Modification of Proteins from Soymilk Residue (Okara) by Trypsin

W.-M. Chan and C.-Y. Ma

ABSTRACT

Okara protein isolates were hydrolyzed by trypsin to about 5% to 14%. Solubility was increased more than twofold by the modification, and water hydration capacity and emulsification activity index were also improved. The okara protein products had good essential amino acid profiles and the trypsin-hydrolysates also had increased in vitro digestibility and available lysine content. The low solubility of okara protein makes it difficult to incorporate it into many food systems. Okara protein hydrolysates, with improved solubility and other functional properties, could be used as a low-cost protein ingredient in processed foods.

Key Words: soymilk, okara, trypsin hydrolysis, protein functionality

INTRODUCTION

SOYMILK AND TOFU MANUFACTURING PRODUCE LARGE AMOUNTS of spent residue (okara) as waste by-product and its disposal poses severe environmental problem. Okara contains about 27% protein (dry basis) and can be extracted with high yield at alkaline pH (Ma et al., 1997). Okara protein has low solubility and its nutritional and functional properties are comparable to commercial soy isolates. To enhance the utilization of okara protein as a food ingredient, chemical modifications can be applied to improve solubility and other functional properties. Enzymatic modification has been an important means of converting food proteins into products with very different, and desirable, properties. It is generally preferred over chemical modification which may alter protein digestibility and cause problems with consumer acceptability and/or government regulations (Panyam and Kilara, 1996). Enzymatic hydrolysis has been extensively applied to improve the solubility and other functional properties of food proteins including soy protein products (Puski, 1975; Zakaria and McFeeters, 1978; Ochiai et al., 1982, Kim et al., 1990). The objective of this investigation was to use enzyme hydrolysis to improve the functional properties of okara protein isolates.

MATERIALS & METHODS

OKARA WAS COLLECTED FROM VITASOY INTERNATIONAL HOLDing Ltd. (Tuen Mun, New Territories, Hong Kong). Protein was extracted from the okara according to Ma et al. (1997). A commercial soy protein isolate was used as a reference protein (Supro 610; Protein Technologies Intl., St. Louis, Mo., U.S.A.). All chemicals used were of reagent grade.

Preparation of hydrolyzed okara protein isolates

Okara protein isolate (OPI) was hydrolyzed according to the method of Nakai et al. (1980) with some modifications. Okara protein (5% w/v) was incubated with 3 levels of trypsin (type II, Sigma Chemical Co., St. Louis, Mo., U.S.A.), 1%, 2% and 5% (vs protein, w/w) in 0.2M phosphate buffer (pH 8.2) at 37 °C for 24 h. The mixtures were continuously mixed in a shaking water bath. A control was prepared with no added enzyme. The mixture was then pasteurized at 63 °C for 30 min to inactivate trypsin, as established by Nakai et al. (1980). After exhaustive dialysis (with molecular cutoff of 6000-8000) against distilled water at 4 °C, the mixture was freeze-dried. The recovery of protein after dialysis was 89% to 94%. The percentage of peptide bonds cleaved during proteolysis (i.e., degree of hydrolysis) was determined by the trinitrobenzenesulfonic acid method (Adler-Nissen, 1979), using L-leucine as standard.

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Chemical analysis

Total solids, ash contents and fat contents were determined according to AOAC standard methods (AOAC, 1984). Nitrogen content was determined by the micro-Kjeldahl method (Concon and Soltess, 1973). A nitrogen-to-protein conversion factor of 6.25 was used. Carbohydrate content was estimated by the phenol-sulfuric acid method (Rao and Pattabiraman, 1989). Amino acid analysis was performed according to Spackman et al. (1958).

Physicochemical properties

Net titratable charge was determined according to Ma et al. (1986). Surface hydrophobicity was determined by the fluorescence probe method (Kato and Nakai, 1980; Hayakawa and Nakai, 1985), using 1-anilino-8-naphthalene sulfonic acid (ANS) as the probe. Raman spectroscopy of solid soy flour and OPI was performed according to Shoute et al. (1998) with the following modifications: laser power, 36 mW; spectral resolution, 8 cm^{-1}; number of scan, 260. Gel filtration chromatography was performed using a Pharmacia FPLC® System and a Superose® 12 HR10/30 column (Pharmacia Biotech AB, Uppsala, Sweden). Elution rate was 0.4 mL/min using 0.01M phosphate buffer (pH 7.4) containing 1.0M NaCl, and the eluant was monitored at 280 nm. The column was calibrated with standard proteins of known molecular weights (Sigma).

