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Original Research Article

Nutritional assessment and effects of heat processing on digestibility of Chinese sweet potato protein

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ABSTRACT

The amino acid composition, *in vitro* and *in vivo* digestibility as well as trypsin inhibitor activity (TIA) of sweet potato protein (SPP) were evaluated. The effects of different types of heat processing on the *in vitro* digestibility and TIA of SPP were also investigated. The results showed that SPP was deficient in lysine, but rich in threonine, valine, tryptophan and aromatic amino acids. SDS–PAGE analysis showed that native SPP was not easily digested by pepsin–pancreatin enzyme system, whereas commercial soy protein isolate (SPI) displayed a good *in vitro* digestibility. Autoclaving (127 °C for 20 min, 0.145 MPa) significantly improved *in vitro* and *in vivo* digestibility of SPP. The *in vivo* digestibility of autoclaved SPP was 95.1%, which was comparable to that of SPI (96.1%) and casein (97.4%), and remarkably higher than that of native SPP (50.4%). PDCAAS of native SPP and autoclaved SPP were 0.36 and 0.66, respectively. In addition, autoclaving also markedly decreased TIA of native SPP from 67.8 to 2.0 mg trypsin/g protein. Autoclaving enhanced *in vitro* digestibility and decreased TIA of native SPP, thereby improving its food qualities. Although SPP was deficient in lysine, autoclaved SPP could be utilized as a good protein source for human consumption.

1. Introduction

In recent years, due to limited supply and increasing cost of animal protein, plant protein from sources like soy bean, lupin bean, chickpea, sorghum and zein have been widely developed and used as nutritional supplements and functional agents in food systems (Siddhuraju et al., 2001). And the search for these unconventional protein sources continues, to help meet the challenge of an ever-growing world population.

Sweet potato is the fourth largest food crop planted in China, with an annual production of 120 millions tons (Mu et al., 2009b; Zhang and Mu, 2011). Sweet potato cultivars contain 20.4–31.8 g/100 g starch (Noda et al., 2001), 0.49–2.24 g/100 g crude protein on a fresh weight basis (Purcell et al., 1972), along with other components such as dietary fibre, pectin, beta-carotene and vitamin C (Mei et al., 2010). A large percentage of the sweet potatoes produced in China is used for production of starch and related products (Mei et al., 2010), and the resultant starch waste water containing useful component-like protein is discarded into the environment. It has been reported that starch waste water effluent contains approximately 1.50 g/100 g crude protein (Mu et al., 2009b). This starch effluent not only constitutes an environmental problem, but also amounts to a waste of resources (Guo and Mu, 2011). Thus, isolation of protein from this sweet potato starch waste water could be an inexpensive way of obtaining SPP for human consumption. Furthermore, isolation of protein from this liquid waste would reduce the organic load of the receiving surface water bodies.

The major storage protein in sweet potato root, which accounts for about 80% of the total root protein called "sporamin", has a molecular mass of approximately 25 kDa under reducing SDS–PAGE conditions. But under non-reducing SDS–PAGE conditions, molecular masses of 31 kDa and 22 kDa were reported, denoting sporamin A and B, respectively (Maeshima et al., 1985).

Although sweet potato protein (SPP) amino acid composition and limiting amino acid vary with cultivar, on the average, its essential amino acid distribution showed that it is nutritionally viable. Nagase (1957) could not find any limiting amino acid for SPP from a Japanese variety; Walter and Catignani (1981) observed sulfur-containing amino acid to be the first limiting amino acid and lysine to be the second limiting amino acid from the Jewel variety, while Mu et al. (2009b) reported lysine to be first limiting amino acids for variety 55-2, which is a table sweet potato variety.

It was reported that SPP has a strong trypsin inhibitor activity (TIA) (Yeh et al., 1997), which could limit effective utilization for human or animal nutrition. Heat processing has been widely used

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to improve the nutritional value of plant proteins. Kiran and Padmaja (2003) reported that heating at 100 °C or microwave boiling destroyed most of the TIA of sweet potatoes. In addition, heat also increases the *in vitro* protein digestibility of some plants, such as soybean protein (Guerrero-Beltrán et al., 2009), African yam bean protein (Adeyeye, 1997) and chickpea vicilin-like protein (Tavano and Neves, 2008), and this could be due to deactivation of trypsin inhibitors (TIs).

Currently, there is no information available on the amino acid composition, antinutritional factors and digestibility of SPP from Mi xuan No. 1 variety, commonly used for starch processing in China. Therefore the objective of this study was to evaluate the amino acid composition, antinutritional factors and digestibility of SPP from Mi xuan No.1. Also the effect of heat processing on the TIA and digestibility of SPP was studied, and SPP was compared with commercial soy protein isolate (SPI) and whey protein isolate (WPI) to confirm nutritional value of SPP. Furthermore, the relationship between the digestibility and TIA of SPP was investigated.

