

ANGIOTENSIN I CONVERTING ENZYME INHIBITORY PEPTIDES FROM SWORD BEAN

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ABSTRACT

Sword bean is a healthy food and herbal medicine in China. In this study, the main components of sword bean were determined. Albumin, globulin, prolamin and glutelin were hydrolyzed by pepsin and then the angiotensin I converting enzyme (ACE) inhibitory activity was evaluated. Our results showed that glutelin peptides manifested the highest ACE inhibitory activity with inhibitory ratio of $22.10 \pm 1.57\%$ followed by prolamin peptides and albumin peptides of $16.77 \pm 0.76\%$ and $16.40 \pm 0.42\%$, respectively, at the final concentration of 0.01 mg/mL. Our results strongly suggest that sword bean at some extent have potential to lower blood pressure.

Keywords: sword bean, main component, angiotensin I converting enzyme, peptides

1. INTRODUCTION

Hypertension, a major risk factor for cardiovascular and renal diseases, has become the most common serious chronic health problem. The rennin-angiotensin system (RAS) is critically involved in the physiological regulation of blood pressure and pathogenesis of hypertension (CAT and TOUYZ, 2011). ACE, as an essential member of RAS, can catalyze the conversion of angiotensin (ANG) I to ANG II by removing a carboxyterminal dipeptide (WYSOCKI *et al.*, 2006). Meanwhile, ACE metabolizes bradykinin (BK), a vasodilator, to inactive BK-(1-7). Therefore, ACE inhibitors are effective first-line treatment against essential hypertension (THOMAS *et al.*, 2004), such as captopril, enalapril and lisinopril. However, these synthetic drugs may also cause obvious side effects including cough, loss to taste, renal impairment, and angioneurotic oedema (ANTONIOS *et al.*, 1995). Thus, peptides with potent ACE inhibitory activity derived from natural food provide an effectively alternative treatment (YU *et al.*, 2006). In recent years, ACE inhibitory peptides from natural protein have been successfully isolated, such as corn (YANG *et al.*, 2007), soybean (MALLIKARJUN *et al.*, 2006) and Coix seed (YUAN *et al.*, 2014). Recently, the antihypertensive peptides from traditional Chinese medicine proteins has drawn considerable attention.

Sword bean, the seed of the leguminous plant *Canavalia gladiata*, also has been treated as traditional medicine for containing canavanine, hemagglutinin, and concanavalin A (EKANAYAKE *et al.*, 2006). It has been reported that sword bean may exhibit antioxidant activity of eliminating free radicals and against oxidative stress. In addition, it also has strong anti-inflammatory and anticarcinogenic effects. It is reported that soybean paste containing sword bean exhibits higher ACE inhibitory effects than other soybean pastes (HAN *et al.*, 2015). In this study, this medicinal food was chose to prepare the ACE inhibitory peptides because of its ACE inhibitory activity and rich protein.

The aims of this study are: (1) to determine the main components and protein content of sword bean. (2) to obtain peptides with low molecular weight (≤ 3 KD) by hydrolyzing protein with pepsin, and estimate their ACE inhibitory activity. (3) to provide some reference for the clinical drug use of sword bean in traditional Chinese medicine.

2. MATERIALS AND METHODS

2.1. Material

Sword bean was purchased from Tongrentang (Beijing, China). The voucher specimen (No. 131121003) was deposited at -20°C . Pepsin, ACE and hippuryl-L-histidyl-L-leucine (HHL) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic acid (TFA, MS grade) and Acetonitrile (HPLC grade) were purchased from Merck KGaA (Darmstadt, Germany) and Fisher Scientific (Pittsburgh, PA, USA) respectively. All other chemicals and reagents were analytical grade.

2.2. Determination of the proximal compositions

Protein, fat, moisture and ash content of sword bean were determined according to the Chinese pharmacopoeia (Commission, 2015). The content of starch was determined by Ji (JI *et al.*, 2016).

2.3. Sequential extraction of seed protein

The seeds were ground into powder by a universal high-speed smashing machine, and then defatted with cooled petroleum ether and dried at 40°C overnight. Albumin, globulin, prolamin and glutelin were then sequentially extracted with deionized water, 0.5 M NaCl, 70% ethanol (containing 0.5% NaAC, 5% β-mercaptoethanol) and 0.0125 M sodium borate buffer (containing 1% SDS, 2% β-mercaptoethanol), respectively. All of the protein solutions were dialysed against deionized water at 4°C for 24 h and then freeze-dried. The samples were stored at -80°C for further analysis.

