Preparation and Composition of Coprecipitated Protein Isolates from Cottonseed, Soybean, and Peanut Flours[1]

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ABSTRACT

Coprecipitated protein isolates were prepared from various combinations of liquid cyclone processed (LCP) cottonseed, soybean, and peanut flours (1:1, w/w) and from each of the flours. Coisolate and isolate methodology involved protein extraction with dilute aqueous NaOH, acidification of the protein extracts to pH 2.5, and adjustment of the resulting mixtures to pH 5.0 to precipitate protein curds. The protein curds were recovered, resuspended in water, neutralized, and lyophilized. Disc-gel electrophoresis showed that some of the proteins in the extracts dissociated into subunits at pH 2.5, then reassociated into their original or new protein forms, or both, as the pH was adjusted to 7.0. The coisolates, containing storage and nonstorage proteins, were high in protein content (95%) and accounted for more than 67 and 43% of total nitrogen and weight, respectively, of the composite flours. The amino acid and chemical scores indicated that addition of soybean flour to LCP cottonseed and peanut flours improved the nutritional value of coisolates. The coisolates might be useful as new, low-cost protein products for food formulations.

Oilseeds are potential sources of low-cost, edible vegetable proteins for supplementing dietary shortages (Altschul 1974, Mattil 1971). World soybean, cottonseed, and peanut cultivation is great enough to contribute vegetable proteins in quantities equivalent to those of edible animal proteins (Altschul 1970). Soybean protein products (meals, grits, flours, concentrates, and isolates) are used commercially as ingredients in foods (Wolf and Cowan 1971); however, processing of cottonseeds and peanuts into similar protein products for food use is still in the experimental stage (McWatters and Cherry 1975, Olsen 1973). Peanuts are consumed in peanut butter, candies, salted nuts, and snack crackers because of their highly acceptable roasted flavor.

Among the many oilseed products developed, protein isolates represent the highest refinement of protein relative to compositional, functional, and nutritional properties. Proper use of protein isolates depends on the source material, denaturation during processing of isolates, method efficiency during preparation, composition, and refining processes.

Just as certain advantages are recognized in using flour blends for human consumption (Dendy et al 1975, Tsen 1976), coprecipitated protein isolates from two or more oilseed flours might offer certain advantages. Wilcke (1974) suggested that coprecipitation of proteins could be used to alter amino acid content, to provide variations in solubility characteristics, and to meet certain functional requirements not met by any one protein source.

This article describes a procedure for preparing coisolates of both storage and nonstorage proteins of oilseeds. The procedure efficiently extracts the proteins from combinations of liquid cyclone processed (LCP) cottonseed, soybean, and peanut flours and yields coisolates that may contain new protein forms in additon to those extracted from the composite flour.

MATERIALS AND METHODS

Flours

Cottonseed meal was prepared at Southern Regional Research Center from glanded cottonseed kernels by the modified liquid cyclone process (Gardner et al 1976). Hexane-extracted soybean flakes of high solubility were obtained from a commercial source. Peanut meal was prepared by hexane extraction of blanched, split-nut kernels with the continuous extractor (Pominski et al 1975). Each of the defatted, desolventized samples was ground to a flour by the Alpine Kolloplex 160 Z mill.[3] The flours exhibited good nitrogen solubility, indicating that proteins had not undergone appreciable denaturation during processing.

Coisolate Formation

Figure 1 summarizes the laboratory procedure for preparing the coprecipitated protein isolates from flour mixtures. The mechanics of the procedure are based on data of previous studies (Berardi et al 1969, Martinez and Berardi 1971, Martinez et al 1970) isolating and characterizing cottonseed proteins. Each mixture contained an equal weight of the two oilseed flours. The flour mixture and 0.034*N* NaOH suspension (15:1, v/w; pH 10.5) were agitated for 30 min with a Burrell wrist-action shaker, then centrifuged to provide a spent flour residue and the protein extract. The extract was decanted and filtered; the residue was lyophilized and stored for analysis.

