ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2008– December 31, 2008

PROJECT TITLE:

RICE UTILIZATION AND PRODUCT DEVELOPMENT

-Development of Functional Polypeptides from Rice Protein

STATUS OF PROPOSAL:

_____/New _____x___/Continuing

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OBJECTIVES AND EXPERIMENTS CONDUCTED BY LOCATION TO ACCOMPLISH OBJECTIVES

The ultimate objective of this research is to develop new ultrasonic-assisted processing methods for producing functional (antihypertensive) polypeptides from rice protein. Broken rice and rice bran are produced in large quantities as by-products from rice processing. Currently they have low values and have not been well utilized. Both of them contain high protein contents and can be used as raw materials for producing high-value functional polypeptides which have great market potential as functional food ingredients and nutritional supplements.

The food protein-based antihypertensive peptides have no or fewer side effects and are safer compared to conventional antihypertensive drugs which are angiotension converting enzyme (ACE) inhibitors. The antihypertensive capability of peptides is normally determined based on their inhibition rate or inhibition ability to ACE. A higher ACE inhibition rate indicates stronger antihypertensive capability.

Ultrasonic technology has many advantages in promoting mass transfer and enzyme modification over conventional extraction and enzymatic hydrolysis methods. It can be used for both protein extraction and enzyme hydrolysis in the production of functional polypeptides for increased functionality and reduced production time.

The objectives of this past year research were to investigate the effect of ultrasonic treatment on the yield of extracted protein and study antihypertensive capability of polypeptides produced with ultrasonic assisted enzymatic hydrolysis.

Experimental Procedures

Effect of ultrasonic treatment on extracted protein yield

Preparation of rice flour

Broken rice was obtained from Pacific International Rice Mill Inc. (Woodland, CA) and ground with a Universal Cutting Mill (NY, USA) equipped with a 1 mm opening sieve. The ground rice flour was sieved through 40 mesh sieve (RO-TAP Testing sieve shaker, W.S. Tyler Co. Cleveland, OH). The chemical composition of rice flour was analyzed and is shown in Table 1.

Table 1. Chemical composition of rice flour (%)

Moisture content	Ash	Total Nitrogen	Total carbon	Total hydrogen	Protein
9.78 (0.01)	0.79 (0.01)	1.33 (0.09)	44.60 (0.08)	6.34 (0.04)	8.91 (0.26)

*Moisture content is on wet basis and others are on dry basis. Protein content was calculated with total nitrogen multiplied by 6.7.

Rice protein extraction experiment

Based on the literature, the basic protein extraction conditions used in this study were the ratio of water to rice flour as 8:1 (48 ml distilled water and 6 g rice flour (<40 mesh) and pH 11.0 which was adjusted by using Na_2HPO_4 and NaOH. The extraction temperature was 50±2 ℃. After the protein slurry was extracted for a specific time period, the protein slurry was centrifuged at 5000 r min^{-1} for 30min and then passed through Whatman filter paper with diameter of 55 mm. The supernatant was diluted to appropriate concentration. Then 1ml solution was pipetted into a clean tube and 1mL dye reagent (Quick Start™ Bradford 1×dye reagent) was added. The sample and reagent were mixed thoroughly using a Vortex mixer and incubated at room temperature (25±2 ℃) for 10 min and then absorbance of the liquid was determined at 595nm. Based on the absorbance results and standard protein concentration curve, the amount of protein in the supernatant was calculated. The extracted protein yield was calculated based on the amount of protein in the flour and expressed as percentage.

To determine the effect of ultrasonic treatment on extracted protein yield, protein was extracted with three different methods, basic method (no stirring), stirring method (600 rpm) and ultrasonic assisted extraction (amplitude 10). The extraction time was from 20 min to 120 min. To determine the effect of temperature and pH, the protein slurry was also extracted with ultrasonic treatment under amplitude of 10 at 20 and 40 ℃ under pH from 7 to 11.

