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The amino acid composition, solubility and emulsifying properties of sweet potato protein

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ABSTRACT

A protein was purified from the high-protein type sweet potato variety 55-2 available in China. The amino acid composition, solubility and emulsifying properties of the sweet potato protein (SPP) were studied. The SPP was rich in aspartic acid (18.5%) and glutamic acid (9.30%) while essential acid amino acids made up approximately 40.7% of the SPP. The SPP was highly soluble in distilled water over a wide range of pH. However, solubility of the SPP in 1.0 M NaCl and 1.0 M $CaCl_2$ solutions was low especially at pH below the pI of the SPP. The SPP in $CaCl_2$ demonstrated emulsifying activity index (EAI) and emulsion stability index (ESI) many folds higher than those in distilled water and NaCl solution ($P < 0.05$).

1. Introduction

Due to increasing market demands on protein ingredients, novel proteins have been purified from various sources (El Nasri & El Tinay, 2007; Lamsal, Koegel, & Gunasekaran, 2007; Løkra, Helland, Claussen, Strækvern, & Egelandsdal, 2007). However, for a novel protein to be useful for food processing application, it should possess desirable functional and nutritional qualities. The functional properties of proteins, such as solubility and emulsifying activities, are in turn highly dependent on many factors, such as pH and the type and amount of salts present. For instance, the presence of NaCl improved the emulsifying properties of cowpea, fenugreek and sesame proteins (El Nasri & El Tinay, 2007; Inyang & Iduh, 1996; Ragab, Babiker, & Eltinay, 2004). On the other hand, addition of $CaC1_2$ prior to emulsification increased the average droplet diameter and reduced creaming stability of the emulsion. (Ye & Singh, 2000).

Sweet potato cultivars contain 0.49% to 2.24% crude protein on a fresh weight basis (Purcell, Swaisgood, & Pope, 1972). With a chemical score of 82, the sweet potato protein (SPP) is of acceptable nutritive value (FAO., 1990). Globally, China is the largest sweet potato producer with an annual yield of approx 120 million tons, which accounted for 80% of the worldwide sweet potato production. In China, sweet potatoes are mainly used for the production of starch and other starchy foods; this activity has generated a huge volume of wastewater effluent. Our preliminary study showed that the effluent contains approx 1.5% crude protein and attempts have been made to recover the protein from the wastewater effluent (Cheng, Xu, & Wang, 2004; Jaw, Chou, Chang, & Duan, 2007). Currently there is no information about the functional properties of SPP. In the present study, a SSP was purified from the high-protein type sweet potato variety 55-2 available in China. The amino acid composition as well as solubility and emulsifying properties of the SPP as influenced by salts and pH were analyzed.

2. Materials and methods

2.1. Materials

Sweet potatoes (*Ipomoea Batatas* L.) of variety 55-2 weighing about 500 g each were purchased from a market in Beijing. Upon arrival to the laboratory, the sweet potatoes were sorted and undamaged sweet potatoes were stored at room temperature until use. Unless otherwise stated, all reagents used in this study were of reagent grade.

2.2. Isolation of sweet potato protein

Sweet potato protein (SPP) was prepared by isoelectric precipitation. Sweet potatoes were washed, cut, mixed with tap water containing 1% sodium bisulfite (1 L/kg of fresh sweet potato), ground and centrifuged at 3000*g* for 15 min. After filtering the supernatant through a double-layer cheese cloth, pH of the filtrate was adjusted to approximately 4 (the pH where, based on our preliminary study, highest amount of the SPP was precipitated)

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using 1 M HCl, and magnetically stirred for 1 h. The slurry was then centrifuged at 3000*g* for 15 min at room temperature. SPP was prepared by resolubilizing the pellet in distilled water, ultrafiltration and lyophilization. The protein powder was kept in a desiccator until use.

2.3. Proximate composition

The moisture, protein, fat and crude fiber contents of the SPP were determined according to the A.O.A.C. (1990).

