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Enzymolysis kinetics, thermodynamics and model of porcine cerebral protein with single-frequency countercurrent and pulsed ultrasound-assisted processing

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ABSTRACT

The present work investigated the enzymolysis kinetics, thermodynamics and model of porcine cerebral protein (PCP) which was pretreated by single-frequency countercurrent and pulsed ultrasound. The kinetic constants for ultrasonic pretreated and traditional enzymolysis have been determined. Results showed that the value of K_M in ultrasonic PCP (UPCP) enzymolysis decreased by 9% over that in the traditional enzymolysis. The values of reaction rate constant (k) for UPCP enzymolysis increased by 207%, 121%, 62%, and 45% at 293, 303, 313 and 323 K, respectively. For the thermodynamic parameters, ultrasound decreased activation energy (E_a), change in enthalpy (ΔH) and entropy (ΔS) by 76%, 82% and 31% in PCP, respectively. However, ultrasound had little change in Gibbs free energy (ΔG) value in the temperature range of 293–323 K. Therefore, a general kinetic equation for the enzymolysis model of UPCP by a simple empirical equation was suggested. The experimental values fits with the enzymolysis kinetic model with a low average relative error (4%) confirmed that the kinetic model was accurate to reflect the enzymolysis process. The positive effect of single-frequency countercurrent and pulsed ultrasound in this study and application of the kinetic model may be useful for the release of bioactive peptides from meat processing by-products.

1. Introduction

Bioactive peptides derived from various proteins by enzymatic hydrolysis are recognized as functional food ingredients in preventing lifestyle-related diseases by their antioxidant, antimicrobial, immunomodulatory activities [1–3]. The isolation of peptides is mainly based on the controlled enzymolysis process [4]. However, traditional enzymolysis has many disadvantages that arise mainly from the low contact frequency and the decreased enzyme activity. Therefore, research have been focused on developing methods to improve the utilization rate of enzyme and the conversion rate of substrate, as well as reduce the enzymolysis time [5–8]. Pig brain, nervous system and spinal cord, has the highest level of cholesterol (13.52–21.95 g kg^{-1}) and also the highest amount of phospholipids compared to other meat by-products. For this reason, the United States Department of Health recommends that limited amounts of these by-products be eaten, because of their associated health concerns [9]. However, there is a growing interest in the utilization and disposal of hydrolysates of porcine cerebral protein (PCP) which are used for the treatment of neural system diseases and prevents the age-dependent dementia [10], characterized by progressive loss of cognitive capability [11], and by pathological changes in the brain [12]. Indeed, one such product; Cerebrolysin (Ebewe Pharmaceutical, Austria), has been in clinical application for more than 40 years. It is known to be a mixture of 75% free amino acids and 25% short-chain peptides, probably only a part of the peptides are physiologically active [13].

The use of ultrasound energy in enhancing the enzyme hydrolysis of proteins is a well known technique [14,15] because it is attributed to mechanical and thermal effects enacted by cavitation, which can result in enhanced mass transfer, increased contact frequency between substrate and enzyme and so on [16–18].

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Moreover, the collapse of cavitation bubbles generates high temperatures (approximately 5500 K) and high pressures (approximately 50 MPa) in very short time (3×10^{-4} s), which results in high intensity of shear forces and shock waves and turbulence [19]. Qu et al. have indicated ultrasound-assisted enzymolysis significantly increased the initial reaction rate by 60% and raised the conversion rate of protein by 35% at substrate concentration of 24 g/L [20]. Single-frequency countercurrent and pulsed ultrasound has many advantages which releases of uniform energy distribution, avoids the energy waste, resonates of treated material easily, generated smaller thermal effect in a pulsed mode with an on-time and an off-time cycle [21]. However, no research has been reported on the kinetics of single-frequency countercurrent and pulsed ultrasound-assisted enzymolysis for producing active peptides from different PCP concentration and times.

The objectives of this research were to (1) study the effects of the single-frequency countercurrent and pulsed ultrasound pretreatment on the kinetic constants of PCP enzymolysis, (2) determine the reaction kinetic parameter k and thermodynamic parameters of ultrasonic PCP (UPCP) enzymolysis at different temperatures and times, and (3) determine the hydrolysis curves of UPCP catalyzed by alcalase in solution, which is modeled by a simple empirical equation from which kinetic parameters can be deduced. This model can predict the release of bioactive peptides from other meat processing by-products.

2. Materials and methods

2.1. The ultrasonic pretreatment of PCP

Fresh porcine brains were purchased from a local market in Zhenjiang, China. After removing hematoma and leptomeninges (pia mater and arachnoid) and rinsing with saline, porcine brains were minced, and added portion-wise to 5 volumes (v/w) of boiling water. They were boiled for 10 min after addition of the final portion. PCP was extracted thoroughly by ethanol treatment with a mince:ethanol ratio of 1:2 at 70 °C for 30 min to obtain a degreased powder. The powder was then mixed with water to form a slurry [22]. The deposited solid was separated from the liquid by centrifuging at 4000×g at room temperature for 15 min. It was then dried and stored in a desiccator for further analysis.