Functional properties

Protein solubility was determined according to Morr et al. (1985). Water hydration capacity was determined by the method of Quinn and Paton (1979) and fat binding capacity was determined according to Sosulski and Jones (1976). Bulk density was determined according to Wang and Kinsella (1976). Emulsifying activity index and emulsion stability index were determined by a turbidimetric method (Pearce and Kinella, 1978). Foamability and foam stability were estimated by the procedure of Yatsumatsu et al. (1972).

Nutritional properties

In vitro digestibility of protein samples was determined by the multienzyme method of Hsu et al. (1977). Available lysine was determined by the TNBS method of Hall et al. (1973), using L-lysine as a standard. Ether extraction was performed prior to the tests to eliminate interfering substances, including the α-amino groups.

Statistical analysis

Statistical analysis was performed using a SAS program (SAS Institute Inc., 1997). Duncan's multiple range test was used to determine significant difference ($p \leq 0.05$) among treatments after initial demonstration of a treatment-related effect by analysis of variance (Steel and Torrie, 1960).

Table 1—Chemical composition of unmodified and trypsin-hydrolyzed okara protein isolates (OPI)[a]

Sample	Protein (%) (N×6.25)	Fat (%)	Carbohydrate (%)	Ash (%)	Moisture (%)
OPI[b]	77.9±0.2	3.2±0.3	6.3±0.1	5.2±0.1	4.4±0.1
OPI (DH=4.9)[c]	76.4±0.4	3.4±0.3	7.4±0.3	4.2±0.1	4.3±0.1
OPI (DH=8.3)[d]	74.2±0.5	4.8±0.4	7.8±0.2	4.6±0.1	4.1±0.1
OPI (DH=13.7)[e]	73.5±0.4	4.3±0.2	8.2±0.1	5.5±0.1	4.1±0.1

[a]Average of duplicate determinations ± S.D.
[b]Unhydrolyzed OPI.
[c]OPI with 4.9% hydrolysis.
[d]OPI with 8.3% hydrolysis.
[e]OPI with 13.7% hydrolysis.

RESULTS & DISCUSSION

Degree of hydrolysis (DH)

The degree of hydrolysis of OPI was compared at different enzyme-to-protein ratios (Fig. 1). There was an initial linear increase in DH followed by a leveling off. The downward curvature of the hydrolysis curve is a common feature in many protein/enzyme systems (Adler-Nissen, 1986) and can be attributed to substrate exhaustion and/or product inhibition (Bombara et al., 1992). The DH levels obtained were similar to those reported in bromelain-hydrolyzed soy protein isolate (Yeom et al., 1994).

Chemical composition

The chemical composition of unmodified and trypsin-hydrolyzed OPI were compared (Table 1). The protein content progressively decreased with an increase in DH. As proteins are broken down during enzyme hydrolysis, the small peptides may be lost during dialysis and this would lead to a decrease in protein content. Since all the OPIs were defatted and dialyzed, the fat and ash contents were low.

Amino acid composition of various soy protein products were compared (Table 2). Trypsin hydrolysis did not cause marked changes in the amino acid profile of OPIs. The most abundant amino acids of OPIs were glutamic acid and aspartic acid but they were also rich in leucine, arginine and lysine. They had a low content of the sulfur-containing amino acids cysteine and methionine. Results indicate that OPIs had amino acid composition similar to that of Supro 610 (Table 2).

Severe treatments of proteins with alkali (high pH and temperature) have been shown to promote β-elimination and racemization of amino acid residues, leading to decreased digestibility and nutritional quality of dietary proteins (Friedman et al., 1984). In our results, enzyme hydrolysis was carried out at mild conditions of alkaline pH and temperature, when compared to those (> 0.1N NaOH and > 60 °C) used to study racemization of proteins. In a previous study (Ma et al., 1990), we found no significant racemization of oat globulin at pH 9.7 and 55 °C over 96 h.