2. Materials and methods

2.1. Materials

Sweet potatoes (*Ipomoea batatas* L.) of variety Mi xuan No. 1 used in this study were cultivated in the area surrounding Beijing (China) and supplied in three different batches of 50 kg each by a sweet potato starch factory in Miyun County (Beijing). They were harvested in the early October harvesting season and then stored at 10–14 °C. The three batches of SPP used in this study were prepared within 2 months after harvesting. SPI was purchased from Shandong Yu Wang Industrial Co. Ltd. (China) and contained 78.5 g protein, 6.4 g moisture and 0.6 g fat per 100 g. WPI was purchased from Le Sueur Cheese Co. (Le Sueur, MN, USA) and contained 95.0 g protein and 5.0 g moisture per 100 g. Ash in both SPI and WPI was less than 3.0 g/100 g. Casein (protein content is 85.0 g/100 g) and other ingredients of diet used in the rat balance experiment were donated by Beijing HFK bioscience Co. Ltd. (China).

Pepsin (7500), porcine pancreatin (p7545), trypsin (T1475, 12,885 units/mg solid), benzoyl-DL-arginine-*p*-nitroanilide (BAPNA), molecular weight marker (from 14,200 to 66,000 Da), SDS, Tris Base and N,N,N′,N′-tetramethylethylenediamine (TEMED) were purchased from Sigma Chemical Company. β-Mercaptoethanol was purchased from Amresco Company (USA). Coomassie Brilliant Blue R-250 was purchased from Fluka Company (USA). All other chemicals were of analytical or better grade.

2.2. Isolation of sweet potato protein

SPP was prepared by isoelectric precipitation as described by Mu et al. (2009a,b) with a minor modification. Briefly, fresh sweet potatoes (~50 kg) were washed with water, cut, sliced and then placed in 10 mg/mL $NaHSO_3$ solution. They were then ground and centrifuged at 10,000 × *g* for 1 h at 5 °C. The supernatant was adjusted to pH 4.0 with 1 mol/L HCl and the precipitate obtained was resolubilized in water; the pH was adjusted to 7.0 with 1 mol/L NaOH and the solution was ultrafiltered and lyophilized. The protein isolate obtained was store at −40 °C prior to analysis. The protein contents were determined by Kjeldhal method (Matissek et al., 1989) using Foss Tecator automatic protein analyser (2300 Kjeltec analyzer unit) after digestion with detection limit of 0.1 mg N. Nitrogen-to-protein conversion factors of 6.38, 5.71 and 6.25 were used for WPI, SPI and SPP, respectively. The fat, ash and moisture contents of SPP were determined using standard AOAC methods (2005).

2.3. Amino acid composition

Amino acid compositions of the protein samples were determined using a high-speed amino acid autoanalyzer (Hitachi L-8800, Japan). Reproducibility of peak retention time with coefficient of variation (CV) = 0.3% (Arg); peak area CV = 1.0% (Gly, His) and detection limit of 3 pmol (Asp) were obtained. Sodium hydroxide (5 mol/L) was used for hydrolysis in the tryptophan determination. Cystine and methionine were determined after performic acid oxidation. Other amino acids were subjected to acid hydrolysis in the presence of 6 mol/L HCl at 110 ± 1 °C for 24 h, and then the pH was adjusted to 2.2 using 0.2 mol/L sodium citrate buffer solution. These analyses were performed in triplicate with three independent protein samples.

2.4. Amino acid scores

Amino acid scores (AAS) were calculated by the DeSantiago et al. (1999) method.

$$\mathrm{AAS}(\%) = \frac{A_x \times 100}{A_s}$$

where A_x is the amino acid content of test protein (mg/g of protein) and A_s is the amino acid content of requirements for children 2–5 years old recommended by FAO/WHO (1991).

2.5. Heat processing

Each protein (SPP, SPI, WPI) was dispersed in distilled water (3 mg/mL). Different methods were used for heat processing, and the conditions were as follows: boiling at 100 °C for 20 min and for 1 h; microwave boiling at 700 W for 3 min; autoclaving at 110 °C (0.048 MPa) for 20 min; autoclaving at 127 °C (0.145 MPa) for 20 min; drying in an oven at 130 °C for 1 h. These analyses were performed in triplicate with three independent protein samples.

2.6. Sequential in vitro protein digestion procedure

In vitro digestibility of protein was determined by the modified method of Vilela et al. (2006). Briefly, three replicate samples of each protein were dispersed in 50 mL distilled water with a final protein concentration of 3 mg protein/mL. The pH value was then adjusted to 1.5 with 1 mol/L HCl. The 10 mL samples of the solution were preheated to 37 °C for 10 min in a water bath and then hydrolyzed with porcine pepsin (5 mol/L with 0.01 mol/L HCl, the enzyme to substrate ratio was 1:100, w/w) at 37 °C for 5, 10, 20 or 30 min with continuous shaking at 10.5 rad/s in a shaking incubator (DSHZ-300A, Tai Cang experimental equipment factory, China). Then the pH of each solution was adjusted to 6.0 with 4 mL 1 mol/L NaOH to stop the reaction and used for SDS–PAGE analysis. Secondly, the aliquot digested with pepsin for 30 min was adjusted to pH 7.8 with 1 mol/L NaOH, heated at 40 °C for 10 min on a water bath and then further hydrolyzed with pancreatin (5 mol/L in pH 7.0 phosphate buffer, the enzyme/substrate ratio was 1:30, w/w) at 40 °C for 30, 60 or 120 min. At the end of the hydrolysis period, the enzymatic reactions were stopped with addition of 100 μL of 0.15 mol/L Na_2CO_3. The digestates were then used for the *in vitro* digestibility and SDS–PAGE analysis.