Antihypertensive capability of polypeptides produced with ultrasonic treatment

The rice protein sample with purity of (70%(w/w)) was used for ultrasonic treatment and enzymatic hydrolysis. The protein solution was prepared by adding water to the protein with water to protein ratio of 25:1. The samples of protein solutions were treated by a GA-99-Ⅱ DB Ultrasonicator (Shangjia Biotech, China) at different powers for various durations (Table 2). After the ultrasonic treatments, the solubility of rice protein was determined by measuring the soluble protein in each sample. A UV spectrometer (Varian Cary100 Conc UV-Visiable spectrophotometer, Varian Company, USA) and a fluorescence spectrometer (Varian Cary Eclipse Fluorescence spectrophotometer, VARIAN Company,USA) were used to measure the UV spectra and the fluorescence spectrometry of the protein solutions to determine the conformation change caused by the ultrasonic treatment.

Table 2. Ultrasonic treatment conditions of rice protein

Power (Watt)	Time (min)	Volume (ml)
0	0	100
750	5	200
1000	10	300
1250	15	400
1500	20	-

The protein solution with significant conformation change was then chosen for the test of enzymatic hydrolysis to produce polypeptides product.

The ultrasonic treated protein solution was hydrolyzed by each of the six different enzymes, Neutrase (hydrolyzed at 50^{o}C, pH 6.0), Protamax (at 40^{o}C, pH 6.0), Trypsin (at 45^{o}C, pH 6.5) and Flavourzyme (at 50^{o}C, pH 7.0) from Novo Enzyme (Denmark), BP071 Altalase (at 50^{o}C, pH8.0), and pepsin (at 45^{o}C, pH 6.5) from Xuemei Enzyme Preparation Science And Technology Co., Ltd (China) with the ratio of enzyme to protein as 2% (w/w) for 3 h. After the enzymatic hydrolysis, the solution was centrifuged and the supernatant was used for determining the ACE inhibition rate of the polypeptides.

It was found that polypeptides produced with alkaline protease had the strongest ACE inhibition rate. Then one kilogram of protein was hydrolyzed with the enzyme and corresponding ultrasonic treatment conditions. The hydrolysate (polypeptides) was dried by spray-drying. The dried rice polypeptides were used for conducting preliminary animal test using spontaneously hypertensive rats (SHR) to prove their antihypertensive function in vivo. The ten weeks old SHRs with body weight 180-200g were selected and stochastically divided into 2 groups with 10 rats each. The two groups were fed with low and high doses of polypeptides (the dose unit was mg/body weight). The tail arterial blood pressures of SHRs were measured before their gavage, and 4 and 6 hours of after feeding.

SUMMARY OF 2008 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES

Effect of ultrasonic treatment on the yield of extracted protein

The extracted protein yields increased with the increase of extraction time for all three methods (Fig. 1). The highest yield among the three methods was obtained from the ultrasonic assisted extraction. The protein yield with ultrasonic assisted was 33.9% at 60 min, which was about 2.2 times higher than that (15.5%) with stirring of 600 r/min. This indicated that ultrasonic processing can significantly promote the extraction of protein due to enhanced mass transfer.

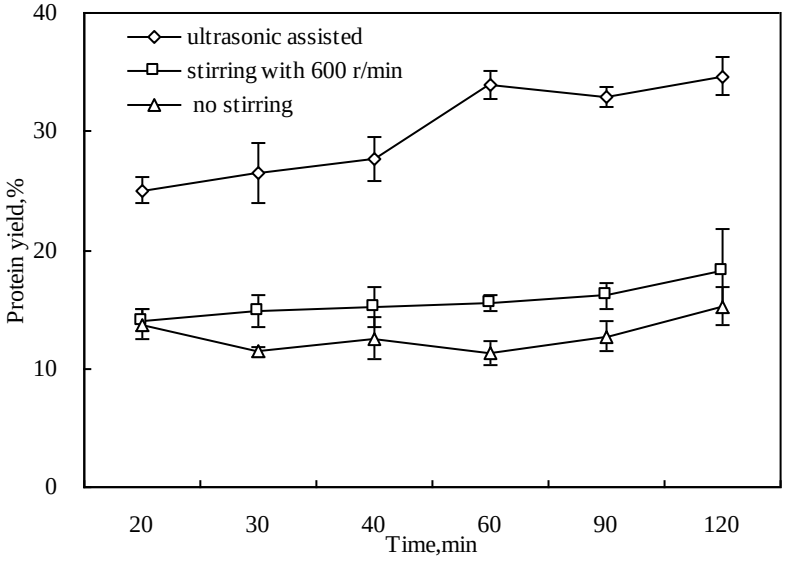

Fig 1. Protein yield at different time with three methods

The extracted protein yields also increased with the increase of pH and the highest yields were obtained at pH 11 (Fig. 2). The yield increased almost linearly when pH was below 10. It appears that temperature did not have much effect on the yield when the pH was below 10. The highest extraction yield was about 40% at pH 11 and temperature of 40 ℃.