2.4. Determination of protein molecular weight distribution

SDS-PAGE was conducted according to the method of Krizkova (KRIZKOVA *et al.*, 2015) with some modifications to determine the molecular weight distribution of all the protein fractions. All the samples were run for approximately 100 min in 3% stacking gel with a electric current of 10 mA and then for another 100 min in 15% separating gel with 30 mA. After that, the gel was dyed with Coomassie brilliant blue overnight and then decolorized with bleaching liquid until the strips were seen clearly.

2.5. Determination of the Amino acid content

For determination of amino acid composition, 100 mg samples were subjected to acid hydrolysis with 20 mL of 6 M HCl at 110°C for 24 h. Then the lyophilized hydrosate was dissolved in 0.02 M HCl and analyzed by a amino acid analyzer (L-8900; Hitachi, Tokyo, Japan) (WANG *et al.*, 2008).

2.6. Preparation of enzymatic hydrolysates

To produce bioactive peptides, enzymatic hydrolysis method was applied. The protein (2%, w/v) was dissolved in 0.01 M HCl, and pepsin was added with enzyme/substrate ratio of 1/10 (w/w). The mixture was incubated at the temperature of 37°C for 48 h. To terminate the reaction, the mixture was heated 95°C for 5 min. The hydrolysates supernatant was collected after the centrifugation (at 10,000 rpm, 10 min, 4°C).

2.7. Ultrafiltration (UF) of protein hydrolysates

To produce low molecular weight peptides, the hydrolysates were passed through ultrafiltration membrane (MWCO, 3 KD). The peptide concentration of each collected fractions was estimated by the Lowry method (SAPAN, and LUNDBLAD, 2015).

2.8. The assay of ACE inhibitory activity

The ACE inhibitory activity was determined according to the method reported by Yuan (YUAN *et al.*, 2014). Briefly, the reaction system contained 10 μL sample, 20 μL ACE (2 mU) and 20 μL HHL (2 mM). Sample and ACE were incubated at 37°C for 10 min prior to adding substrate HHL, and then for an additional incubation for 80 min at the same temperature. To terminate the reaction, 100 μL acetonitrile was added. Captopril and borate buffer solution was used as positive and blank control, respectively. ACE inhibitory activity was confirmed by monitoring the formation of HA which was generated by HHL under enzymatic hydrolysis. HA was detected by RP-HPLC on a C₁₈ column (250 × 4.6 mm, 5 μm, Tianhe). The column was eluted by a mobile phase of acetonitrile/water (0.05%

TFA) at a volume ratio of 25 : 75 with the flow rate of 1 mL/min. The elution was monitored at 228 nm. The ACE inhibitory ratio of each sample was calculated as follows:

$$\text{Inhibitory activity (\%)} = [(A-B)/A] \times 100\%$$

where A was the HA peak area of blank control; B was the HA peak area in the presence of the sample.

2.9. Statistical analysis

All data were conducted in triplicate and expressed as the mean±SD. The SAS 9.3 program was used for Multiple comparison, and $P < 0.05$ were considered to be significant.

3. RESULTS

3.1. Proximal compositions of sword bean

The proximal compositions of sword bean were presented in Table 1. The starch content of sword bean ranked first (36.59±2.93%). As the member of Leguminosae, the protein content of sword bean accounted for 31.95±0.24%. The moisture and ash contents of this medicinal food all conformed to the requirements of the Chinese pharmacopoeia.

Table 1. Proximal compositions (%) of sword bean.

	Protein	Crude fat	Moisture	Ash	Starch
Sword bean	31.95 (±0.24)	0.71 (±0.04)	8.33 (±0.01)	3.16 (±0.04)	36.59 (±2.93)

Results were expressed as the mean±SD (n = 3).

3.2. Protein fractions distribution of sword bean

Protein patterns of sword bean were shown in Table 2. Considerable variability among albumin, globulin, prolamin and glutelin was observed. Albumin had the highest percentage of 70.93±0.25% followed by globulin of 16.75±0.51%. The prolamin and glutelin contents of this leguminous seeds were rather low. Insoluble protein in the residue only accounted for 5.83±0.04% indicating effective extraction of protein.

Table 2. Protein pattern of sword bean (% of total protein).

	Albumin	Globulin	prolamin	Glutelin	Residual
Sword bean	70.93 ^a (±0.25)	16.75 ^b (±0.51)	1.48 ^e (±0.02)	7.02 ^c (±0.26)	5.83 ^d (±0.04)

Results were expressed as the mean±SD (n = 3). Different letters of indicated having significantly different ($P < 0.05$).

