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ACE-inhibitory peptides production from defatted wheat germ protein by continuous coupling of enzymatic hydrolysis and membrane separation: Modeling and experimental studies

Wenjuan Qu [a], Haile Ma [a,b,*], Weirui Zhao [a], Zhongli Pan [a]

[a] *School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China*
[b] *Key Laboratory for Physical Processing of Agricultural Products, Zhenjiang, Jiangsu 212013, China*

HIGHLIGHTS

- An efficient technique of continuous CEH-MS and its kinetic model were developed.
- The CEH-MS method was beneficial to the production of ACE-inhibitory peptides.
- This method was more efficient than the traditional EH and offline MS one.
- This method gave a high protein conversion rate, and yield and activity of peptides.

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ABSTRACT

To increase the yield and activity of angiotensin converting enzyme (ACE) inhibitory peptides from defatted wheat germ protein (DWGP), an efficient method of continuous coupling of enzymatic hydrolysis and membrane separation (CEH-MS) and its kinetics were systematically studied. The results showed the steady state theory fitted the continuous CEH-MS kinetics study well and the kinetic model was successfully established with K_m of 8.163 g/L and V_{max} of 0.790 g/L min. Both the degree of hydrolysis and the conversion rate of protein were significantly affected by substrate concentration. By the kinetic model prediction, the optimum substrate concentration was 10.65 g/L. Considering the safe pressures of flushing and backflushing ultrafiltration, the maximum runtime of the continuous CEH-MS reactor was 300 min. Under the optimum conditions of substrate concentration of 10.65 g/L, Alcalase quantity of 0.51 g, temperature of 50 °C, pH of 9.0, permeation flux of 0.011 L/min, effective volume of 0.4 L, and runtime of 300 min, the continuous CEH-MS method showed high conversion rate of protein (65.21%), yield of peptides (34.10 g/g), and IC_{50} of peptides (0.452 g/L). The conversion rate of protein and yield of peptides were significantly increased by 63.4% and 7.1 times, and the IC_{50} of peptides was significantly reduced by 13.6% compared with the traditional EH and offline MS one. Therefore, the continuous CEH-MS reactor is beneficial to the efficient production of ACE-inhibitory peptides from DWGP.

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1. Introduction

Wheat germ is a potential plant-based protein because of its high protein content of 30% [1]. Researchers found that enzymatically produced peptides from wheat germ protein have the antihypertensive activities [2,3]. This was due to their function in angiotensin converting enzyme (ACE) inhibition. It is known that ACE, an important endogenous enzyme in the human body, can cause a blood pressure increase commonly called hypertension [4,5]. Therefore, ACE-inhibitory peptides have the antihypertensive function and the ACE-inhibitory activity can be used as a critical indicator to evaluate their antihypertensive capability *in vitro*. Our previous research has demonstrated that defatted wheat germ protein (DWGP) has a good potential to enzymatically produce ACE-inhibitory peptides [6]. At present, the usual preparation methods of ACE-inhibitory peptides include autolysis, fermentation and enzymatic hydrolysis (EH) [7–9], in which the EH method is widely used because of its mild production conditions and high specificity [10,11]. However, the EH method exerts some limitations in practical applications such as low conversion rate of substrate, low utilization rate of enzyme, and low yield of peptides [12]. This is partially because the hydrolyzate cannot be timely

* Corresponding author at: School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang, Jiangsu 212013, China. Tel./fax: +86 511 88790958.

E-mail address: mhl@ujs.edu.cn (H. Ma).

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Nomenclature

v	reaction rate (g/L min)
K_m	Michaelis constant (g/L)
V_{max}	maximum reaction rate (g/L min)
V	effective volume (L)
J	permeation flux (L/min)
B	amount of base consumed to keep the pH constant during the reaction (mL)
Nb	normality (mM) of the base
MP	mass of protein (g)
h_{tot}	mole number of peptide bond per gram of protein (mmol/g)
α	average degree of dissociation of the α-NH_2 groups released during the hydrolysis
pH, pK	values at which the proteolysis was conducted
t	enzymolysis time (min)
P_0, P	initial concentration of protein and concentration of unhydrolyzed protein at a given time t in the substrate (g/L)
IC_{50}	concentration of peptides when its ACE-inhibitory activity reached 50% (g/L)
DH	degree of hydrolysis (%)

separated from the EH reaction system and cause an inhibitory effect of hydrolyzate on the EH process. Thus, there is a great demand for developing more efficient methods to overcome these shortcomings.

