Effect of Lipids on Soy Protein Isolate Solubility

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ABSTRACT: Reduced-lipid soy protein isolate (SPI), prepared from soy flour treated so that most of the polar lipids have been removed, exhibited an increase in protein solubility of 50% over that of the control SPI prepared from hexane-defatted flour. Adding lipids from a commercial SPI during processing of reduced-lipid SPI decreased SPI solubility by 46%. The 19% decreased solubility caused by the lipids (primarily phospholipids) was largely recovered by treating the protein with a reducing agent (2-mercaptoethanol). The balance of protein insolubility, caused by the lipids, was attributed to a smaller lipid fraction (approximately 5% of the total lipids). Adding lipids during SPI processing contributed to both the formation of oxidized protein sulfhydryls, incapable of being reduced by 2-mercaptoethanol, and to oxidative deterioration of protein as determined by protein carbonyl contents.
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KEY WORDS: Lipid oxidation, lipids, phospholipids, protein oxidation, protein solubility, soy protein isolate, sulfhydryl.

Protein solubility in water is an important functional characteristic for most applications of soy protein isolates (SPI) and is indicative of water–protein interactions that relate to other functional characteristics. Nash and Wolf (1) examined the solubilities of both laboratory-prepared and commercially manufactured SPI in 0.5 ionic strength buffer (pH 7.6) with and without 0.01 M 2-mercaptoethanol. SPI insolubility was due partly to the formation of disulfide-linked polymers of the 7S and 11S proteins. A subsequent investigation (2) indicated that the fraction remaining insoluble in the buffer with the reducing agent was the result of protein denaturation during isoelectric precipitation.

Lipid peroxides cause oxidation of thiol groups in enzymes (3), nonenzymic proteins (4,5), and low-molecular-weight thiols (6). Treatment of cytochrome *c* with lipid peroxides has been shown to modify tyrosine, tryptophan, cystine, histidine, and methionine residues (7). Roubal and Tappel (8) demonstrated that free radicals, derived from peroxidizing lipids, induce polymerization of proteins and consequently, decrease protein solubility. In this investigation, we examined the lipids associated with SPI and their effects on protein solubility and protein oxidation.

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MATERIALS AND METHODS

Defatted soybean flour with a particle size ≤0.25 mm was prepared from seed-grade Forrest var. soybeans. Dehulling was done with a blender (about 3 min on high speed), and the hulls were removed with a vertical aspiration unit (Seedburo Equipment Co., Chicago, IL). Full-fat flour was produced with a UDY Cyclone Sample Mill (UDY Corp., Fort Collins, CO) without an outlet screen. Sieving full-fat flour was accomplished with an Alpine AirJet Sieve (Alpine American Corp., Natick, MA) through a 60-mesh screen. One part of full-fat flour was mixed with 5 parts hexane, agitated by hand for 5 min, and centrifuged at 1000 × *g* for 10 min at 20°C. The hexane micella (supernatant) was decanted and discarded. The extraction was repeated three more times. The hexane was evaporated from the defatted flour in a fume hood for 6 h at 20°C, and the residual hexane was evaporated overnight in a forced-air oven at 30°C overnight.

The reduced-lipid flour was prepared by suspending approximately 75 g hexane-defatted flour in 500 mL American Chemical Society-grade glycerol by using a blender with a lid at 25°C. This was done by using pulsations of high speed (for approximately 3 min) to minimize incorporating large amounts of air into the slurry. After standing 30 min, 300 mL 2-propanol was blended into the mixture. This slurry was centrifuged at 1,000 × *g* for 15 min. The pellet was resuspended in 400 mL 2-propanol, followed by centrifuging to remove residual glycerol from the pellet. Washing with 2-propanol was repeated two more times. The bulk of 2-propanol was evaporated from the reduced-lipid flour in a fume hood at 20°C for 6 h and the residue 2-propanol was evaporated overnight in a forced-air convection oven at 30°C.

