Effects of synthetic and natural extraction chemicals on yield, composition and protein quality of soy protein isolates extracted from full-fat and defatted flours

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Abstract With increasing preference for all-natural foods to those involving synthetic chemicals, native isoelectrically precipitated soy protein isolate (SPI) was prepared using amaranth (*Amaranthus tricolor* L.) lye (pH>12.5) and lemon extract, (pH<2.5) as natural, food-plant-based chemicals. Protein content (91.21 %), yield (43.62 %) and digestibility correlation amino acid score (0.77) were obtained and were comparable to those of SPI prepared using synthetic chemicals (NaOH and HCl). Methionine and cysteine-s were significantly higher in the natural SPI while glutamine and serine were higher in synthetic SPI ($p<0.01$). Most of the determined minerals were higher in the natural SPI with potassium being the highest. Sodium was very high in the synthetic SPI. The rest of the minerals including phosphorus, iron and nickel, showed no significant difference. Anti-nutritional factors (trypsin inhibitors and phytic acid) were considerably lower in the natural SPI. Thus, a quality all-natural SPI can be produced using amaranth lye and lemon extract to address concerns regarding use of synthetic chemicals.

Keywords Soy protein isolate · Amaranth lye · Lemon extract · Natural chemicals · Synthetic chemicals

Abbreviations

AA	Amino acid
AAS	Amino acid score
ANOVA	One way analysis of variance
BAPA	Benzoyl-DL-arginine-p-nitroanalide hydrochloride
DF	Defatted soybean flour
FAO	Food and agriculture organization
FF	Full-fat soybean flour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
INAPP	International association of natural product producers
NaOH	Sodium hydroxide
NC	Natural chemical
NCDF	Natural chemical—deffated soybean flour prepared soy protein isolate
NCFE	Natural chemical—full-fat soybean flour prepared soy protein isolate
PDCAAS	Protein digestibility correlation amino acid score
RP-HPLC	Reverse phase high performance liquid chromatography
SC	Synthetic chemical
SCDF	Synthetic chemical—deffated soybean flour prepared soy protein isolate
SCFF	Synthetic chemical—full-fat soybean flour prepared soy protein isolate
SPI	Soy protein isolate
TCA	Trichloroacetic acid
TIs	Trypsin inhibitors
TIA	Trypsin inhibitor activity
IVPD	In vitro protein digestibility
USDA	United States department of agriculture
WHO	World health organization
UNU	United nations university

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Introduction

Recent years have witnessed fast growth of natural, organic and environmentally friendly or “green” food market. Skepticism about highly processed foods is increasing as many people are becoming more conscious about their health. Studies on food preferences have shown fear of exposure to non-food synthetic chemicals and environmental consciousness as main factors influencing this new food consumer behavior (Dickson-Spillmann et al. 2011). During the past few years, farmers have tried to produce food naturally without using synthetic chemicals. Likewise, manufacturers are exploring new food processing techniques to address the need for safer food and compete for consumer acceptance (Zink 1997).

Several modern and partially traditional methods are currently being employed commercially to process plant agricultural products, such as soybeans, into protein-enriched, fat-reduced compositions for use in food manufacturing. They include solvent extraction and a variety of press-based methods, e.g., extruder, expeller, continuous and cold presses, to separate at least a portion of the fat from the remaining plant material. Nevertheless, at some stage strong non-food synthetic chemicals are still used to finalize the process. Some of these chemicals are not natural, or plant based and cannot be used to produce certified organic food products under United States Department of Agriculture (USDA) guidelines for organic food labeling (Gold 2012). As such, a lot of improvements are yet to be made on highly processed foods.

Soy protein isolate (SPI) is one of the most important ingredients in many processed foods replacing animal based protein. However, processing of SPI itself requires application of strong food and non-food synthetic chemicals such as 95 % alcohol, hexane, sodium hydroxide (NaOH) and hydrochloric acid (HCl). Although these chemicals are removed during the process, having some small residues in the final SPI cannot be completely overruled. Additionally, a health conscious natural food consumer can hardly be convinced that foods processed using these chemicals are safe to eat. Dickson-Spillmann et al. (2011) reported that laypeople view chemicals as either safe or dangerous, and think that even minor doses of chemicals are likely to cause harm. To such people, ‘synthetic equals dangerous. The challenge lies on identifying natural and food-based reagents and chemicals that would produce similar or improved results as the synthetic ones. Amaranth ash solution has been used traditionally for food preparation as an alternative to bicarbonate of soda (alkaline). Nevertheless, information on the same can hardly be found. Lemon juice as citric acid is a well known food acid, but rarely mentioned in soybean products studies.