2.7. Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE)

SDS–PAGE under reducing conditions was performed according to the method described by Laemmli (1970) using an AE-6450 electrophoresis system (Atto Corporation, Tokyo, Japan). The digestates were directly dissolved (4:1, v/v) in the sample buffer,

containing 5 mL 0.5 mol/L Tris–HCl buffer (pH 6.8), 2.5 mL β-mercaptoethanol, 12.5 mL 60 mmol/L Na_2EDTA, 2.5 g SDS, 250 μL bromophenol blue and 30 g glycerol in 50 mL distilled water (pH 6.8). After boiling at 100 °C for 2 min, the digested samples were centrifuged at 10,000 × *g* for 20 min. The 15-μL digestibility samples and 10-μL marker solutions were placed into wells. Gel electrophoresis was run on 5% loading gels and 15% separating gels using a discontinuous buffer system at 30 mA until the tracking dye reached the bottom of the gel. The gels were stained using Coomassie Brilliant Blue R-250 method and distained with methanol wash solution. The appearances of the gels were recorded by an image forming system (Fluor Chem. FC2, Alpha Innotech Corporation, USA).

2.8. In vitro digestibility

The aliquot that was digested by pepsin for 30 min and pancreatin for another 120 min was used for the *in vitro* digestibility test. The digested aliquot was put into Millipore centrifugal ultrafiltration filters (10 kDa cut-off, 15 mL and 29.7 mm × 122 mm) and centrifuged at 3500 × *g* for 50 min. The protein content in the supernatant was determined by the modified Lowry method (Peterson, 1977; Markwell et al., 1978). Bovine serum albumin (BSA) was used for the standard calibration curve with linear range for BSA equals 10–100 μg/mL (r^2 = 0.9996). The detection limit was 5 μg/mL.

In vitro protein digestibility (%) was calculated as follows: Digestibility (%) = the protein content in the supernatant/total protein content × 100. These analyses were performed in triplicate for each sample.

2.9. In vivo rat assay for true protein digestibility

The modified method of Wong et al. (2004) was used for the *in vivo* digestibility. The rats (Wister, 3 weeks, 50–70 g) were divided into five groups, each consisting of eight rats (4 male and 4 female), and were housed in individual metabolic cages kept under the conditions of 18–26 °C, 40–70% relative humidity and with 12 h light/dark cycle. All animals had free access to water and diets, but restrict diets to 15 g/day. All diets contained 10.0 g of protein (except for protein-free group), 13.2 g dextrinzed cornstarch, 10.0 g sucrose, 3.5 g AIN minerals mixed, 7.0 g corn oil, 1.0 g AIN vitamins mixed, 5.0 g cellulose, 0.2 g choline bitartrate, 0.3 g L-cystine, and corn starch to equal 100 g.

The rat balance method (28 d) used in this study consisted of a 5-day preliminary period, during which the rats were allowed to adapt to the diets and experimental conditions, followed by a 5-day balance period. During the balance period, faeces, urinary output and spilled food were collected daily and separately for each rat. The percent nitrogen contents of faeces, urinary output and spilled food were analyzed using Kjeldhal method. The endogenous or metabolic nitrogen loss was determined from the faeces of the rats fed the protein-free diet.

Nitrogen retention (NR), apparent digestibility (AD), true digestibility (TD) and net protein utilization (NPU) of protein were calculated according to the following formulas:

$$\mathrm{NR} = \mathrm{I} - (\mathrm{FN} + \mathrm{UN})$$

$$\mathrm{AD} = \frac{[\mathrm{I} - \mathrm{FN}] \times 100}{\mathrm{I}}$$

$$\mathrm{TD} = \frac{[\mathrm{I} - (\mathrm{FN} - \mathrm{EFN})] \times 100}{\mathrm{I}}$$

$$\mathrm{NPU} = \frac{[\mathrm{I} - (\mathrm{FN} - \mathrm{EFN}) - (\mathrm{UN} - \mathrm{EUN})]}{\mathrm{I}}$$

$$\mathrm{PDCAAS} = \text{true protein digestibility} \times \text{lowest limiting amino acid score},$$

where I is nitrogen intake of the test group; FN is faecal nitrogen of the test group; EFN is endogenous faecal nitrogen excreted by the protein-free group; UN is urinary nitrogen excreted; EUN is endogenous urinary nitrogen excreted by the protein-free group; PDCAAS is the protein digestibility-corrected amino acid score calculated according to the method of El and Kavas (1996) using the limiting essential amino acid composition of the test sample and the amino acid pattern suggested by FAO/WHO (1990) for children 2–5 years old.

