JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

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Evaluation of Bamboo Shoot Peptide Preparation with Angiotensin Converting Enzyme Inhibitory and Antioxidant Abilities from Byproducts of Canned Bamboo Shoots

Lianliang Liu, Lingyi Liu, Baiyi Lu, Meiqin Chen, and Ying Zhang*

Department of Food Science and Nutrition, School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, 310058 Zhejiang, PR China

ABSTRACT: In this paper, aqueous extract fractions from byproducts of the processing of canned bamboo shoots, including boiled water, filled liquid, and squeezed juice, were obtained by 5 kDa molecular cutoff membranes and marcoporous resin DA201-C treatment. The enriched bamboo shoot angiotensin converting enzyme (ACE) inhibitory peptide preparation fraction (called BSP for short) was extracted with ethyl acetate and *n*-butanol. The ethyl acetate fraction and *n*-butanol fraction exhibited higher antioxidant capacities than the leaving water fraction (BSML), which was attributed to the higher phenolic acid and flavonoid content of both fractions, while BSML exhibited the strongest ACE inhibitory activity. Sephadex G-15 gel filtration and semipreparative high-performance liquid chromatography were used for further purification of bamboo shoot ACE inhibitory peptide from BSML. Asp-Tyr was identified as the key active component by ultra-high-performance liquid chromatography and electrospray ionization tandem mass spectrometry. A short-term antihypertensive assay stated that both Asp-Tyr [10 mg day^{-1} (kg of body weight)$^{-1}$] and BSP [50 mg day^{-1} (kg of body weight)$^{-1}$] could significantly reduce the systolic blood pressure of spontaneously hypertensive rats (3−6 h). This study provides further examples of utilization of byproducts from the processing of canned bamboo shoots for the prevention of hypertension and attenuation of oxidative stress.

KEYWORDS: *bamboo shoot ACE inhibitory peptide, byproducts of canned bamboo shoots, phenolic compounds, antioxidant capacity, antihypertension*

INTRODUCTION

Hypertension is a leading cause of cardiovascular disease (CVD), which is the world's leading cause of death each year.[1] According to a survey by the Ministry of Public Health of China in 2010, nearly 200 million adults suffer from hypertension, accounting for one-fifth of global hypertensive patients. Angiotensin converting enzyme (ACE, EC 3.4.15.1) is an important factor in the pathogenesis of hypertension. It converts inactive decapeptide angiotensin I (Ang I) into angiotensin II (Ang II). Ang II exercises a powerful vasoconstrictive action and stimulates the secretion of aldosterone, while aldosterone would favor the retention of sodium and consequent increase in artery pressure.[2] Thus, ACE has been considered as a key point in the prevention and therapy of hypertension. Since the first ACE inhibitor was discovered in snake venom in the 1970s, a series of ACE inhibitors have been synthesized, such as Captopril, Lisinopril, and Enalapril. However, the side effects always associated with these synthetic ACE inhibitors have forced researches to pay attention to food-derived antihypertensive peptides.[3] Many peptides with ACE inhibitory activity have been identified and characterized after their release by fermentation or enzymatic hydrolysis.[4] Generally, potent ACE inhibitory peptides are generally short peptides (2−12 amino acids), which usually contain aromatic or hydrophobic residues at their C-termini, such as Pro, Tyr, Val, and Ala residues.[5]

Bamboo, the giant grass of subfamily Bambusoideae of Graminales, covers wide areas of Asia, Africa, the Caribbean, and Latin America.[6] Bamboo shoots, low in fat, high in dietary fiber, and rich in mineral content, are common vegetables and traditional herbal medicines with an antihypertensive effect in China. *Phyllostachys pubescens,* which is widely distributed in China, is the main bamboo producing bamboo shoots. The antioxidant of bamboo leaves (AOB), an extract from *P. pubescens* that is enriched with phenolic acids and flavonoid, has been reported to exhibit multiple biological activities, including scavenging oxygen radicals and anticancer, antibacterial, and antiviral activity, especially for its antioxidant activity.[7] Methanol extracts of bamboo shoots were reported to possess ACE inhibitory activity.[8] Aqueous extract fractions of bamboo shoots were proven to have an antihypertensive effect on spontaneously hypertensive rats (SHRs).[9] However, the key active ingredients and antihypertensive mechanisms of bamboo shoots in both of the studies were not clear.

Fresh bamboo shoots have limitations for storage or consumption as they easily become lignified (woody) within 2−3 days of being harvested. An approximately 40% output of bamboo shoots is consumed directly and 60% processed in China, with canning being the main form.[10] According to the latest data, more than 1700000 tons of canned bamboo shoots were manufactured in 2011 in China. During this process, the excessive aqueous extract fractions (AEBS), including boiled water, filled liquid, and squeezed juice, are wasted. Nearly 1 ton of canned bamboo shoots comes along with 2 tons of wastewater. AEBS, the byproducts of the canned bamboo

Received: November 27, 2012
Revised: May 5, 2013
Accepted: May 6, 2013
Published: May 6, 2013

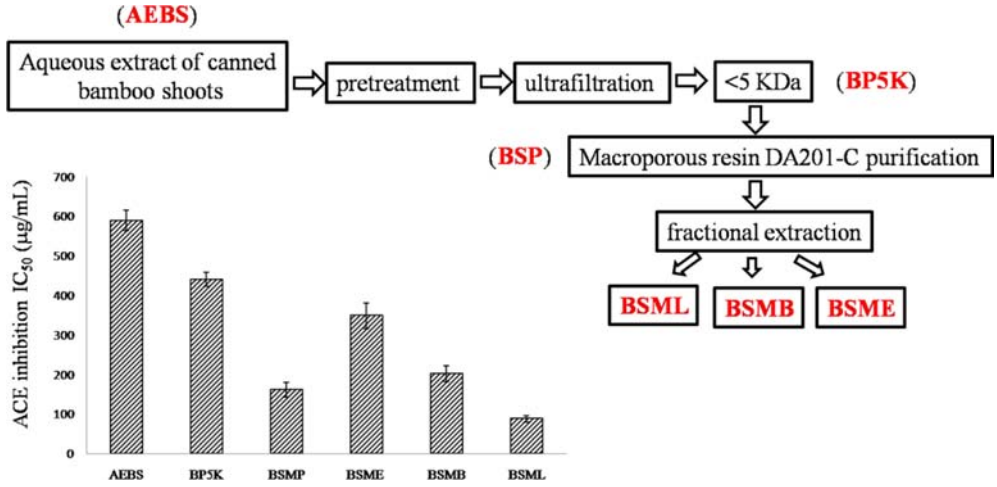

Figure 1. Sample preparation scheme and ACE inhibitory activity detection for byproducts of canned bamboo shoots. Abbreviations: AEBS, which included boiled water, filled liquid, and squeezed juice, which were byproducts of the processing of canned bamboo shoots; BP5K, aqueous extract fractions from byproducts of canned bamboo shoots, with a molecular mass of <5 kDa; BSP, purified fraction obtained via macroporous resin DA201-C from BP5K; BSME, ethyl acetate fraction of BSP; BSMB, *n*-butanol fraction of BSP; BSML, remaining water residual fraction of BSP.

