Ultrasonics - Sonochemistry 42 (2018) 704–713

Contents lists available at ScienceDirect

Ultrasonics - Sonochemistry

journal homepage: www.elsevier.com/locate/ultson

# Impact of ultrasound pretreatment on hydrolysate and digestion products of grape seed protein

Qingzhi Ding[a,b,c,d], Ting Zhang[a,c], Shuai Niu[a], Feifan Cao[a], Ricardo Antonio Wu-Chen[a,c], Lin Luo[a,c,d], Haile Ma[a,b,*]

[a] School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[b] Institute of Agricultural Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[c] Key Laboratory for Physical Processing of Agricultural Products, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China
[d] Jiangsu Provincial Research Center of Bio-Process and Separation Engineering of Agri-Products, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China

A R T I C L E I N F O

*Keywords:*
Grape seed protein
$IC_{50}$
Energy-gather counter flow ultrasound
Frequency

A B S T R A C T

The effects of ultrasound pretreatment with different working modes, including mono frequency ultrasound (MFU), simultaneous dual frequency ultrasound (SDFU) and alternate dual frequency ultrasound (ADFU) using energy-gather counter flow ultrasound equipment, on the degree of hydrolysis (DH) of grape seed protein (GSP) hydrolysate and $IC_{50}$ of GSP digestion products were studied. Amino acid composition analysis (AACA), ultraviolet–visible (UV) spectroscopy and atomic force microscopy (AFM) of GSP with different ultrasound pretreatments were measured. The results showed that MFU, SDFU and ADFU pretreatments improved the DH and reduced the $IC_{50}$ of GSP significantly ($P < .05$). The MFU of 20 kHz and SDFU of 20/40 kHz showed higher ACE inhibitory activity within the MFU and SDFU groups, respectively. ADFU of 20/35 kHz produced the highest ACE inhibitory activity among the three working modes (MFU, SDFU and ADFU). AACA showed that all the working modes of the ultrasound pretreatment could increase the amount of hydrophobic amino acids and the total amino acids. The changes in UV spectra and amino acid analysis indicated the unfolding of protein structure and exposure of more hydrophobic groups by SDFU and ADFU pretreatments. AFM analysis of the GSP indicated that the microstructures were destroyed and the particle size reduced after dual-frequency ultrasound pretreatments. Therefore, energy-gather counter flow ultrasound pretreatment is an effective method to improve the DH and reducing the $IC_{50}$ due to the changes of molecular conformation and effects on the microstructure by sonochemistry of GSP. In conclusion, it is necessary to select the frequency and working modes of ultrasound pretreatment for the preparation of ACE inhibitory peptide of GSP.

## 1. Introduction

Grape seed, the by-product in wine production with protein content about 10% (w/w), is treated as a waste in many factories. Grape seed protein contains all essential amino acids, especially valine, arginine, methionine and phenylalanine which are the limiting amino acids in our staple foods [1,2]. In recent years, many researchers have been trying to make full use of grape seeds which were selected to be the raw material for producing tannins, galloylated compounds and non-galloylated procyanidins. Those compounds are important parts of human diet because most of them are concentrated in fruits and vegetables [3,4]. Bioactive compounds of grape seed hydrolysate [5–8] and extract [9–13] have become a hot topic and been focused on the fields of food, biochemistry, nutrition, etc. However, most of the grape seed hydrolysates were not dissolved in water and resulted in a huge loss of bioactive compounds. Enzymatic hydrolysis and ultrasound pretreatment were chosen to improve the solubility of the functional compounds, enzymatic hydrolysis speed, protein conversion rate, enzyme utilization and peptide bioactivity [14].