While being constantly stirred, the protein extract was acidified to pH 2.5 by dropwise addition of 1.0*N* HCl (Fig. 1). At pH 2.5, the protein mixture was stirred for an additional 5 min to aid dissociation of proteins. The stirred extract was adjusted to pH 5.0 with 1.0*N* NaOH to precipitate the proteins. Centrifugation of the pH 5.0 mixture provided the whey and protein curd fractions; the whey was decanted, filtered, and stored for analysis. The protein curd was resuspended in deionized water, adjusted to pH 7.0 with 1.0*N* NaOH, and lyophilized to yield the coisolate. Centrifugations were conducted at 14,000 rcf and 24° C for 30 min. All other steps in the laboratory procedure were done at room temperature (23–26° C).

The procedure outlined in Fig. 1 also was applied to each of the flours to make the corresponding isolates.

Methods of Analysis

The macro-Kjeldahl procedure was used to determine nitrogen content. Determinations of moisture, lipid, free and total gossypol, crude fiber, and ash contents were made with recommended procedures (AACC 1961, AOCS 1976). Sugar content was determined by the modified procedure of Larson et al (1974), and amino acids by the gas-liquid chromatographic procedure of Kaiser et al (1974) at a commercial laboratory. Spies and Chambers' colorimetric procedure (1948) was used to determine tryptophan.

To demonstrate qualitative changes in soluble protein during specific steps of coisolate or isolate formation, polyacrylamide disc-gel electrophoresis was performed on these fractions according to the procedures of Cherry et al (1970) and Canalco (1973). Electrophoretic patterns were obtained under nondissociating conditions with the Ames Model 1200 Bath apparatus. Disc-polyacrylamide gels (10%) were set with Tris-glycine buffer (pH 8.9) and run at pH 9.5. Proteins (200, 400, 600 μg) were evaluated in the following preparations: pH 10.5 extract; pH 2.5 dissociated

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[3]Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

proteins; and pH 7.0 coisolate or isolate.

Essential amino acid and chemical scores of proteins in flours, coisolates, and isolates were calculated (FAO/WHO 1973). Statistical evaluation of amino acid composition and calculated nutritional scores were performed with one-way analysis of variance and the Newman-Kuel's ranking of the mean values (Steele and Torrie 1960).

RESULTS AND DISCUSSION

Flours

Proximate compositions of the LCP cottonseed, soybean, and peanut flours are given in Table I. Gossypol content (0.02% free, 0.14% total gossypol) of the LCP cottonseed flour met Food and Drug Administration standards (Federal Register 1974) for an edible product. Comparison of the essential amino acid composition of the three flours (Table II) showed that LCP cottonseed and soybean flours contain more threonine, lysine, and tryptophan than does peanut flour. The LCP cottonseed flour has the highest half-cystine, and the peanut flour was intermediate for phenylalanine. These data suggested the possibility of forming coisolates (especially with LCP cottonseed and soybean proteins) with an essential amino acid composition of higher quality than that of the corresponding isolates.

Coisolate Formation

The new procedure outlined in Fig. 1 permitted preparation of coisolates possibly containing some new protein forms as well as the original nonstorage and storage proteins in the flours. It differed from a previously reported process (Martinez et al 1973) that also involved acid-dissociation and reassociation of proteins but provided coisolates containing only the storage proteins of the oilseed flours. The new procedure presented in Fig. 1 was designed for use with mixtures of defatted oilseed flours (1:1, w/w). It should, however, be readily applicable with mixtures containing various ratios of two or more oilseed flours, provided the volume and normality of the aqueous NaOH (extraction solvent) are sufficient to rupture membranes and cell walls and solubilize proteins.

