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Heat-pretreatment and enzymolysis behavior of the lotus seed protein

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ABSTRACT

Lotus seed protein (LSP) was heat-pretreated before enzymolysis in order to seek a greater degree of hydrolysis (*DH*) during enzymatic hydrolysis. The parameters including substrate concentration, temperature, pH, and papain concentration were optimized by response surface methodology in the enzymolysis of the heat-pretreated LSP. The influence of substrate concentration on the non-pretreated LSP enzymolysis was assessed, and the enzymolysis was found to obey the Haldane model with inhibition by LSP substrate. The initial concentration of non-pretreated LSP was inferred theoretically to be 11.07 g/L in order to avoid substrate inhibition. On the other hand, Chrastil model was fitted and the diffusion resistance constant values were in the range of 0.5–0.6 for the diffusion-controlled encounter of enzyme and substrate, implying that diffusion was a rate-limiting step. The heat-pretreatment at 60 °C for 60 min could increase the *DH* of the LSP, which enhanced the efficiency of the enzymolysis by papain.

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1. Introduction

Lotus seed, widely used as food in China (by the name of lianzi), is rich in protein, amino acids, unsaturated fatty acids and minerals. The seed contains approximately 19.85% protein on a dry weight basis, and shows a well-balanced amino acid composition compared with FAO/WHO pattern, with nutritive properties similar to soybean (Zeng, Cai, Cai, Wang, & Li, 2013). The interest in the lotus seed protein (LSP) as a functional food has been increased due to the potential health benefits (Bhat & Sridhar, 2008; Wu et al., 2007; Yen, Duh, & Su, 2005; Zeng et al., 2013).

Hydrolysis of proteins by proteases has undergone development in recent years. Enzymolysis can improve the functional properties of food without affecting its nutritive value. And the degree of hydrolysis (*DH*) is an important parameter for the determination of functional properties of protein hydrolysates (Adler-Nissen, 1979). Hence, protein hydrolysates are extensively incorporated as food ingredients in energy drinks, hypoallergenic formulae and enteral diets for children and sick adults due to their functional, nutritional and immunological properties (Moure, Sineiro, Domínguez, & Parajó, 2006; Prieto, Guadix, & Guadix, 2008; Schmidl, Taylor, & Nordlee, 1994). For the preparation of protein hydrolysates, it is essential to apply pretreatments to increase the accessibility of the enzyme to cleavage sites and consequently to raise the *DH*. Heat-pretreatment is effective for improving enzymatic hydrolysis and considered to be an eco-friendly processing technology since no chemicals are added and decreasing toxic compound formations (Cao, Sun, Liu, Yin, & Wu, 2012; Saha, Yoshida, Cotta, & Sonomoto, 2013). In food industry, heat-pretreatment of proteins before enzymolysis can lead to rearrangements of the inter- and intra-molecular linkages with concomitant changes in protein conformation, making proteins more susceptible to enzymolysis, due to the exposure of previously hidden cleavage sites, thereby enhancing enzymolysis (Adjonu, Doran, Torley, & Agboola, 2013; Boye, Ma, Ismail, Harwalkar, & Kalab, 1997; Chao, He, Jung, & Aluko, 2013; Tang, Chen, & Ma, 2009). In spite of the merits of the proposed approach, the application of heat-pretreatment to the LSP is scarcely described in the literature. In our preliminary work, the *DH* in the enzymolysis of

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non-pretreated LSP by papain was about 11%, which was too low to improve its functional properties. In order to enhance the *DH*, it is necessary to heat-pretreat the LSP.

The traditional "one-factor-at-a-time approach" is an operation frequently used in optimization to obtain high yields of the desired products. But that method disregards the complex interactions among various physicochemical parameters. The response surface methodology (RSM) is defined as a statistical method using quantitative data from an appropriate experimental design to determine and simultaneously solve multivariate equations. RSM can evaluate the effects of multiple parameters, alone or in combination, on response variables and also predict their behavior under given sets of conditions (Pan, Zeng, Foua, Alain, & Li, 2016).

In the present study, LSP was firstly heat-pretreated, and then the pretreated LSP was enzymolyzed by papain. In order to obtain high *DH*, the effect of the pretreatment temperature on the *DH* was investigated, and the enzymolysis parameters of the pretreated LSP including temperature, initial pH, substrate concentration and papain concentration were also optimized using RSM. Finally, the enzymatic hydrolysis process of non-pretreated LSP was analyzed in terms of kinetic parameters.