shoot industry, are a sort of good functional food source that contain amino acids, peptides, and phytochemicals. However, it has not been well utilized. This study was aimed at the separation and purification of bamboo shoot ACE inhibitory peptide by 5 kDa molecular mass cutoff membranes, marcoporous resin DA201-C, fractional extraction, Sephadex G-15 gel filtration, and semipreparative high-performance liquid chromatography (HPLC). Ultra-high-performance liquid chromatography and electrospray ionization mass spectrometry (UPLC−ESI-MS/MS) were used for the structural identification of the key active component of bamboo shoot ACE inhibitory peptide. Combined with antioxidant activities and ACE inhibition assessment *in vitro* and antihypertensive activity (0−8 h) *in vivo*, the active ingredients for the prevention of hypertension were evaluated, which would justify the utilization of byproducts from canned bamboo shoots.

MATERIALS AND METHODS

Materials. Bamboo (*P. pubescens*) shoots were provided by Hangzhou U-mate Technology Co., Ltd. (Hangzhou, Zhejiang, China). DPPH [1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl], $ABTS^{\bullet+}$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)], Trolox (6-hydroxy-2,5,7,8-tetramethychroman-2-carboxylic acid), ACE (angiotensin converting enzyme, EC 3.4.14.1) from rabbit lung, and HHL (hippuryl-L-histidyl-L-leucine) were purchased from Sigma-Aldrich (St. Louis, MO). Captopril was provided by Sino American Shanghai Squib Pharmaceutical Co., Ltd. (Shanghai, China).

Animals. SHRs (male, $n = 30$), 13−14 weeks old and weighing 250 ± 10 g, were provided by the Shanghai laboratory animal center (Certificate SOXK. 2007−0005, Shanghai, China). All procedures were approved by the Care and Use Ethics Committee of Zhejiang University and conducted according to the guidelines established by the Experimental Animal Center of Zhejiang University.

Sample Preparation. AEBS were collected during the production of canned bamboo shoots, with a water:bamboo shoot ratio of 2:1 (w/w). AEBS were filtered by a 5 kDa molecular mass cutoff membrane (Millipore Corp., Billerica, MA), and the permeated fraction (BP5K) was collected by macroporous resin DA201-C (Jiangsu Suqing Water Treatment Engineering Group, Jiangyin, China). Thus, a crude bamboo shoot ACE inhibitory peptide preparation (BSP) was obtained. Then, BSP was partitioned successively with ethyl acetate, *n*-butanol, and the remaining residual water extracts, with the fraction marked as the ethyl acetate fraction of BSP (BSME), the *n*-butanol fraction of BSP (BSMB), and the remaining fraction of BSP (BSML), respectively. Each fraction was vacuum-freeze-dried for further study (Figure 1).

Macroporous Resin DA201-C Purification. Prior to the adsorption test, macroporous resin DA201-C was pretreated according to the macroporous resin activation process. The adsorption test was conducted as follows. A 25 mg/mL BP5K solution was loaded onto the pretreated column and adsorbed at a flow rate of 60 mL/min. After adsorption, the column was eluted with 4 bed volumes of MilliQ water to remove the polar impurities. The loading volume was collected at the penetration point when the absorbance of elution at 220 nm showed little alteration ($A_{220} = 0.05$). The fraction containing BSP was eluted with 8 bed volumes of 70% ethanol [ethanol:water ratio (v/v)]. The collected fractions were freeze-dried for further study.

Purification of BSP via Sephadex G-15 Gel Filtration. BSP was dissolved in MilliQ water at a concentration of 100 mg/mL and loaded onto a gel Sephadex G-15 column (1.6 cm × 100 cm; GE Healthcare Life Sciences, Shanghai, China), eluted with MilliQ water at a flow rate of 60 mL/h. Fractions were collected every 5 min. The ACE inhibitory activity of each gel chromatographic profile was obtained by monitoring the absorbance at 220 nm. Fractions associated with each peak showing ACE inhibitory activity were freeze-dried.

Purification by Preparative Reverse-Phase High-Performance Liquid Chromatography (RP-HPLC). The sample was dissolved in distilled water to a concentration of 4.0 mg/mL, filtered through a 0.22 μm filter, and then separated by RP-HPLC. For the Hedera ODS column (10 mm × 300 mm, 5 μm), the mobile phase consisted of solvent A (0.05% trifluoroacetic acid aqueous solution) and solvent B (0.05% trifluoroacetic acid/acetonitrile solution). The following gradient elution program was used: from 95 to 80% A over 5 min, from 80 to 70% A over 5 min, from 70 to 10% A over 5 min, and from 10 to 95% A over 5 min. HPLC analysis was performed at a flow rate of 1 mL/min, a DAD wavelength of 220 nm, and a detection temperature of 35 °C.