The aim of this study was to explore the possibility of producing soy protein isolate using purely natural, food-plant-based chemicals, and examine its yield, composition and

nutritive quality, with reference to one similarly prepared using the conventional synthetic chemicals.

Materials and methods

Materials

Edible green and purple colored amaranth (*amaranthus tri-color L.*) mature plants were obtained from local farmers in the outskirts of Wuxi city, Jiangsu province, China. Tender amaranth plants, lemons fruits and soybeans (single butch) were purchased from local supermarket. The soybeans were sorted, dehulled and ground into full-fat flour to pass through an 80 mesh sieve. On dry basis, the flour had about 35 % protein (N x 6.25), 23 % lipid, 5 % ash, 7 % moisture and protein dispersibility index (PDI) of 72 %. Amaranth ash solution (pH > 12.50) was prepared by filtering distilled water through burnt amaranth plant ash at the ash to water ratio of 1:5 (w/v). Lemon juice (pH < 2.20) was squeezed from lemon fruit and vacuum filtered. All other reagents and chemicals were of analytical grade.

Preparation of soy protein isolate

Four SPI samples namely; natural chemical full fat flour (NCFF), synthetic chemical full fat flour (SCFF), natural chemical defatted flour (NCDF) and synthetic chemical defatted flour (SCDF) were prepared using full-fat (FF) or defatted flour (DF), with either amaranth ash solution and lemon juice as natural chemicals (NC), or NaOH and HCl as synthetic chemicals (SC) by modified method of Li et al. (2007). The flour was suspended in distilled water at the ratio of 1:10 (w/v). The pH of the suspension was adjusted to and maintained at 7.0 with amaranth ash solution or NaOH, constantly stirred for 1 h at ambient temperature (around 23 °C). Supernatant was recovered after centrifugation at 10,000 × g, 4 °C for 30 min. Where the full-fat was used, the creamy, butter-like fat layer formed after centrifugation, was easily separated from the supernatant using a 100 mesh sieve. Soy protein was precipitated by adjusting pH to 4.5 with lemon juice or HCl then centrifuged at same conditions. The precipitate was washed and centrifuged twice before resuspended in distilled water and neutralized to pH 7.0 with amaranth ash solution or NaOH. The resolubilized protein was then freeze dried, sealed in polythene bags and stored at 4 °C until further analysis. Clark and Snyder (1989) rapid equilibrium extraction method, in the manner described by Clark and Proctor (1994) was used to defat the flour. Distilled or deionized water was used in sample preparation and all laboratory procedures.

Proximate analysis and protein yield determination

Crude protein and protein solubility were determined by micro-Kjeldahl method with the common conversion factor of 6.25 (ISO 5983 2005; AOAC 2000). Crude lipid was extracted by FOSS Soxtec™ 2043 fat extraction system (China) and determined according to AOAC official method (AOAC 2000). Total ash was analyzed using the conventional method by dry-ashing in muffle furnace at 550 °C. Moisture content was calculated by drying weighed samples for 3 h in an oven at 120 °C (Smith et al. 1966). Protein yields of all samples were calculated on dry basis, as proportion of protein in the final SPI as a function of total protein available in the initial full-fat soy flour (formula 1) (Russin et al. 2007).

$$\text{Protein yield(\%)} = \frac{\text{Mass of Protein in SPI}}{\text{Mass of protein in FFSF}} \times 100 \quad (1)$$

Amino acid determination

Seventeen amino acids and tryptophan were determined using the conventional procedure. Samples were incubated in 6 M HCl (for total amino acids) and 5 M NaOH (for tryptophan) in air forced oven at 110 °C for 24 h. They were automatically injected into Agilent octyldecylsilane (ODS) analytical HPLC column (4.6×250 mm, 5 µm particle size) (Agilent Technologies, Palo Alto, CA, USA) system with o-phthalaldehyde (OPA) precolumn derivation. The Determination was made by reverse phase high performance liquid chromatography (RP-HPLC) (HP-Agilent 1100 model, Agilent Technologies, Palo Alto, CA, USA) assembly system at 338 nm detection, 1.0 mL/min flow rate and 40 °C column temperature. Mobile phase A was 7.35 mM/L sodium acetate/triethylamine/tetrahydrofuran (500:0.12:2.5, v/v/v), adjusted to pH 7.2 with acetic acid, while mobile phase B (pH 7.2) was 7.35 mM/L sodium acetate/methanol/acetonitrile (1:2:2, v/v/v). The amino acid composition was expressed as g per 100 g of the SPI.

