protein for Chinese people, egg is widely used in various foods. EWP are refined products made of fresh eggs with the characteristics of desugarization, deodorization, high purity, and rapid dissolution. It can be used to improve the quality, extend the shelf life, and strengthen nutrition of products during the food processing. For the reason of multi-functional properties, it can be used widely as additives in food industry. But the egg white proteins are very heat sensitive proteins and can be undesirably coagulated by standard temperature pasteurization process. Therefore, during the conventional pasteurization for EWP the egg whites destined for drying are pasteurized only mildly and the heat treatment for inactivation of present bacteria is postponed until drying. In addition, as a kind of high-quality protein EWP was widely used in the preparation of active peptides. But due to the problem of less hydrolysis degree by enzymolysis, the production efficiency of EWP peptides is relatively low. Therefore, the aim of this study was to investigate the effect of EBI treatment on the quality characteristics of EWP to provide scientific basis for the EBI application on EWP production, meanwhile, to solve the problem of the low production efficiency of EWP peptides. The effect on degree of hydrolysis (DH), moisture state, and denaturation temperature of EWP were investigated. The changes of microcosmic surface structure and secondary structure of EWP were measured by using scanning electron microscope (SEM) and mid-infrared spectroscopy (MIR) technology to reveal the reason of EBI effects on the quality characteristics of EWP.

2. Materials and methods

2.1. Materials

EWP powder with protein content of 80.96% was purchased from Jinjiangli Co. (Peking, China).

2.2. Electron beam irradiation (EBI) treatment on EWP

Four EWP samples of 500 g were packed in polyethylene bags. These samples were subjected to EBI provided by a 10Mev/15 kW electron linear accelerator (YIFU Electronic Accelerator Co. Ltd, Changchun, China). All irradiations were carried out at ambient temperature (25 ± 0.5 °C). The samples were irradiated with 1.08 kGy/s by single neutron beam to adjust the conveyer speed when each of the sample batches was passed under the beam. The delivered doses of each sample were strictly controlled and calculated to be 0, 1.08, 3.24, and 5.40 kGy. Then, these samples were saved standby at room temperature for further tests.

2.3. EWP hydrolysis by alcalase

To further evaluate the effect of EBI on EWP, the EBI-treated samples were hydrolyzed with the previous method of Lin et al. (2013). Briefly, EWP solution at specified concentration of 5.16% was heated at 90 °C to denature the protein. The pH value of the EWP solution was adjusted to 10.66 and the temperature of the proper hydrolysis parameters was adjusted to 50 °C. After adding 3% of Alcalase (W/W), the hydrolysis began and the pH value of solution was kept within 10.66 ± 0.05 by adding NaOH solution. After three hours' hydrolysis, the enzyme was inactivated in 90 °C water bath for 10 min and the hydrolysate was vacuum-concentrated, freeze-dried, and stored at−20 °C until used. The degree of hydrolysis (DH) was calculated during the hydrolysis process by the following equation (1):

$$\mathrm{DH} = \frac{h}{h_{tot}} \times 100\% = \frac{V \times C}{M_p \times h_{tot} \times \alpha} \times 100\% \quad (1)$$

where, h is the number of peptide bonds hydrolyzed; V is the volume of NaOH (mL); C is the concentration of NaOH (mol/L); M_p is the protein mass of hydrolysis (g); h_{tot} is hydrolysis equivalents per gram protein (mmol/g) (For egg white, h_{tot} is 8.38 mmol/g protein); α is the calibration factors for pH-stat ($1/\alpha = 1.01$ for Alcalase).

2.4. Low-field nuclear magnetic resonance (LF-NMR) test

LF-NMR was performed with a 21.96 MHz NMR Analyzer PQ001 (Niumag Electric Corp., Shanghai, China). Approximately 1 g of EWP samples were placed into 15 mm LF-NMR glass tubes and determined in the NMR probe coil. Transverse relaxation, T_2, was measured using the Carr–Purcell–Meiboom–Gill (CMPG) sequenceat (Carr & Purcell, 1954; Meiboom & Gill, 1958). The time (τ) between 90° and 180° pulses was 200 μs Data were acquired from the 3000 echoes by 16 scan repetitions. The interval time between two successive scans was 3 s. Thus, the influence of field inhomogeneity, diffusion, and chemical exchange on the relaxation can be minimized (Hills, Takacs, & Belton, 1990). MultiExp Inv Analysis software (Niumag Electric Corp., Shanghai, China) was employed for data analysis and distributed exponential curve fitting. In the time domain, spin–spin relaxation data is presumed to be a sum of exponentials (2):

$$M(t) = \sum_{i=1}^{n} \mathrm{p}_{2i} \exp\left(\frac{-t}{T_{2i}}\right) + \mathrm{d}(t) \quad (2)$$

where, M is the residual magnetization as a function of acquisition time t; n the number of exponential functions or components in the sample; T_{2i} and p_{2i} are the transverse relaxation time and the relaxation signal amplitude, respectively, of the ith component; and $d(t)$ is residual error. According to the above relaxation model, the plots of relaxation amplitude versus relaxation time were obtained by multiexponential fitting analysis and inversion according to a modified method (Xiao, Wang, & Liu, 2004). The relaxation time T_{2i} and its corresponding signal area (A_{2i}) and ratio R_{2i} were recorded.

2.5. Effect of EBI treatment on EWP denaturation

To further investigate the effect of EBI treatment on EWP, the denaturation status of EWP at different temperatures was measured by LF-NMR. EWP solutions of 1 g/mL were adjusted to 0, 25, 50, 75 and 90 °C for 5 min in a water bath respectively. Then the denaturation statuses of EWP solution were determined by LF-NMR. Unlike the solid sample, the time (τ) between 90° and 180° pulses in CMPG sequence test was 800 μs Data were acquired from the 15 000 echoes by 4 scan repetitions. Other parameters used in LF-NMR test were the same as described in section 2.4.