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Enzymolysis kinetics and structural characteristics of rice protein with energy-gathered ultrasound and ultrasound assisted alkali pretreatments

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ABSTRACT

This research investigated the structural characteristics and enzymolysis kinetics of rice protein which was pretreated by energy-gathered ultrasound and ultrasound assisted alkali. The structural characteristics of rice protein before and after the pretreatment were performed with surface hydrophobicity and Fourier transform infrared (FTIR). There was an increase in the intensity of fluorescence spectrum and changes in functional groups after the pretreatment on rice protein compared with the control (without ultrasound and ultrasound assisted alkali processed), thus significantly enhancing efficiency of the enzymatic hydrolysis. A simplified kinetic equation for the enzymolysis model with the impeded reaction of enzyme was deduced to successfully describe the enzymatic hydrolysis of rice protein by different pretreatments. The initial observed rate constants ($K_{in,0}$) as well as ineffective coefficients (k_{imp}) were proposed and obtained based on the experimental observation. The results showed that the parameter of $k_{in,0}$ increased after ultrasound and ultrasound assisted alkali pretreatments, which proved the effects of the pretreatments on the substrate enhancing the enzymolysis process and had relation to the structure changes of the pretreatments on the substrate. Furthermore, the applicability of the simplified model was demonstrated by the enzymatic hydrolysis process for other materials.

1. Introduction

With the improvement of people living standard and social rhythm speeding up, many people are in the sub-health state. Functional food can improve this state, which relies on the biological activity of food. Rice protein is a kind of functional food, which is hydrolyzed by enzymolysis has been largely studied because of their activity polypeptide, preventing lifestyle-related diseases by their hypertension, antioxidant, hypoallergenic activity [1–3]. The controlled enzymolysis process can make the isolation of peptides. However, traditional enzymolysis has many disadvantages such as the low degree of hydrolysis (DH) and the long time of hydrolysis. As a result, many studies have been devoted to developing methods to improve the conversion rate of substrate and reduce the enzymolysis time [4–6]. The use of ultrasound assisted enzymolysis of proteins is a well known technology, because it is attributed to mechanical and thermal effects enacted by cavitation [7,8]. Many authors reported that ultrasound can improve the protein properties [9], enhance the enzyme activity [10], extract the polysaccharides [11]. Recently, the interest of food researcher has turned to change the design of the ultrasonic equipments in improving functional properties of food materials. The equipments of dual-frequency, single-frequency [12], ultrasonic horn and ultrasonic bath [13] and sweep frequency [14] were compared the efficacy of various acoustic and hydrodynamic cavitation. In our experiments, the apparatus of the single-frequency energy-gathered ultrasound equipment (SFEGU) was developed by our team and has many advantages. "Energy-gathered" is realized by avoiding the waste of energy, due to the direction of the ultrasound wave contrary to that of the solution [15]. In addition this kind of the machine belongs to the low frequency high-energy ultrasound in the kHz range relative to the MHz range [10]. Therefore the SFEGU can improve functional properties of food materials using minor energy.

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The rice protein is mainly composed by glutelin (~80%), which is alkali-soluble [9]. Therefore ultrasound assisted alkali pretreatment can enhance mass transfer, increase contact frequency between substrate and enzyme [16]. In our previous study, the pretreatment of rice protein ultrasound assisted alkaline was investigated, and the enzymatic hydrolysis was also significantly enhanced.

For an enzymolysis process, a kinetic model which may appropriately describe the enzymatic reaction is essential to the controlled hydrolysis and the design of the reactor. The classical Michaelis–Menten modeling has been used for enzyme kinetics of proteins with different enzymes. However, enzymatic hydrolysis of proteins is a highly complex process due to the different pretreatment to substrates and leading to the undefined nature of substrates, a heterogeneous reaction system and so on [17]. But no study has been reported on the kinetics of SFEGU assisted alkali enzymolysis for producing active peptides from different rice protein concentration and time.

The aims of this study were to (1) further explore the structural changes of rice protein before and after pretreatment with SFEGU-assisted and SFEGU-assisted alkali at a mild condition, including the comparison of surface hydrophobicity and Fourier transform infrared (FTIR), (2) deduce a simplified kinetic model which could indicate the efficiency of the different pretreatments, avoiding solving complicated ordinary differential equations and using many uniquely determined parameters, (3) the hydrolysis equation could predict the enzymatic hydrolysis process for other materials.