Protein isolates. Laboratory control isolates were prepared by dispersing the hexane-defatted soybean flour in water (1 part flour to 10 parts water), followed by additions of 1 N sodium hydroxide, as needed, until pH 9 was achieved and maintained for 15 min. After centrifuging at 1,500 × *g* for 10 min, the supernatant was adjusted to pH 4.5 with 1 N HCl to precipitate proteins. After centrifuging at 1,500 × *g* for 10 min, the precipitate was washed once with water, and the protein isolate was adjusted to pH 7 with 1 N sodium hydroxide. Samples were freeze-dried after being frozen overnight at −15°C.

The reduced-lipid SPI was prepared by the same method as was the control SPI, except that it was started with re-

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duced-lipid flour. To evaluate the effect of various types of lipids, SPI was prepared from reduced-lipid flour, and lipids were added to the alkaline protein extract prior to isoelectric precipitation at a ratio of 0.75 g lipid to 50 g starting reduced-lipid flour. The lipids, in either chloroform or methanol/chloroform, were brought to dryness in a rotary evaporator at 60°C, and 508 mm Hg vacuum and then suspended in water by sonicating with a Sonorex Super RK106 sonicator (Bandelin Electronics, Berlin, Germany). The suspension was then added to the aqueous reduced-lipid flour slurry.

The commercial SPI was Pro Fam 970, obtained from the Archer Daniels Midland Co. (Decatur, IL), and Supro 760 from Protein Technologies International (St. Louis, MO).

Fractionation of lipid extracts. Lipid extracts from the commercial SPI were fractionated by CM-cellulose column chromatography by the method of Comfurius and Zwaal (9) with preswollen CM-cellulose, CM-52 (Whatman, Clifton, NJ).

Protein solubility. Assays of protein solubility were performed according to the method of Morr *et al.* (10), with the following modifications: (i) sample pH (6.8 ± 0.2) was not adjusted; (ii) one drop of Antifoam B Silicone Emulsion (J.T. Baker, Phillipsburg, NJ) was added to the 50-mL volumetric flasks prior to bringing the solution to volume; and (iii) centrifugation was accomplished at 20,000 × *g* for 10 min. Unless otherwise noted, 0.1 M NaCl was the solvent, and soluble nitrogen was determined by micro-Kjeldahl.

Peroxide value (PV). PV of lipid extracts was determined by the ferric thiocyanate method (11).

Sulfhydryl and disulfide content of protein. The sulfhydryl (free and buried SH) and total sulfhydryl (SH and reduced SS) groups of protein were determined in triplicate by titrating with 5,5′-dithiobis(2-nitrobenzoate) (DNTB), using the general procedure of Ellman (12). SPI samples (75 mg) were suspended in 10 mL 0.1 M phosphate buffer (pH 8.0), 1 mM disodium ethylenediaminetetraacetic acid (EDTA), and 1% sodium dodecyl sulfate (SDS) by stirring with a 1.25-cm stir bar in a 20-mL beaker on medium speed for 30 min at 20°C.

For SH determinations, 3 mL 0.1 M phosphate buffer (pH 8.0), 1 mM EDTA, 1% SDS, and 0.1 mL DTNB reagent were added to 3 mL protein solution, which was then vortexed and incubated at 25°C for 1 h (13). This mixture was then centrifuged at 10,000 × g for 30 min. For total SH determination, the method of Beveridge *et al.* (14) was modified by using 0.1 M phosphate buffer (pH 8.0), 1 mM EDTA, and 1% SDS instead of *Tris*-glycine buffer, and 0.08 mL DTNB reagent was added. Absorbance was measured at 412 nm against reagent blanks. Calculations were based on an extinction coefficient of 13.6 mM^{-1} cm^{-1} for the thiolate chromogen (12).