2.10. Determination of TIA

TIA was determined by the method of Smith et al. (1980) using trypsin and the synthetic substrate BAPNA. Briefly, 1 g of protein sample was extracted with 50 mL of 0.01 mol/L NaOH. The pH was adjusted to 9.5. After stirring for 3 h at room temperature, 1 mL of each protein extract was respectively placed in test tube and 1 mL distilled water was added as sample blank (c), followed by addition of 2 mL of trypsin solution (2 mg/100 mL of 0.001 mol/L HCl) as sample solution (d). Distilled water (2 mL) was as a reagent blank (a), and standard trypsin solution (2 mL trypsin solution and 2 mL distilled water) was as a standard (b). After pre-heating to 37 °C for 10 min, 5 mL BAPNA solution (previously warmed to 37 °C) was pipetted into each tube and mixed. After exactly 10 min incubation at 37 °C, each tube received 1 mL acetic acid (5.2 mol/L) to stop the reaction. Trypsin solution (2 mL) was then added to the reagent blank (a) and sample blank (c) tubes. The solution was filtered through Whatman No. 2 paper, and the absorbance was measured at 410 nm. These analyses were performed in triplicate with three independent protein samples.

$$\mathrm{TIA(mg\ trypsin/g\ protein)} = \frac{2.632 \times D \times A_\mathrm{I}}{w},$$

where D is dilution factor; $A_\mathrm{I} = (A_\mathrm{b} - A_\mathrm{a}) - (A_\mathrm{d} - A_\mathrm{c})$; A_a is absorbance of reagent blank; A_b is absorbance of standard trypsin solution; A_c is absorbance of sample blanks; A_d is absorbance of sample solution; w is the protein weight of each sample. The percentage inhibition in each sample tube is given by $100A_\mathrm{I}/(A_\mathrm{b} - A_\mathrm{a})$. Percent inhibition values <40% or >60%, were repeated to make a more suitable dilution of the sample suspension.

2.11. Statistical analysis

The results presented in tables and figures were reported as means ± standard deviation (SD). The differences between means were established with Duncan's Multiple Range Tests ($P < 0.05$) using SAS software (version 8.1).

3. Results and discussion

3.1. Amino acid composition analysis

The protein content of SPP was 69.0 ± 0.6 g/100 g. The fat, ash and moisture contents of SPP were 0.6 ± 0.0, 3.9 ± 0.8 and 8.0 ± 0.1 g/100 g, respectively. Amino acid compositions of SPP, SPI and WPI are shown in Table 1. The total content of essential amino acids (EAA) in SPP was 402 mg/g protein and accounted for 40% of the total amino acid content, which is slightly higher than the reference value of 40% recommended by FAO/WHO (1991), higher than the value of 35% for SPI but lower than the value of 46% for WPI. In addition, the ratio of EAA to non-essential amino acid (NEAA) for SPP

Table 1
Amino acid compositions of three proteins from different sources (mg/g protein).

EAA	SPP	SPI	WPI	NEAA	SPP	SPI	WPI
Ile	48.5	44.2	54.4	Asp	163	114	113
Met	21.3	11.8	21.9	Ser	64.2	53.5	39.4
Val	72.8	47.5	51.5	Glu	87.5	197	172
Leu	68.6	80.0	129	Pro	30.2	30.1	30.0
Trp	16.8	17.7	14.7	Gly	45.4	40.6	16.8
Phe	71.3	53.0	35.0	Ala	46.9	40.1	50.6
Thr	61.3	38.1	48.1	Cys	14.7	8.9	26.7
Lys	41.3	60.2	103	Tyr	52.7	37.6	36.1
total	402	352	458	His	34.4	43.0	31.6
				Arg	59.5	82.7	26.3
				total	598	647	542
EAA/(EAA + NEAA)	40%	35%	46%				
EAA/NEAA	67%	54%	84%				

EAA, essential amino acid participation; NEAA, non-essential amino acid participation; SPP, sweet potato protein; SPI, soy protein isolate; WPI, whey protein isolate. Values are means of three determinations.

was 67%, which is markedly higher than the reference protein pattern of 60% recommended by FAO/WHO (1991) and SPI (54%) but lower than that of WPI (84%). SPP had higher contents of threonine, valine and total aromatic amino acids (phenylalanine + tyrosine) than SPI and WPI. The tryptophan content of SPP was also higher than WPI. In SPI, sulfur-containing amino acids were the first limiting amino acid. Similar results were reported by Wang et al. (2010), who indicated that the limiting amino acid in SPI was sulfur-containing amino acids. The AAS values for WPI were not less than 100%, which suggests WPI has nutritionally adequate amino acid composition with no limiting amino acids.

The AAS values (Table 2) showed that lysine was the limiting amino acid in SPP. This result is in accordance with the previous report of Mu et al. (2009b) on SPP from 55-2 sweet potato variety, in which lysine was the first limiting amino acid. This is however different from the report of Walter and Catignani (1981), who found that sulfur-containing amino acids were the first limiting amino acid of SPP from Jewel sweet potato variety. This variation could be due to varietal differences or reduction in the protein lysine content induced by Millard reaction during storage. Lysine is an ε-amino group in protein which is particularly susceptible to conjugation with the reducing end of polysaccharide carbonyl group through Millard reaction (Hurrell and Carpenter, 1981).