Selection of pH 5.0 for coprecipitation of the proteins (Fig. 1) was based on previous work with cottonseed proteins (Berardi et al 1969, Martinez and Berardi 1971, Martinez et al 1970). Each of the oilseed flours used as protein sources in the present study demonstrates (Berardi et al 1972, Martinez and Berardi 1971) a characteristic pH value for the maximal, simultaneous precipitation of the nonstorage and storage proteins, but each pH value is sufficiently close to pH 5.0 to provide a representative composite of both protein classes in the centrifuged curds of coprecipitated proteins. The recovered curds were neutralized and dried by lyophilization. The procedure outlined in Fig. 1 proved equally feasible for preparing isolates from each of the oilseed flours.

TABLE I
Proximate Compositions of Defatted Oilseed Flours[a]

Component	LCP Cottonseed (%)	Soybean (%)	Peanut (%)
Protein[b]	68.1	57.1	67.2
Lipid	0.26	0.22	0.56
Carbohydrate	24.0	35.5	27.7
Crude fiber	2.2	3.2	3.4
Ash	7.6	7.1	4.5
Free gossypol	0.02	0.00	0.00
Total gossypol	0.14	0.00	0.00

[a] Dry weight basis.
[b] % Crude protein = % N × 6.25.

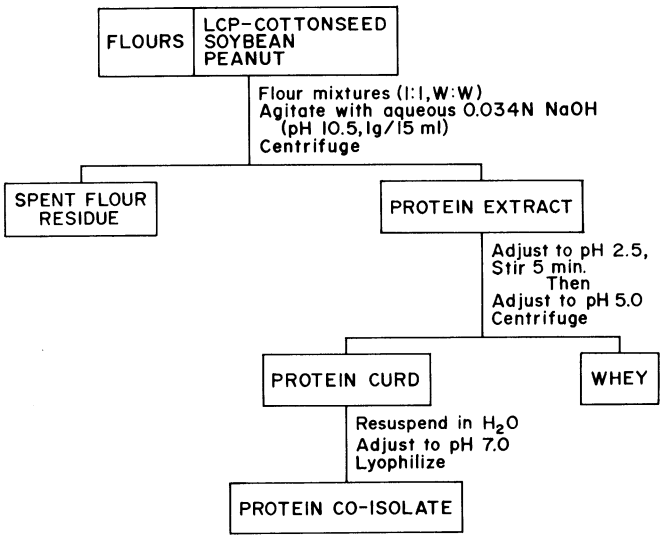

Fig. 1. Laboratory method for preparation of protein coisolates.

TABLE II
Amino Acid Composition of Oilseed Flours, Coisolates, and Isolates[a]

Amino Acid[b][c]	Flour			Coisolate			Isolate		
	LCP[d] Cottonseed	Soybean	Peanut	LCP Cottonseed-Soybean	LCP Cottonseed-Peanut	Soybean-Peanut	LCP Cottonseed	Soybean	Peanut
Glycine	4.39a	4.45a	5.65b	3.98a	4.21a	3.95a	4.27a	4.35a	4.36a
Threonine	3.65ed	3.70ed	2.61a	3.96e	3.06bc	3.40cd	3.56d	3.71de	2.86ab
Serine	5.00a	5.96bc	6.13d	5.41abc	5.21ab	4.83a	5.10a	5.62abc	5.50abc
Methionine	1.41bc	1.25abc	1.15ab	1.54c	1.22abc	1.29abc	1.37bc	1.16abc	0.96a
Phenylalanine	5.85bcd	4.75a	5.22ab	6.01cd	5.97cd	5.48bc	6.56d	6.20cd	5.48bc
Aspartic acid	9.76a	12.44de	12.82de	10.88b	11.40bc	11.95cd	9.75a	11.03bc	13.08e
Tyrosine	3.56ab	3.31a	4.30bc	3.55ab	4.11abc	4.20abc	3.44ab	3.57abc	4.54c
Lysine	5.10bcd	6.90d	3.66abc	5.08bc	3.54ab	4.52abc	4.10abc	5.28cd	3.22a
Histidine	2.66abc	2.26ab	2.22ab	3.10cd	2.96cd	2.81bc	3.58d	2.74bc	2.04a
Half-cystine	1.85b	1.29a	1.33a	1.37a	1.28a	1.61ab	1.15a	1.46ab	1.30a
Tryptophan	1.60d	1.60d	1.37abc	1.62d	1.40c	1.25ab	1.39bc	1.57d	1.23a