Protein oxidation. Protein oxidation was measured in triplicate as the carbonyl content of the protein by the method of Levine *et al.* (15). SPI samples were suspended in deionized water by stirring with a 1.25-cm stir bar on medium speed for 30 min at 20°C to provide a solution that contained from 1.0 to 1.5 mg protein per 0.35 mL. In 2.0-mL capped polyethylene centrifuge tubes, 0.35 mL protein solution was mixed with 1 mL 10 mM 2,4-dinitrophenylhydrazine in 2 N HCl and incubated at 20°C for 2 h. A matching aliquot was mixed in 1 mL 2 N HCl as an absorbance blank. Then 0.45 mL 40% trichloroacetic acid was added to each tube. The tubes were then vortexed, allowed to stand for 20 min, and centrifuged. The supernatant was discarded, and the pellet was washed three times with 1.5 mL ethanol/ethyl acetate solution (1:1, vol/vol). The protein, free of unreacted reagent, was suspended in 1.0 mL 6 M guanidine hydrochloride solution by incubating at 37°C for 20 min, with vortexing every 5 min. The absorbance at 367 nm was corrected for the absorbance in the HCl blank, and the moles of carbonyl derivative per mg protein were calculated by using the extinction coefficient of 22,000 M^{-1} cm^{-1}.

Analytical. Protein isolates were analyzed for moisture in triplicate by drying at 130°C for 1 h. Nitrogen was determined in triplicate by micro-Kjeldahl, and values were multiplied by 5.71 to calculate protein (16). Values were reported as percentages by weight on dry basis.

Lipids were extracted by either the modified method of Bligh and Dyer (17) or by the modified method of Folch *et al.* (18), as previously described (19). Lipids reintroduced into SPI during processing or fractionated by CM-cellulose column chromatography were extracted in a blender with a 5:1 ratio of solvent to lipid-bearing material. This was repeated twice, and the extracts were combined prior to phase separation. Larger-scale SPI extractions were accomplished by using the modified method of Bligh and Dyer (17), and hexane-defatted flour extractions were performed by the modified Folch *et al.* (18) procedure.

Total lipids were determined in triplicate from each duplicate extraction as the weight of total solids in the chloroform phase after evaporating the solvent. The samples were placed on a hot plate on low setting in a fume hood, and the solvent was allowed to evaporate until the samples appeared dry. They were then placed into a forced-air convection oven at 130°C for 15 min. Values were reported as percentages by weight on dry basis.

Phospholipid contents were determined in triplicate from the phosphorus content (20) of the chloroform phase multiplied by 25 (21). Two-dimensional thin-layer chromatography (TLC) was accomplished as previously described (19).

Statistical analysis. Statistical evaluation of data was conducted by the Statistical Analysis System (22) with general linear model (GLM) analysis of variance. Least significant difference (LSD) values were computed at the 5% level.

RESULTS AND DISCUSSION

Composition and protein solubility data of the commercial SPI, designated as "highly soluble," are presented in Table 1. The solubility of each commercial SPI in 0.1 M NaCl is low. Chen and Morr (23) reported similar results (12% protein solubility) for Supro-620 SPI at pH 6.0 in 0.1 M NaCl. The 9–24% increases in protein solubilities with the addition of 0.01 M 2-mercaptoethanol to 0.1 M NaCl were similar to

TABLE 1
Compositions and Protein Solubilities of Commercial Soy Protein Isolate

	Pro Fam 970[a]		Supro 760[b]	
	Sample A	Sample B	Sample A	Sample B
Moisture, %	5.5 (0.02)[c]	7.6 (0.12)	5.6 (0.17)	5.3 (0.01)
Protein, % ($N \times 5.71$)	83.5 (0.43)	83.4 (0.37)	82.2 (0.55)	83.5 (0.20)
Protein solubility, % (pH 6.8 ± 0.2)	13.0 (0.3)	12.0 (0.1)	11.0 (0.2)	14.0 (0.2)
Protein solubility, % (pH 6.8 ± 0.2)[d]	22.0 (0.8)	26.0 (0.2)	24.0 (0.2)	38.0 (0.2)
Total lipids, %[e]	3.9 (0.03)	4.2 (0.04)	4.8 (0.02)	4.8 (0.02)
Phospholipids, % (lipid $P \times 25$)	2.6 (0.07)	2.5 (0.08)	2.9 (0.07)	3.3 (0.08)