2.4. Amino acid composition

A 75 mg portion of the SPP was placed in a 20 ml ampoule and mixed with 10 ml of 6 M HCl. After sealing the ampoule, the SPP was hydrolyzed at 110 °C for 24 h under vacuum. The hydrolysate was evaporated to dryness under vacuum at 60 °C. The dried sample was dissolved in 3–5 ml of sodium citrate buffer (pH 2.2) to yield an amino acid concentration of 50–250 nmol/ml, filtered and loaded on a Hitachi L-8800 amino acid analyzer (Tokyo, Japan) for amino acid analysis.

2.5. Protein solubility

Protein solubility of the SPP was measured according to the method of Casella and Whitaker (1990). The SPP was solubilized in distilled water, 1.0 M NaCl or 1.0 M $CaCl_2$ solutions (1% SPP, w/v) by mixing with a vortex. After adjusting the pH from 1 to 10 using 0.5 M HCl or 0.5 M NaCl, the SPP solutions were magnetically stirred for 1 h and centrifuged at 3000*g* for 15 min. Protein concentrations of the supernatants were measured according to Peterson (1977) and Markwell, Haas, Bieber, and Tolbert (1978) with bovine serum albumin as the standard. Protein solubility was calculated as (protein content in the supernatant)/(protein content in the SPP solution) × 100.

2.6. Emulsifying properties

SPP solutions (1%, w/v) in distilled water, 1 M NaCl and 1 M $CaCl_2$ solutions of different pH were prepared as described above before forming emulsion with peanut oil.

Emulsifying activity index (EAI) of the SPP was determined according to the method of Pearce and Kinsella (1978). For emulsion formation, 3 ml aliquot from each of the three SPP solutions was homogenized with 1 ml peanut oil in a FJ-200 High-Speed Homogenizer (Shanghai Specimen Model Co., China) for 30 s at 15,000 rpm. Immediately after homogenization, an aliquot (1 μl) of emulsion was taken from the bottom of each homogenized emulsion, and diluted with 5 ml 0.1% sodium dodecyl sulfate (SDS) solution. After vortex mixing, the absorbance of the diluted emulsions was read at 500 nm using a spectrophotometer. EAI (m^2/g) value was calculated by the following equation:

$$\mathrm{EAI} = \frac{2 \times 2.303 \times A \times \mathrm{DF}}{c \times \phi \times (1-\theta)}$$

where A is the absorbance of the diluted emulsions, DF is the dilution factor, c is the initial concentration of protein, ϕ is the optical path, θ is the fraction of oil used to form the emulsion.

The emulsion stability index (ESI) of the SPP was estimated by measuring the turbidity of the emulsion at 500 nm immediately after emulsion formation and after heating at 100 °C for 30 min (Aluko & Yada, 1993). ESI was calculated as the turbidity ratio (%) between heated emulsion and newly-formed emulsion.

2.7. Statistical analysis

Data were analyzed by analysis of variance (ANOVA) using the general linear model (Version 8.0; SAS Institute Inc., NC, USA). Duncan's multiple range test was used to determined the differences among samples. Significant levels were defined as probabilities of 0.05 or less. All treatments were triplicated.

3. Results and discussion

3.1. Proximate composition

Proximate composition of the sweet potato protein (SPP) was determined. The SSP was composed of 87.0% protein, 0.6% fat, 0.16% crude fiber, 2.19% ash and 1.56% sugar.

3.2. Amino acid composition

The amino acid composition of the SPP is shown in Table 1. Aspartic acid and glutamic acid (the amino acids with negatively charged side chains), were the most abundant amino acids found in the SPP, making up about 18.5% and 9.30% of the total amino acid of SPP, respectively.