Prior to enzymolysis, PCP were pretreated by ultrasound (UPCP) with a 2.0 cm flat tip probe operating in a pulsed on time and off-time of both 2 s. A probe ultrasonic reactor (SC-II, Chengdu Jiuzhou Ultrasonic Technology Co., LTD.) working with a single frequency of 20 kHz and a maximum power of 80 W was used in the ultrasonication experiments for 5 min [23]. 2% (w/v) PCP solution was prepared by dispersing a predetermined weight of sample into 250 mL of deionized water in a beaker and it was placed in a thermostatic water bath at different initial temperatures. The pretreated sample solution passed through the probe by countercurrent method which two peristaltic pumps used to keep the material solutions in a counter-current flow state. The pH of the solution was then corrected to 8.5 using 0.5 M NaOH [20].

2.2. Enzyme hydrolysis

UPCP solution obtained from Section 2.1 reacted with alcalase (E/S ratio, 2000 U/g). The traditional enzymolysis was prepared from PCP and alcalase using the same procedure with UPCP enzymolysis. The pH of the reaction solution was adjusted to 8.5 and then the solution was incubated in a water bath at 20, 30, 40 and 50 °C, respectively. During the whole period of hydrolysis, the pH was maintained at 8.5 by frequent addition of 0.5 M NaOH. At the end of the incubation period, the enzymatic hydrolysis was terminated by boiling the mixture for 10 min. Then the mixture of the protein and enzyme was centrifuged at 4000×g for 15 min at 4 °C. The supernatant of hydrolysate was collected and stored at 4 °C.

2.3. Determination of polypeptide concentration

The polypeptide concentration (10^{-3} g/L) was determined using the reported Folin-phenol colorimetric method as explained by Wenjuan Qu [24]. 4 mL Folin-phenol (reagent A) was mixed with 0.5 mL sample and incubated for 10 min at room temperature, after which 0.5 mL Folin-phenol (reagent B) was added, and the absorbance was read at 500 nm on a spectrophotometer (Unic 7200, Unocal Corporation, Shanghai, China) after 30 min incubation at room temperature.

2.4. Test of effect of ultrasound on enzymolysis kinetic constants

2.4.1. Enzymolysis reaction condition

The traditional and UPCP enzymatic hydrolysis were performed at substrate concentrations of 7.48, 9.98, 14.97, 19.96 and 29.94 g/L, respectively. Each treatment was replicated three times. The other hydrolysis conditions were: pH, 8.5; temperature, 50 °C; E/S ratio, 2000 U/g; hydrolysis time, 5 min.

2.4.2. Michaelis–Menten constant and maximum initial velocity (K_M and V_{max})

The classic Michaelis–Menten equation was applied in effect of ultrasonic pretreatment on enzymolysis kinetic constants of porcine cerebral protein. In order to estimate the two constants K_M and V_{max}, the experimental data can be plotted according to the double-reciprocal transformation of Eq. (1):

$$\frac{1}{V} = \frac{K_M}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}} \tag{1}$$

where V is the initial reaction rate (g/mL s), $[S]$ is the initial protein concentration (g/mL), K_M is Michaelis constant, and V_{max} is the maximum initial velocity (g/mL s). The K_M and V_{max} values were determined by Eq. (1) from the slope and intercept by plotting $1/V$ against $1/S$. In order to calculate the initial reaction rate of enzymolysis, the polypeptide concentration (g/mL) was determined at enzymolysis time of 5 min. The initial reaction rate was express as:

$$V(\text{g/mL s}) = \frac{Q_t}{5 \times 60(s)} \tag{2}$$

Q_t is the polypeptide concentration in the reaction solution at a given time t min.

2.5. Test of effect of ultrasound on the kinetics and thermodynamics

2.5.1. Enzymolysis reaction kinetics

The kinetic models of PCP were fitted to the first-order kinetics [23,25]. The kinetic model was written as:

$$\frac{dC_t}{dt} = -kC_t \tag{3}$$

where k is the total reaction rate constant, and C_t is the PCP or UPCP concentration in reaction solution at a given time t min (μg/mL).

After integrating Eq. (3), the kinetic model was expressed as:

$$\ln\left(\frac{C_t}{C_0}\right) = -kt \tag{4}$$

where C_0 is the initial concentration of PCP, t is time. As it is difficult to measure the decrement of PCP, the reaction rate can be determined by the increased amount of polypeptide released by PCP.