[a]Archer Daniels Midland Co. (Decatur, IL).
[b]Protein Technologies International (St. Louis, MO).
[c]Figures in parentheses are standard errors.
[d]0.1 M NaCl/0.01 M 2-mercaptoethanol with protein determined by the biuret method (Ref. 26).
[e]Total lipids extracted with the modified Bligh and Dyer procedure (Ref. 17).

those observed by Nash and Wolf (1) in 0.5 M NaCl. The amounts of lipids extracted from these isolates (3.9–4.8%) was much greater than the 0.1% total fat reported for SPI extracted with petroleum ether (24,25). Our lipid extracts contained from 59–69% phospholipids.

Composition and solubility data for SPI prepared in our laboratory, along with composition data for the corresponding soy flours, are presented in Table 2. The modified Folch *et al.* procedure (18) extracted lipids more effectively from the defatted flour than did the modified Bligh and Dyer procedure (17), and the control SPI lipids were best extracted by the modified Bligh and Dyer procedure (17). The lipid contents of our control SPI were in the range of lipid contents found in the commercial SPI samples. The protein solubility of our control SPI, while better than that of commercial samples, was poor. Extraction of hexane-defatted flour with glycerol and 2-propanol produced a reduced-lipid flour with total lipids and phospholipids contents approximately 90% lower than that of hexane-defatted flour. The protein solubility of SPI, prepared from the reduced-lipid flour, increased 147% over that of the control. The decrease of 4.4% lipids in the reduced-lipid SPI, compared with the control SPI, corresponds to a 6% increase in protein. The difference in total phosphorus content between our control and the reduced-lipid SPI can be accounted for by the nearly complete removal of phospholipids. The remaining phosphorus in the reduced-lipid SPI indicates that this procedure had little effect on other sources of phosphorus, such as phytate.

To further investigate the effect of lipids on the soy protein solubility, additional reduced-lipid SPI was prepared, except that, prior to isoelectric precipitation, lipids extracted from the commercial SPI and from the hexane-defatted flour were returned to the alkaline protein extracts at a level similar to that of the control SPI. Composition and protein solubility data for these SPI are presented in Table 3.

The addition of lipids from the commercial SPI resulted in a 46% decrease in protein solubility compared with the reduced-lipid SPI. Despite the increased level of binding to the protein, the lipids from the hexane-defatted flour were less detrimental to protein solubility (23% decrease). These results suggest that some form of lipid degradation occurred during SPI processing, which contributed to reduced protein solubility. While the addition of 2-mercaptoethanol to the solvent increased the percentage of soluble protein in the SPI with added hexane-defatted flour lipids, the solubility of the SPI with added commercial SPI lipids decreased slightly. All

TABLE 2
Compositions and Solubilities of Control and Reduced-Lipid Soy Protein Isolates (SPI) with Compositions of Corresponding Defatted and Reduced-Lipid Flour

	Hexane-defatted flour	Control SPI	Reduced-lipid flour	Reduced-lipid SPI
Moisture, %	10.4 (0.02)[a]	6.3 (0.19)	8.8 (<0.01)	2.7 (0.03)
Protein, % ($N \times 5.71$)	48.3 (0.56)	83.2 (0.53)	54.6 (0.60)	89.1 (0.14)
Protein solubility, % (pH 6.8 ± 0.2)	—	34.0 (1.4)	—	84.0 (1.3)
Protein solubility, % (pH 6.8 ± 0.2)[b]	—	76.0 (0.3)	—	97.0 (0.0)
Total lipids, %[c]	1.60 (0.10)A[e]	4.63 (0.05) A	—	0.26 (0.01)
Total lipids, %[d]	3.69 (0.02)B	3.09 (0.06)B	0.39 (0.01)	—
Phospholipids, % (lipid $P \times 25$)	2.40 (< 0.01)[d]	3.20 (0.05)[c]	0.20 (< 0.01)[d]	0.04 (< 0.01)[c]
Total phosphorus, %	—	0.78 (0.04)	—	0.69 (0.02)