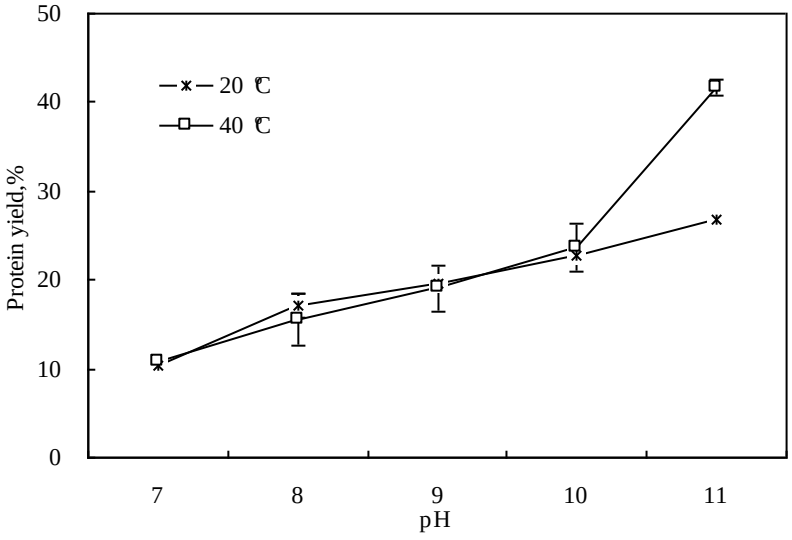

Fig 2. Extracted protein yields at different pH and temperatures

Effect of ultrasonic treatment on protein structure and antihypertensive capability of polypeptides

Effect of ultrasound treatment on solubility of rice protein

The solubility of rice protein treated under different powers for 10 min is shown in Fig. 3. It is clear that the solubility increased with the increase of ultrasonic power. When the power was increased from 1250 W to 1500 W, the degree of solubility increase was reduced. Therefore, the 1250 W power was selected to study the treatment time effect on solubility. It is observed that the solubility increased quickly when the time increased from 5 to 15 min and then the increase was level off (Fig. 4).

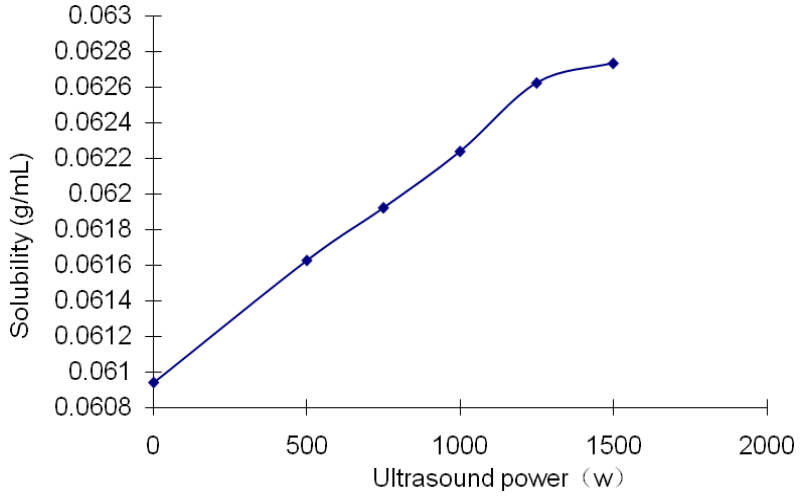

Fig.3 Rice protein solubility under different powers of ultrasonic treatments (treated for 10min)