[a] Grams of amino acid/total grams of amino acids recovered.
[b] No significant differences were noted among flours, coisolates, and isolates for alanine (4.04–4.56%), valine (3.74–4.66), isoleucine (2.93–3.87), leucine (6.15–7.35), proline (4.02–5.30), glutamic acid (19.04–22.28), and arginine (7.86–11.90).
[c] Means having any letters in common are not different at the 0.05 level of probability.
[d] LCP = liquid cyclone processed.

Gel Electrophoresis

Proteins dissociated at pH 2.5 during isolate and coisolate preparation could be distinguished on the polyacrylamide disc-gels made under the nondissociating electrophoretic conditions (Fig. 2). An increase in the number of bands differing in mobility from those in gels of the 0.034*N* NaOH extracts typified most of the gel patterns that compared the steps of coisolate formation, especially during formation of LCP cottonseed-soybean and LCP cottonseed-peanut coisolates. On the other hand, both the coisolate and corresponding isolate preparations contained bands that are common for both pH 2.5 and 0.034*N* NaOH preparations. This similarity was demonstrated mainly in the steps forming the LCP cottonseed and soybean isolates.

The gel patterns of soluble proteins in the pH 7.0 preparations of coisolates and isolates suggested that the proteins in the pH 2.5 fraction reassociate into new or similar components. These major changes were noted with the LCP cottonseed and peanut isolates and in all three of the coisolate preparations. Adjusting the pH of the 0.034*N* NaOH extracts of individual or blended flours produces patterns either resembling those of individual extracts or, in the case of LCP cottonseed flour preparations, containing bands typical of water-soluble proteins (unpublished data).

These data suggest that the proteins were dissociated and reassociated into new combinations of components when the pH of the 0.034*N* NaOH extract was adjusted to 2.5, to 5.0, and finally to 7.0. Verification of these changes will have to await future characterization of each protein band in the gel patterns of preparations at the various steps of the method. Presence of some new protein forms, as well as original proteins, suggests that they may impart functional properties to the coisolates that may differ from those of isolates.

Efficiency of Method

Between 87.1 and 92.0% of total nitrogen in individual and blended flours was extracted with 0.034*N* NaOH (Table III). The lyophilized coisolates contained 14.7–15.2% nitrogen and accounted for recovery of 67.4–72.0% of the total nitrogen, or 43.7–48.6% of the total weight of the initial blends. These data agreed closely with those of the isolates. The yields of the coisolate preparations, which were quite similar to those of the isolates, suggested that the procedure outlined (Fig. 1) might be economically feasible for preparing protein derivatives on a pilot-plant scale.

The wheys produced during coisolate and isolate formation contained 0.4–1.0 mg nitrogen per milliliter. They accounted for similar recoveries (5.5–16.7%) of the total nitrogen in composite or individual flours. Recovery in the LCP cottonseed-soybean coisolate whey was comparable with that of the LCP cottonseed isolate (14.4 vs. 16.7%); recovery of the LCP cottonseed-peanut coisolate and the soybean isoalte whey were similar (10.7 vs. 7.9%); and the soybean-peanut coisolate and peanut isoalte wheys showed comparable recoveries (6.9 vs. 5.5% of the total nitrogen).