[a]Figures in parentheses are standard error.
[b]0.1 M NaCl/0.01 M 2-mercaptoethanol with protein determined by the biuret method (Ref. 26).
[c]Total lipids extracted with the modified Bligh and Dyer procedure (Ref. 17).
[d]Total lipids extracted with the modified Folch procedure (Ref. 18).
[e]The same letter in columns indicates no significant difference at $P \geq 0.05$.

TABLE 3
Compositions and Protein Solubilities of Reduced-Lipid Soy Protein Isolates (SPI) with Added Lipid Extracts

	Type of lipid added			
	Commercial SPI lipids	Hexane-defatted flour lipids	Commercial SPI Fraction A	Commercial SPI Fractions A and B
Moisture, %	2.3 (0.01)[a]	2.4 (0.08)	2.1 (0.07)	2.7 (0.03)
Protein, % ($N \times 5.8$)	86.8 (0.13)	85.0 (0.15)	84.4 (0.36)	85.1 (0.35)
Protein solubility, % (pH 6.8 ± 0.2)	38.0 (0.3)	61.0 (0.9)	65.0 (1.3)	24.0 (0.9)
Protein solubility, % (pH 6.8 ± 0.2)[b]	33.0 (0.0)	70.0 (0.0)	88.0 (0.1)	29.0 (0.2)
Total lipids, %[c]	4.7 (0.02)	5.7 (0.02)	3.7 (0.02)	3.3 (0.01)

[a]Figures in parentheses are standard errors.
[b]0.1 M NaCl/0.01 M 2-mercaptoethanol with protein determined by the biuret method (Ref. 26).
[c]Total lipids extracted with the modified Bligh and Dyer procedure (Ref. 17).

solubility analyses after employing 2-mercaptoethanol were performed approximately six months after the evaluations in 0.1 M NaCl with samples that were stored in the dark at 4°C. The solubility of the SPI with added commercial SPI lipids (see Table 3) in 0.1 M NaCl after the same storage time was 28%, which indicates protein degradation in this material during storage. The addition of 2-mercaptoethanol contributed only a 5% increase in the solubility of the SPI with added commercial SPI lipids.

Lipid extracts from the commercial SPI and hexane-defatted flour were examined after separation by two-dimensional TLC (Fig. 1). The two most apparent types of lipid degradation in the SPI extract (compared with the defatted flour extract) were large increases in the phosphatidic acid (PA) content and in material that remained at or near the origin. Removal of the majority of PA from the commercial SPI lipid extract by CM-cellulose column chromatography, prior to adding these lipids in the processing of the reduced-lipid SPI, had approximately the same effect on protein solubility as did the addition of the PA fraction. The addition of the entire lipid extract, eluted from the CM-cellulose with 50% methanol in chloroform (Fraction A), made a similar contribution to protein solubility in the prepared isolate (19% decrease compared with the reduced-lipid SPI), as did the addition of the total lipid extract from the hexane-defatted flour (23% decrease)(see Table 3).

Triplicate determinations on each of three preparations of the lipids, designated as Fraction A, accounted for 89.9 ± 0.72% by weight of the total material loaded on the CM-cellulose column. Subsequent elution with 100% methanol through the column produced an additional 4.7 ± 0.04% weight of nonvolatile material, which was designated as Fraction B and corresponded to the material that did not migrate from the origin during two-dimensional TLC. Analyses of SPI prepared with either 100% lipid Fraction A or with 95.2% Fraction A and 4.8% Fraction B are presented in Table 3. The detrimental effect of Fraction B on protein solubility is evident. The SPI with added Fractions A and B lipids, such as the SPI with the total commercial SPI lipid extract added, exhibited poor solubility in 0.1 M NaCl (24 and 38%, respectively), and little improvement in protein solubility was obtained with the addition of 2-mercaptoethanol.