3.2. In vitro digestibility of SPP analyzed by SDS–PAGE

The *in vitro* digestibility of native SPP in comparison with that of commercial SPI, using the digestive pepsin–pancreatin system for different digestion times, are shown on reducing SDS–PAGE electrophoretogram (Fig. 1). SPP had one main band (Fig. 1a, Lane 2) which is similar to the report of Maeshima et al. (1985). After pepsin hydrolysis for 30 min, the main band of native SPP remained. On further hydrolysis with pancreatin, the main bands of native SPP still had no visible change, although the bands of pancreatin hydrolysates became less dense. These results indicated that SPP could not be easily digested by pepsin–pancreatin enzymatic treatment.

The SDS–PAGE pattern of SPI differed from that of native SPP (Fig. 1b). Under reducing conditions, the SPI bands corresponding to 7S (α, 68 kDa; β, 52 kDa) and 11S (AS, 35 kDa; BS, 20 kDa) were detected (lane 2). After pepsin digestion for 30 min, only the β fraction was digested completely (Fig. 1b, lane 6). Although α, AS and BS were not easily digested by pepsin, they were digested completely after pancreatin digestion for 30 min (Fig. 1b, lane 7).

The results above suggest that native SPP was resistant to digestion than SPI. Thus, to ensure better utilization of SPP, improvement of the *in vitro* digestibility of SPP is quite necessary.

3.3. Effect of heat on in vitro digestibility of SPP

To improve the *in vitro* digestibility of native SPP, different heat processing methods were investigated. Table 3 shows the effects of different types of heat processing on the *in vitro* digestibility of SPP compared with SPI and WPI.

The *in vitro* digestibility of SPP improved significantly ($P < 0.05$) by boiling, microwave boiling, drying and autoclaving, among which autoclaving was the most effective method (Table 3). After autoclave treatment, the *in vitro* digestibility of SPP increased from 52.8% to 99.2%, to give approximately the same digestibility value as obtainable with SPI and WPI. Autoclaving was also the best way to improve the *in vitro* digestibility of commercial SPI, increasing its digestibility by 7.8%. Drying significantly ($P < 0.05$) increased SPP *in vitro* digestibility, but reduced that of commercial SPI by 5.2% and had no effect on WPI. For the boiling process, the *in vitro*

Table 2
Amino acid scores (AAS,%) of three proteins from different sources.

Amino acids	SPP	SPI	WPI	FAO/WHO/UNU requirements for children 2–5 years old (mg/g protein)
Phe + Tyr	197	144	113	63
Ile	174	158	194	28
Leu	104	121	195	66
Lys	71[a]	104	178	58
Met + Cys	144	83[a]	195	25
Thr	180	112	141	34
Trp	152	161	134	11
Val	208	136	147	35
His	181	226	166	19

SPP, sweet potato protein; SPI, soy protein isolate; WPI, whey protein isolate.
[a] Represent first limiting amino acid.

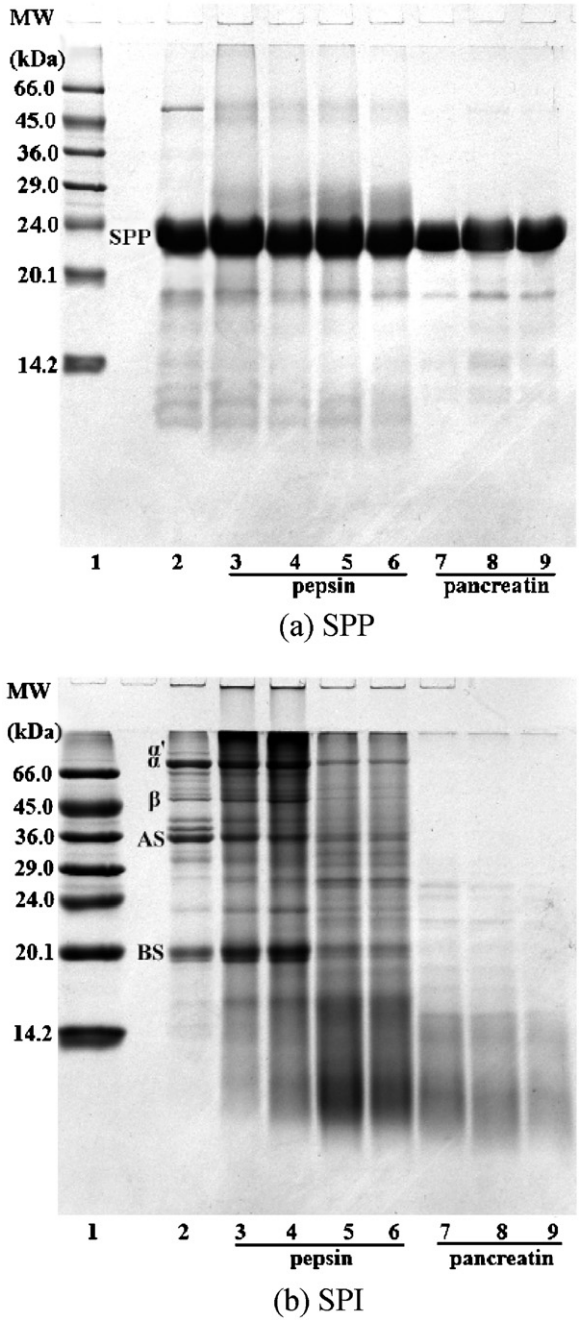

Fig. 1. Reducing SDS–PAGE profiles for sweet potato protein (SPP) and soy protein isolate (SPI) digested with successive pepsin and pancreatin. Lane 1, marker; lanes 2–6, protein digested by pepsin for 0, 5, 10, 20 and 30 min, respectively; lanes 7–9, the protein pepsin-hydrolysate further digested by pancreatin for 30, 60 and 120 min, respectively.