In vitro protein digestibility

Pepsin digestibility: Pepsin in vitro protein digestibility (IVPD) was determined according to the method of Maliwal (1983), in the manner described by Elkhailil et al. (2001) with some modifications. A known weight of the sample containing 16 mg nitrogen was digested with 15 mg pepsin in 15 mL of 0.1 M HCl (pH 1.8) at 37 °C for 2 h in water bath under mild agitation. The reaction was stopped by the addition of 15 mL 10 % trichloroacetic acid (TCA), immediately cooled in ice bath and contents centrifuged at 10,000×g, 4 °C for 20 min. A blank containing everything, except the sample,

was also prepared. TCA-soluble fraction was assayed for nitrogen using the micro-Kjeldahl method.

Pepsin-pancreatin digestibility: AOAC procedure as optimized by Calsamiglia and Stern (1995) with minor modifications, was used to measure the pepsin-pancreatin digestibility. Sample containing 16 mg nitrogen was put into a 50 mL centrifugation tube. A 10 mL HCl 0.1 N, pH 1.9 solution containing 1 g/L of pepsin (Sigma P-7012, Sigma Aldrich) was added, vortexed, and incubated for 2 h in a 38 °C shaker water bath. Then, 0.5 mL of 1 N NaOH solution and 13.5 mL (of 3 g/L pancreatin (Sigma P-7545, Sigma Aldrich) in 0.5 M of KH₂PO₄ buffer solution, adjusted to pH 7.8, containing 50 ppm of thymol) were added, vortexed and incubated under same condition for 24 h. Finally, 3 mL of a 100 % (w/v) TCA solution was added then centrifuged at 10,000×g, 20 °C for 15 min. TCA-insoluble nitrogen was measured by micro-Kjeldahl method. Blank containing the same except sample was also prepared. Digestibility was calculated as in formula 2.

$$\text{Protein digestibility(\%)} = \frac{N_{\text{supernatant}} - N_{\text{blank}}}{N_{\text{sample}}} \times 100 \quad (2)$$

Protein digestibility-corrected amino acid score (PDCAAS)

Considering that in animal or human, protein digestion process uses multiple enzymes, the pepsin-pancreatin digestion value was used in the calculation of PDCAAS, and the FAO/WHO prescribed formulas (3 and 4) were employed (WHO/FAO/UNU 2002).

$$\text{Amino acid score(AAS)} = \frac{\text{AA in test protein (mg/g)}}{\text{AA in reference pattern}} \quad (3)$$

$$\text{PDCAAS} = \text{AAS of the most limiting AA}$$

$$\times \text{In vitro digestibility} \quad (4)$$

where reference AA pattern is the amino acid requirement for 2–5 years preschool children.

Minerals determination

Twelve mineral elements were determined according to the slightly modified method of Shahidi et al. (1999). A 1 g of each SPI samples was weighed into clean porcelain crucibles and dry ashed at 550 °C in a muffle furnace. The ash was dissolved in 5.0 ml of HNO₃/HCl/H₂O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. A 5 ml of de-ionized water was added and heated until colorless. The solution in each crucible was then transferred into separate 50 ml volumetric flasks by filtration through Whatman

No.42 filter paper and the volume was made to the mark with pure water. This solution was used for elemental analysis by Varian Spectra AA-220 FS atomic absorption spectrophotometer (Varian Inc., Australia). Phosphorus content of the digest was determined colorimetrically at 690 nm (after adding ammonium molybdate solution and aminonaphtholsulfonic acid reagent to the solution) by a Cary 50 UV-Visible spectrophotometer (Varian Inc., Australia). The mineral elements were calculated as mg/100 g of original dry sample.

