Waibel, P. E., Cuperlovic, M., Hurrell, R. F., Carpenter, K. J., *J. Agric Food Chem.* **25**, 171 (1977).
Walker, J. F., "Formaldehyde", 3rd ed, Reinhold, New York, N.Y., 1964.
Warren, J. R., Spero, L., Metzger, J. F., *Biochim. Biophys. Acta* **365**, 434 (1974).
Wiechers, S. G., Laubscher, H., *Annu. Rep. Fish. Ind. Res. Inst. (Cape Town)* **16**, 97 (1962).

Received for review December 19, 1977. Accepted April 10, 1978. The work was supported by a grant from the Agricultural Research Council and carried out at the Department of Applied Biology, University of Cambridge, England.

Utilization of Protein from Soy Flour and Soy Isolate by Adult Women

L. Janette Taper,* Nina L. Marable, Mary K. Korslund, and S. J. Ritchey

Two nitrogen balance studies with adult women subjects were conducted to assess the effect of alkaline processing of soy products on protein nutritive value. Two soy flours (Soyafluff 200W and Soyafluff 200T) were compared with soy isolate (Promine D) alone, soy isolate supplemented with amino acids to match the flour, and in one study, soy isolate supplemented with sulfur amino acids. At nitrogen intakes of 5.2–5.3 g/day, nitrogen retentions were −0.06 ± 0.16, −0.30 ± 0.27, and −0.21 ± 0.71 g of nitrogen/day for Soyafluff 200W, Promine D, and Promine D plus amino acids, respectively, in study I, and −0.09 ± 0.65, 0.17 ± 0.38, 0.04 ± 0.98, and −0.32 ± 0.33 g of nitrogen/day for Soyafluff 200T, Promine D, Promine D plus matching amino acids, and Promine D plus sulfur amino acids, respectively, in study II. PER values determined for Soyafluff 200W, Promine D, and Promine D plus amino acids were 1.6 ± 0.2, 1.4 ± 0.2, and 1.5 ± 0.2, respectively (casein: 2.7 ± 0.3). Nitrogen retentions and PER values were not significantly different from each other.

The use of protein from soybean has increased dramatically in the last decade. This relatively abundant, inexpensive and good quality protein is being utilized in human foods in a variety of forms, including flour, soy protein concentrates, soy protein isolate, and spun soy fibers.

The preparation of soy protein isolates includes exposure to heat and mild alkali (Horan, 1974). Isolated soy protein may undergo more severe alkaline processing in the production of spun fibers used in the fabrication of meat analogues (Horan, 1974; Rosenfield and Hartman, 1974). In addition, during processing, certain protein fractions may be discarded. These treatments may influence the nutritive value of the final product. Several investigators have reported decreases in the nutritive value of soy protein isolated by an alkaline process (Bressani et al., 1967; Cogan et al., 1968; Badenhop and Hackler, 1970, 1973). The destruction of certain amino acids has been associated with alkaline processing (Cogan et al., 1968; DeGroot and Slump, 1969; Badenhop and Hackler, 1970; Robinson et al., 1971; and Woodard and Short, 1973). However, amino acid destruction alone does not sufficiently explain the decrease in protein quality in all cases. For example, DeGroot and Slump (1969) found that a decreased net protein utilization (NPU) resulting from alkaline treatment could not be completely alleviated by supplementation with either methionine or methionine plus lysine. Supplementation with threonine, which had not been destroyed on processing, plus methionine and lysine did improve NPU relative to supplementation with methionine and lysine. DeGroot and Slump (1969) suggested that a decreased utilization of threonine might be the result of isomerization from the L to D form. It seems clear that the availability of amino acids can be decreased under conditions of alkaline processing, and that the basic cause may be either racemization to less readily utilized forms (DeGroot and Slump, 1969) and/or destruction of amino acids and changes in the overall amino acid pattern (Harper, 1956; Kofranyi, 1972).