digestibility of SPP increased with increasing heating time. Whereas boiling at 100 °C for 20 min increased the *in vitro* digestibility of SPP by 46.1% compared with that of native SPP, boiling same for 1 h further increased *in vitro* digestibility to 62.2%. On the overall, the effects of heat processing on the *in vitro* digestibility of SPP were in the order: autoclaving > microwave boiling > boiling > drying.

The electrophoretograms of *in vitro* digestion of different heat treated SPP are shown in Fig. 2. *In vitro* digestion of SPP showed varying degrees of improvement by different heat processing methods. After drying (at 130 °C for 1 h) and boiling (at 100 °C for 20 min), the main bands of SPP (lanes 5 and 6, respectively) still

Table 3
In vitro digestibility (%) of three proteins treated with different heat processing methods.

Heat processing methods	SPP	SPI	WPI
Native	52.8 ± 0.7^{f}	92.3 ± 0.7^{b}	97.0 ± 0.7^{c}
Boiling at 100 °C for 20 min	77.2 ± 0.1^{d}	88.3 ± 0.2^{c}	99.8 ± 0.1^{a}
Boiling at 100 °C for 1 h	85.7 ± 1.4^{c}	92.8 ± 0.6^{b}	98.8 ± 0.0^{b}
Microwave boiling at 700 W for 3 min	94.1 ± 1.8^{b}	92.7 ± 0.7^{b}	98.8 ± 0.2^{b}
Drying at 130 °C for 1 h	54.7 ± 0.4^{e}	87.5 ± 0.5^{c}	97.0 ± 0.9^{c}
Autoclaving at 110 °C for 20 min	98.7 ± 0.2^{a}	98.9 ± 0.3^{a}	99.3 ± 0.4^{a}
Autoclaving at 127 °C for 20 min	99.2 ± 0.1^{a}	99.4 ± 0.2^{a}	99.8 ± 0.2^{a}

SPP, sweet potato protein; SPI, soy protein isolate; WPI, whey protein isolate. Values are means ± standard deviation of three determinations ($n = 3$). a–f indicate significant differences ($P < 0.05$) in the digestibility of the same protein by different processing methods.

remained, though they became smaller. However, after autoclaving (lane 4), boiling at 100 °C for 1 h (lane 7) or microwaving (lane 8), the main bands of SPP disappeared completely. These results further corroborates our earlier claim on *in vitro* digestibility of heat processed SPP.

3.4. In vivo digestibility of SPP

In the *in vitro* experiment described above, native SPP could not be hydrolyzed completely by pepsin–pancreatin, but heat treated SPP was easily digested, especially autoclaving processing. Therefore, to confirm these results, we carried out an *in vivo* experiment using rat to determine the true digestibility of native and autoclaved SPP (Table 4). True digestibility and biological values of native and autoclaved SPP (ASPP) are shown in Table 4.

The results of the animal studies showed that the TD of ASPP increased significantly ($P < 0.05$) when compared with native SPP. TD of native SPP and ASPP were 50.4% and 95.1%, respectively. As

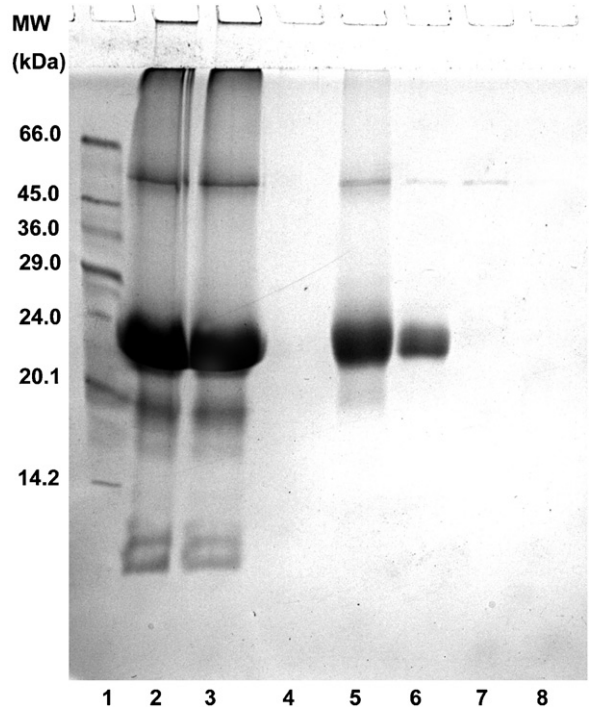

Fig. 2. Reducing SDS–PAGE profiles of *in vitro* digestibility of different heat treated sweet potato protein (SPP) after pepsin digested for 30 min and further digested by pancreatin for 120 min. Lane 1, marker; lane 2, native SPP; lane 3, hydrolysate of native SPP; lane 4, hydrolysate of autoclaved SPP at 127 °C (0.145 MPa) for 20 min; lane 5, hydrolysate of dried SPP at 130 °C for 1 h; lane 6, hydrolysate of boiled SPP at 100 °C for 20 min; lane 7, hydrolysate of boiled SPP at 100 °C for 1 h; lane 8, hydrolysate of microwave boiled SPP at 700 W for 3 min.