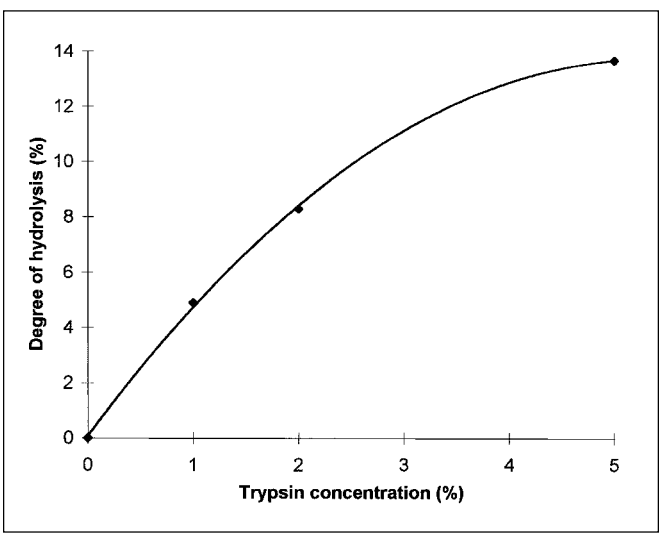

Fig. 1—Effect of trypsin concentration on the degree of hydrolysis of okara protein isolate.

Table 2—Amino acid composition (mg/g protein) of unmodified and trypsin-hydrolyzed okara protein isolate (OPI) and suggested patterns of human amino acid requirement[a]

Amino acid	OPI[b]	OPI (DH=4.9)[c]	OPI (DH=8.3)[d]	OPI (DH=13.7)[e]	Supro 610	FAO scoring pattern[f]
Aspartic acid	117	119	118	120	116	—
Threonine	41	43	43	43	38	34
Serine	49	47	48	50	52	—
Glutamic acid	192	195	195	194	191	—
Glycine	46	47	48	47	42	—
Alanine	47	47	47	47	43	—
Methionine + Cysteine	29	29	30	31	26	25
Valine	52	56	56	56	50	35
Isoleucine	53	55	56	56	49	28
Leucine	81	85	85	85	82	44
Tyrosine + Phenylalanine	94	91	91	91	90	63
Lysine	69	65	64	60	63	44
Histidine	27	28	28	28	26	19
Arginine	76	64	60	58	76	—
Proline	28	30	31	33	51	—
Tryptophan	ND[g]	ND	ND	ND	ND	11

[a]Average of duplicate determinations.
[b]Unhydrolyzed OPI.
[c]OPI with 4.9% hydrolysis.
[d]OPI with 8.3% hydrolysis.
[e]OPI with 13.7% hydrolysis.
[f]Amino acid requirements from FAO/WHO/UNU, 1985.
[g]Not determined.

Physicochemical properties

Net titratable charge. The net titratable charge of OPI was determined (Table 3) by titration of protein dispersions from pH 10.0 to 3.0. The net charge was progressively increased by trypsin hydrolysis. With increase in DH, more free amino and carboxyl groups were produced at the newly hydrolyzed peptide bonds (Vojdani and Whitaker, 1994). At pH 10.0, carboxyl groups were the predominating charged species and more acid was needed to decrease the pH to 3.0 for the trypsin-treated samples, leading to an increase in H^+ and titratable charge.

Surface hydrophobicity. Surface hydrophobicity of OPI was progressively decreased with an increase in DH (Table 3). Since okara protein was extensively denatured during soymilk production, most hydrophobic groups were exposed. However, extensive hydrolysis of okara protein may lead to the exposure of large numbers of hydrophilic groups which could affect the binding of the fluorescence probe to the hydrophobic groups resulting in an apparent lower hydrophobicity reading (Ma et al., 1986).

Raman spectroscopy. The Raman spectra of soy flour, unmodified and enzyme-hydrolyzed OPI were compared (Fig. 2). Normalized intensity and peak area of OPI against shift in wave number were also determined (Fig. 3). Soy flour, the raw material for preparation of soymilk and, subsequently, OPI, was used as a reference. Amide I and amide III bands of soy flour were located at 1658 and 1266 cm^{-1}, respectively, indicating the presence of α-helical structure (Li-Chan et al., 1994). The amide I and amide III bands of unmodified and enzyme-hydrolyzed OPIs were centered around 1665-1675 and 1245-1255 cm^{-1}, indicating that β-sheet and random coils were the predominant structures (Li-Chan et al., 1994). Progressive rise in both normalized intensity and peak area at amide I and amide III bands with increase in DH (Fig. 3a and c) demonstrated that both β-sheet structures and random coils were increased by tryptic hydrolysis. The decrease in helix accompanied by increases in β-sheet