The eight essential acid amino acids, namely, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine, made up approximately 40.7% of the SPP. The percentage of some essential amino acids or amino acid pairs of the SPP in the present study fulfilled or exceeded their respective percentages stated in the 'ideal protein' of WHO (1985). Specifically, isoleucine, methionine + cysteine, phynylalanine + tyrosine, threonine and valine of the SPP were 188%, 194%, 179%, 197% and 213% of the their counterparts stated in the WHO standard, respectively (WHO, 1985). However, the overall quality of the SPP was slightly compromised by the low level of lysine and tryptophan, which accounted to only 64.5% and 89.7% of lysine and tryptophan requirement for children as recommended by FAO/WHO (WHO, 1985) (Table 2). The lysine levels in other sweet potato varieties, namely Jewel and Centennial, were higher than that stated in FAO reference protein (FAO, 1990). The lysine levels in the chromoplast proteins extracted from Jewel and Centennial sweet potato roots were

Table 1
Amino acid composition of protein derived from sweet potato variety 55-2[a]

Amino acid	Content	
	(mg/g dry weight)	% of SPP
Aspartic acid (Asp)	154	18.5
Threonine (Thr)[b]	55.8	6.70
Serine (Ser)	60.3	7.24
Glutamic acid (Glu)	77.5	9.30
Glycine (Gly)	34.3	4.12
Alanine (Ala)	13.4	1.61
Cystine (Cys)	24.3	2.92
Valine (Val)[b]	62.0	7.44
Methionine (Met)[b]	16.1	1.93
Isoleucine (Ile)[b]	43.7	5.25
Leucine (Leu)[b]	57.9	6.95
Tyrosine (Tyr)	40.1	4.81
Phenylalanine (Phe)[b]	54.3	6.52
Lysine (Lys)[b]	43.3	5.20
Tryptophan (Trp)[b]	5.85	0.71
Histidine (His)	12.9	1.55
Arginine (Arg)	44.2	5.30
Proline (Pro)	32.9	3.95
Total	833	100
% Essential amino acid	339	40.7

[a] The values reported represent the average of three determinations.
[b] Essential amino acids.

Table 2
Essential amino acid composition of SSP compared to the WHO 'ideal protein'

Amino acid	WHO ideal protein (% of total protein)	SPP	
		% of total amino acid	% amino acid/ ideal protein × 100
Isoleucine	2.8	5.25	188
Leucine	6.6	6.95	105
Lysine	5.8	5.20	89.7
Methionine + cysteine	2.5	4.85	194
Phynylalanine + tyrosine	6.3	11.3	179
Threonine	3.4	6.70	197
Tryptophan	1.1	0.71	64.5
Valine	3.5	7.44	213

7.03 g amino-acid/16 g N and 6.43 g amino-acid/16 g N respectively, while the lysine levels in white protein extracted from Jewel and Centennial sweet potato roots were 5.16 g amino-acid/16 g N and 5.21 g amino-acid/16 g N respectively (Walter & Catignani, 1981).

3.3. Protein solubility

Protein solubility of the SPP in distilled water, 1.0 M NaCl and 1.0 M $CaCl_2$ solutions as a function of pH is shown in Fig. 1. Generally, without the presence of salts, the SPP demonstrated very high solubility over a wide range of pH. In distilled water, the lowest solubility of 47.5% was observed at pH 4, indicating the isoelectric point of the SPP. As pH shifted away from the pI, solubility of the SPP in distilled water increased drastically ($P < 0.05$); the SPP demonstrated a solubility exceeding 95% in distilled water when the pH was reduced to 2 and lower or increased to 5 and above ($P < 0.05$). The net positive and negative charges acquire by proteins at highly acidic and alkaline regions, respectively, promote intermolecular repulsion and thus increase the solubility (Seena & Sridhar, 2005).

The presence of NaCl and $CaCl_2$ exerted 'salting-out' effect, i.e. reduction in protein solubility, on the SPP (Fig. 1). At pH lower than the pI, solubility of the SPP in $CaCl_2$ and NaCl solutions was at least 40% lower than that in distilled water ($P < 0.05$). As pH rose, solubility of the SPP in NaCl or $CaCl_2$ solutions increased. At pH higher than 8, maximum solubility of 80% and 100% was observed for the SPP solubilized in $CaCl_2$ and NaCl solutions, respectively ($P < 0.05$).