Table 4
In vivo digestibility and biological values of rats fed with SPP and ASPP diets.

	SPP	ASPP	Casein	SPI
NI (g/rat day)	0.94 ± 0.47^{a}	1.45 ± 0.50^{a}	1.46 ± 0.41^{a}	1.31 ± 0.67^{a}
UN (mg/rat day)	130.9 ± 71.9^{a}	75.9 ± 35.3^{a}	78.0 ± 33.1^{a}	94.6 ± 13.2^{a}
FN (mg/rat day)	517.5 ± 21.7^{a}	120.2 ± 46.4^{b}	87.4 ± 6.8^{b}	100.8 ± 28.1^{b}
NR (g/rat day)	0.30 ± 0.28^{b}	1.25 ± 0.42^{a}	1.30 ± 0.36^{a}	1.11 ± 0.63^{a}
NPU (%)	39.9 ± 0.8^{c}	92.0 ± 1.0^{b}	94.2 ± 1.1^{a}	91.2 ± 1.1^{b}
AD (%)	45.1 ± 0.7^{c}	91.7 ± 0.9^{b}	94.0 ± 1.0^{a}	92.3 ± 0.9^{b}
TD (%)	50.4 ± 1.2^{b}	95.1 ± 3.1^{a}	97.4 ± 0.4^{a}	96.1 ± 2.5^{a}
PDCAAS	0.36 ± 0.04^{d}	0.66 ± 0.02^{c}	0.97 ± 0.03^{a}	0.80 ± 0.01^{b}

SPP, sweet potato protein; ASPP, autoclaved sweet potato protein; SPI, soy protein isolate; NI, nitrogen intake; UN, urinary nitrogen; FN, fecal nitrogen; NR, nitrogen retention; NPU, net protein utilization; AD, apparent digestibility; TD, true digestibility; PDCAAS, protein digestibility corrected amino acid score. Values are means ± standard deviation of eight rats, each in triplicate ($n = 8$). Different letters in the same row indicate different levels of significance ($P < 0.05$).

expected, rats fed with casein (control) diet utilized the dietary protein more efficiently when compared to their counterparts fed with the native SPP and autoclaved SPP (Table 4). But there is no significantly ($P < 0.05$) difference among the TD of casein (97.4%), SPI (96.1%) and ASPP (95.1%).

The nitrogen intake (NI) pattern indicated that there was no significantly ($P < 0.05$) difference among rats fed with the different diets. Rats fed with ASPP and SPI had significantly ($P < 0.05$) the same apparent digestibility (AD), nitrogen retention (NR), net protein utilization (NPU) values but significantly ($P < 0.05$) higher than that of rats based on native SPP diet.

3.5. Protein Digestibility Corrected Amino Acid Score (PDCAAS)

The PDCAAS method is the most appropriate to estimate the food protein quality for humans. Because it allows evaluation of food protein quality based on both the amino acid requirements of humans and their ability to digest it (Schaafsma, 2000). The highest PDCAAS value that any protein can achieve is 1.0. This score means that, after digestion of the food protein, one unit of protein provides 100% or more of the indispensable amino acids required by the 2–5 years old child (Usydus et al., 2009).

As shown in Table 4, autoclaving process significantly ($P < 0.05$) increased PDCAAS value of native SPP with the PDCAAS value of ASPP running to twice that of native SPP. In addition, the PDCAAS values showed that SPI had more nutritionally available protein than native SPP. This is contrary the impression gained from the earlier AAS values in which both native SPP and SPI had comparatively the same AAS values. This therefore further confirms PDCAAS which takes true digestibility into consideration as a better predictor of protein nutritional value and the empirical nature AAS as protein nutritional value determinant.

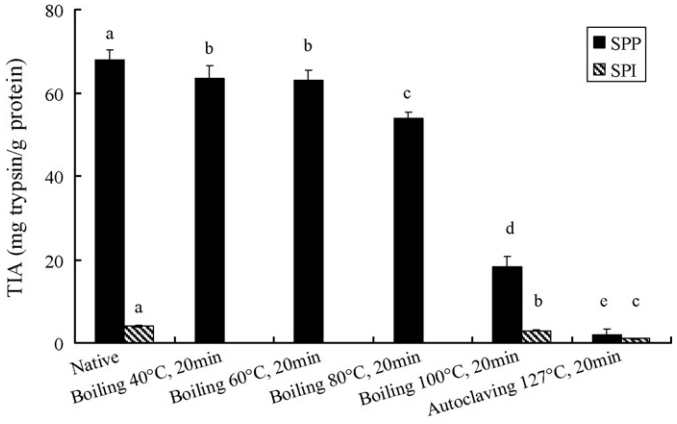

Fig. 3. Effects of different heat processing methods on the TIA (mg trypsin/g protein) of sweet potato protein (SPP) and soy protein isolate (SPI). a–e indicates different significance levels for different processing methods for the same protein ($P < 0.05$).