Data on the protein nutritive value of soy products, as measured in human subjects, are not as readily available as data from rat studies (Bressani, 1975). Furthermore, in studies with humans, the various forms of soy are usually tested in comparison to a non-soy protein source (Parthasarathy et al., 1964; Bressani et al., 1967; Kies and Fox, 1971, 1973; Korslund et al., 1973). These cited studies tested soy flour, textured vegetable protein, and a spun soy fiber product. Soy protein isolate has also been tested in human subjects, but again not in direct comparison to other soy protein sources (Zezulka and Calloway, 1976a,b).

The present studies were designed to compare, with human subjects, the relative protein quality of two soy flours which had not been processed with alkali and a soy protein isolate, which had received alkaline treatment. The subjects were young adult women, and nitrogen retention was used as the criterion for comparing the sources of protein. Study I compared Soyafluff 200W, which according to the manufacturer is a minimally moist-heat-treated flour, with a soy isolate (Promine D). Study II compared Soyafluff 200T, a toasted flour, to Promine D. These products were supplied by Central Soya Co., Chicago, Ill. Since changes in amino acid pattern could account for potential nutritive differences in flour and isolate, some experimental treatments were designed to obtain information about this possibility. In both studies, one group of subjects was fed soy isolate plus essential amino acids and a nonspecific nitrogen source. The combination of isolate and amino acids was designed to provide an essential amino acid pattern from isolate and supplemental amino acids identical with the soy flour pattern. In study II, one experimental group received

Department of Human Nutrition and Foods, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061.

0021-8561/78/1426-0802$01.00/0

Table I. Experimental Design and Sources of Nitrogen Fed during Two Studies

Source of nitrogen	Study I Treatments A	Study I Treatments B	Study I Treatments C	Study II Treatments A	Study II Treatments B	Study II Treatments C	Study II Treatments D
Basal diet[b]	1.27	1.28	1.28	1.16	1.20	1.28	1.20
Soy flour	4.00[c]			4.00[d]			
Soy isolate[e]		4.00	3.09		4.00	3.64	4.00
Amino acids			0.21[f]			0.22[f]	0.01[g]
Diammonium citrate			0.70			0.16	
Total	5.27	5.28	5.28	5.16	5.20	5.30	5.21

[a] Nitrogen expressed as g/day. Subjects received these diets during 5-day adjustment and 5-day experimental periods. [b] Includes nitrogen from ingredients of bread. [c] Soyafluff 200W; Central Soya Co., Chicago. [d] Soyafluff 200T; Central Soya Co., Chicago. [e] Promine D; Central Soya Co., Chicago. [f] Amino acid mixture to match pattern of soy flour. [g] Methionine and cystine.

Table II. Mean Weights of Subjects on Each Experimental Treatment of the Two Studies[a]

Source of nitrogen	Study I No. of subjects	Study I Initial	Study I Final	Study II No. of subjects	Study II Initial	Study II Final
Soy flour	5	56.9 ± 6.4	57.4 ± 6.1	4	57.7 ± 10.6	58.2 ± 9.5
Soy isolate	6	57.6 ± 7.3	57.9 ± 7.2	3	58.4 ± 4.6	58.3 ± 4.9
Soy isolate + amino acids	6	57.0 ± 6.3	57.2 ± 6.2	6	60.3 ± 5.4	59.5 ± 5.6
Soy isolate + sulfur amino acids				5	58.8 ± 5.0	58.2 ± 4.8

[a] Weights, in kg, ± SD on the first and last days of the experimental period.

supplements of sulfur amino acids in addition to soy isolate. The general experimental design and sources of nitrogen for the human studies are shown in Table I. In connection with study I, protein efficiency ratios (PER's) were determined for Soyafluff 200W, Promine D, and Promine D plus essential amino acids.