3.3. SDS-PAGE pattern of protein fractions of sword bean

The molecular weight (MW) distributions of different protein fractions for sword bean were detected by SDS-PAGE, which was shown in Fig. 1. Albumin and globulin resolved into similar subunits ranging from 97 KD to 19 KD, with the major subunit of 50 KD. The bands of prolamin and glutelin were heterogeneous ranging from 57 to 14.4 KD and 97 to 19 KD, respectively.

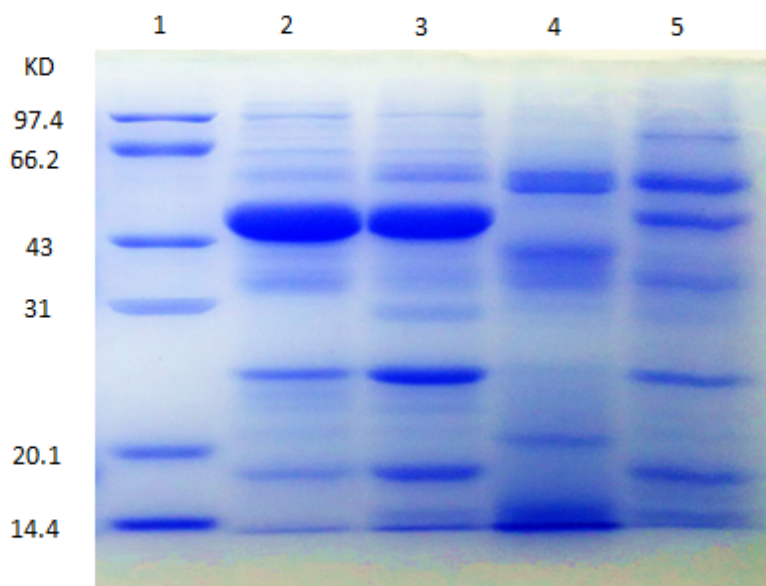


Figure 1. The molecular weight distribution of proteins extracted from sword bean. Lane 1, marker; Lane 2, albumin; Lane 3, globulin; Lane 4, prolamin; Lane 5, glutelin.

3.4. Amino acid composition of seeds flour

For sword bean, a total of 13 kinds of amino acid were detected. Including almost all the essential amino acids and semi-essential amino acids for human beings. From Table 3, it could be seen that Phe had the highest percentage of 4.55 ± 0.11 mg/100 mg, while His recorded the lowest (0.50 ± 0.01 mg/100 mg) in sword bean.

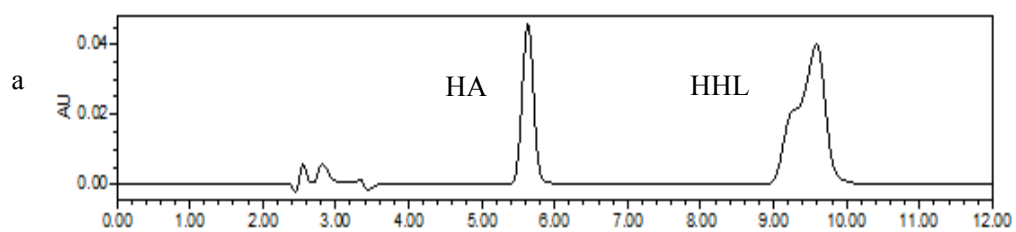
3.5. ACE inhibitory activity assay

The RP-HPLC method was utilized to estimate ACE inhibitory activity of peptide mixtures (≤ 3 KD). The blank control displayed a strong peak area of HA (Fig. 2a), while the positive control (captopril, final concentration of 2×10^{-6} mol/L) manifested a strong ACE inhibition ratio of $91.64 \pm 0.07\%$ (Fig. 2b). The glutelin peptides (≤ 3 KD) revealed the highest ACE inhibitory activity at the final concentration of 0.01 mg/mL with 22.10 ± 1.57 (Fig. 2c). All results were showed in Table 4.

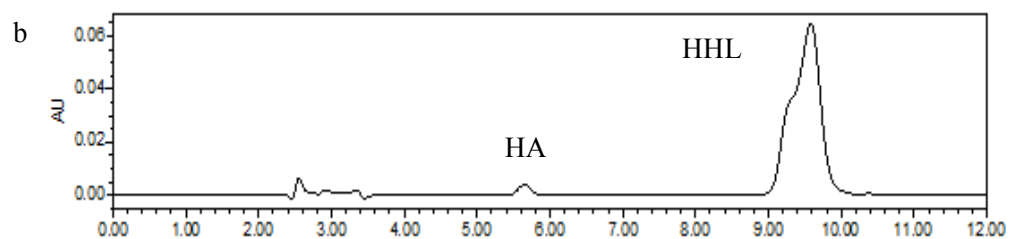
Table 3. The amino acid content of sword bean (mg/100 mg).