The lyophilized residues of spent flours of flour mixtures resulting from the initial protein solubilization step (0.034*N* NaOH extraction) contained similar nitrogen content ranging from 2.12 to 3.54% (Table III). Less than 10% of the total nitrogen and 16.2% (soybean) to 33.8% (soybean-peanut composite) of the total weight of the starting material were present in the residues. In addition to protein, the residues contained 51.7–67.5% carbohydrate, 9.3–11.1% crude fiber, 6.0–12.9% ash, and 1.7% or less of lipids.

Coisolates and Isolates

The coisolates and isolates contained more than 95% protein, small amounts of lipid, ash, and sugars, and less than 0.1% crude fiber (Table IV). Concentration of the gossypol pigments in the cottonseed isolate (0.03% free and 0.17% total gossypol) still permitted an acceptable level (Federal Register 1974) of free gossypol. Coprecipitation of the LCP cottonseed proteins with the soybean or peanut proteins provided coisolates with lower gossypol content (0.004–0.006% free, and 0.05% total gossypol) and lighter color than the isolates.

The coisolates and isolates contained only small amounts of the sugars originally present in the flours (Table V). Centrifugation of the protein precipitation mixtures at pH 5.0 (Fig. 1) allowed 83–92% recovery of the soluble carbohydrates in the wheys, thereby eliminating most of the sugars, believed to be involved in flatulence (soluble oligosaccharides), from the coisolates and isolates.

The content of many amino acids in the coisolates was similar to or intermediate between that of the corresponding isolates (Table II). Combining soybean flour with LCP cottonseed, however, significantly increased content of threonine, aspartic acid, and tryptophan in coisolates over that of LCP cottonseed isolate; soybean-peanut coisolate had significantly higher content of methionine and histidine than the peanut isolate.

The amino acid and chemical scores indicated that additon of soybean flour to LCP cottonseed and peanut flours improved the