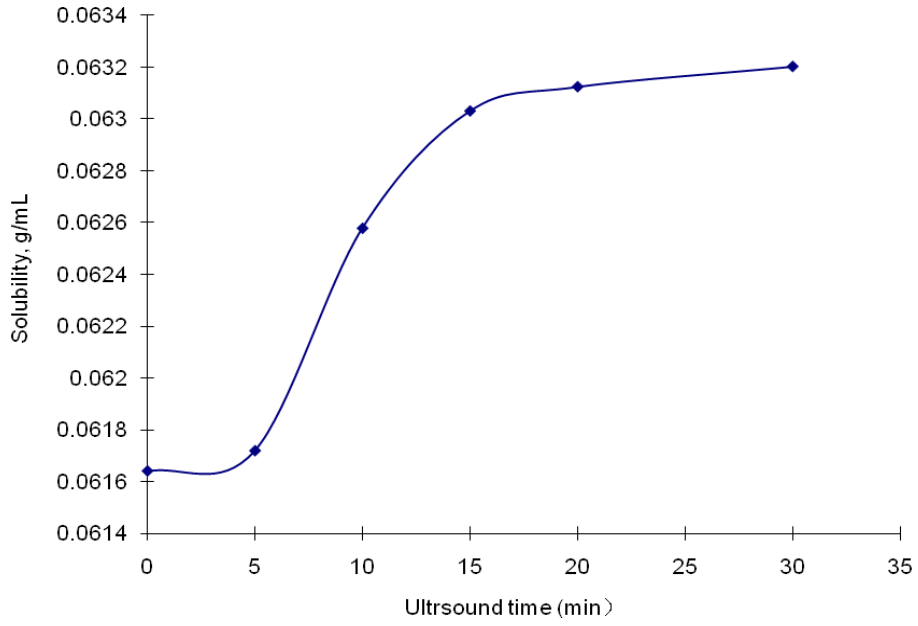

Fig.4 Rice protein solubility under different treatment durations (treated with 1250W power)

Effect of ultrasound treatment on conformation of rice protein

Based on literatures, ultrasonic pretreatment of substrate could accelerate protease hydrolysis reaction because it may modify the spatial conformation to make it easily to conjugate with protease and accelerate the hydrolysis reaction. It was reported that the change of UV spectrometry indicates the structure degradation of protein and the change of fluorescence spectrometry of the protein reflects the conformation change of protein.

The UV and and fluorescence spectra of rice protein treated with ultrasound at different power levels are shown in Figs. 5 and 6. There was no significant change in UV spectra, indicating that the structure of protein did not degrade. However, the remarkable change in fluorescence spectra demonstrates that the spatial conformation of protein has been modified by ultrasonic treatment, which may allow accelerated reaction in enzyme hydrolysis. The change increased with the increase of ultrasonic power.

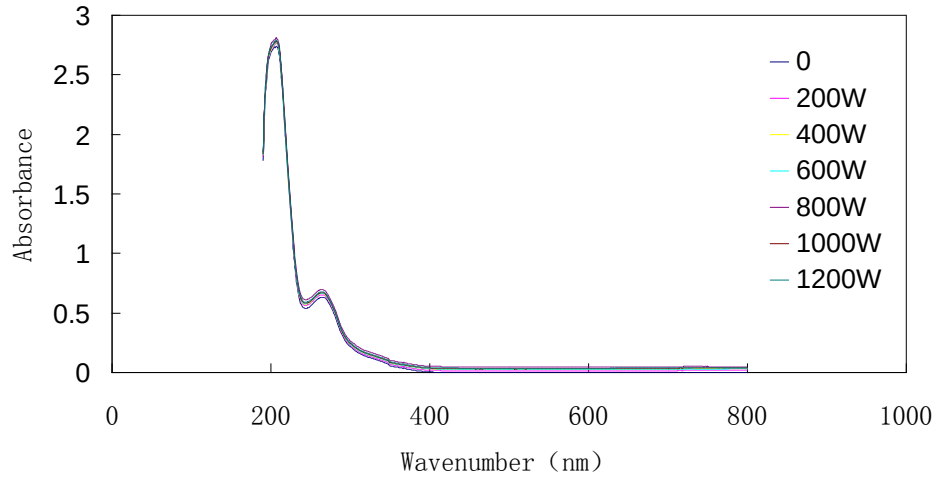

Fig. 5 UV spectra of rice proteins treated with ultrasound at different powers (treated for 10min)