In the SPI prepared with added lipids, the free SH content appears to have little relationship to protein solubility (Table 4). The total SH and SS contents of the control SPI and the reduced-lipid SPI were similar to Pro Fam 970 (Table 5). The Supro 760, with the higher lipid content, had lower total SH and SS contents, similar to the SPI examined by Voutsinas and Nakai (27). The lipids from the hexane-defatted flour (PV of 7.9 ± 0.4 meq/kg) contributed to a decrease in the amount of disulfide bonds reduced by 2-mercaptoethanol, and consequently, to a decline in the total SS and SH contents as compared with the reduced-lipid and the control SPI (see Table 4). The lipid extract from the commercial SPI (PV of 8.8 ± 0.2) resulted in an even larger decrease in disulfide bonds available for reduction by 2-mercaptoethanol. Little and O'Brien (4) demonstrated that lipid peroxides can react with protein thiols to form monomeric oxidation products, such as sulfonic acid, which are not reduced by 2-mercaptoethanol and may be responsible for the loss of total sulfhydryl content (SH and SS) in the SPI with added lipids. PVs of lipid Fraction A and Fraction B were 26.7 ± 0.3 and 9.5 ± 0.3 meq/kg, respectively, indicating greater lipid oxidation for lipid Fraction A during column fractionation. Because these combinations of fractions both contributed similar reductions in total SH and SS, as did the defatted flour lipid extract, the detrimental effect of lipid Fraction B appears to be due to some other reason than its ability to oxidize protein sulfhydryls.

The measurements of protein oxidation (see Table 4) demonstrate that the reduced-lipid SPI contained the lowest level of carbonyls. The addition of the defatted flour lipids contributed to increased protein oxidation, while the commercial SPI lipid extract and its fractions resulted in further increases. These increases in protein oxidation with the addition of lipids and the carbonyl contents of the control SPI and the commercial SPI (see Table 5) suggest that protein oxidation may have an important effect on protein solubility. The detrimental effect of lipid Fraction B, again, appears to be related to some other property than its ability to contribute to the level of protein oxidation products.

It is likely that reintroducing lipids during SPI processing presents lipids to the proteins in a different manner than normally occurs during SPI processing, where the lipids are pre-