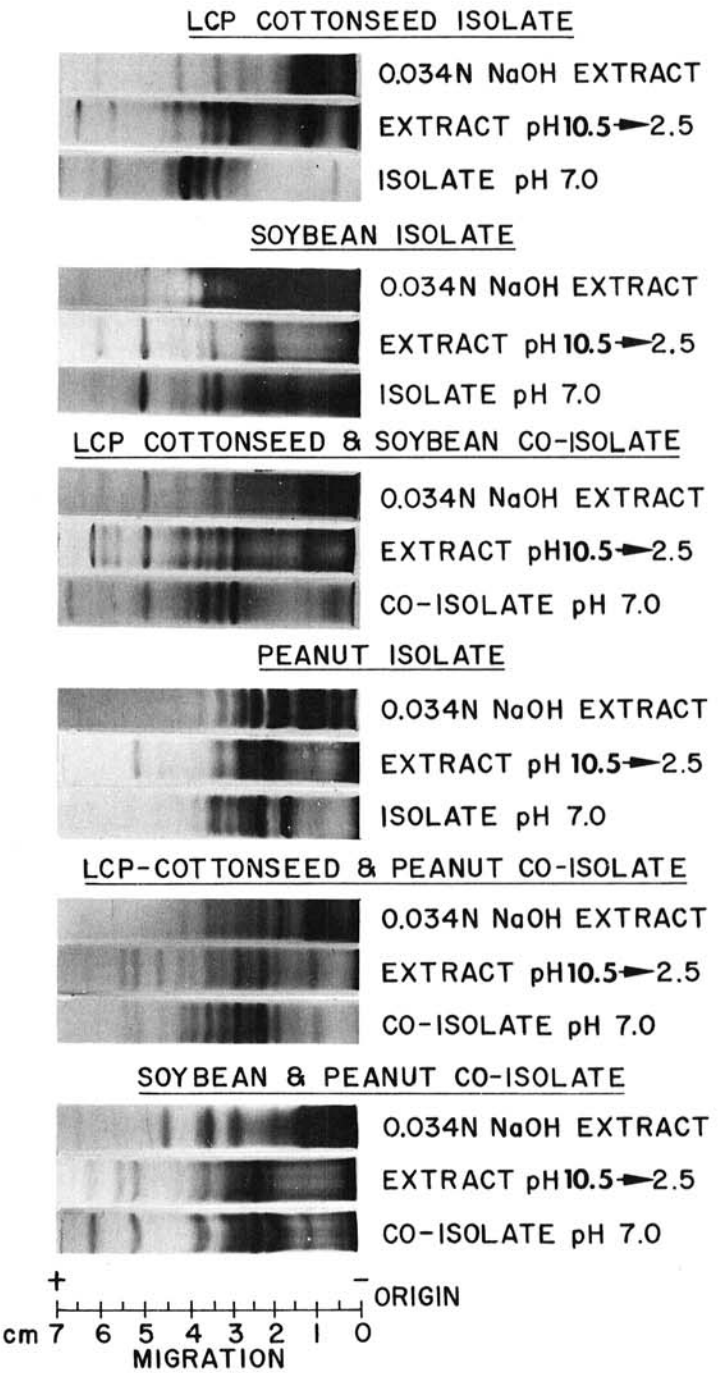

Fig. 2. Protein changes detected by gel electrophoresis during preparation of coisolates and isolates.

TABLE III
Recovery of Total Nitrogen and Total Weight of Flours and Flour Mixtures in Process Stream[a]

	Coisolate			Isolate		
	Cottonseed-Soybean	Cottonseed-Peanut	Soybean-Peanut	Cottonseed	Soybean	Peanut
0.034*N* NaOH Extracts						
% of total N	92.0	89.7	89.0	89.8	87.1	89.7
Coisolates-Isolates[b]						
Content of N (%)	14.68	15.15	15.05	15.01	14.43	15.37
% of total N	67.4	72.0	71.9	67.8	71.8	71.6
% of total wt	43.7	48.6	44.5	45.4	41.2	48.5
Wheys						
% of total N	14.4	10.7	6.9	16.7	7.9	5.5
Residues[b]						
Content of N (%)	3.28	2.43	2.78	2.12	3.60	3.54
% of total N	9.5	6.2	9.2	4.6	6.7	12.1
% of total wt	26.8	26.1	30.8	21.8	16.2	33.1

[a] Average values from two or more trials.
[b] Results based on analysis of lyophilized as-is coisolates, isolates, and residues.

TABLE IV
Proximate Composition of Coisolates and Isolates[a,b]

Component	Coisolate (%)			Isolate (%)		
	Cottonseed-Soybean	Cottonseed-Peanut	Soybean-Peanut	Cottonseed	Soybean	Peanut
Protein[c]	95.3	95.5	96.4	96.2	95.5	96.8
Lipid	0.22	0.60	0.85	0.25	0.16	0.31
Ash	3.2	2.7	1.6	2.7	3.2	2.1
Carbohydrate	1.2	1.1	1.1	0.7	1.1	0.7
Free gossypol	0.004	0.006	0.000	0.030	0.000	0.00
Total gossypol	0.05	0.05	0.00	0.17	0.00	0.00

[a] Percentage composition of lyophilized products on dry weight basis.
[b] Less than 0.1% crude fiber in products.
[c] Percentage protein = 100% − percent (sum of other components).

