Food Hydrocolloids 87 (2019) 933–942

Contents lists available at ScienceDirect

Food Hydrocolloids

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

Effects of different proteases on the emulsifying capacity, rheological and structure characteristics of preserved egg white hydrolysates

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ARTICLE INFO

Keywords:
Preserved egg white
Emulsifying capacity
Rheology
Intermolecular interaction

ABSTRACT

This paper investigated the effects of six proteases on the properties of preserved egg white gel, including emulsifying capacity, rheological properties and intermolecular interactions. The results showed that the hydrolysis degree (DH) and nitrogen solubility index (NSI) of preserved egg white gel hydrolysates increased, and the surface hydrophobicity declined due to the embedding and destruction of the hydrophobic groups. The determination of zeta potential and particle size indicated that the hydrolysate peptides treated by flavourzyme occurred to aggregation to exhibit a larger particle size in contrast to other proteases. The emulsifying ability of preserved egg white hydrolysates dropped after the hydrolysis of proteases, except the flavourzyme at pH 3.0, 5.0, and 7.0, and the emulsifying stability declined obviously, in addition to the pepsin at pH 5.0 and 7.0 and the neutrase at pH 9.0, which were similar to the results of rheological characteristics determined by rheometer. Additionally, the enzymatic hydrolysis caused significant increase in the total sulfhydryl and disulfide bond contents of hydrolysates compared with those of the untreated preserved egg white. The results of intermolecular interactions and Fourier transform infrared spectrometry (FTIR) indicated that preserved egg white hydrolysates were mainly supported by the hydrogen bonds and hydrophobic interactions. These results suggested that the hydrolysates of preserved egg white possessed different physicochemical and functional characteristics, which were attributed to the different effects of six proteases on the structure of preserved egg white proteins.

1. Introduction

Preserved egg (Pidan in Chinese) is a kind of traditional Chinese food with a unique flavour. The brown and transparent egg white and dark green egg yolks were obtained by curing in NaOH, salt, black tea and $CuSO_4$. The proteins in the egg white were denatured after the penetration of alkali through egg shell pores and the membrane, resulting in degradation occurring to re-gather the high elasticity and low viscosity gel via ionic bonds and disulfide bonds (Zhao et al., 2016). The gel structure presented a uniform, loose, and fine filamentous structure of regular voids (Zhao, Tu, Xu, Li, & Du, 2014). There are more than 100 types of proteins in the egg white, and those present in the highest proportions are albumin, transferrin, mucin, and lysozyme, which are almost 90% of the protein (Mine, 2008). After alkali treatment, the protein in egg white decomposed and produced lots of polypeptide fragments. Therefore, it was difficult to explain the specific mechanism of gelation thus far due to the complex protein components in the egg white.

Proteases, such as flavourzyme, pepsin, trypsin, neutrase, alcalase, and papain, are usually used to prepare peptide (Feng, Ruan, Jin, Xu, & Wang, 2018; Xu et al., 2016). Many proteins from animals or plants are processed by enzymes to produce antioxidant or functional peptides, such as whey protein, flaxseed protein, and walnut protein (Jahanbani et al., 2017; O'Keeffe, Conesa, & Fitzgerald, 2017; Udenigwe, Lin, Hou, & Aluko, 2009). The use of multiple enzymes has also been adopted to hydrolyse proteins to enhance some acquirable properties. Meanwhile, the hydrolysates obtained from different proteases have different characteristics, such as the emulsifying capacity, antioxidant or antibacterial properties related to the characteristics of the proteins and the active sites of the enzymes (Chakka, Elias, Jini, Sakhare, & Bhaskar, 2015; Chen, Chen, Yu, & Wu, 2016; Jemil et al., 2014). Interestingly, the decomposed proteins in preserved egg white may have some special

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https://doi.org/10.1016/j.foodhyd.2018.09.023
Received 29 May 2018; Received in revised form 13 September 2018; Accepted 17 September 2018
Available online 20 September 2018
0268-005X/

effects, such as anti-inflammatory and digestive. For example, the digested preserved egg white hydrolysate might have an anti-inflammatory effect and could be used as a novel preventative food (Zhao et al., 2017). Furthermore, egg and egg by-products have always been used as the raw material to purify functional peptides (Nyemb et al., 2016). However, there are few studies on the use of different proteases to treat preserved egg whites as raw materials, which lays a foundation for the later study of the functional properties and development of deep-processed products of preserved eggs, such as the development of antioxidant polypeptides and angiotensin converting enzyme inhibitory peptides using preserved egg whites. It is very interesting for the authors to investigate the variation in the preserved egg white gel after hydrolysis action to explain the hydrolysis mechanism.

The main components in egg white are water and proteins, while the proteins in preserved eggs have been alkaline-denatured. In this paper, the effects of different proteases on preserved egg white gels were studied, including the emulsifying capacity, rheological properties, intermolecular interactions and structure characteristic of preserved egg white hydrolysates. The mechanism of the change of preserved egg whites before and after enzymatic hydrolysis has been discussed, which provided a reference for the utilization of preserved egg whites.