Amino acid	Sword bean (mg/100 mg)
Asp	2.84±0.03
Thr ^a	1.32±0.03
Ser	1.49±0.02
Glu	3.26±0.07
Gly	0.88±0.02
Ala	0.68±0.01
Cys	-
Val ^a	2.74±0.06
Met ^a	-
Ile ^a	1.07±0.02
Leu ^a	1.69±0.05
Tyr	-
Phe ^a	4.55±0.11
Lys ^a	1.47±0.02
His ^a	0.50±0.01
Trp ^a	-
Arg ^a	1.56±0.02
Pro	-

^a(semi-) essential amino acid for human, - not detected. The data was expressed as the mean±SD (n = 3).



Elution time (min)



Elution time (min)

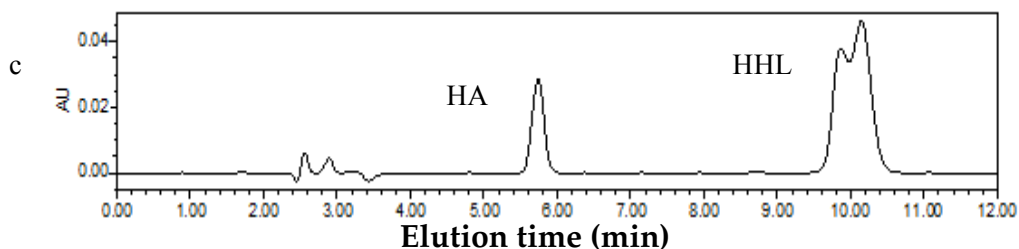


Figure 2. RP-HPLC chromatograms of (a) blank control, (b) positive control (captopril, final concentration of 2×10^{-9} mol/L), (c) glutelin peptides (≤ 3 KD) of 0.01 mg/mL. The mobile phase consisted of 25% acetonitrile (containing 0.05% TFA), eluting at a flow rate of 1.0 mL/min and the absorbance of eluent was detected at 228 nm.

Table 4. ACE inhibition rate (%) of peptides (≤ 3 KD) from different protein fractions.

Peptide	ACE inhibition rate (%)
Albumin peptides	16.40 \pm 0.42 ^b
Globulin peptides	12.72 \pm 0.29 ^c
Prolamin peptides	16.77 \pm 0.76 ^b
Glutelin peptides	22.10 \pm 1.57 ^a

Results were expressed as the mean \pm SD (n = 3). The samples were measured at the final concentration of 0.01 mg/mL. Different letters of indicated having significantly different (P < 0.05).

4. DISCUSSION

Food-derived ACE inhibitory peptides can provide an effectively alternative treatment for hypertension. There are different methods to produce ACE inhibitory peptides from precursor proteins, such as enzymatic hydrolysis (CHEN *et al.*, 2007), microbial fermentation (YAMAMOTO *et al.*, 1994) and chemical synthesis. Among these methods, enzymatic hydrolysis is the most commonly used method (YUAN *et al.*, 2014). There are a great number of studies have proved that food-derived protein hydrolysates and peptides possess ACE inhibitory activity (BALTI *et al.*, 2010; LASSISSI *et al.*, 2014; LEE *et al.*, 2010). Soybean paste containing sword beans exhibits higher ACE inhibitory effects (HAN *et al.*, 2015). Research has shown that the presence of hydrophobic amino acids can increase ACE inhibitory activity (LI *et al.*, 2004). Our study showed that sword bean included all the essential amino acids (except Met) and semi-essential amino acids for human beings. Moreover, sword bean protein may become effective sources for preparation of ACE inhibitory peptides because of its high proportion of hydrophobic amino acid and proline, with 44.58% of total amino acid. Albumin, globulin, prolamin and glutelin were sequentially extracted, which is of benefit to study different proteins activity. The high levels of protein and starch make sword bean good sources of these nutrients.

5. CONCLUSION

Our study mainly focused on the ACE inhibitory activity of protein hydrolysates. The result showed that glutelin peptides manifested the highest ACE inhibitory activity with

inhibitory ratio of $22.10 \pm 1.57\%$ followed by prolamin peptides and albumin peptides of $16.77 \pm 0.76\%$ and $16.40 \pm 0.42\%$, respectively, at the final concentration of 0.01 mg/mL. After further separation, purification and structural identification of hydrolysates (≤ 3 KD), bioactive peptides with better antihypertensive activity might be obtained. Our data might contribute to further research into food derived antihypertensive compounds, meanwhile it also provides some reference for the clinical drug use of sword bean in traditional Chinese medicine.

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