Structural Identification by ESI-MS/MS. Structural identification of the purified compound was conducted by UPLC−ESI-MS/MS analysis and structural identification. UPLC analysis was performed at a flow rate of 0.3 mL/min, with an Agilent Zorbax SB C_{18} column [2.1 mm × 150 mm (inside diameter), 3.5 μm], and a detection temperature of 30 °C by Agilent 1290 UPLC. The mobile phases consisted of solvent A [0.01:99.9 (v/v) methane acid/distilled water mixture] and solvent B [0.01:99.9 (v/v) methane acid/acetonitrile mixture]. Mass detection was performed via Agilent 6460 triple quadrupole liquid chromatography and mass spectrometry with an ESI ion source scanning range from 50 to 800 amu.

dx.doi.org/10.1021/jf305064h | J. Agric. Food Chem. 2013, 61, 5526−5533

Amino Acid Composition and Protein Content Detection. The protein content was measured according to the Kjeldahl determination method, and the protein content was calculated as nitrogen × 6.25. The amino acid composition of bamboo shoot extracts was analyzed using the method of Norziah and Ching, using Waters Associates AccQ Tag.[11] Materials were dissolved in 6 mol/L HCl at 110 °C (24 h). Precolumn derivatization of samples with Waters Associates AccQ Tag was analyzed by reverse-phase HPLC.

Assay of ACE Inhibitory Activity *in Vitro*. The ACE inhibitory activity was determined, using an adapted RP-HPLC method by Wu and others.[12] The substrate, HHL, was dissolved (5 mmoL/L) in 0.1 mol/L sodium borate buffer (pH 8.3) containing 0.3 mol/L NaCl. The assay was performed by mixing 50 μL of a substrate solution with 20 μL of an inhibitor solution (or borate buffer for control). After incubation at 37 °C for 10 min, 20 μL of the ACE solution (100 milliunits/mL) was added and further incubated at 37 °C for 30 min. The reaction was stopped by addition of 85 μL of 1 mol/L HCl, and the solution was filtered through a 0.45 μm nylon syringe filter membrane before reversed-phase HPLC analysis. HPLC analysis was performed on a Waters chromatographic system with a C_{18} column [250 mm × 4.6 mm (inside diameter), particle size of 5 μm]. Analysis was conducted at a wavelength of 228 nm, using Captopril as a reference drug.

A blank group was also prepared using the procedure described above, but the diluents were replaced with 0.1 mol/L sodium borate buffer (pH 8.3). The mobile phases consisted of solvent A [0.5:99.5 (v/v) acetic acid/distilled water mixture] and solvent B [0.5:99.5 (v/v) acetic acid/acetonitrile mixture].

The ACE inhibitory activity was calculated as follows

$$R = (A - B)/A \times 100\%$$

where R is the ACE inhibitory activity (percent), A is the peak area of hippuric acid in the blank, and B is the peak area of hippuric acid in the diluents. IC_{50} is defined as the concentration of the inhibitor required to reduce the magnitude of the hippuric acid peak by 50%.

Determination of Total Flavonoid and Phenolic Acid Contents. The total flavonoid content was measured using a modified method;[13] 2 mL of 5 mg/mL sample solutions was mixed with 3 mL of a 30% (v/v) ethanol solution and subsequently with 0.3 mL of a 5% (w/v) $NaNO_2$ solution. The mixture was allowed to react for 5 min. Then 0.3 mL of a 10% $Al(NO_3)_3$ solution was added and allowed to react for an additional 6 min before 2 mL of a 1 mol/L NaOH solution was added. A 30% (v/v) ethanol solution was added to bring the final volume of the mixture to 10 mL. The absorbance of the mixture was immediately measured at 510 nm against a prepared blank. The total flavonoid content was determined by the Rutin standard curve and expressed as the mean grams of Rutin equivalents (RE) per 100 g of sample ± the standard deviation (SD) for three replications.

The total phenolic content of the sample was measured by the Folin-Ciocalteu reagent method with modifications;[14] 1 mL of 5 mg/mL sample solutions was diluted to 10 mL with distilled water and mixed with 1 mL of Folin-Ciocalteu reagent and 2 mL of a 15% $NaCO_3$ solution. Distilled water was added to bring the final volume to 25 mL. The absorbance was measured at 760 nm after 60 min at room temperature. Absorbance values were compared with the concentration of the standard (gallic acid). All values were expressed as the mean grams of gallic acid equivalents (GAE) per 100 g of sample ± SD for three replications. The phenolic acid content was calculated as follows: phenolic acid content = total phenolic acid content − total flavonoid content

RP-HPLC Detection of Phenolic Compounds. Chromatographic separation was performed on a Waters 2996 photodiode array detector. Phenolic compounds were measured according to the method of Zhang.[9] Samples were dissolved in methanol and filtered through a 0.45 μm membrane filter. Standard solutions of orientin, homoorientin, *p*-coumaric acid, chlorogenic acid, caffeic acid, and ferulic acid were prepared at a concentration of 1 mg/mL in a 50:50 (v/v) methanol/water mixture. The mobile phase consisted of solvent A (0.05% trifluoroacetic acid aqueous solution) and solvent B (0.05% trifluoroacetic acid/acetonitrile solution). The following gradient elution program was used for preparative separation: 15% A over 15 min, from 15 to 40% A over 10 min, 40% A over 9 min, and from 40 to 15% A over 6 min. HPLC analysis was performed on a Waters chromatographic system with a C_{18} column [250 mm × 4.6 mm (inside diameter), particle size of 5 μm], a flow rate of 1 mL/min, a DAD wavelength of 330 nm, and a detection temperature of 40 °C.

Antioxidant Capacities of Bamboo Shoot Extracts. *Scavenging Capacity on DPPH• Radical.* DPPH• radical scavenging capacities were determined by the method of Turkoglu et al. with some modifications;[15] 0.3 mL of sample solutions was added to 3.7 mL of a DPPH• solution (0.2 mmol/L). The absorbance was measured at 517 nm against a prepared blank after incubation for 1 h at room temperature. Trolox was considered as a reference.

Scavenging Capacity on ABTS•+ Radical. The $ABTS^{\bullet+}$ scavenging assay was conducted by a modified method.[16] An aqueous solution of 7 mmol/L $ABTS^{\bullet+}$ and 2.45 mmol/L potassium persulfate was used, with the absorbance adjusted to 0.70 ± 0.02 at 734 nm; 0.1 mL of a sample solution and 3.9 mL of an $ABTS^{\bullet+}$ solution were mixed. The absorbance was measured at 734 nm after 6 min. Ascorbic acid was considered as a reference.