Enzymolysis is an efficient method to modify the properties of food proteins in many studies [15]. GSP hydrolysate, which contains many hydrophobic amino acids (HAA), has many bioactive functions on preventing diseases such as antihypertensive effect, because of the powerful inhibition of ACE activity. Ultrasound pretreatment [16–19], which is widely used to improve the efficiency of extraction and protein enzymolysis, is able to make the proteins more sensitive to enzymolysis and change the molecular structure of proteins [20]. Ultrasound pretreatment of substrate suspension before enzymatic hydrolysis is widely applied to improve the ACE inhibitory activity [2,21]. Yang et al. [2] reported that the main reasons of improving the ACE inhibitory activity

* Corresponding author at: School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China.
*E-mail address:* mhl@ujs.edu.cn (H. Ma).

https://doi.org/10.1016/j.ultsonch.2017.11.027
Received 28 September 2017; Received in revised form 19 November 2017; Accepted 20 November 2017
Available online 21 November 2017
1350-4177/ © 2017 Elsevier B.V. All rights reserved.

were the changes in UV spectra, free sulfhydryl and disulphide bond contents, surface hydrophobicity and hydrophobic protein content after ultrasound pretreatment of defatted wheat germ protein. However, few reports focused on the frequencies of ultrasound and $IC_{50}$ of 50% ACE inhibition systematically. Energy-gather counter flow ultrasound (ECFU) is an advanced ultrasound technology in the physical processing of food production. It is characterized by high-energy (high-power and high-intensity) using intensities higher than 1 $W/cm^2$ with frequencies from 20 to 500 kHz. ECFU provides a continuous flow of substrate suspension to achieve continuously industrialized production when compared with the traditional high energy ultrasound. Besides, the suspension was stirred with a high-speed stirrer in the ultrasonic vessel, which induced by the reversed flow. Ultrasonic frequency of ECFU, including mono frequency (one probe) and dual frequency (simultaneous pulsed on-time/off-time mode and alternate non-interval mode), is one of the most important parameters for different raw materials. However, there are few systematic researches on the enzymolysis efficiency impacts, ultrasonic frequency and simulated digestion of ECFU pretreatment.

Therefore, the aims of this research were to (1) optimize the enzymolysis conditions on the $IC_{50}$ of pancreatic digestion products; (2) study the sensitive ultrasound frequencies (such as MFU, SDFU and ADFU) using ECFU pretreatment on the $IC_{50}$ of pancreatic digestion products; (3) reveal the mechanism of ultrasound pretreatment on the GSP through analyzing the characters of protein structure. These studies would provide more information about the effect of ECFU pretreatment on the enzymolysis efficiency and structure characterization of GSP. It is expected that the results of this research will be of great value to provide ideas for the development of ultrasonic equipment in the proteolysis industry.

## 2. Materials and methods

### 2.1. Materials and chemicals

Grape seed (GS, protein content, 9.21 g/100 g) was purchased from COFCO China Great Wall Wine Co., Ltd (Hebei, China). Alcalase 2.4 L with an activity of $1.979 \times 10^5$ U/g (by Folin Phenol method), was purchased from Novozymes Co. Ltd. (Tianjin, China). ACE was extracted from the pig lung according to the method of Maruyama [22]; Hippuryl-His-Leu (HHL) was purchased from Sigma-Aldrich Corp (USA). All other reagents used in the experiment were of analytical grade and solutions were prepared with distilled water.

### 2.2. Enzymolysis of GSP

The volume of enzyme added, substrate concentration and enzymolysis time were optimized one by one. The experiment was conducted under the following conditions: the amount of enzyme added was 0.5, 1, 1.5, 2, 2.5 and 3 mL with 50 g GS powder; substrate concentration of 40, 60, 80, 100, 120 and 140 g/L; enzymolysis time between 0 and 90 min. For the first 20 min, the usage of NaOH was recorded every 5 min, then every 10 min for the following 70 min. The GSP suspensions were preheated for 20 min up to 50 °C. The suspensions were mixed using an electric stirrer (KMO 2, IKA, Germany) at a speed of 300 rpm. During the hydrolysis, pH of suspensions was maintained at 8.0 by addition of 1.0 M NaOH. At the end, the enzymatic hydrolysis was stopped by boiling hydrolysate for 10 min. The hydrolysate was stored at 4 °C for the following analysis.

