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Heat and/or ultrasound pretreatments motivated enzymolysis of corn gluten meal: Hydrolysis kinetics and protein structure

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ABSTRACT

The purpose of this study was to investigate the effect of pretreatments of heat (HP), ultrasound (UP), and combinations of heat/ultrasound (HPUP) and ultrasound/heat (UPHP) on protamex-catalyzed enzymolysis of corn gluten meal (CGM). Enzymolysis kinetics, molecular conformation and microstructure of CGM protein were monitored. Enzymolysis reaction rate increased with increasing substrate concentration and 80 g/L resulted in the highest hydrolysis reaction rate. The best improvement in CGM enzymolysis was obtained by HPUP (K_M value decreased by 11.56%) and then followed by UPHP (K_M value decreased by 9.97%). Fluorescence spectra and fourier transform infrared (FTIR) spectra indicated that the CGM pretreatments induced molecular unfolding, exposing the hydrophobic groups. HPUP and UPHP pretreatments both decreased the α-helix content by 12.2% and increased the random coil content by 2.23 and 2.60%, respectively. Scanning electron microscopy and atomic force microscopy showed that HPUP and UPHP changed surface topography and distribution of protein particles, enhancing the rate of enzymolysis. In conclusion, combined ultrasound and heat pretreatment and controlled enzymolysis could be an effective method for the functionality modification of globular proteins.

1. Introduction

Corn gluten meal (CGM) is a high protein ingredient produced as a byproduct during corn starch processing with protein content ranged between 60% and 62% (Zhou et al., 2015). In its native form, CGM has poor functional characteristics. It is not easy to be absorbed by the body. Enzymolysis is the most common method used to overcome the low utilization of CGM protein, resulting from its poor solubility in water (Zhou, Yu, Zhang, He, & Ma, 2012). Traditional enzymolysis has many disadvantages such as low utilization rate of the enzyme, low conversion rate of the substrate, and long enzymolysis time (Li, Han, & Chen, 2008).

Power ultrasound is a clean technology, which has been widely used in the laboratory or food processing factory to improve food physicochemical properties (Zhang et al., 2015). The periodic mechanical motions of a probe can transfer ultrasonic energy into a fluid medium and lead to formation of small rapidly growing bubbles. The ultrasound-assisted technology has been applied to solving the drawbacks of traditional enzymolysis (Jin et al., 2010). Ultrasound pretreatment has been reported to improve enzymatic hydrolysis (Zhou et al., 2013a). The protein structural changes associated with ultrasound application enhance enzyme hydrolysis, by weakening interactions and disrupting quaternary as well as tertiary structures (Arkell, Krawczyk, & Jönsson, 2013). Heat pretreatment is used extensively in food industry to improve quality and functional properties of processed food products (Arkell et al., 2013). For instance, heat pretreatment of food or its components can reduce microbial counts and enzyme activity, and preserve as well as enhance sensory properties such as taste, flavor, color and texture (Girgih et al., 2013). Heat pretreatment of proteins before enzymatic hydrolysis rearranges the intermolecular and intramolecular linkages, especially disulfide and hydrophobic bonds, leading to changes in protein conformation and digestibility (Ding, Zhang, & Li, 2015).

A combination of heat and sonication has been used to improve the heat stability of whey protein (Zisu, Bhaskaracharya, Kentish, & Ashokkumar, 2010). In this context, changes in protein structure

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due to sonication and heat treatments need more investigations (Jin et al., 2015a; Orsini Delgado, Tironi, & Añón, 2011). The conversion rate of protein and degree of hydrolysis are used to evaluate the feasibility of ultrasound and heat pretreatment enzymolysis, which can provide the theoretical basis and technological support for further research in production of functional peptides. The purpose of this paper is to study the effects of heat and/or ultrasound pretreatments on enzymolysis of CGM by evaluating hydrolysis kinetics, and protein conformation and morphology.

2. Materials and methods

2.1. Material and chemicals

CGM, with 58.6–60% protein, was purchased from Yishui Earth Corn Development Co., Ltd. (Shandong, China). Protamex (activity of 3.0×10^5 U/g) was purchased from Nanning Doing Higher Biotech Co., Ltd. (Guangxi, China). All other chemicals used were of analytical grade.

2.2. Heat (HP) and ultrasound pretreatments (UP) of CGM

Prior to enzymolysis reaction, different CGM weights (16–32 g) were dispersed in 400 mL deionized water, preparing dispersions of different concentrations ranged between 40 and 80 g/L (w/v). These dispersions were divided into four parts. Two parts were preheated at 121 °C in an oil bath for 40 min. One part of the heated CGM of different concentrations was kept (designated as HP) and the other part was subjected to sonication (designated as HPUP). The other two parts of non-heated CGM dispersions (40–80 g/L) were both prone to sonication. One part of the sonicated CGM was kept (designated as UP) and the other part was heated as before (designated as UPHP). Sonication was performed using an ultrasonic device equipped with a titanium probe (Shangjia Biotechnology Co., Wuxi, China; Model GA92-II DB) of 2.0 cm flat tip (Fig. 1). Sonication conditions were fixed frequency of 40 kHz, pulsed on-time 10 *s* and off time 3 *s*, duration of 40 min and temperature of 20 °C.

