Food Research International 100 (2017) 850–857

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Effects of electron beam irradiation (EBI) on structure characteristics and thermal properties of walnut protein flour

Yue Zhao[a], Na Sun[a], Yong Li[a], Sheng Cheng[b], Chengyao Jiang[a], Songyi Lin[a,*]

[a] National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China
[b] Analysis and Test Center, Dalian Polytechnic University, Dalian 116034, PR China

ARTICLE INFO

Keywords:
Walnut protein flour
Electron beam irradiation (EBI)
Low-field nuclear magnetic resonance (LF-NMR)
Structure characteristics
Thermal properties

ABSTRACT

The effects of electron beam irradiation (EBI) on structure characteristics and thermal properties of walnut protein flour (WPF) were evaluated. The WPF was irradiated by 0–15.0 kGy of the EBI. Scanning electron microscopy and X-ray diffraction analysis revealed that the EBI irradiation could not change the amorphous structure of WPF but resulted in puncture pores and fragmentation on microcosmic surface of WPF. Besides, low-field nuclear magnetic resonance results showed the EBI irradiation had effects on increasing denaturation temperature of WPF to 70 °C, and the particle size of WPF hydrolysates (WPFHs) irradiated by EBI at dose of 5.0 kGy significantly ($P < 0.05$) increased to 753.8 ± 21.0 nm. The molecular weight of WPFHs at dose of 5.0 kGy increased compared with that of non-irradiated sample. These revealed that EBI irradiation led to aggregation or cross-linking of the walnut protein. In addition, thermogravimetric analysis and zeta potential values indicated that the EBI enhanced thermal stability of WPF and didn't affect the physical stability of the WPFHs. Therefore, these results provided a theoretical foundation that the EBI applies on improving the properties of protein in the future.

1. Introduction

In recent years, tree nuts are increasingly popular and valued for their desirable sensory and nutritional attributes. Walnuts are one category of tree nuts and have high economic value due to the high level of lipids (67% on dry basis) (Sabate et al., 1993). The increasing market need of walnut lipids results in a large amount of by-products: walnut meal containing nutritional proteins. Therefore, it is essential to improve the economic value of the defatted walnut meal (Gu et al., 2015). Especially, the hydrolysates of walnut protein have crucial biological activities including anti-inflammatory, antiatherogenic, antimutagenic properties (Martínez, Labuckas, Lamarque, & Maestri, 2010; Taheri, Farvin, Jacobsen, & Baron, 2014). However, without modification, the application of food proteins is limited because of their certain functional properties. To obtain ideal or prefect properties, many technologies have been applied to improve the properties of proteins. Enzymatic modifications, physical or chemical methods were applied to protein processing (Qin et al., 2013). Food irradiation is a physical method of food preservation to maintain the freshness and modify the food properties (Kuan, Bhat, Patras, & Karim, 2013).

Food irradiation makes food expose non-ionizing and ionizing irradiation to damage microorganisms, viruses or bacteria in the food and modify food protein (Farkas & Mohacsifarkas, 2011). Studies about the application of irradiation technology in the fields of food preservation and protein modification were gradually increasing, which were primarily focused on the physical and chemical properties (Fan, Niemira, & Sokorai, 2003; Soliman & Furuta, 2009; Su, Venkatachalam, Teuber, Roux, & Sathe, 2004). The UV-C irradiation decreases growth of psychrotrophic bacteria, and coliform and extends the shelf life (Allende & Artes, 2003). The irradiated fresh cilantro retained its sensorial quality and shelf life (Fan et al., 2003). The application of irradiation is not only limited to food preservation, but also widely used in other applications such as modification of proteins. The γ-irradiation had enormous effects on the properties of soy protein flour. The ordered molecular structure of soybean protein flour was disrupted after the irradiation, as well as degradation, cross-linking, and aggregation of the polypeptide chains occurred (Lee, Lee, & Song, 2005). It was applied to the inclusion of soybean meal and protein concentrate. Macromolecular proteins were degraded and the irradiation could improve the performance of the inclusion of soybean meal and soybean protein concentrate (Wu, Wang, Ren, Qin, & Kim, 2016). Furthermore, the irradiation was effective in attenuating allergenicity of walnut proteins and

* Corresponding author at: No. 1 Qinggongyuan, Ganjingzi District, School of Food Science and Technology, Dalian Polytechnic University, National Engineering Research Center of Seafood, Dalian 116034, PR China
E-mail address: linsongyi730@163.com (S. Lin).

http://dx.doi.org/10.1016/j.foodres.2017.08.004
Received 12 May 2017; Received in revised form 1 August 2017; Accepted 2 August 2017
Available online 10 August 2017

decreased the anaphylaxis of walnut proteins (Su et al., 2004). The irradiation includes many categories and electron beam irradiation (EBI) belongs one of irradiation technologies. Comparing with other irradiation methods, the equipment of EBI does not generate radioactive waste or hazards. In addition, the EBI is much higher utilization rate and has a low cost of operation. The electron beam with good concentricity cans generare high energy within a short time (Xue, Zhao, Wen, Cheng, & Lin, 2017). The application of EBI has also been reported by Jin et al. (2017) that the EBI changed the properties of egg white protein. Moreover, Xue et al. (2017) reported the EBI technology was applied to the research of the properties of corn flour. EBI affected physicochemical properties of corn flour and caused the change of corn flour particle surfaces and molecular chains. The walnut protein has abundant nutrition. However, the effect of EBI on walnut protein flour (WPF) hasn't been studied yet. To date, there is not sufficient understanding of characteristics and thermal properties of WPF irradiated by EBI. This study concentrated on elucidating the effects of EBI on WPF. Therefore, the research should be performed in order to provide scientific theoretical basis for the EBI application on WPF productions.