Anti-nutritional factors determination

The two common anti-nutritional factors (trypsin inhibitor and phytic acid) were determined. Trypsin inhibitors (TI) were determined using benzoyl-DL-arginine-p-nitroanalide hydrochloride (BAPA, Sigma Aldrich) as synthetic substrate (Kakade et al. 1974; Hamerstrand et al. 1981). SPI sample (1 g) was extracted with 50 ml of 0.01 N NaOH, pH adjusted to 8.95 for 2 h. The extract was diluted to inhibit between 40 and 60 % the trypsin used as standard. Four aliquots (2 ml) of each sample were pipetted into glass tubes. A fifth tube containing 2 ml distilled water was prepared as standard. To three of the four tubes containing sample and one containing water only, 2 ml solution of 4 mg trypsin (298 U/mg, Worthington Biochemical) in 200 ml of 0.001 N HCl was added and placed at 37 °C constant temperature. After 10 min, BAPA (dissolved in 2 ml dimethyl sulfoxide, diluted to 200 ml with 0.05 M, pH 8.2 Tris buffer containing 0.02 M CaCl_2) solution, prewarmed to 37 °C in a water bath, was rapidly blown into. Immediately, the tubes were vortexed to mix and incubated again at the same constant temperature (37 °C). Exactly after 10 min, 1 ml of 30 % acetic acid was added to each tube to terminate the reaction and immediately vortexed. Trypsin solution (2 ml) was added to the fourth tube (which previously did not contain it) as a blank, then vortexed. Absorbance was read at 410 nm against blank. TI (mg/g) was calculated as function of differential absorbance of the standard and sample (Hamerstrand et al. 1981) using formula 5.

$$\text{TI (mg/g) sample} = \frac{A_{\text{std}} - A_{\text{sample}}}{19} \times \text{dilution factor} \quad (5)$$

Chromophore method of Mohamed et al. (1986) was used to determine phytic acid in the SPIs. A 1 g of each sample was extracted with 20 ml of 3 % TCA in 125 ml erlemeyer flask. The extract was centrifuged at 19,000×g, 20 °C for 15 min. The supernatant (5 ml) was drawn into clean glass tube and 3 ml of 1 % $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in 1.0 N HCl was added. The mixture was heated in boiling water for 45 min, let to cool and centrifuged at same conditions for 10 min. The supernatant was discarded. The ferric phytate precipitate was suspended in 5 ml of 0.5 N HCl and incubated at ambient (about 25 °C) for 2 h. the supernatant was discarded and precipitate washed twice with 0.5 M HCl. To the final

precipitate, 3 ml of 1.5 N NaOH and 7 ml water were added. The contents were heated in boiling water for 15 min, cooled and centrifuged (19,000×g, 20 °C, 15 min.). The supernatant (20 µl) was drawn into a clean graduated glass tube. Water was added to 4.8 ml level then 0.2 ml of the chromogenic solution was added. The mixture was heated to for 15 min at 95 °C. The absorbance of the final blue colored product was read at 830 nm by UV-vis spectrophotometer (UV-2450, Shimadzu Corporation, Japan). The standard curve ($y = -0.00371 + 0.04949x$) was prepared from six tubes containing 1, 2, 4, 8, 12 and 16 µg/ml phytic acid (Sigma Aldrich).

Statistical analysis

One way analysis of variance (ANOVA), with Duncan's multiple range test, using a SAS program (version 8.1, SAS Institute Inc., Cary, NC, USA) was conducted to assess significance of differences ($P < 0.05$) among statistics obtained from various variable.

Results and discussion

Amaranth ash solution optimization

At 1:5 ash to water (w/v) ratio, the average water pH rose from initial 6.50 to 12.56 (mature plants) and 13.10 (tender plants). Variation of mixing water temperatures (range 25–90 °C) did not show statistically significant difference ($p < 0.05$) in the resultant solution pH levels (pH 12.36 and 12.56). Nevertheless, at 70 °C the lye showed a higher pH as compared to all other temperatures. Tender plants solutions registered significantly higher average pH (13.10) and were clearer than those obtained from mature plants (pH 12.56). Much as the results on temperature optimization agree with random online discussions, the lack of significance indicates that any mixing temperature can be used. Ash to water ratio of 1:5 provided highest lye yield with no significant pH difference with the solutions obtained from the lower ratios. The higher pH observed in tender plants may be associated with the fact that they contained relatively more leaves where most of the plant food, including salts, which are responsible for the pH, are found. The tender amaranth plants are therefore more desirable for preparation of this alkaline solution. Lemon juice was used as extracted without changes.