2. Materials and methods

2.1. Materials

Preserved eggs, which were stored at normal temperature and were produced 1–2 weeks before the experiment, were made by the immersion method and were purchased from a local supermarket in Guangzhou, China. Sodium hydroxide (NaOH), ethylenediamine tetraacetic acid (EDTA), 5,5′-dithiocarbamate (2-nitrobenzoic acid) (DTNB), disodium hydrogen phosphate ($Na_2HPO_4.7H_2O$) and sodium dihydrogen phosphate ($NaH_2PO_4.2H_2O$) were obtained from the Tianjin Fuyu Fine Chemical Co., Ltd. (Tianjin, China). Potassium bromide (KBr), trichloroacetic acid (TCA) and glycine were bought from the Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Protease (flavourzyme (50,000 u/g), neutrase (102,786 u/g), alcalase (217,434 u/g), papain (71,461 u/g), pepsin (3000 u/g), trypsin (3500 u/g)), trimethylolaminomethane (Tris), sodium dodecyl sulfate (SDS), β-mercaptoethanol (β-Me), 8-aniline-1-naphthalenesulfonic acid (ANS) were supplied from Sigma (St. Louis, MO, USA).

2.2. Hydrolysates preparations

Some whole preserved eggs were selected to prepare preserved egg white gel gently after peeling the egg shells gently and separating egg white and yolk thoroughly by a knife. Ten grams of preserved egg white and 90 mL of deionized water were homogenized at speed of 12,000 × rpm (Ultra Turrax homogeniser, IKA T18 digital, IKA Works Guangzhou Co., Ltd., China), and they were adjusted to the optimum temperature and pH (PB-10, Sartorius, Berlin, Germany) for different enzymes. The optimum pH values of trypsin, pepsin, alcalase, neutrase, flavourzyme and papain were 8, 2, 9, 6.8, 6.2 and 7.5, respectively, and the optimum temperatures of these enzymes were 55 °C, 37 °C, 55 °C, 55 °C, 50 °C and 50 °C, respectively. Then, adjustments were made to achieve 2000 U/g of enzyme activity. The optimum pH was regulated every 20 min via 1 moL/L HCl or 1 moL/L NaOH. After being hydrolysed up for 4 h, the hydrolysates was placed in a boiling water bath for 10 min to deactivate the protease activity. Hydrolysates were leached by using a 0.45 μm filter when the temperature dropped to 25 °C, and then, they were placed in −18 °C freezer before adopting the Kjeldahl method to determine the protein content.

2.3. Determination of DH

The degree of hydrolysis was determined by the pH-stat method according to Adler-Nissen (1986). The DH was calculated by the following Eq. (1):

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

where B is the volume of base (mL), N_B is the molarity of the base (mol/L), α is the average degree of dissociation, M_p is the amount of protein used (g), and h_{tot} is the theoretical number of peptide bonds per mass unit (mmol/g, with reference to the collected protein of 6.77 mmoL/g). The value α of a was calculated by Eq. (2):

$$\alpha = \frac{10^{pH-pK_a}}{1 + 10^{pH-pK_a}} \times 100\% \quad (2)$$

where the pH is the value at which enzymatic hydrolysis was conducted, and the pKa values are for a $-NH_3+$ groups.

2.4. Determination of NSI

The soluble nitrogen content of the hydrolysate at different times was appropriately modified according to the method described by Jang and Lee (2005). Ten millilitres of hydrolysates was added into 10 mL of 10% (w/v) TCA solutions, and centrifuged at a speed of 8000 × g for 15 min (Polytron, PT 2100, Kinematica AG, Luzern, Switzerland). After 30 min of standing the supernatant was discarded. The precipitate was washed with 10 mL of 10% (w/v) TCA solutions twice, and was centrifuged again to obtain the precipitate. The soluble nitrogen content of the precipitate was determined by the Kjeldahl method. The NSI (%) is calculated as follows by Eq. (3):

$$NSI\,(\%) = \frac{N_0}{N_t} \times 100\% \quad (3)$$

where N_0 is the soluble nitrogen of the precipitate and N_t is the total nitrogen of the hydrolysate.

2.5. Turbidity and solubility analysis

Solubility and turbidity analysis was performed by the method described by Damodaran and Kinsella (2002) with some modifications. The freeze-dried sample was dissolved in deionized water at a concentration of 1 mg/mL, and the pH (3, 5, 7, 9, 12) was adjusted with using 0.1 moL/L HCl and NaOH, respectively. The absorbance was measured at 590 nm (UV-2600, Shimadzu, Tokyo, Japan). After centrifugation at 10,000 × g for 15 min at 4 °C, the protein concentration in the supernatant was expressed as A_{280}, and the solubility of the protein was expressed as the percentage of the supernatant protein concentration in comparison to the corresponding total protein concentration.

2.6. Emulsifying capacity

The emulsifying capacities of different hydrolysates were determined according to the method described by Pearce and Kinsella (1978) with some modifications. The pH values of 30 mL of samples were adjusted to 3.0, 5.0, 7.0 and 9.0 after adding 10 mL of corn oil, respectively. Each mixture was homogenized at a speed of 10,000 × rpm for 1min, and then, 100 μL of the emulsion was pipetted from the bottom of the mixture and diluted to 10 mL with 0.1% SDS solution at 0 min and 10 min after homogenization. After 5 s of vortex mixing using a vortex mixer (RE-52A, Shanghai, China), the absorbance values was measured at 500 nm (UV-2600, Shimadzu, Tokyo, Japan). The absorbance value of A_0 and A_{10} were used to calculate the EAI and ESI as shown in Eqs. (4) and (5).