Table 3—Net titratable charge and surface hydrophobicity of unmodified and trypsin-hydrolyzed okara protein isolates (OPI)[a]

Sample	Net titratable charge (mmoles H^+/g protein)	Surface hydrophobicity (S_0)
OPI 3[c]	1.38±0.02a[b]	483±8a
OPI (DH=4.9)[d]	1.54±0.02b	168±4b
OPI (DH=8.3)[e]	2.15±0.01c	145±6b
OPI (DH=13.7)[f]	2.82±0.03d	106±7c

[a]Average of duplicate determinations ± S.D.
[b]Means in a column bearing the same letter are not different ($p \geq 0.05$) as determined by Duncan's multiple range test.
[c]Unhydrolyzed OPI.
[d]OPI with 4.9% hydrolysis.
[e]OPI with 8.3% hydrolysis.
[f]OPI with 13.7% hydrolysis.

Table 4—Water and fat binding properties of different soy protein products[a]

Sample	Water-holding capacity (mL/g)	Fat-binding capacity (mL/g)	Bulk density (g/mL)
OPI[c]	4.01±0.09a[b]	9.29±0.05a	0.09±0.01a
OPI (DH=4.9)[d]	5.33±0.10bc	6.56±0.03b	0.24±0.01b
OPI (DH=8.3)[e]	5.83±0.10b	6.34±0.04c	0.26±0.01bc
OPI (DH=13.7)[f]	5.86±0.12b	6.29±0.04c	0.30±0.01d
Supro 610	5.13±0.03c	1.52±0.01d	0.41±0.01e

[a]Average of duplicate determinations ± S.D.
[b]Means in a column bearing the same letter are not different ($p \geq 0.05$) as determined by Duncan's multiple range test.
[c]Unhydrolyzed okara protein isolate (OPI).
[d]OPI with 4.9% hydrolysis.
[e]OPI with 8.3% hydrolysis.
[f]OPI with 13.7% hydrolysis.

and random coil structures indicate protein denaturation (Sawyer et al., 1971; Lin and Koening, 1976; Clark et al., 1981). Enzymatic hydrolysis of proteins is accompanied by the cleavage of peptide bonds, an increase in the number of ionizable groups and the exposure of previously concealed hydrophobic groups (Panyam and Kilara, 1996). These processes may all induce protein unfolding and lead to the observed changes in Raman spectra.

Aliphatic amino acid residues show C-H stretching and bending modes near 2800 to 3000 cm^{-1} and 1440 to 1465 cm^{-1}, respectively (Li-Chan et al., 1994; Li-Chan, 1996). When compared to soy flour, a shift to higher wave numbers was observed in the unmodified and enzyme-hydrolyzed OPI (Fig. 2), again suggesting protein denaturation (Arteaga, 1994). Progressive increases in the normalized intensity and peak area of aliphatic amino acid bands with an increase in DH (Fig. 3b) indicate further protein denaturation, similar to that reported in ribonuclease (Verma and Wallach, 1977).