Ferric-Reducing Antioxidant Power (FRAP) Assay. The reducing ability of the sample was determined by Yazdanparast with modifications.[17] FRAP reagent was freshly prepared from 300 mmol/L acetate buffer (pH 3.6) and 10 mmol/L tripyridyltrizine (TPTZ) made up in 40 mmol/L HCl and 20 mmol/L $FeCl_3$, at a ratio of 10:1:1 (v/v/v); 0.1 mL of the tested sample was mixed with 3.9 mL of FRAP reagent. The absorbance was measured at 593 nm as soon as the reaction had proceeded for 10 min at 37 °C. The reducing ability was expressed as the ferric-reducing ability equivalent concentration of 1 mmol/L $FeSO_4$.

Short-Term (0−8 h) Antihypertensive Effect on SHRs. SHRs (n = 30) with a systolic blood pressure (SBP) higher than 180 mmHg were randomly divided into six groups after being acclimated for 1 week, each group consisting of five rats: blank control group orally administrated distilled water and positive control group orally administered Captopril [10 mg day^{-1} (kg of body weight)$^{-1}$]. Asp-Tyr groups were administered at a dose of 10 mg day^{-1} (kg of body weight)$^{-1}$. BSP were diluted with distilled water at different solution concentrations [20, 50, and 100 mg day^{-1} (kg of body weight)$^{-1}$]. SBP was measured before administration and after administration for 1, 2, 3, 4, 5, 6, 7, and 8 h using a tail-cuff apparatus (ALC-NIBP Non-Invasive Blood Pressure Monitoring and Analyzing System). SHRs were kept at 37 ± 1 °C for 10 min before the measurement to make the pulsations of the tail artery detectable.

Statistical Analysis and Experiment Replicates. Data were analyzed by one-way analysis of variance (ANOVA) using statistical software SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Significant effects were determined using Duncan's multiple-comparison tests, and differences at the 5% level were considered significant. All experiments were conducted in a completely randomized design with three replicates for each treatment.

RESULTS

Amino Acid Composition and Protein Content Changed by Macroporous Resin DA201-C Purification. Macroporous resin DA201-C was chosen for purification of BP5K and control desalination. The yield of BSP from BP5K by treatment of macroporous resin was about 66.43 ± 0.32 g/100 g. The total amino acid content and protein content significantly increased after macroporous resin DA201-C treatment ($p < 0.01$) as shown in Table 1 and Figure 2. Bamboo shoot has a high protein content (amino acids), ranging from 2.31 to 3.72 g/100 g of fresh weight in juvenile shoots, of which hydrophobic amino acids, such as Tyr, account for amounts of total amino acids.[10] Results indicated that the contents of Tyr, Glx, and Asx significantly increased from 78.45 to 130.80 mg/g, from 51.95 to 103.05 mg/g, and from 34.4 to

Table 1. Amino Acid Composition of Bamboo Shoot Peptides

amino acid	content (mg/g)	
	BP5K	BSP
Tyr	78.45 ± 0.93	130.80 ± 1.07**
Ser	20.89 ± 0.14	19.45 ± 0.71
Glx	51.95 ± 0.37	103.05 ± 0.72**
Pro	2.18 ± 0.03	2.56 ± 0.08
Gly	7.63 ± 0.13	3.70 ± 0.21**
Ala	18.01 ± 0.09	23.56 ± 0.16**
Cys	8.36 ± 0.18	16.15 ± 0.23
Asx	34.4 ± 0.29	60.23 ± 0.74**
His	4.21 ± 0.04	3.39 ± 0.07*
Arg	5.93 ± 0.08	5.37 ± 0.15
Val*	15.26 ± 0.36	23.70 ± 0.55**
Met*	0.67 ± 0.05	0.95 ± 0.08
Ile*	4.12 ± 0.13	5.36 ± 0.24*
Leu*	10.68 ± 0.18	11.28 ± 0.31
Phe*	5.19 ± 0.32	6.74 ± 0.43*
Lys*	6.06 ± 0.52	10.48 ± 0.53**
Thr*	0.73 ± 0.05	1.05 ± 0.07*
total amino acid content	274.72 ± 4.01	427.82 ± 5.75

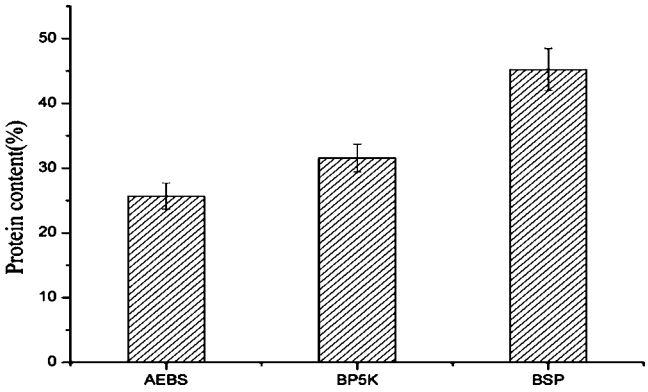

Figure 2. Protein content of fractions from byproducts of canned bamboo shoots. Abbreviations: AEBS, which included boiled water, filled liquid, and squeezed juice, which were byproducts of the processing of canned bamboo shoots; BP5K, aqueous extract fractions from byproducts of canned bamboo shoots, with a molecular mass of <5 kDa; BSP, purified fraction via macroporous resin DA201-C treatment of BP5K.

60.23 mg/g, respectively, because of the macroporous resin DA201-C treatment (Table 1).

ACE Inhibitory Activity *in Vitro* of Fractional Extractions. The ACE inhibitory activities *in vitro* of all fractions were assayed. The ACE inhibitory activity of BP5K (IC_{50} = 440.06 ± 18.03 μg/mL), the fraction obtained by filtration via a 5 kDa molecular mass cutoff membrane, was enhanced compared with that of AEBS (IC_{50} = 588.29 ± 51.7 μg/mL). Macroporous resin DA201-C was used to improve the ACE inhibitory activity of BSP (IC_{50} = 160.54 ± 13.52 μg/mL). The fractional extract proved to be an effective way to separate the active components for ACE inhibition. Thus, BSP was partitioned successively with ethyl acetate (yield of 16.93 ± 0.79 g/100 g), *n*-butanol (yield of 27.75 ± 0.94 g/100 g), and the remaining residual water extracts (yield of 53.18 ± 1.21 g/100 g). Results indicated that BSML (IC_{50} = 88.08 ± 7.75 μg/mL) exhibited the best ACE inhibitory activity, followed by BSMB (IC_{50} = 201.96 ± 20.33 μg/mL) and BSME (IC_{50} = 348 ± 30.10 μg/mL), which may be closely related to the components of each fraction.