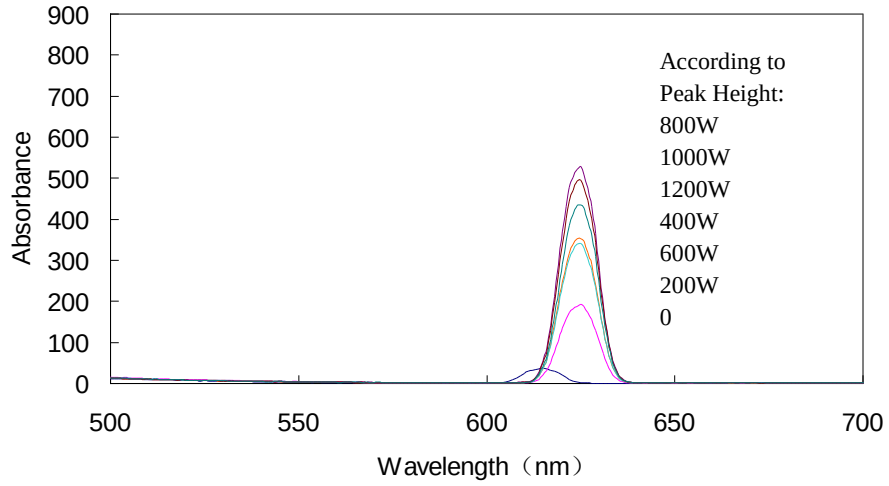

Fig. 6 Fluorescence spectrometry of rice proteins treated with ultrasound at different powers (treated for 10min)

The UV and fluorescence spectra of rice protein treated by using ultrasound at 1000W for different sample volumes for 10 min are shown in Figs. 7 and 8. Since the power level was fixed, a larger volume indicates a lower power density. There was no significant change in UV spectra, indicating that the structure of protein did not degrade. However, the spatial conformation of rice protein was changed by ultrasonic treatment at the power density of higher than 2.5W/mL or volume less than 400 ml.

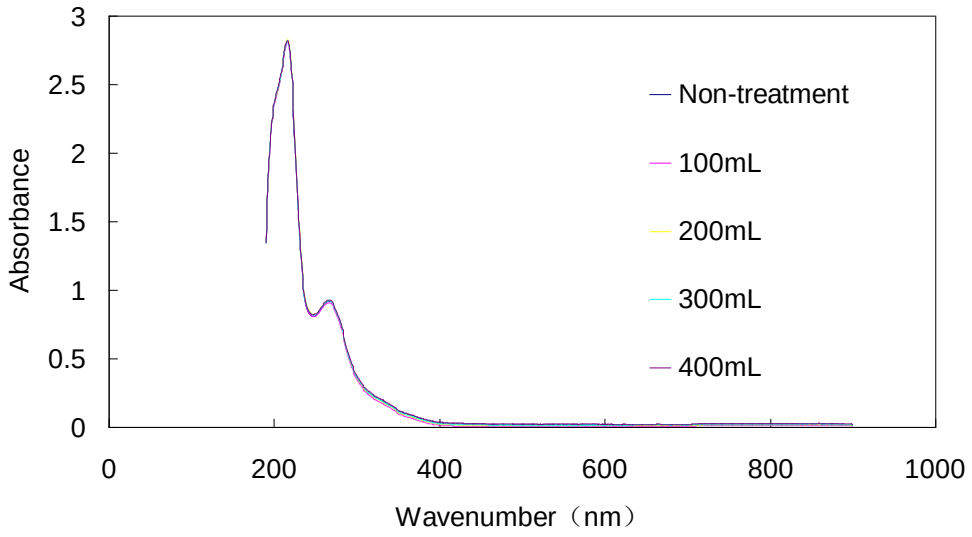

Fig. 7　UV spectra of rice proteins treated with 1000W ultrasound with different sample volumes (treated for 10min)