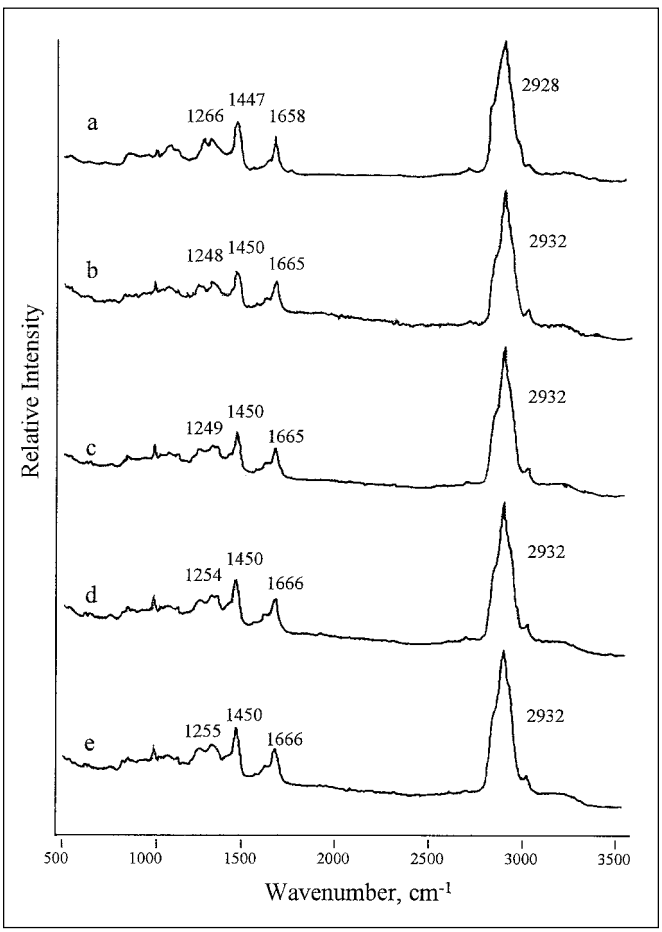

Fig. 2—Raman spectra of unmodified and trypsin-hydrolyzed okara protein isolate. (a) soy flour; (b) unhydrolyzed okara protein isolate (OPI); (c) OPI with 4.9% DH; (d) OPI with 8.3% DH; (e) OPI with 13.7% DH.

The Raman spectroscopic data indicate that proteins in the OPIs were extensively denatured, probably during soymilk manufacturing. Some residual native structures could still be observed in OPI. Trypsin hydrolysis led to further protein unfolding, and results were in partial agreement with the reported circular dichroism data of β-lactoglobulin (Vojdani and Whitaker, 1994) which showed marked protein unfolding by enzyme hydrolysis. However, the β-sheet and β-turn structures of β-lactoglobulin hydrolysates were largely intact, indicating that the large polypeptides could refold (Vojdani and Whitaker, 1994).

Fast protein liquid chromatography (FPLC). The molecular weight distribution of unmodified and trypsin-hydrolyzed OPI was monitored by FPLC (Fig. 4). Unmodified OPI exhibited a profile with both high- and low-molecular-weight components (Fig. 4b). When subjected to tryptic digestion, the high-molecular-weight polypeptides were broken into smaller fragments. The mildly hydrolyzed OPI exhibited a broad spectrum of medium-molecular-weight polypeptides (Fig. 4c). At high DH, the hydrolysates were found to contain mainly low-molecular-weight components (Fig. 4d, e).

Functional properties

Solubility. The pH-solubility curves of unmodified and enzyme-hydrolyzed OPI were compared (Fig. 5). A typical bell-shaped curve was observed for all protein samples, and the pH of minimum solubility was not shifted by modification. When compared to soy flour, OPI had much lower solubility, particularly at acidic and alkaline pH (Ma et al., 1997), and the poor solubility has been attributed to protein denaturation. The solubility of OPIs was progressively improved by trypsin hydrolysis over a wide range of pH (Fig. 5). This may be attributed to both a reduction in the molecular size of the proteins, and an increase in net charge in the hydrolyzed OPI The cleavage of peptide bonds adjacent to basic amino acids could dissociate the protein complexes to expose more charged and polar groups to the surrounding water (Jones and Tung, 1983). Similar to acylation, this could promote protein-water interactions and enhance solubility (Kinsella and Shetty, 1979).

Water and fat absorption. The water hydration capacity (WHC) and fat binding capacity (FBC) of unmodified and enzyme hydrolyzed OPI were compared (Table 4). Results indicate that WHC was progressively increased by trypsin treatment whereas FBC was decreased. There was no difference ($p \geq 0.05$) between the WHC of Supro 610 and that of the 4.9% DH OPI, but the more extensively hydrolyzed OPI (8.3% and 13.7%) had higher WHC than Supro 610. Proteolytic modification improved the water binding of OPIs since water uptake by proteins is related to the liberation of amino and carboxyl groups (Phillips and Beuchat, 1981). The bulk density of OPIs was markedly increased by enzyme treatment (Table 4). Since the mechanism of fat absorption has been attributed mostly to phys-