2. Materials and methods

2.1. Materials and ultrasonic equipment

The rice protein used in this study was purchased from Zhengzhou Tianshun food additives Co., Ltd. (Henan, China). The particle size of rice protein was 0.15 mm and crude protein content was 813 g/kg. Commercial Alcalase 2.4 L purchased from Novozymes Co., Ltd. (Tianjin, China) (activity of 23,400 U/mL) was used in the enzymatic hydrolysis. All reagents used in the experiment were of analytical grade. In our experiment the single-frequency energy-gathered ultrasound equipment (developed by our team, Fig. 1) was equipped with a 2 cm flat tip probe (Fanbo Biological Engineering Co., Ltd., Wuxi, China; Model FBTQ 2000), which was dipped to a depth of 2 cm in the rice protein suspension. The ultrasonic generator can deliver a maximum power of 300 W. This machine provides a continuous flow of raw materials by peristaltic pump and the direction of the solution moved counter currently to that of the ultrasound wave. The temperature of the reaction is kept constant by water bath.

2.2. Pretreatments of rice protein with SFEGU-assisted and SFEGU-assisted alkali

The solution containing 32 g rice protein and 800 mL deionized water was stirred at 50 °C for 15 min. After pretreatment, the solution was divided into two equal parts. One part (400 mL) solution was centrifuged at 5030 (×g) to remove the supernatant, and the precipitate was subjected vacuum freeze drying for 36 h to obtain the treated rice protein; the other part (400 mL) was used for enzymatic hydrolysis. This method was called method 1.

The solution was prepared as mentioned previously. The difference is the solution was pretreated by ultrasound at 50 °C for 15 min. Based on our previous result, the optimal ultrasonic parameters were 58 W/L (power density), pulsed on-time 3 s and off-time 2 s and the frequency was set at 28 kHz. After ultrasound application, the solution was subjected to the same treatment as stated in method 1 above. This was named method 2.

The rice protein solution was first adjusted to pH 8.0 using 1 M NaOH before ultrasound. Ultrasonic parameters were the same with the method 2. After ultrasound, the way of dealing with the solution was at the same with method 1. The ultrasound-alkali assisted pretreatment of rice protein was denoted as method 3.

2.3. Enzymatic hydrolysis and analysis

After pretreatment by three different methods, the solution was subjected to enzymatic hydrolysis. The equipment consisted of a pH meter (FE-20, Mettler Toledo Co., Shanghai, China), a digital thermostat water bath (DK-S26, JingHong experimental apparatus Co., Shanghai, China) and an impeller-agitator (JJ-1, Zhongda instrument Co., Jiangsu, China) at a speed of 100 rpm. Rice protein solution was stirred at 50 °C and 1 M NaOH was added to adjust the pH value to 8.5, then Alcalase enzyme (E/S = 1638 U/g) was

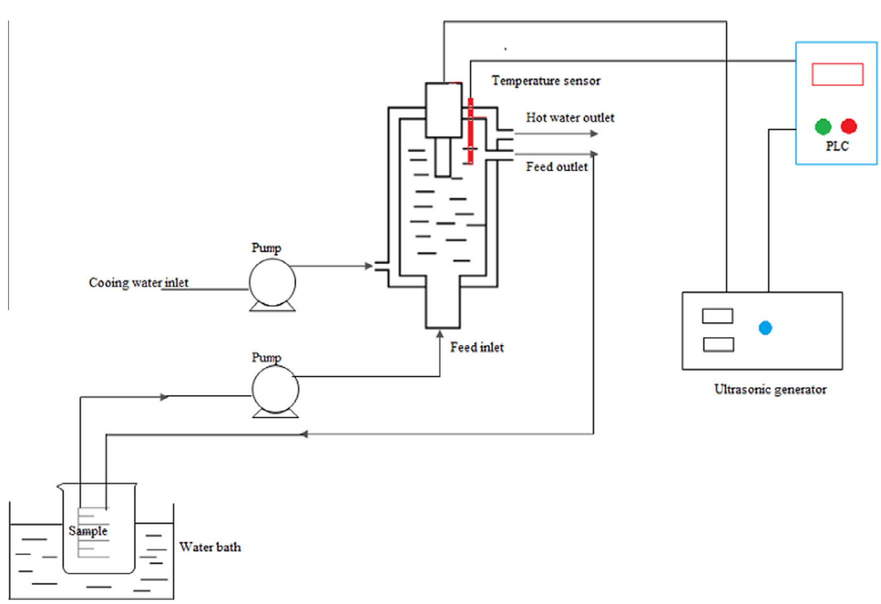

Fig. 1. Schematic diagrams of the single-frequency energy-gathered ultrasound equipment (SFEGU).