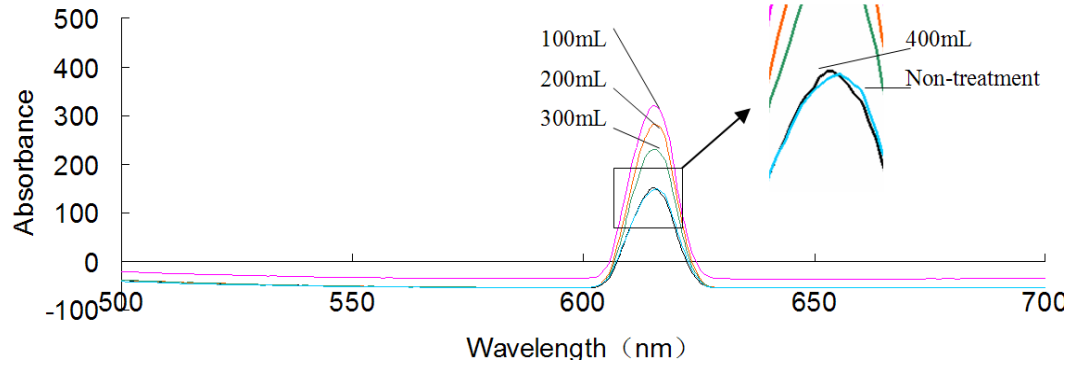

Fig. 8 Fluorescence spectra of rice proteins treated by 1000W ultrasound with different sample volumes (treated for 10min)

Enzymatic hydrolysis of rice protein for producing functional polypeptides

Based on the ultrasonic treatment results, we treated rice protein slurry sample with 1250W for 20 min. Then the treated solution was hydrolyzed by different proteases at each recommended condition by the manufacturers. The ACE inhibitory rate of each hydrolysate was determined to show the in vitro antihypertensive activity (Fig. 9). It was found that hydrolysate by Alkaline protease hydrolysis had the highest activity and was used for producing sample for in vivo test.

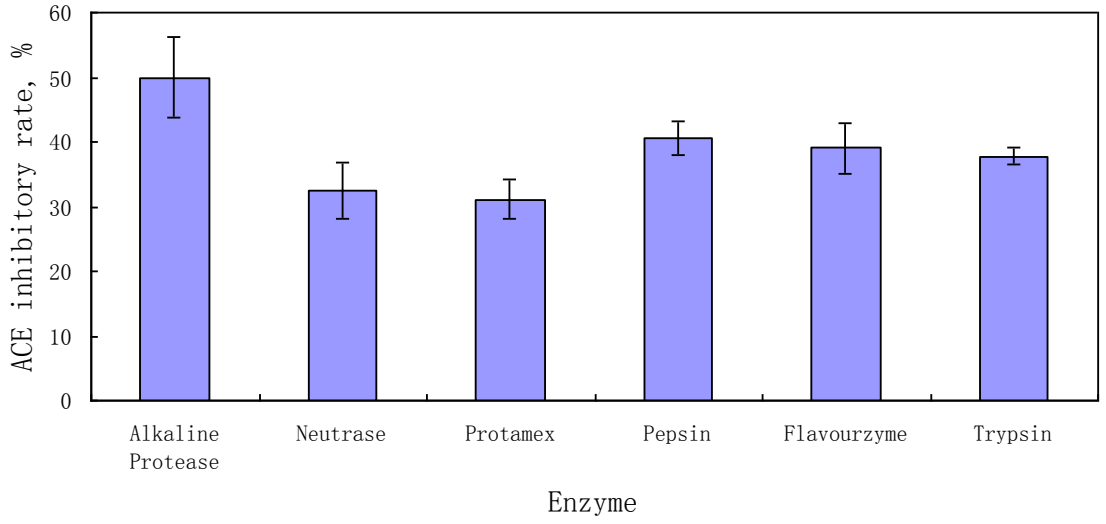

Fig. 9 ACE inhibitory rate of polypeptides produced using different proteases

One kilogram rice protein was hydrolyzed using Alkaline protease to produce polypeptides according to above used conditions. The polypeptides were condensed and dried by spray-drying to produce powder product. The yield of dried rice polypeptides is 15.4% (based on the weight of rice protein). The artery blood pressure changes of rats before and after oral feed of rice polypeptides (with 150mg/kg body weight and 5000mg/kg body weight) are shown in Figs. 10 and 11.