### 2.3. In vitro digestion

Gastric and intestinal fluids consisted of simulated digestion conditions, which were conducted according to the description of Martinez et al. [23] with some modifications. Pepsin (5g) was mixed with 8.2 mL of 1 M HCl and distilled water until a volume of 500 ml which was named artificial stomach digestive juice (ASDJ). The hydrolysis products (HP) were stirred evenly, mixed with the ASDJ (volume ratio was 1:1) and then putted in the constant temperature shaker at 37 °C and 180 rpm for 3 h. Afterwards, it was putted in 100 °C boiling water for 10 min. After cooled to room temperature, the stomach digestive product (SDP) was used for subsequent pancreas digestion analysis. For the preparation of simulated intestinal fluid (SIF), 5g pancreatin with 50 ml distilled water was mixed with 3.4g $KH_2PO4$ which was dissolved in 250 ml distilled water and adjusted to the pH of 6.8 by adding 0.1 M NaOH to a final volume of 500 ml. The final liquid was named artificial pancreatic digestive juice (APDJ). The SDP was mixed with ASDJ while stirring evenly, and the same volume of APDJ was added (3ml respectively). Then the mixture was putted in the constant temperature shaker at 37 °C and 180 rpm for 3 h. Finally, it was putted in 100 °C boiling water for 10 min. After cooled to room temperature, the pancreatic juice digestion product (PJDP), SDP and hydrolysate were all centrifuged at 5000 rpm for 10 min at 4 °C, and the supernatant was used for subsequent analysis.

### 2.4. Pretreatment of GSP using ultrasound

Mono-frequency ultrasound (Fig. 1A), simultaneous dual-frequency ultrasound and alternate dual-frequency ultrasound (Fig. 1B) were used in this research, which were developed by our research team and manufactured by Meibo Biotechnology Co., Ltd (Zhenjiang, China). The details of the equipment have been described in our previous researches [24–26]. All of the ultrasonic power density should be kept at 150 W/L when we used different working modes. Simultaneous working mode works with different ultrasound frequencies simultaneously pulsed on-time and off-time. Alternate working mode works in the term of different ultrasound frequencies generating a successive mode without interval. To obtain the optimization of the ultrasonic frequency and working mode, different frequencies were discussed. The experiment was executed under the following conditions: ultrasonic power density of 150 W/L; pretreatment time of 20 min; temperature of 30 °C; substrate concentration of 60 g/L; mono ultrasonic frequency of 20, 28, 35, 40, 50 kHz and dual frequency of 20/28, 20/35, 20/40, 20/50 kHz in simultaneous and sequential working modes with the pulsed on-time and off-time of 5 s and 5 s respectively during ultrasound processing. The $IC_{50}$ and the DH were set as index for the optimization of ultrasonic frequencies. All experiments were carried out in triplicate.

### 2.5. Determination of the DH

The DH was calculated according to the pH-stat method described by Mat et al. [27]:

$$DH = \frac{h}{h_{tot}} \times 100\% = \frac{V \times N_b}{M_p \times h_{tot} \times \alpha} \times 100\% \quad (1)$$

$$\alpha = [10^{(pH-pK)}]/[1 + 10^{(pH-pK)}] \quad (2)$$

where, $N_b$ is the concentration of NaOH (mol/L); V is the volume of NaOH used (mL); Mp is the hydrolyzed protein (g); $h_{tot}$ is the total moles of peptide bond per gram of protein substrate, which is 6.35 mmol/g for grape seed protein; α is the average dissociation degree of the α-amino groups in substrate influenced by particular pH and temperature, which is 0.909 at pH 8.0 and 50 °C by Eq. (2).

### 2.6. Evaluation of the ACE inhibitory activity of hydrolysate and digestion product

The ACE inhibitory activity was measured according to the method of Zhang [28] and Abdelhedi et al. [29] with some modifications which is closely related to the antihypertensive activity. The hydrolysate and digestion products were diluted 8-fold by borate buffers (BB, 0.1 mol/L borate buffers containing 0.3 mol/L NaCl, pH 8.3). A sample diluent

Download English Version:

https://daneshyari.com/en/article/7703391

Download Persian Version:

https://daneshyari.com/article/7703391

Daneshyari.com