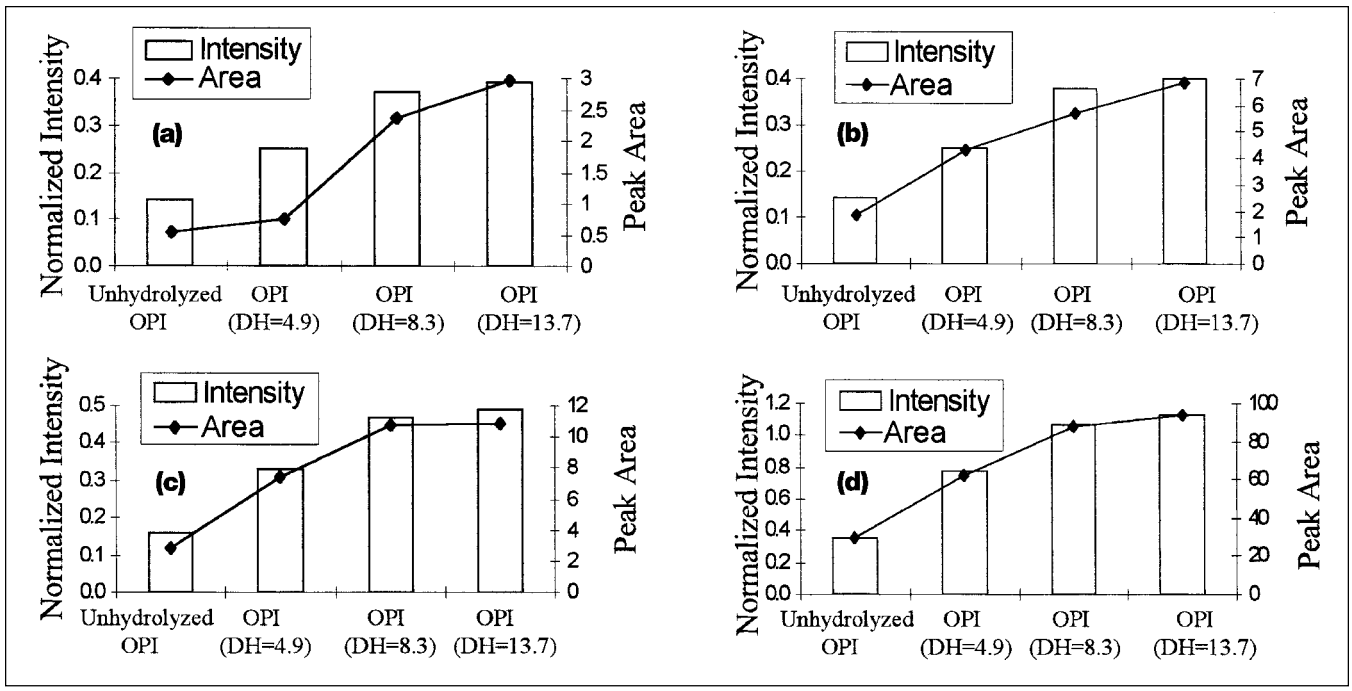

Fig. 3—Normalized intensity and peak area of (a) 1245-1255; (b) 1445-1455; (c) 1665-1675 and (d) 2930-2940 cm^{-1} bands in unmodified and trypsin-hydrolyzed okara protein isolates.