Proximate and protein yield analyses

Values for proximate analysis and protein yield are presented in Table 1. SPIs prepared from full-fat soybean flour and natural chemicals (ash solution and lemon juice) showed slightly lower protein yield as compared to those from defatted flour and

Table 1 Proximate analysis of NCFF, SCFF, SCDF, SCFF SPIs and full fat and defatted soybean flours

	Soy protein isolate				Soybean flour	
	NCFF	NCDF	SCFF	SCDF	DF	FF
Crude SPI (g)	32.93	36.97	37.63	35.78		
Crude SPI yield (%)	21.95	24.65	25.09	23.85		
Crude protein (%)	91.21	93.64	88.12	98.45	52.72	45.90
Protein yield (%)	43.62	50.28	48.16	51.16		
Crude lipid	0.71	<0.05-	0.65	<0.05-	1.24	21.32
Ash	5.42	5.91	3.83	2.08	6.08	4.70
Moisture	3.38	3.24	2.49	2.84	11.05	7.34

synthetic chemicals (NaOH and HCl) respectively. The experimental SPI (NCFF) had a lower protein yield of 43.62 % compared to that of the main control (SCDF SPI), which was 51.16 %, representing a difference of 7.54 %.

Soy product is recognized as an isolate only if it contains ≥ 90 % protein (Preeti et al. 2008). Thus all samples, including the NCFF, qualified to be SPIs. Although NCFF was prepared from full-fat flour (21.32 % fat), it showed significant reduction in fat content up to 0.7 %, which is within the recommended range of a quality SPI according CODEX STAN 175–1989 general standards. This reduction may be attributed to the lower density and higher freezing point of fat (compared to water) that enabled it to form a creamy butter-like layer during centrifugation at 4 °C, which was easily removed from the supernatant by mere sieving. The rest of the major constituents were also within the recommended ranges. The lower yield observed in NCFF may be attributed to high fat in the starting material (FF) and ash composition in the solvent (ash solution), may hinder dispersibility of protein. Thus, the yield may increase if mechanically defatted flour is used.

Amino acid profile

Comparison of 18 amino acids of the two main SPIs (NCFF and SCDF), only five differed significantly ($p < 0.01$). Glutamine, serine and arginine were higher in SCDF SPI, while cysteine-s and methionine were higher in NCFF SPI (Table 2). The rest of the amino acids and the total did not show significant difference and were similar to other studies (Fernández-quintela et al. 1997).

In the determination of quality of food protein for human nutrition, amino acid, which were higher in SCDF SPI (glutamine, serine and arginine) are considered non-essential, as they can be synthesized by the body. Cysteine-s and methionine, which are essential, were higher in NCFF SPI. Although its crude protein composition seems lower, NCFF SPI showed higher protein quality than SCDF SPI. Additionally, it seems that use of synthetic chemicals for processing of

SPI, lowers the nutritional quality of the product. Nevertheless, it was not known at which processing stage (flour defatting or protein extraction) methionine and cysteine were lost.

In vitro protein digestibility

In vitro pepsin-pancreatin digestibility of all samples showed much higher values (96.24 ± 0.03 % for NCFF and 97.33 ± 0.41 % for SCDF) with no significant difference,

Table 2 Amino acid composition of NCFF and SCDF SPIs

Amino acid	NCFF SPI	SCDF SPI
Non-essential		
Asp	9.77 ± 0.36^a	10.55 ± 0.37^a
Glu	18.68 ± 0.66^b	21.53 ± 0.67^a
Ser	4.18 ± 0.12^b	4.73 ± 0.01^a
Gly	3.73 ± 0.16^a	3.92 ± 0.07^a
Arg	6.79 ± 0.24^b	7.78 ± 0.19^a
Ala	3.43 ± 0.12^a	3.40 ± 0.09^a
Tyr	2.96 ± 0.12^a	3.22 ± 0.14^a
Cys-s	0.79 ± 0.02^a	0.53 ± 0.01^b
Pro	4.77 ± 0.81^a	5.26 ± 1.03^a
Essential		
Leu	6.89 ± 0.21^a	7.37 ± 0.24^a
Thre	3.03 ± 0.09^a	2.99 ± 0.07^a
His	2.23 ± 0.10^a	2.41 ± 0.14^a
Val	4.62 ± 0.18^a	4.44 ± 0.15^a
Met	1.54 ± 0.07^a	1.10 ± 0.02^b
Phe	4.60 ± 0.15^a	4.91 ± 0.13^a
Ile	4.39 ± 0.16^a	4.54 ± 0.17^a
Lys	6.08 ± 0.17^a	6.41 ± 0.30^a
Try	0.88 ± 0.05^a	0.98 ± 0.02^a
Total	89.36 ± 2.41^a	96.06 ± 1.83^a