To sufficiently understand the effect of EBI irradiation on structure characteristics and thermal properties of WPF, scanning electron microscope (SEM), X-ray diffraction, low-field nuclear magnetic resonance (LF-NMR), thermogravimetric (TG) analysis and particle size analyzer were used. These results provided some new theoretical basis that the EBI applies on improving the properties of protein in the future.

2. Materials and methods

2.1. Materials

The walnut was purchased in Changchun (China). The protein content of walnut protein flour was 64.02%. The 2.4 L of Alcalase was donated by Novozyme (Bagsvaerd, Denmark) and the enzyme activity was 170,000 U/g. All other chemicals and reagents used in this study were analytical grade and commercially available.

2.2. Electron beam irradiation (EBI) of walnut protein flour (WPF)

The WPF was irradiated with the previous method described by Xue et al. (2017) with some modifications. The WPF was crushed to pass through a 100-mesh standard sieve. 100 g of WPF was vacuum sealed in plastic bags, flatted and kept the thickness of WPF < 8 mm. Then the WPF was placed on a conveyor belt to subject electron beam irradiation chamber. The irradiation treatments were performed by a 10 MeV/15 kW electron linear accelerator (YIFU Electronic Accelerator Co. Ltd., Changchun, China). Samples were irradiated at room temperature and the dose levels were 0 (non-irradiated), 2.5, 5.0, 7.5, 10.0, and 15.0 kGy. The dose rate was 0.5 kGy/h. After EBI treatment, the samples were cooled down to room temperature and stored − 80 °C for further tests.

2.3. Scanning electron microscopy (SEM)

The WPF was scanned with the previous method described by Jin et al. (2017). The surfaces of WPF were observed with a JSM-6700F field emission SEM (JEOL Ltd., Japan) by the E-1045 ion beam sputtering instrument (Hitachi Co. Ltd., Japan). The WPF was adhered to a circular aluminum specimen stub by an adhesive tape and covered with gold-palladium for 90 s under the condition that the current was 15 mA. The samples were photographed under a potential of 5 kV. The SEM micrographs magnification was 6000 ×.

2.4. X-ray diffraction technology

The X-ray diffraction was performed with the previous method described by Abbott, Capper, McKenzie, and Ryder (2007) with some modifications. The X-ray diffraction was measured by X-ray diffraction system (Shimadzu XRD-7000S, Dalian, China). The diffraction total power and circulating pump were opened. The appropriate diffraction conditions and parameters were set. The normal current operating conditions were 40.0 kV and 30.0 mA. Scans mode was continuous scan at a speed of 5.0°/min in range of 10° and 70°. The WPF was filled into the sample area. Then the X-ray diffraction procedure was performed.

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

The LF-NMR measurements were carried out according to the method (Lin, Yang, Li, Chen, & Zhang, 2016) with some modifications. LF-NMR was performed with a 22 MHz NMR Analyzer PQ001 (Niumag Electric Corp., Shanghai, China). 3 mL of WPF solution (0.1 g/mL) was placed into a diameter of 10 mm of the nuclear magnetic tube for testing. Transverse relaxation, T_2, was monitored by the Carr-Purcell-Meiboom-Gill (CPMG) sequence under the conditions that the proton resonance frequency was 22 Hz, the half-echo time t-value (time between 90° pulse and 180° pulse) was 200 ms, the number of sampling (TD) was 1,984,972, the spectral width (SW) was 200 kHz, the repetitive sampling latency (TR) was 10,000 ms, the number of repetitive scans (NS) was 6, and the number of echoes was 12,000. The experimental data were dealt with by Multi Inv Analysis soft. The T_2 attenuate curve was substituted into the relaxation model by the iterative optimization method (formula (A)) and the multi-samples relaxation time spectrum was obtained.

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

where, $M(t)$ is the signal intensity at an involved time (t) after adhibition of radio frequency pulse for the first time, n is the quantity of the exponential functions in the specimen, A_{2i} and T_{2i} are the signal intensity of relaxation amplitude and time of the ith component, and $d(t)$ is the residual error. In order to better fit, multi-exponential fitting analysis was applied on the relaxation data analysis in line with an altered inversion method (Li et al., 2015). The WPF solutions of 0.1 g/mL were placed into 30, 50, 70, and 90 °C for 5 min in a water bath kettle, respectively. Then the denaturant statuses of WPF solutions were measured by LF-NMR.

2.6. Preparation of walnut protein flour hydrolysates (WPFHs) by Alcalase

For a more in-depth understanding of the effect of EBI, the WPFHs were prepared with the previous method described by Jin et al. (2017) with some modifications. The preparation process of WPFHs was shown in Fig. 1.

2.7. Particle size determination of WPFHs

The particle size distribution of the WPFHs was measured using a Zetasizer Nano ZS90 particle size analyzer (Malvern Instruments Ltd., Malvern, UK) with a 633 nm red laser. The particle size analysis was performed using dynamic light scattering. The average value and standard deviation of 3 measurements per sample was reported. 1 mL of WPFHs solution (1 g/mL) was used to measure the particle size distribution. The particle size was obtained via the translational diffusion coefficient by using the Stokes Einstein equation (formula (B)):

$$d_h = kT/3\pi\rho\eta D \quad \text{(B)}$$

where, d_h is the hydrodynamic diameter; k is the Boltzmann's constant; T is the absolute temperature; D is the translational diffusion coefficient and η is the viscosity. Protein solution was filtered by 0.45 μm before the measurement and then all WPFHs were measured via this method.