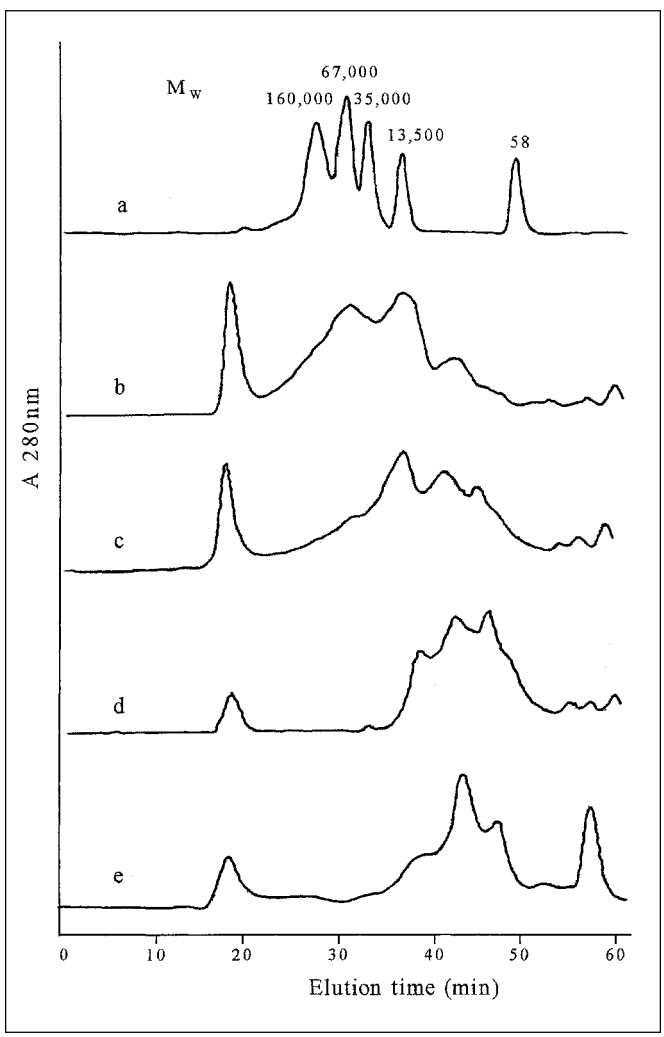

Fig. 4—FPLC profiles of unmodified and trypsin-hydrolyzed okara protein isolates. (a) markers; (b) unhydrolyzed okara protein isolate (OPI); (c) OPI with 4.9% DH; (d) OPI with 8.3% DH; (e) OPI with 13.7% DH.

ical entrapment of oil (Kinsella, 1976), the increase in bulk density would decrease FBC. However, it is not known why the unhydrolyzed control had such low bulk density. A previous study (Ma et al., 1997) showed that OPI had a much higher bulk density and lower FBC. When compared to Supro 610, hydrolyzed OPI exhibited higher FBC which may be attributed to the lower bulk density of the hydrolyzed samples.

Emulsifying properties. Emulsification activity index of OPIs was increased ($p \leq 0.05$) by trypsin digestion (Table 5). Hydrolysis of proteins increased the number of polar groups and hydrophilicity, decreased the molecular weight, altered the globular structure of proteins, and exposed previously buried hydrophobic regions. All these changes would affect the emulsifying properties of proteins (Nielsen, 1997; Schwenke, 1997). With increases in solubility and surface hydrophobicity by trypsin hydrolysis, a better hydrophilic-hydrophobic balance for emulsification could be attained (Phillips and Beuchat, 1981). Trypsin hydrolysis of OPI also increased the number of peptide molecules available at the oil-water interface, and a larger area may be covered resulting in the emulsification of more oil and an increase in EAI (Puski, 1975).

The emulsification stability index (ESI) of OPIs was markedly reduced by trypsin digestion (Table 5). Although the increases in both solubility and surface hydrophobicity would improve ESI, excessive increase in net charge may reduce protein-protein interac-

Table 5—Emulsifying and foaming properties of different soy protein products[a]

Sample	EAI (m²/g)	ESI (min)	Foamability (%)	Foam stability (%) 30min	60min
OPI[c]	37.2 ± 0.9[ab]	3.1 ± 0.3[a]	128 ± 3[a]	73 ± 1[a]	38 ± 3[a]
OPI ([D]H=4.9)[d]	51.7 ± 1.1[b]	1.2 ± 0.2[bc]	38 ± 3[b]	23 ± 2[b]	9 ± 1[bc]
OPI ([D]H=8.3)[e]	56.6 ± 0.8[c]	0.7 ± 0.4[b]	78 ± 4[c]	20 ± 1[b]	6 ± 1[bd]
OPI ([D]H=13.7)[f]	79.4 ± 1.3[d]	0.6 ± 0.2[b]	90 ± 4[c]	0[c]	0[d]
Supro 610	37.3 ± 1.1[a]	2.2 ± 0.3[ac]	105 ± 5[d]	93 ± 3[d]	84 ± 4[e]

[a]Average of duplicate determinations ± S.D.
[b]Means in a column bearing the same letter are not different ($p \geq 0.05$) as determined by Duncan's multiple range test.
[c]Unhydrolyzed okara protein isolate (OPI).
[d]OPI with 4.9% hydrolysis.
[e]OPI with 8.3% hydrolysis.
[f]OPI with 13.7% hydrolysis.

Table 6—In vitro protein digestibility and available lysine contents of different soy protein products[a]

Sample	Digestibility (%)	Available lysine (g/100g protein)
OPI[c]	79.61 ± 0.22[ab]	1.83 ± 0.03[a]
OPI (DH=4.9)[d]	75.00 ± 0.19[b]	1.97 ± 0.02[b]
OPI (DH=8.3)[e]	74.14 ± 0.23[bc]	2.10 ± 0.03[c]
OPI (DH=13.7)[f]	73.48 ± 0.28[c]	2.41 ± 0.02[d]
Supro 610	80.73 ± 0.19[d]	4.47 ± 0.02[e]

[a]Average of duplicate determinations ± S.D.
[b]Means in a column bearing the same letter are not different ($p \geq 0.05$) as determined by Duncan's multiple range test.
[c]Unhydrolyzed okara protein isolate (OPI).
[d]OPI with 4.9% hydrolysis.
[e]OPI with 8.3% hydrolysis.
[f]OPI with 13.7% hydrolysis.

tion and prevent the formation of an elastic film at the oil-liquid interface. The detrimental effect of peptide bond hydrolysis was related to the loss of globular structure and optimum size of peptides, resulting in the formation of a thinner protein layer around the oil droplets and, hence, less stable emulsion (Puski, 1975; Phillips and Beuchat, 1981).