2. Materials and methods

2.1. Materials

Papain (EC 3.4.22.2, ⩾99%) was purchased from Sigma–Aldrich Company Ltd in China. All other reagents used in experiments were of analytical grade without further purification. All solutions were made with redistilled and ion-free water. The lotus seeds without seed coats and embryos, cultivated in Hunan Province of China, were purchased from a retail outlet in Xiangtan (China). Prior to experiments, the seeds were milled, and ground to pass through a 100-mesh screen to produce the lotus seed powder. The lotus seed protein (LSP) was extracted as previously described by Zeng et al. (2013). Briefly, the LSP was extracted in the 0.1 mol/L NaCl solution, where 5.0 g lotus seed powder was placed into a 1000 mL flat bottom flask containing 500 mL NaCl solution. The reaction mixture was blended for 1 h at 40 °C under atmospheric pressure and constant magnetic stirring (100 rpm). The slurry was centrifuged using a L535R tabletop refrigerated centrifuge (Xiangyi Centrifuge Instrument Co., Hunan, China) at room temperature (2800 g, 20 min). The supernatant containing the LSP was adjusted to pH 4.2 by adding 0.1 mol/L HCl solution (acid precipitation), and then re-centrifuged (4800 g, 25 min) in order to obtain the crude LSP product (residue). The crude product was washed with deionized water (pH 4.2) three times, and subsequently freeze-dried to give the LSP powder. The LSP powder contained 88.9% protein content, 1.1% fat, 1.8% total sugar content and 2.9% moisture content.

2.2. Differential scanning calorimetric (DSC) analysis

The heat denaturation temperature of the non-pretreated LSP was monitored by differential scanning calorimetry (DSC) in a TA Q100-DSC thermal analyzer (Shimadzu DT-40) (Kumar, Ganesan, Selvaraj, & Rao, 2014). 2.0 mg of the LSP powder was weighed into aluminium liquid pans (Dupont), and 10 μL PBS (0.05 mol/L, pH 7.5) was added. The sealed pans containing the LSP and buffer were equilibrated at 4 °C for more than 12 h. The pans were sealed and heated at a rate of 10 °C/min from 20 to 100 °C in 150 mL/min flow of helium, and subsequently cooled to 20 °C at the same rate. A sealed empty pan was used as a reference. The denaturation temperature, corresponding to the maximum of the transition peak, was determined. Experiments were carried out in triplicate and varied not more than 0.25 °C.

2.3. Heat-pretreatment of the LSP

The assay included two parts of heat-pretreatment and enzymolysis by papain. Firstly, the heat-pretreatment was carried out, where the LSP powder was prepared into a suspension containing 34 g/L LSP concentration. The suspension was pretreated in a rotating vessel (50 rpm) at a temperature range of 50–80 °C for 75 min. Five vessels were used at one time at a specific temperature, where a vessel was removed at each 15 min interval to stop the pretreatment for gaining the pretreated LSP suspension. Secondly, the enzymolysis of the pretreated LSP was performed. All the pretreated LSP suspensions at different temperatures were separately diluted to 15 g/L. Enzymolysis of the pretreated LSP by papain was performed under the conditions of pH 5.5, 45 °C and papain concentration 0.5 g/L for 45 min. All experiments were carried out in triplicate. It was found that the degree of hydrolysis (*DH*) at 60 °C for 60 min was the highest among all the pretreated LSP (Fig. 1B).

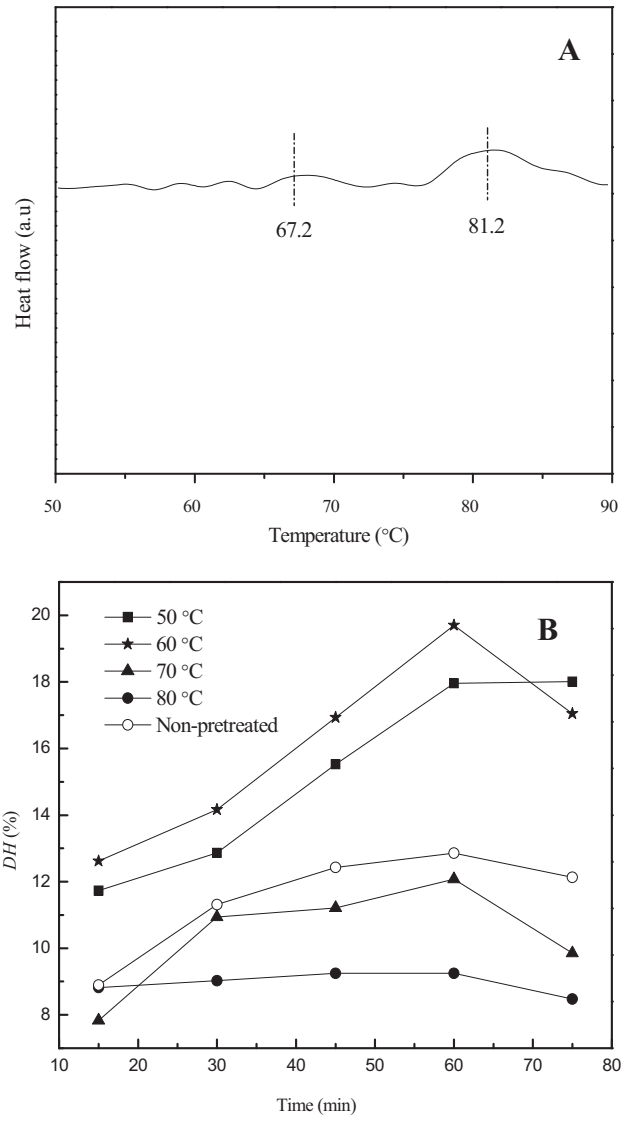

Fig. 1. (A) DSC thermograms of the non-pretreated LSP. (B) Influence of heat-pretreatment temperature on the enzymolysis of the LSP.

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