2.3. Enzymolysis of CGM

After pretreatment, the temperature of CGM suspension was maintained constant at 55 °C in a water bath, and then the pH was adjusted to 7.5. Protamex enzyme (0.6 g/L) was added to the suspension and hydrolysis was done, aided by constant agitation, for a total period of 7 h. The pH of the mixture was maintained constant during the hydrolysis period by frequent addition of 1.0 mol/L NaOH. At the end of the reaction, the enzyme was deactivated by heating the mixture for 10 min at 85 °C. The mixture was then centrifuged at 5000 × g for 15 min and the supernatant was lyophilized (protein hydrolysate), and kept for subsequent analysis. An enzymolysis experiment of untreated CGM, to serve as a control, was also performed. All experiments were repeated three times.

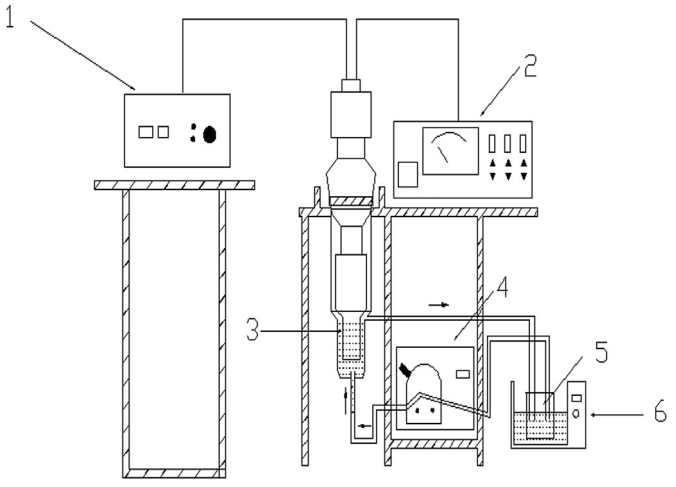

Fig. 1. The ultrasonic equipment. 1: Ultrasonic controller; 2: Ultrasonic power supply; 3: Shaped ultrasonic probe; 4: Peristaltic pump; 5: Protein solution; 6: Water bath.

2.4. Determination of degree of hydrolysis (DH)

The DH was calculated as a proportion (%) of free amino groups (AG) released after hydrolysis with respect to total AG in each sample (Huang, Ma, Peng, Wang, & Yang, 2014; Qu et al., 2012). The DH was calculated as follows:

$$\mathrm{DH}(\%) = (L_t - L_0)/(L_{max} - L_0) \times 100 \quad (1)$$

where *Lt* is the amount of α-AG released at time *t*, L_0 is the amount of α-AG in original homogenate and *Lmax* is the total α-AG in original homogenate obtained after acid hydrolysis (6 N HCl at 110 °C for 24 h).

2.5. Measurement of the hydrolyzed protein concentration

In order to describe the enzymolysis kinetics of CGM, the hydrolyzed protein concentration was calculated using the following equation (Jin et al., 2015b):

$$C = S \times \mathrm{DH} \times 0.01 \quad (2)$$

Where *C* is the hydrolyzed protein concentration (g/L), *DH* is the degree of hydrolysis (%) and *S* is the initial protein concentration (g/L).

2.6. Enzymolysis kinetics of the corn gluten meal

The model applied for studying the enzymolysis kinetics of corn gluten meal was stated by Zbigniew et al. (2013) and Jin et al. (2015b). The kinetic model is expressed as:

$$V = \frac{K_A \times E_T \times S}{K_M + S} \quad (3)$$

where, *V* is the initial reaction rate (g/L·min), K_A is the average value of apparent breakdown rate constant, representing binding frequency between substrate (corn gluten meal) and enzyme (protamex protease) (1/min), E_T is the protamex protease concentration (g/L), *S* is the initial substrate concentration (g/L) and K_M is the apparent constant (g/L) analogous to Michaelis–Menten constant.

Eq. (3) can be rearranged to obtain a linear form (Lineweaver–Burk equation):

$$\frac{1}{V} = \frac{K_M}{K_A \times E_T} \times \frac{1}{S} + \frac{1}{K_A \times E_T} \quad (4)$$

However, $1/V$ against $1/S$ was plotted (Fig. 3), resulting in a straight line with a slope K_M/K_AE_T and intercept $1/K_AE_T$. The values of K_M and K_A were obtained from the plot.

2.7. Measurement of the molecular conformation of CGM

The intrinsic fluorescence emission spectra of CGM protein (0.1 mg/mL, w/v) samples were measured at room temperature