Purification and Identification of the Bamboo Shoot ACE Inhibitory Peptide. BSML, which exhibited a relatively higher ACE inhibitory activity *in vitro*, was separated into eight fractions (P_I–P_{VIII}) by size exclusion chromatography on a Sephadex G-15 column. Each fraction was pooled and lyophilized, and the ACE *in vitro* inhibitory activity of each is shown in Figure 3. Fraction P_{II}, the yield of which accounted

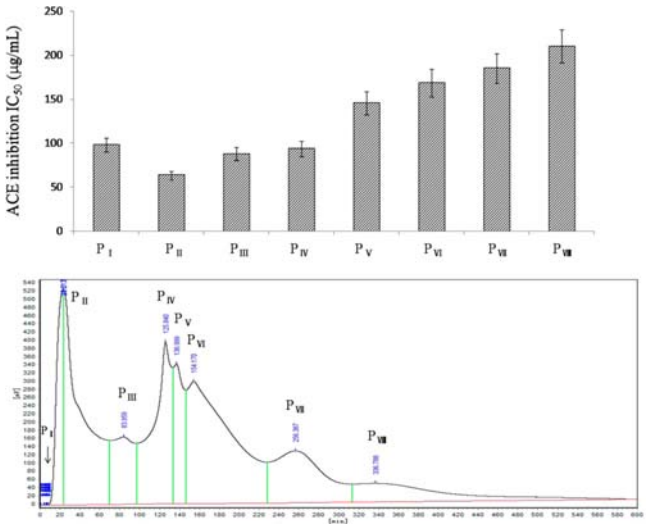

Figure 3. ACE inhibitory activities of the bamboo shoot ACE inhibitory peptide purified by Sephadex G-15 chromatography.

for 7.29% of the BSP content, possessed the highest ACE inhibitory activity among all fractions. Two major subfractions, $P_{II\text{-}I}$ and $P_{II\text{-}II}$, were fractionated after the separation of preparative RP-HPLC (Figure 4), collected separately, and freeze-dried to measure the ACE inhibitory activity. Subfraction $P_{II\text{-}II}$ possessed a higher ACE inhibitory activity (IC_{50} = 32.31 ± 3.09 μg/mL), which was further identified by UPLC–ESI-MS/MS. Asp-Tyr was identified through the mass spectrum as shown in the bottom panel of Figure 4.

Determination of Flavonoid and Phenolic Acid Compound Content. The total flavonoid and phenolic acid contents of all fractions of AEBS are listed in Figure 5. Total flavonoid and phenolic acid contents of AEBS were 1.41 ± 0.12 g of RE/100 g and 4.78 ± 0.24 g of GAE/100 g, respectively. Similar to the ACE inhibitory activities, total flavonoid and phenolic acid contents increased after filtration via 5 kDa molecular mass cutoff membranes and purification with macroporous resin DA201-C. However, total flavonoid and phenolic acid contents of BSME, BSMB, and BSML seemed to be inconsistent with ACE inhibitory activities. BSME possessed the highest total flavonoid and phenolic acid contents, while BSML possessed the lowest (Figure 5).

Subsequently, analytic RP-HPLC detection was used to identify the phenolic compound of BSME, BSMB, and BSML. Chlorogenic acid, caffeic acid, orientin, homoorientin, *p*-coumaric acid, and ferulic acid were involved in the RP-HPLC chromatogram. To be specific, a high content of chlorogenic acid was detected in BSP (2.15 ± 0.05 g/100 g). Contents of *p*-coumaric acid and ferulic acid in BSMB (0.88 ± 0.02 and 0.14 ± 0.01 g/100 g, respectively) and BSME (3.08 ± 0.06 and 0.51 ± 0.02 g/100 g, respectively) . In this text, BSME

dx.doi.org/10.1021/jf305064h | J. Agric. Food Chem. 2013, 61, 5526–5533

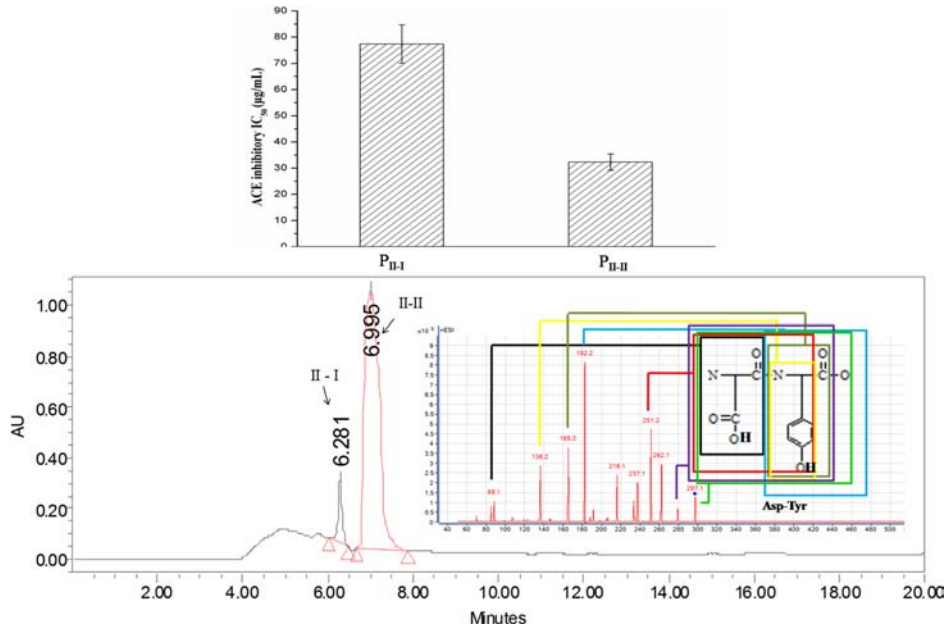

Figure 4. Purification of the bamboo shoot ACE inhibitory peptide by preparative HPLC and identification by UPLC–ESI-MS/MS. Fraction P_{II} was obtained by semi-HPLC (bottom), and the ACE *in vitro* inhibitory activity was assayed (top). UPLC–ESI-MS/MS was used to identify structure of the bamboo shoot peptide.