At pH lower than the pI, the reduction of solubility for the SPP in NaCl and $CaCl_2$ solutions could be explained by the predominant electrostatic screening of the positively charged protein and/or by adsorption of chloride ions by the protein (Retailleau, Riès-Kautt, & Ducruix, 1997). However, changes in protein solubility are mainly caused by anions and the effects from cations are minimal (Reis-Kautt & Ducruix, 1989). Hence, the effect of NaCl and $CaCl_2$ on the SPP solubility was less apparent at pH higher than the pI, the region where the SPP was negatively charged.

3.4. Emulsifying properties

Some studies have suggested that the good emulsifying activity of a protein is related to its high solubility (Inyang & Iduh, 1996; Nasri et al., 2007; Ragab et al., 2004). However, our results are not in agreement with these studies as the trends for both EAI and ESI did not follow those of protein solubility (Figs. 1–3). Pre-solubilization of the SPP in salts solutions seems to modify the emulsifying properties of the protein as the SPP in distilled water, NaCl and $CaCl_2$ solutions demonstrated different emulsifying properties as detailed below.

The emulsifying activity index (EAI) measures the area of interface stabilized per unit weight of protein (m^2/g) and hence relates the ability of a protein to coat an interface (Pearce & Kinsella, 1978). Compared to the SPP solubilized in distilled water where EAI increased from 108 m^2/g to 643 m^2/g ($P < 0.05$), only slight fluctuations in EAI values were observed for SPP solubilized in $CaCl_2$ (1290–1360 m^2/g) and NaCl solutions (220–380 m^2/g) ($P < 0.05$) when pH rose from 1 to 10. Moreover, the SPP in $CaCl_2$ solution demonstrated EAI value many folds higher than those in distilled water or NaCl solution ($P < 0.05$).

Emulsion stability index (ESI) of the SPP solubilized in distilled water, NaCl and $CaCl_2$ solutions as a function of pH is shown in Fig. 3. Compared to the SPP solubilized in distilled water and $CaCl_2$ solution, the SPP solubilized in NaCl solution reduced the stability of the emulsion at most of the pH studied except at pH 4 and pH 5 ($P < 0.05$). The instability of the SPP solubilized in NaCl against heat treatment was more pronounce at the extreme acidic region; at pH 1 and 2 ($P < 0.05$), the ESI was only 13% for the SPP solubilized in NaCl solution. The ESI values of the SPP in both distilled water and NaCl solution improved sharply as pH approaching the pI, and a maximum ESI of approx 76% was attained at the pI ($P < 0.05$). Besides elevating sharply the ESI value, the SPP solubilized in $CaCl_2$ solution also reduced the influence of pH on the emulsion stability against heat treatment. Emulsion containing the SPP solubilized in $CaCl_2$ exhibited strong stability exceeding 88% over the pH range studied (Fig. 3).

Some studies have shown that addition of salts to denatured proteins could alter the proteins' emulsifying properties. Singh

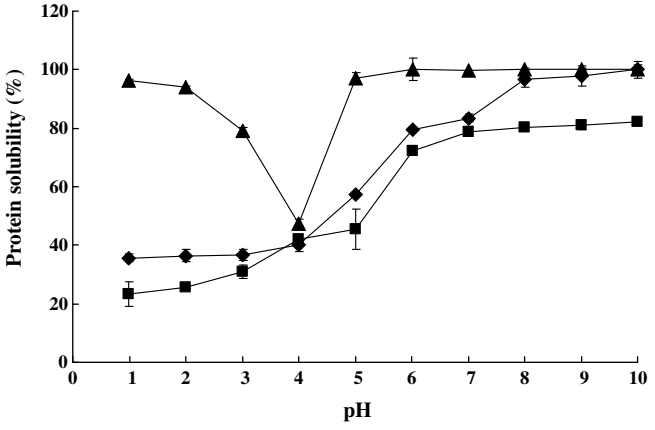

Fig. 1. Protein solubility determined in SPP solubilized in distilled water (▲), 1 M $CaCl_2$ (■), and 1 M NaCl (♦) (1%.SPP, w/v). Each data point is composed of three replicates. Error bars represent the standard deviations.