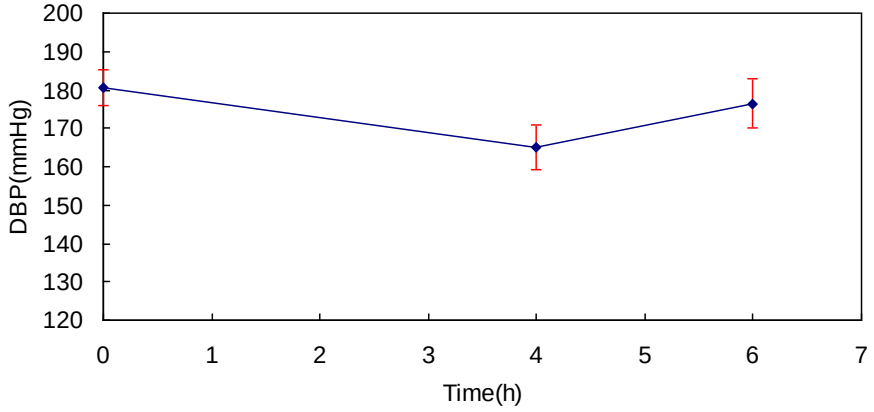

Fig. 10 Artery blood pressure of SHR by the administration of 150mg/kg rice antihypertensive peptides

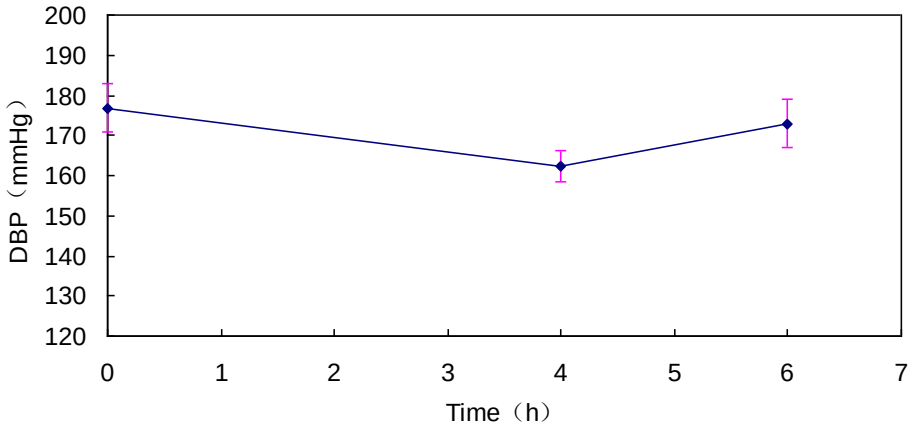

Fig. 11 Artery blood pressure of SHR by the administration of 500mg/kg rice antihypertensive peptides

It was found that the artery blood pressures of rats significantly decreased after 4 hours of oral feed of rice polypeptides. This result indicates that rice polypeptides have significant antihypertensive effect in vivo.

PUBLICATIONS OR REPORTS

N/A

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GENERAL SUMMARY OF CURRENT YEAR'S RESULTS

In this research, we studied the effect of ultrasonic treatment on extracted rice protein yield, conformation of protein, and antihypertensive capability of rice protein based polypeptides. When ultrasonic treatment was applied to protein extraction from rice flour, the extracted protein yield increased with the increase of its power level and extraction time. The highest extraction yield was about 34% when pH and temperature were 11 and 50 ℃, respectively. The yield was 2.2 times higher than that conventional extraction. This indicated that ultrasonic assisted extraction is an effective processing method to achieve high protein yield with reduced processing time.

The research results also showed that the UV spectra of ultrasonic treated protein did not change which indicates that the protein structure was not degraded. However, the florescence spectrometry of the treated protein varied after the protein was treated with different levels of ultrasonic power. The results confirmed that the confirmation of protein changed after the treatment. The solubility of rice protein was also increased after the ultrasonic treatment. The conformation change and increased solubility could promote enzymatic hydrolysis when the protein is used for producing polypeptides.

After rice protein treated using ultrasound at power level of 1250 W for 20 min, the protein was hydrolyzed using six different proteases. The polypeptides produced with Alkaline Protease had the highest ACE inhibitory rate of 50% among the products produced using six different enzymes. The result demonstrated that the rice protein based polypeptides had strong antihypertensive capability. When the polypeptides were fed to the spontaneously hypertensive rats (SHR), the blood pressure of SHR was significantly lowered. The in vivo tests also demonstrated that rice protein based polypeptides had strong antihypertensive capability.