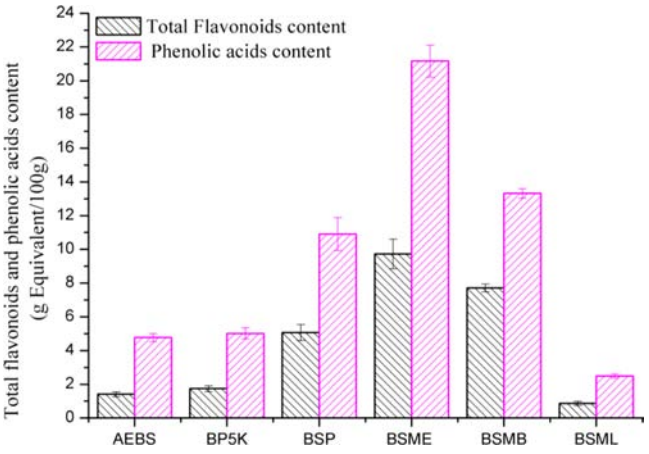

Figure 5. Total flavonoid and phenolic content of all fractions from byproduct of canned bamboo shoot. Abbreviations: AEBS, which included boiled water, filled liquid, and squeezed juice, which were byproducts of the processing of canned bamboo shoots; BP5K, aqueous extract fractions from byproducts of canned bamboo shoots, with a molecular mass of <5 kDa; BSP, purified fraction by macroporous resin DA201-C from BP5K; BSME, ethyl acetate fraction of BSP; BSMB, *n*-butanol fraction of BSP; BSML, remaining water residual fraction of BSP.

was obtained by repeated ethyl acetate fractional extraction in order to enrich phenolic acid compounds, which were higher than that in BSP obtained by ethyl acetate extraction treatment published previously. The RP-HPLC chromatogram of BSP was similar to that of AOB,[7] including homoorientin, orientin, chlorogenic acid, caffeic acid, *p*-coumaric acid, and ferulic acid. However, isovitexin and vitexin were not detected in BSP. The correlation between phenolic acid content and antioxidant capacity of each fraction needs to be assessed further.

Antioxidant Capacities of Bamboo Shoot Extract Fractions. Antioxidant capacities of all fractions of AEBS were assayed (shown in Figure 6). The DPPH$^{\bullet}$ assay has been widely used to provide basic information about the antioxidant ability of extracts from plant, food material, or a single compound. DPPH$^{\bullet}$ is a stable free radical with a characteristic absorption, which decreases significantly upon exposure to proton radical scavengers.[18] The order of the capability of scavenging DPPH$^{\bullet}$ radicals is as follows: BSME > BSMB > BSP > BP5K > AEBS > BSML (Figure 6).

ABTS$^{\bullet+}$ is another synthetic radical and more versatile than DPPH$^{\bullet}$, as it can assess the scavenging activity of both polar and nonpolar samples. The order of the capability of scavenging ABTS$^{\bullet+}$ radicals is as follows: BSME > BSMB > BSP > BP5K > BSML (Figure 6).

FRAP measures the antioxidant effect of a detected substance in the reaction medium by the ability to reduce TPTZ–Fe(III) to TPTZ–Fe(II). The order of reducing ability is as follows: BSME > BSMB > BSP > BP5K > BSML (Figure 6).

Short-Term (0–8 h) Antihypertensive Effect of BSP on SHRs. As shown in Figure 7, the short-term antihypertensive effect of BSP has been shown to be dose-dependent. Asp-Tyr possessed effective antihypertensive activity (3–6 h) compared with the blank control group ($p < 0.01$). The antihypertensive effect of Asp-Tyr [10 mg day^{-1} (kg of body weight)$^{-1}$] was similar to that of BSP [50 mg day^{-1} (kg of body weight)$^{-1}$] on SHRs. The SBP of SHRs fed with 50 mg of BSP day^{-1} (kg of body weight)$^{-1}$, 100 mg of BSP day^{-1} (kg of body weight)$^{-1}$, and 10 mg of Captopril day^{-1} (kg of body weight)$^{-1}$ significantly decreased after the mice had been fed for 2–6 h compared with that of the blank control group ($p < 0.01$). No significant differences were presented between the SBP of SHRs that were fed 100 mg of BSP day^{-1} (kg of body weight)$^{-1}$ and 10 mg of Captopril day^{-1} (kg of body weight)$^{-1}$ for 4–6 h, while Captopril [10 mg day^{-1} (kg of body weight)$^{-1}$] could reduce the SBP in less time. The SBP in all treated groups returned close to baseline values after oral administration for 8 h.