Values with different superscript letters along the same row indicate significant difference ($p < 0.01$) between means of triplicates of the two samples

Table 3 Amino acid score, digestibility and PDCAAS of the SPIs

Amino acid	FAO/WHO amino acid (mg/g) reference pattern ^a	SPI Amino Acid content (mg/g)		SPI amino acid score	
		NCFF	SCDF	NCFF	SCDF
Histidine	19	22.26	24.05	1.17	1.27
Isoleucine	28	43.86	45.35	1.57	1.62
Leucine	66	68.85	73.72	1.04	1.12
Lysine	58	60.79	64.07	1.05	1.10
Methionine + Cysteine ^b	25	23.31	16.32	0.93	0.65
Phenylalanine + Tyrosine	63	75.62	52.31	1.20	0.83
Threonine	34	30.30	29.85	0.89	0.88
Tryptophan ^c	11	8.84	9.83	0.80	0.89
Valine	35	46.22	44.41	1.32	1.27
In vitro pepsin-pancreatin digestibility (%)				96.24±0.03 ^d	97.33±0.41 ^d
PDCAAS				0.77	0.63

^a Reference amino acid pattern of preschool children (2–5 years) (FAO/WHO/UNU, 1985)

^b Most limiting amino acid in SCDF SPI

^c Most limiting amino acid in NCFF SPI

^d No significant difference ($p<0.05$) between means of triplicates values of the two samples

although the in vitro pepsin digestibility alone showed lower values (Table 3). These low values in pepsin digestibility may be due to the nativity of the samples, which is well known to be associated with high levels of enzyme inhibitors. This is supported by the higher composition of anti-nutritional factors observed in the samples in comparison to those observed by other authors (Honig et al. 1984; Ellis and Morris 1982; Kakade et al. 1974). However, considering that in animal or human, protein digestion process uses multiple enzymes, the pepsin-pancreatin digestibility is a better reflection of in vivo digestibility than does the pepsin digestibility.

Table 4 Mineral composition (mg/100 g as is basis) of NCFF and SCDF SPIs

Mineral	NCFF SPI	SCDF SPI
Calcium	11.52±0.04 ^a	9.38±1.44 ^a
Sodium	347.60±0.71 ^b	1280.50±432.28 ^a
Iron	13.80±1.08 ^a	9.59±0.37 ^b
Magnesium	3.11±0.32 ^a	1.74±0.07 ^b
Copper	4.18±0.36 ^a	2.00±0.03 ^b
Potassium	3756.01±112.46 ^a	8.08±4.11 ^b
Manganese	0.34±0.00 ^a	0.24±0.00 ^b
Phosphorus	89.22±3.51 ^a	94.19±3.51 ^a
Zinc	1.25±0.00 ^a	0.65±0.04 ^b
Nickel	<0.05±0.00 ^a	<0.05±0.00 ^a
Lead	44.22±3.51 ^a	1.97±0.00 ^b
Cobalt	<0.005±0.00 ^a	<0.005±0.00 ^b
Total	4271.3±113.47 ^a	1408.4±423.52 ^b

Values with different superscript letters along the same row indicate significant difference ($p<0.05$) between means duplicates of the two samples

Protein digestibility-corrected amino acid score (PDCAAS)

To calculate PDCAAS, true protein digestibility and score of the most limiting amino acid are required (WHO/FAO/UNU 2002). However, studies have shown in vitro digestibility to be similar with true digestibility and use it as alternative (Geisert et al. 2007; Wu et al. 1999). In this study, the latter was used. Scores of the essential amino acids are indicated in Table 3. Tryptophan and methionine + cysteine-s were the most limiting in NCFF and SCDF SPIs respectively. The PDCAAS of NCFF SPI was 22 % higher than that of SCDF SPI. Nevertheless, PDCAAS and specific essential amino acids values were lower than those reported on commercial SPIs (Hughes et al. 2011).