TABLE V
Sugar Content of Flours, Coisolates, Isolates, and Spent Flour Residues

Protein Product	Content of Sugars (Total, %)	% Recovery of Sugars from Flours	Content (%) of Sugars in Protein Products				
			Raffinose	Sucrose	Stachyose	Fructose	Glucose
Flour							
LCP cottonseed	12.90	100.0	11.17	1.73	Trace	0.00	Trace
Soybean	8.12	100.0	0.64	6.60	0.79	0.06	0.03
Peanut	9.95	100.0	0.14	9.26	0.36	0.11	0.08
Coisolate[a]							
LCP cottonseed-soybean	1.22	5.1	0.54	0.45	0.23	0.00	0.00
LCP cottonseed-peanut	1.04	4.4	0.44	0.56	0.04	0.00	0.00
Soybean-peanut	1.03	5.1	0.06	0.74	0.23	0.00	0.00
Isolate[a]							
LCP cottonseed	0.70	2.4	0.44	0.26	0.00	0.00	0.00
Soybean	1.06	5.4	0.12	0.62	0.28	0.00	0.04
Peanut	0.70	3.0	0.01	0.69	Trace	0.00	0.00
Spent Flour Residue[a]							
LCP cottonseed-soybean	3.23	8.23	1.46	1.16	0.61	0.00	0.00
LCP cottonseed-peanut	1.98	4.52	0.85	1.04	0.09	0.00	0.00
Soybean-peanut	3.24	11.04	0.20	2.04	0.71	0.05	0.04
LCP cottonseed[b]	3.21	5.4	3.11	0.00	0.00	0.00	0.00
Soybean	5.10	10.2	0.50	2.57	1.96	0.03	0.04
Peanut	3.07	10.2	0.07	2.78	0.22	0.00	0.00

[a] Lyophilized products.
[b] Residue also contained 0.1% lactose.

TABLE VI
Nutritive Quality of Flours, Coisolates, and Isolates Based on Essential Amino Acid Content[a]

Amino Acid[c,d]	Flour			Coisolate			Isolate		
	LCP[b] Cottonseed	Soybean	Peanut	LCP Cottonseed-Soybean	LCP Cottonseed-Peanut	Soybean-Peanut	LCP Cottonseed	Soybean	Peanut
Leucine	73.0a	87.0a	86.0a	95.0ab	86.0a	94.0ab	90.0ab	99.5b	89.5ab
Lysine	77.0cd	109.0f	59.0ab	85.0de	58.0ab	78.0cd	69.0bc	90.5e	53.5a
Threonine	76.0cd	80.0de	58.0a	91.5g	69.5bc	80.0de	83.5ef	88.0fg	66.0b
Tryptophan	130.0d	139.0e	121.0b	150.0e	126.5c	127.0c	130.5d	149.0f	112.5a
Cystine-Methionine	77.5c	63.0ab	63.0ab	77.0c	65.0ab	78.0c	68.0b	70.5bc	59.0a
Tyrosine-Phenylalanine	130.0b	116.0a	140.0c	138.5c	152.0de	152.0de	156.5e	148.5d	153.0de
Most limiting amino acid	isoleucine	cystine-methionine	threonine	cystine-methionine	lysine	lysine, cystine-methionine	cystine-methionine	cystine-methionine	lysine
Chemical score	64.0	63.0	58.0	77.0	58.0	78.0	68.0	70.5	53.5

[a] Amino acid score and chemical score were calculated according to FAO/WHO (1973).
[b] LCP = liquid cyclone processed.
[c] No significant differences were noted among flours, coisolates, and isolates for isoleucine (64.0–92.0) and valine (66.0–87.0).
[d] Means having any letters in common are not different at the 0.05 level of probability.

nutritional value of isolated proteins (Table VI). The LCP cottonseed-peanut coisolate had a chemical score intermediate to the LCP cottonseed and peanut isolates. The most limiting amino acids in both coisolates and isolates were cystine-methionine and lysine.

SUMMARY

A new, simple procedure for preparing coprecipitated protein isolates from mixtures of LCP cottonseed, soybean, and peanut flours involves acid dissociation and reassociation of polypeptides into their original or new protein forms. The lyophilized coisolates contained more than 95% protein and accounted for more than 67% of the nitrogen and 43% of the weight of the flour composites. LCP cottonseed-soybean and soybean-peanut coisolates had better amino acid content and nutritive value than their corresponding isolates. Use of this coprecipitation procedure should allow formulation of coisolates from composites containing different flour ratios to meet specific food requirements, especially in view of increasing computerization of low-cost, high-quality edible products in food formulations (Hsu et al 1977).

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