dx.doi.org/10.1021/jf305064h | J. Agric. Food Chem. 2013, 61, 5526–5533

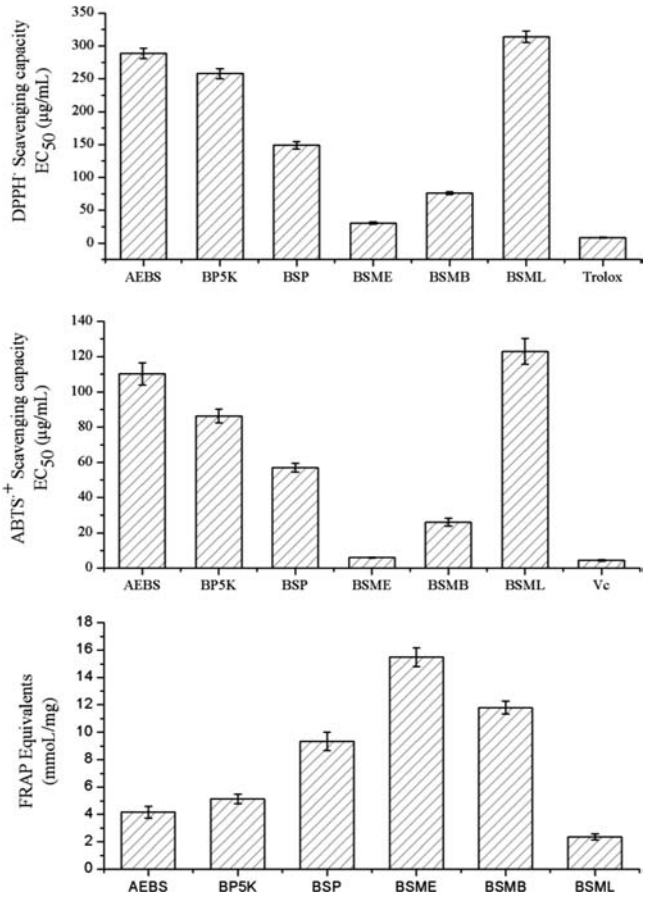

Figure 6. Antioxidant capacities of all fractions of byproduct from canned bamboo shoot. Abbreviations: AEBS, which included boiled water, filled liquid, and squeezed juice, which were byproducts of the processing of canned bamboo shoots; BP5K, aqueous extract fractions from byproducts of canned bamboo shoots, with a molecular mass of <5 kDa; BSP, purified fraction by macroporous resin DA201-C from BP5K; BSME, ethyl acetate fraction of BSP; BSMB, *n*-butanol fraction of BSP; BSML, remaining water residual fraction of BSP.

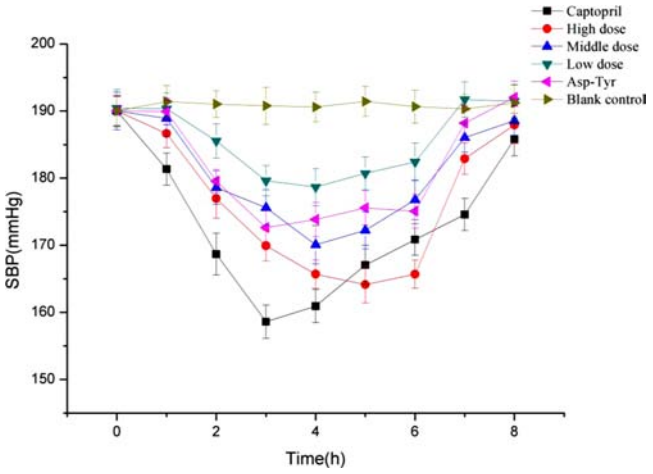

Figure 7. Short-term antihypertensive effect of Asp-Tyr on SHRs. SHRs (n = 30) were randomly divided into six groups after being acclimated for 1 week, each group consisting of five rats: blank control group orally administrated distilled water and positive control group orally administered Captopril [10 mg day^{-1} (kg of body weight)$^{-1}$]. The Asp-Tyr group was administered at a dose of 10 mg day^{-1} (kg of body weight)$^{-1}$. BSP was diluted with distilled water with different solution concentrations [20, 50, and 100 mg day^{-1} (kg of body weight)$^{-1}$]. The SBP was measured before administration and after administration for 1, 2, 3, 4, 5, 6, 7, and 8 h.

DISCUSSION

ACE, a key target of hypertension, is involved in blood pressure-related systems. Potent ACE inhibitory peptides are generally short peptides (2−12 amino acids), which usually contain aromatic or hydrophobic residues at their C-termini, such as Pro, Tyr, Val, and Ala residues.[5] Research of the antioxidant peptide pointed out that His, Leu, Tyr, and Met could enhance the scavenging free radical capacity.[19] The amino acid composition of BSP with a high content of Tyr (130.80 ± 1.07 mg/g), Glx (103.05 ± 0.72 mg/g), Asx (60.23 ± 0.74 mg/g), and Val (23.70 ± 0.55 mg/g) in bamboo shoots provided the possible foundation of both ACE inhibitory activity and antioxidant capacity. Several peptides containing amino acids mentioned above have been considered to possess potent ACE inhibitory activity, such as Asp-Tyr-Gly-Leu-Tyr-Pro and Asp-Leu-Thr-Asp-Tyr.[20,21] Bioactive peptides should be absorbed to exert their biological functions *in vivo*. Thus, dipeptides are very valuable in therapeutic usage, as dipeptides are easily absorbed in the intestine. Yokoyama et al. have previously indicated Asp-Tyr may be the ACE inhibitory center of Asp-Tyr-Gly-Leu-Tyr-Pro from dried bonito (*katsuobushi*).[21] Asp-Tyr has also been identified as the real effector of peptide Asp-Leu-Thr-Asp-Tyr after digestion. Wu reported Asp-Tyr with an ACE IC_{50} value of 29.6 μg/mL,[22] while Asp-Tyr, as one possible real effector of bovine gelatin hydrolysate (Bh2t), was reported to have an ACE IC_{50} value of 26.6 μg/mL.[23] It was in line with our result that Asp-Tyr possessed an ACE IC_{50} value of 32.31 ± 3.09 μg/mL. The differences between the data provided above may be based on the methodology with different substrate and enzyme ratios.

The dietary approach pattern to lower blood pressure has attracted a growing amount of attention, including the decrease in the level of salt intake and the increase in the level of potassium intake.[24] Many peptides with ACE inhibitory activity were identified and characterized from fermentation foods, enzymatic hydrolysis proteins, or natural peptides.[4] Bamboo shoot is a kind of ideal vegetable, being low in fat, high in dietary fiber, and rich in mineral content.[6] The edible part consists of meristematic cell tissue with regions of rapid cell division, differentiation, and lignification.[25] During this period, nutrients in bamboo shoots such as protein, carbohydrates, amino acids, and fiber changed with enzymatic browning,[10] and protein may be hydrolyzed into small peptides and amino acids. AEBS showed a limited ACE inhibitory activity with an IC_{50} value of 588.29 ± 51.7 μg/mL, while the further fraction (BSP) that was obtained by membrane filtration and macroporous resin DA201-C purification exhibited higher inhibition activity. Asp-Tyr was identified as the key active peptide of BSP by UPLC−ESI-MS/MS, with an ACE IC_{50} value of 32.31 ± 3.09 μg/mL.[22] IC_{50} values of ACE inhibitory activity of the methanol extract of bamboo shoot extracts were reported to be 4.7 ± 0.3 mg/mL (*P. pubescens*) and 5.3 ± 0.8 mg/mL (*Phyllostachys nigra*), which were much higher than our results.[9] This is attributed to 5 kDa molecular mass cutoff membrane and macroporous resin DA201-C purification were applied in our study. The molecular mass cutoff membrane is commonly used to concentrate ACE inhibitory active materials,[26] and macroporous resin purification is also commonly used in the purification and desalination of the peptide.[27] In addition, a

dx.doi.org/10.1021/jf305064h | J. Agric. Food Chem. 2013, 61, 5526−5533

high rate of salt intake is an important factor for increasing blood pressure.[28] Macroporous resin DA201-C desalination provided a certain security guarantee for desalination.