An efficient method was designed by continuous coupling of enzymatic hydrolysis and membrane separation (CEH-MS) and applied in producing ACE-inhibitory peptides from DWGP in this research. This CEH-MS method can continuously accelerate the conversion of substrate toward hydrolyzate in EH by using MS technology to break the reaction equilibrium. Therefore this method can greatly improve the conversion rate of substrate, utilization rate of enzyme and yield of peptides compared with the traditional single EH. In addition, the CEH-MS system can achieve continuous operation by the integration of substrate enzymolysis, enzyme recovery and hydrolyzate separation compared with the traditional EH and offline MS method [12]. However, little information is available about the practical application and kinetics theory of the continuous CEH-MS reactor for the production of ACE-inhibitory peptides from DWGP, which needs to be studied.

The objectives of this research were to establish an efficient continuous CEH-MS method and compare it with the traditional EH and offline MS method. The conversion rate of protein, yield of peptides and antihypertensive activity of peptides were determined to evaluate the feasibility of continuous CEH-MS reactor. In addition, the kinetic model of CEH-MS was required to design an industrial scale continuous CEH-MS system in order to predict the production of ACE-inhibitory peptides from DWGP.

2. Materials and methods

2.1. Materials

Defatted wheat germ was obtained from An-yang Mantianxue Food Manufacturing Co., Ltd. (Henan, China). Alcalase 2.4LFG with the activity of 2.670 U/g, recommended temperature of 50 °C and pH value of 9.0 was purchased from Novozymes Co., Ltd. (Shanghai, China). Angiotensin converting enzyme (ACE) was extracted from the pig lung according to the reported method [13], which had the activity of 0.1 U/mL. N-[3-(2-Furyl)acryloyl]-Phe-Gly-Gly (FAPGG) and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were ordered from Sigma–Aldrich Company (Shanghai, China). Diastase, sodium hydroxide (NaOH), sodium chloride (NaCl) and hydrochloric acid (HCl) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All the reagents were of analytical grade.

2.2. Methods

2.2.1. Defatted wheat germ protein preparation

The defatted wheat germ protein (DWGP) was produced by the alkaline extraction and subsequent isoelectric precipitation. This procedure is described in detail in our previous research [6]. The produced DWGP was vacuum dried at 50 °C and then the DWGP powder was stored in a desiccator until use. The protein content of DWGP powder measured by a Kjeldahl method [14] was 76.67% (w.b.).

2.2.2. Traditional EH and offline MS method

The traditional EH and offline MS method was performed according to the following steps. Prior to EH, a mass of 4.0 g DWGP was well mixed with 400 mL water. Subsequently, the DWGP solution was adjusted to pH value 9.0 using 0.1 M NaOH and then reacted with Alcalase added at 6.367 g per 100 g DWGP in a water bath (SHZ-88A, Taicang Laboratorial Equipment Factory, Jiangsu, China) at 50 °C for 120 min. The reaction solution in the whole process of EH was kept constant at the pH value of 9.0. After the enzymolysis, the Alcalase in the hydrolyzate was inactivated in a water bath at 100 °C for 10 min and then the hydrolyzate was cooled to ambient temperature (25 °C). The liquid hydrolyzate was separated from the solid by centrifugation (Avanti J-25, Beckman Coulter Inc., Brea, CA, USA) at 10,000 rpm at ambient temperature for 10 min and used for subsequent offline MS. The ACE-inhibitory peptides with molecular weight (MW) less than 5 kDa in the liquid hydrolyzate was separated from the other ingredients by an offline MS system, including an ultrafiltration device (Pellicon, Millipore Corporate, Billerica, MA, USA) equipped with a 5 kDa membrane (working area of 0.5 m^2). The ACE-inhibitory peptides after traditional EH and offline MS were collected for subsequent analysis.

2.2.3. Continuous CEH-MS method

The schematic diagram of continuous CEH-MS set-up is displayed in Fig. 1. Masses of DWGP (2.0, 4.0, 6.0 and 8.0 g) were separately mixed with 400 mL water in the stock tank to obtain initial solutions with different substrate concentrations of 5, 10, 15 and 20 g/L with the aid of magnetic stirring at 100 rpm. The temperature and pH of each DWGP solution were kept constant at 50 °C and 9.0 in the whole process. Then, 0.51 g of Alcalase was added to react with the DWGP solution in the stock tank. At the same time, a circulation pump was opened and the timing started. The DWGP solution prepared with the same concentration as the initial solution was continuously pumped into the stock tank at the rate of 0.011 L/min. The ACE-inhibitory peptides separated by an online

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