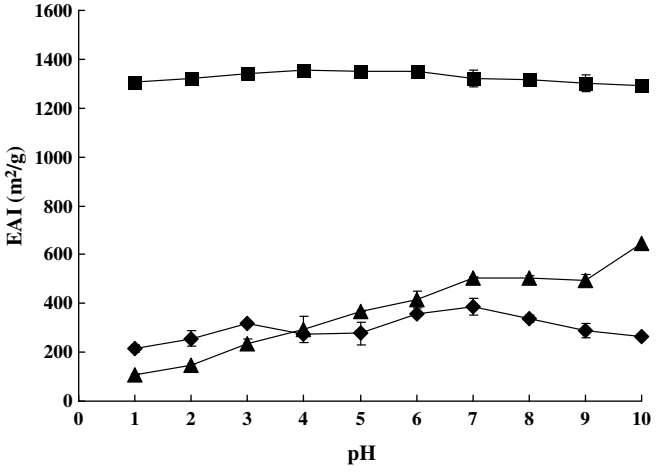

Fig. 2. EAI determined in SPP solubilized in distilled water (▲), 1 M $CaCl_2$ (■), and 1 M NaCl (♦) (1%.SPP, w/v). Each data point is composed of three replicates. Error bars represent the standard deviations.

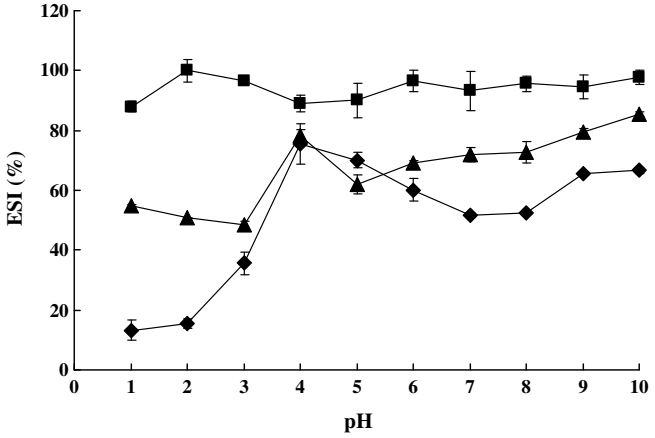

Fig. 3. ESI determined in SPP solubilized in distilled water (▲), 1 M $CaCl_2$ (■), and 1 M NaCl (♦) (1%.SPP, w/v). Each data point is composed of three replicates. Error bars represent the standard deviations.

and Queiroga (2004) shows that addition of NaCl to denatured cashew nut kernel protein isolates at the isoelectric pH (a point where proteins show poor functionality) improves the emulsifying properties of the protein. Similarly, Palazolo, Mitidieri, and Wagner (2003) suggest that emulsions stabilized by denatured soy isolate were stable in presence of salt, due to the formation of rigid flocs resistant to agitation. As adding salt and changing pH are among causes leading to protein denaturation, there could be interaction between pH and salts in affecting the denaturation and subsequently the emulsifying properties of the SPP. However, at present, the reason on how pH, NaCl and $CaCl_2$ salts affect the emulsifying properties of the SPP is not yet clear. Our laboratory is currently undertaking research to elucidate the actual mechanism behind the phenomenon observed.

4. Conclusion

Results from the present study show that sweet potato could be a good source of protein ingredient for food processing as it possesses good solubility and emulsifying properties. The solubility and emulsifying properties of the sweet potato protein could be modified by changing pH and addition of salts.

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