It was reported that heat treatment could increase the accessibility of proteins to enzymatic attack (Zhou and Han, 2006), which might be the reason for the improved ASPP digestibility. Liener (1976) also indicated that the inactivation of antinutritional factors is one of the main reasons for the increase in protein digestibility after heat treatment. This prompted our further investigation of the antinutritional factors of SPP before and after heat treatment.

3.6. SPP trypsin inhibitor activity

Trypsin inhibitor (TI) is one of the common antinutritional factors present among plant proteins that inhibit digestion and absorption of plant proteins and may even be harmful to human health (Valle et al., 1983).

TIAs of native SPP and commercial SPI are shown in Fig. 3. TIA of native SPP (67.8 ± 2.6 mg trypsin/g protein) was higher than that of SPI (4.0 ± 0.2 mg trypsin/g protein). Some researchers reported that there is a Kunitz trypsin inhibitor in sweet potato (Suguria et al., 1973; Yao et al., 2001; Yeh et al., 1997), which demonstrates a strong TIA. Lin (1993) proposed that the water soluble protein sporamin in sweet potato tuberous roots could be one form of trypsin inhibitor, which was confirmed later by Yeh et al. (1997). In addition, Yao et al. (2001) reported that two inter-chain disulfide bonds of sporamin are very important for TI stabilization and that the two acidic residues in the sporamin A4-B1 loop, Asp70 and Glu72, are critical for inhibitory function. It has been reported that soybean has two major types of trypsin inhibitor, the Kunitz trypsin inhibitor and the Bowman-Birk trypsin inhibitor (Huang et al., 2008), which impede the use of soybean in human food. According to the European Federation of Feed Manufacturers (Monari, 1993), the threshold value for feeding soy products is 4 mg/g for residual TIA. TIA of commercial SPI in this paper met this standard, but native SPP required some treatment to inactivate TIs.

3.7. Effect of heat processing on TIA of SPP

The effects of heat processing on TIA of native SPP and commercial SPI are shown in Fig. 3. Similar trends were observed for SPP and SPI, and there were significant differences in TIA between heated samples and unheated samples ($P < 0.05$). TIA of SPP decreased with increasing temperature. After boiling at 40, 60 or 80 °C for 20 min, TIA of SPP displayed a small but significant decrease. Whereas TIA was remarkably reduced to 18.42 mg trypsin/g protein after boiling at 100 °C for 20 min. Kiran and Padmaja (2003) also found that TI of sweet potatoes could be rapidly inactivated upon heating at 100 °C. Furthermore, autoclaving (127 °C for 20 min, 0.145 MPa) was more effective in inactivating TI than boiling. TIA of the autoclaved SPP was just 2.05 mg trypsin/g protein, reaching the standard of the European Federation of Feed Manufacturers (Monari, 1993). In addition, TIA of SPI also decreased significantly ($P < 0.05$) after boiling and

autoclaving. According to Hout et al. (1998), heat treatment at high temperatures is an effective physical method for deactivation of TI.

In this study, high temperature heat treatment had significant effect on digestibility and TIA of SPP and SPI. The *in vitro* digestibility (Table 3) and TIA (Fig. 3) of SPP were inversely related, which was similar to results on soybean protein (Guerrero-Beltrán et al., 2009) and African yam bean protein (Adeyeye, 1997). It was suggested that the increase of digestibility was related to the destruction of TIA, which was corroborated by the results of this study.

The improvement in the digestibility of SPP after heat treatment could be due to the reduction in TIA, denaturation and unfolding of the protein. High temperature heat treatment might not only destroy the reactive site loop that is responsible for inhibiting trypsin to eliminate TIA of SPP, but also increase the accessibility of the protein to enzymatic attack. However, further study is needed to clarify the mechanism underlying the effect of heat on SPP digestibility.

4. Conclusions

The present study showed that the percentage of EAAs in total amino acids of SPP was 40% and the ratio of EAAs to non-EAAs was 67%, which shows the nutritional value of SPP. Lysine was the first limiting amino acid in SPP. Although the *in vitro* digestibility of native SPP was lower than commercial SPI and WPI, it was significantly ($P < 0.05$) enhanced by different heat treatments. After the autoclaving process, the *in vitro* digestibility of SPP was comparable to that of SPI and WPI. True digestibility of autoclaved SPP reached 95.1%, which was significantly ($P < 0.05$) higher than 50.4% of native SPP. This was attributed to destruction of the TIA of this SPP during heat processing. In addition, autoclaving process improved PDCAAS of SPP from 0.36 to 0.66. This study may provide valuable information on the potential application of SPP as novel protein resource in the food industry.

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