The lower PDCAAS and specific essential amino acids values, in comparison to the reported commercial SPIs, may be attributed to other processing mechanisms and soybean cultivars used because digestibility was relatively the same.

Minerals composition

Twelve mineral elements were determined and compared between the two main samples (NCFF and SCDF). Those

Table 5 Trypsin inhibitor activity and phytic acid content

SPI Sample	Trypsin inhibitor		Phytic acid content	
	(mg/g)	(%)	(mg/g)	(%)
SCDF	98.03±0.08 ^a	9.80	37.81±2.80 ^a	3.77
NCFF	90.88±0.46 ^b	9.09	27.07±1.02 ^b	2.70
NCDF	98.68±0.50 ^a	9.87	29.45±2.41 ^b	2.93
SCFF	73.42±1.54 ^c	7.34	27.57±2.58 ^b	2.75

Superscript letters in the same column indicate significant differences ($p<0.01$) of mean values ($n=3$)

from the other samples were not determined. Table 4 shows that most mineral elements were significantly higher in NCFE (potassium being the highest) except sodium, which was significantly higher in SCDF SPI. The rest of the minerals including calcium, phosphorus, iron and nickel, showed no significant difference.

The differences observed in mineral composition between NCFE and SCDF was not surprising, considering the composition of the solvents (ash and NaOH solutions) used to prepare the samples. In general, both samples showed lower values relative to those reported by other studies (Aletor 2010). The difference with the commercial samples may be attributed to the variation in processing parameters such as amount of washing done to the SPIs and the soybean cultivar used. Thus, this natural SPI cannot pose toxic danger of introducing excessive minerals in food products if used as an ingredient.

Anti-nutritional factors composition

The two common anti-nutritional factors (trypsin inhibitor and phytic acid) were determined and the results are presented in Table 5. Trypsin inhibitor was significantly lower ($p < 0.01$) in SCFF than the rest of SPI samples. NCFE, SCDF and NCDF had significantly higher compositions and no difference was observed among them.

Results of phytic acid composition of the four SPI samples are presented in Table 5. It was significantly lower in NCFE, NCDF and SCFF than in SCDF SPI. Similar to TI composition, general observation showed samples prepared from full-fat soybean flour to have lower phytic acid than those from defatted flour.

All TI values were within the ranges reported by Kakade et al. (1974) on commercial SPIs. Generally, SPIs prepared from full-fat soybean flour showed lower composition. Although TIs are proteins in nature, this variation may not be related to the crude protein composition in the samples. This is so because SCFF, NCFE and NCSF contained similar percentages of protein (Table 1) but their TI content differed significantly. Those of SCDF and NCDF were different but their TIs were similar. It seemed that defatting soybean flour with n-hexane and 95 % alcohol purified TIs thereby rendering them more active to substrate.

Compared to those reported by other studies (Honig et al. 1984; Ellis and Morris 1982), all samples showed slightly higher percentages of phytic acid. Phytic acid is known to bind to non-heme iron thereby lowering its absorption. On the other hand, it is considered to be an antioxidant (Hurrell et al. 1992; Graf et al. 1987; Graf and Eaton 1990). However, considering its anti-nutritional capacity, phytic acid is not considered as an appropriate antioxidant, and hence need to be in lowest amounts possible in human food. Thus, the lower value observed in NCFE makes it a relatively better

product compared to SCDF, whose composition was significantly higher than the rest of the SPI samples.

Conclusion

The findings of this study have shown that amaranth ash solution and lemon juice as plant-food-based chemicals may be used to process a natural soy protein isolate, with nutritional quality similar to those of conventionally or commercially processed SPIs. It has also been observed that using these natural chemicals together with full-fat flour conserved some essential amino acids such as cysteine-s and methionine, which were significantly reduced when the conventional synthetic chemicals (n-hexane, HCl and NaOH) were used to defat the flour and process the actual SPI. Additionally, the natural SPI contained lower amounts of anti-nutritional factors (trypsin inhibitor and phytic acid) compared to the conventional one. Thus, if well developed, this method may potentially be applied in food industry to address some of the fears of consuming residues of synthetic chemicals by the health-conscious organic and natural food consumers. Nevertheless, lower protein yield was observed in the natural SPI, which signifies that the technique would require further improvement in order to achieve a more efficient and economic production.

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