However, ACE inhibitory activity *in vitro* is not always directly related to an antihypertensive effect.[23] Thus, an antihypertensive effect of BSP *in vivo* was required. A short-term (0−8 h) antihypertensive test on SHRs indicated that the antihypertensive effect of Asp-Tyr [10 mg day^{-1} (kg of body weight)$^{-1}$] was similar to that of BSP [50 mg day^{-1} (kg of body weight)$^{-1}$] on SHRs. Captopril [10 mg day^{-1} (kg of body weight)$^{-1}$] could reduce the SBP in less time, indicating that Captopril played a more effective antihypertensive role than BSP. Nevertheless, BSP was a natural food source material for antihypertensive prevention, and until now, more attention has been focused on food-derived antihypertensive peptides. No significant differences were observed between SBP of SHRs fed 100 mg of BSP day^{-1} (kg of body weight)$^{-1}$ and 10 mg of Captopril day^{-1} (kg of body weight)$^{-1}$ for 4−6 h.

Phenolic compounds contained in food are thought to be the major bioactive compounds for health benefits.[29,30] Ferulic acid and *p*-coumaric acid are the major hydroxycinnamic acids in cell walls of bamboo shoots, which participate with lignin in generating polysaccharide−lignin complexes.[31] The two phenolic compounds in cowpea have been reported to exhibit excellent antioxidant properties.[32] Furthermore, *p*-coumaric acid could be absorbed and detected in rats and exhibited a biological function.[33] Besides the ACE inhibitory peptide, BSP contained a variety of phenolic compounds, which could possess antioxidant function.[34] Pathogenesis of hypertension is usually associated with oxidative stress, a state of excessive reactive oxidative species activity. Antioxidant therapy has a beneficial effect on the prevention and treatment of hypertension.[35,36] Phenolic compounds and ACE inhibitor in BSP may exert synergistic antihypertensive effect on SHRs. However, this explanation needs to be investigated further.

A linear relationship was presented between antioxidant capability and total flavonoid and phenolic acid content, in which the former was better. A linear relationship was presented between antioxidant capability and total flavonoid content (for DPPH$^{\bullet}$, $Y = -22.24X + 321.6$ and $R^2 = 0.986$; for ABTS$^{\bullet+}$, $Y = -8.686X + 121.062$ and $R^2 = 0.963$; for FRAP, $Y = 0.9476X + 2.278$ and $R^2 = 0.98$). A linear relationship was presented between antioxidant capability and phenolic content (for DPPH$^{\bullet}$, $Y = -8.628X + 344.5$ and $R^2 = 0.859$; for ABTS$^{\bullet+}$, $Y = -3.465X + 131.7$ and $R^2 = 0.886$; for FRAP, $Y = 0.377X + 1.113$ and $R^2 = 0.902$). BSME and BSMB contained higher phenolic compound content and exhibited higher antioxidant capabilities (Figures 5 and 6). This was in agreement with the results of Park et al., who reported that ethyl acetate and *n*-butanol extract of bamboo shoot (*Sasa borealis*) exhibited significant antioxidant capacity related to phenolic compound content.[8] Correlations between the antioxidant capacity of plant extracts and phenolic content were observed.[37] However, some results seem controversial,[38] possibly because of the method adopted. The Folin-Ciocalteu assay is a simple and widely used way to determine the total phenolic content of fruits and vegetables. However, this agent is specific for not only phenolic compounds but also active hydroxyl groups, including sugars, amino acids, and aromatic amines.[39] Some interfering substances such as protein and amino acid may affect its detection and provide an explanation for this result.

In recent years, there has been considerable interest in the potential for using natural food components as functional foods to treat hypertension, especially for patients with borderline to mild high blood pressure that does not warrant the prescription of antihypertensive drugs.[30] Bamboo shoot has been regarded as a kind of ideal vegetable and medicinal material for the prevention of hypertension. BSP derived from byproducts of canned bamboo shoots showed both antihypertensive and antioxidant activity *in vitro* and short-term (0−8 h) antihypertensive activity *in vivo*. Asp-Tyr was isolated and identified as the key active component of bamboo shoot ACE inhibitory peptide. Antioxidant capacities were attributed to phenolic compounds, such as ferulic acid and *p*-coumaric acid. Combined with the 4 week assay of BSP on SHRs in our previous work,[9] these findings suggested that BSP, exerting both ACE inhibitory and antioxidant activity, could be a good source of multifunctional food for the prevention of hypertension and attenuation of oxidative stress.

AUTHOR INFORMATION

Corresponding Author

*E-mail: yzhang@zju.edu.cn. Phone: +86-571-88982164. Fax: +86-571-88982164.

Funding

This work was financially supported by the National Natural Science Foundation of China (31271847) and the National Basic Research Program of China (973 Program) (2012CB720806).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

ACE, angiotensin converting enzyme; Ang, angiotensin; DPPH$^{\bullet}$, 1,1-diphenyl-2-picrylhydrazyl radical 2,2-diphenyl-1-(2,4,6-trinitrophenyl)hydrazyl; ABTS$^{\bullet+}$, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid); TPTZ, 2,4,6-tri(2′-pyridyl)-1,3,5-triazine; HHL, hippuryl-L-histidyl-L-leucine; FRAP, ferric-reducing antioxidant power; SEC, size exclusion chromatography; HPLC, high-performance liquid chromatography; UPLC, ultra-high-performance liquid chromatography; MS, mass spectrometry; AEBS, aqueous extract fractions from byproducts of processing of canned bamboo shoots, which included boiled water, filling liquid, and squeezed juice; BP5K, aqueous extract fractions from byproducts of canned bamboo shoots, with a molecular mass of <5 kDa; BSP, purified fraction by macroporous resin DA201-C from BP5K; BSME, ethyl acetate fraction of BSP; BSMB, *n*-butanol fraction of BSP; BSML, remaining water residual fraction of BSP

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