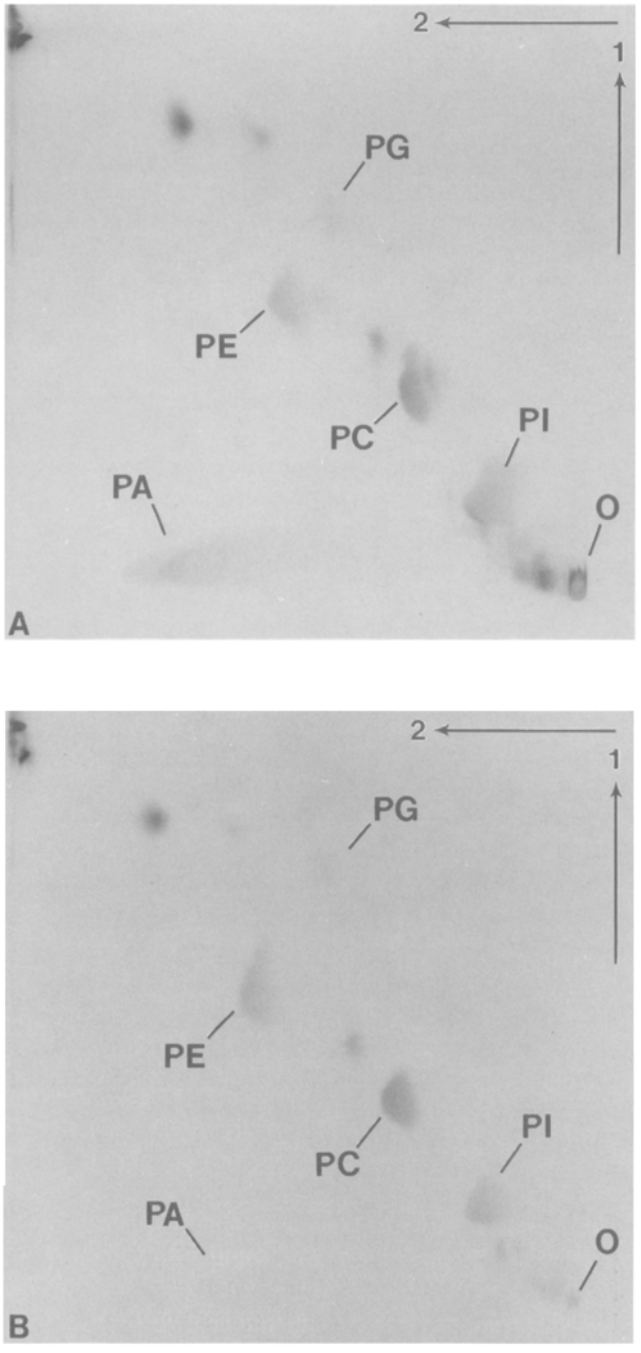

FIG. 1. Lipid extracts from commercial soy protein isolate and hexane-defatted flour examined after two-dimensional thin-layer chromatography; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; PA, phosphatidic acid; O, origin; PI, phosphatidylinositol.

dominantly associated with membranes prior to cell disruption. This difference in presentation may account for the increased level of sulfhydryi oxidation products that are not reduced with 2-mercaptoethanol in the SPI with added lipids, compared with our control SPI and the commercial SPI. Also, the higher carbonyl content of our control SPI and the commercial SPI, compared to the SPI with added lipids, suggests that this type of protein modification occurs more readily in

TABLE 4
Effects of Added Lipids on the Sulfhydryl (SH) and Carbonyl Contents of SPI

	Free SH[a]	Total SS and SH[a]	Carbonyls[b]
Control SPI	8.32 (0.09)[c]A[d]	61.23 (0.37)A	4.73 (0.06)A
Reduced-lipid SPI	3.59 (0.03)B	60.09 (1.06)A	2.32 (0.02)B
SPI with added defatted flour lipids	2.86 (0.04)C	52.82 (1.96)B	2.63 (0.03)C
SPI with added commercial SPI lipids	2.72 (0.03)C	47.83 (1.23)C	2.93 (0.02)D
SPI with added Fraction A lipids	4.29 (0.04)D	54.72 (0.85)B	2.86 (0.08)D
SPI with added Fraction A and B lipids	4.56 (0.07)E	53.98 (0.41)B	2.90 (0.09)D

[a]μmoles SH/g protein. SPI, soy protein isolates.
[b]nmoles Carbonyl/mg protein.
[c]Values in parenthesis are standard errors.
[d]The same letter in columns indicates no significant difference at $P \geq 0.05$.

TABLE 5
Sulfhydryl and Carbonyl Contents of Commercial SPI

	Free SH[a]	Total SS and SH[a]	Carbonyls[b]
Pro Fam 970[e], sample A	4.07 (0.01)[c]A[d]	62.06 (1.24)A	4.48 (0.15)A
Pro Fam 970, sample B	5.55 (0.03)B	62.34 (0.41)A	6.42 (0.07)B
Supro 760[e], sample A	6.15 (0.06)C	57.78 (0.43)B	6.25 (0.24)B
Supro 760, sample B	5.59 (0.21)B	57.92 (0.90)B	6.15 (0.16)B

[a]μmoles SH/g protein. Abbreviations as in Table 4.
[b]nmoles carbonyl/mg protein.
[c]Values in parenthesis are standard errors.
[d]The same letter in columns indicates no significant difference at $P \geq 0.05$.
[e]See Table 1 for company sources.