Foaming properties. Trypsin hydrolysis of OPI led to decreases ($p \leq 0.05$) in foamability and foam stability (Table 5). However, foamability was progressively increased with an increase in DH, and foamability of the moderately and extensively hydrolyzed OPI was similar to that of the unmodified sample. Increases in solubility would enhance foamability since soluble proteins contribute to foaming (Kinsella, 1976), but excessive increases in net charge may reduce protein-protein interaction and prevent the formation of elastic film at the air-liquid interface, hence decreasing foam stability.

Nutritional properties

All the essential amino acids of the OPIs met or exceeded (Table 2) the essential amino acid pattern for FAO/WHO/UNU (1985). The in vitro digestibility of OPIs decreased progressively with increase in DH (Table 6). Since OPIs were broken down by trypsin hydrolysis, less unhydrolyzed proteins were available for digestion, leading to apparently lower digestibility readings. The in vitro digestibility of all the soy protein products was relatively high (over 74 %), indicating that heating and tryptic digestion could improve the digestibility of soy proteins by deactivating protease inhibitors and opening the protein structure through denaturation (Hsu et al., 1977; Kamata and Shibasaki, 1979). The available lysine content of OPIs was increased by trypsin hydrolysis (Table 6). Enzyme hydrolysis may expose the buried lysine in the protein matrix which then becomes available. The nutritional quality of OPIs was assessed by chemical means. A better evaluation would require animal feeding experiments and the determination of indices such as protein efficiency ratio and true digestibility.

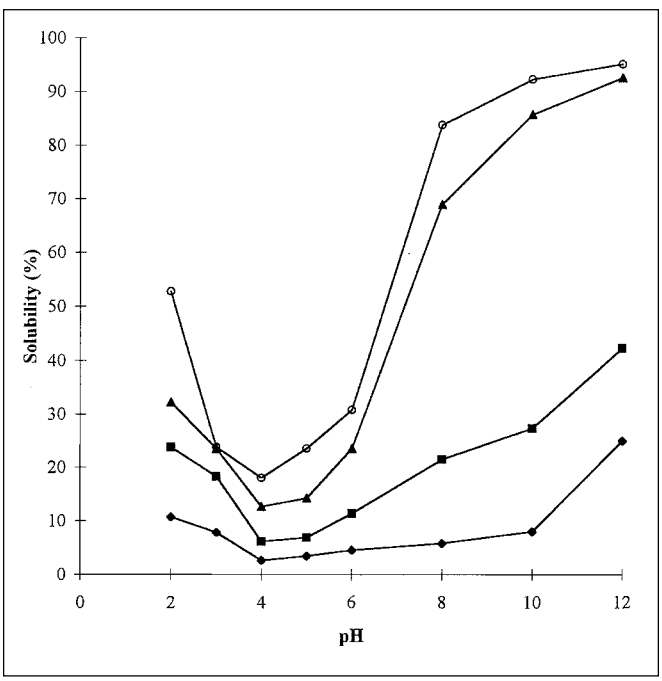

Fig. 5—pH-solubility curves of unmodified and trypsin-hydrolyzed okara protein isolates. ◆ unhydrolyzed okara protein isolate (OPI); ■ OPI (DH = 4.9); ▲ OPI (DH = 8.3); ○ OPI (DH = 13.7). (DH = 13.7).

CONCLUSION

WITH GOOD NUTRITIONAL QUALITY, OPI IS A POTENTIAL LOW-cost ingredient for formulated foods. Its improvement in solubility and some functional properties by enzyme hydrolysis would further enhance its utilization. As okara is a waste by-product of soymilk and tofu manufacturing, the cost of the raw material would be very low. The cost of producing hydrolyzed OPIs could be reduced by combining the extraction of protein from wet okara with enzyme treatment, instead of using dried isolates.

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