SPI processed from hexane-defatted soybean. The corresponding decreases in protein solubility and increases in protein oxidation products with the addition of lipids suggest that controlling lipid and protein oxidation during SPI processing may result in soy protein products with improved solubility characteristics. Identification of the components of lipid Fraction B may elucidate the reason for the detrimental effect of this material on SPI solubility.

REFERENCES

1. Nash, A.M., and W.J. Wolf, *Cereal Chem. 44*:183 (1967).
2. Nash, A.M., W.F. Kwolek, and W.J. Wolf, *Ibid 48*:360 (1971).
3. Lewis, S.E., and E.D. Willis, *Biochem. Pharmacol. 11*:901 (1962).
4. Little, C., and P.J. O'Brien, *Arch. Biochem. Biophys. 122*:406 (1967).
5. Little, C., and P.J. O'Brien, *Biochem. J. 106*:419 (1968).
6. Little, C., and P.J. O'Brien, *Ibid. 102*:10P (1967).
7. O'Brien, P.J., *Ibid. 101*:12P (1966).
8. Roubal, W.T., and A.L. Tappel, *Arch. Biochem. Biophys. 113*:150 (1966).
9. Comfurius, P., and R.F.A. Zwaal, *Biochim. Biophys. Acta, 488*:36 (1977).

10. Morr, C.V., B. German, J.E. Kinsella, J.M. Regenstein, J.P. Van Buran, A. Kilara, B.A. Lewis, and M.E. Mangino, *J. Food Sci. 50*:1715 (1985).
11. Chapman, R.A., and K. Mackay, *J. Am. Oil Chem. Soc. 26*:360 (1949).
12. Ellman, G.L. *Arch. Biochim. Biophys. 82*:70 (1959).
13. Girotti, A.W., *J. Biol. Chem. 253*:7186 (1978).
14. Beveridge, T., S.J. Toma, and S. Nakai, *J. Food Sci. 39*:49 (1974).
15. Levine, R.L., D. Garland, C.N. Oliver, A. Amici, I. Climent, A.-G. Lenz, B.-W. Ahn, S. Shaltiel, and E.R. Stadtman, in *Methods of Enzymology,* Vol. 186, edited by L. Packer, and A.N. Glazer, Academic Press, San Diego, 1990, pp. 464-478.
16. Morr, C.V., *J. Food Sci. 47*:1751 (1982).
17. Bligh, E.G., and W.J. Dyer, *Can. J. Biochem. Phys. 37*:911 (1959).
18. Folch, J., M. Lees, and G.H. Stanley, *J. Biol. Chem. 226*:497 (1957).
19. Boatright, W.L., and H.E. Snyder, *J. Am. Oil Chem. Soc. 70*:623 (1993).
20. Bartlett, G.R., *J. Biol. Chem. 234*:466 (1959).
21. Chapman, G.W., *J. Am. Oil Chem. Soc. 57*:299 (1980).
22. Statistical Analysis System, *SAS User's Guide,* SAS Institute Inc., Cary, 1987.
23. Chen, B.H.-Y., and C.V. Morr, *J. Food Sci. 50*:1139 (1985).
24. Horan, F.E., *J. Am. Oil Chem. Soc. 51*:67A (1974).
25. Alais, C., and G. Linden, in *Food Biochemistry,* Ellis Horwood, New York, 1990, p. 132.
26. Gornall, A.G., C.G. Bardwill, and M.M. David, *J. Biol. Chem. 177*:751 (1949).
27. Voutsinas, L.P., and S. Nakai, *Can. Inst. Food Sci. Technol. J. 16*:185 (1983).

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