MATERIALS AND METHODS

Human Studies. *Subjects.* Subjects for the two studies were healthy young adult women who volunteered from the university community. Subjects maintained their normal schedules throughout the study, but consumed all meals in the metabolic unit of the department. The mean age of subjects in study I was 22.6 (range of 20–25) years; the mean weight on the first day of the experimental period was 57.2 (range of 47.3 to 67.3) kg. Subjects in study II weighed an average of 58.8 (range of 42.2–66.7) kg. Subjects were randomly assigned from weight groups to dietary treatments at the end of a 2-day nitrogen-depletion period. Mean initial and final weights of subjects for all experimental treatments are shown in Table II.

Experimental Treatments and Diets. Each study was divided into a 2-day nitrogen-depletion period, a 5-day adjustment period, and a 5-day experimental period. The 2-day period was utilized to reduce the time necessary for adjustment to a low-protein diet (Kies and Fox, 1970). During this period the subjects consumed a basal diet of low nitrogen fruits and vegetables and a yeast bread made with wheat starch. The basal diet provided approximately 1.2 g of nitrogen and 1800 kcals daily. The total caloric intake during both studies was approximately 2300 kcals, except for individuals whose intake was adjusted to maintain weight.

During the adjustment and experimental periods, the daily nitrogen intake was maintained at approximately 5 g. This level was selected because other studies (Kies, 1972; Kies et al., 1975) have indicated that this amount is borderline in meeting protein needs for human adults and therefore would be a sensitive level for evaluating responses to the dietary treatments. Total nitrogen was provided from the basal diet and from either soy flour, isolated soy protein, or isolated soy proteins plus amino acids or a nonspecific nitrogen source (Table I).

After the 2-day depletion period, soy flour and soy isolate were incorporated into the wheat starch bread; amino acids and the nonspecific nitrogen source were mixed into juices or applesauce. Except for snacks, approximately one-third of the nitrogen was given to subjects at each meal. Subjects were provided vitamin and mineral supplements throughout the studies. In study I, One-A-Day Plus Iron, Miles Laboratories, Elkart, Ind., provided supplemental vitamins and iron. An additional supplement provided 464 mg of Ca, 360 mg of P, and 10 μg of vitamin D. In study II OsCal Plus, Marion Laboratories, Inc., Kansas City, Mo., was used to provide supplemental vitamins and minerals.

Supplements of amino acids or nonspecific nitrogen were based on amino acid analysis of soy flours and soy isolates. Duplicate amino acid analyses were accomplished using a Model NC-1P or TSM Technicon Amino Acid Analyzer; cystine and methionine were determined following conversion to cysteic acid and methionine sulfone (Moore, 1963); tryptophan was determined by the method of Graham et al. (1947). The amino acid composition of the flours and the isolate is summarized in Tables III and IV. The amino acid which was present in the largest excess in the soy isolate compared to the flour was used to determine the level of nitrogen to be supplied from the isolate. In study I, isoleucine was the amino acid used as the basis of nitrogen supplied; in study II, phenylalanine was the amino acid utilized for that purpose (Tables III and IV).

In study I, the soy isolate provided 3.09 g of nitrogen in the amino acid supplement group; essential amino acids and cystine were added to match the soy flour pattern (Tables I, III). Diammonium citrate was added to make the diets isonitrogenous (Table I). In study II, a similar manipulation was accomplished to achieve the desired intakes of nitrogen and amino acids. In addition, a fourth treatment was introduced to demonstrate the response to additions of only the sulfur-containing amino acids, the limiting amino acids in soy protein (Tables I, IV).

Table III. Essential Amino Acid Intake Patterns from Soy Flour, Soy Isolate and Amino Acids Added to Achieve Desired Patterns of Intake for Study I[a]

	Daily intake levels for three treatment groups			
	A	B	C[b]	
	Soyafluff 200W, g/4 g of N	Promine D, g/4 g of N	Promine D, g/3.09 g of N	Added amino acids, g
Lysine	1.50	1.58	1.22	0.28
Methionine	0.31	0.25	0.19	0.12
Cystine	0.30	0.30	0.23	0.07
Threonine	1.00	0.95	0.73	0.27
Leucine	1.93	2.00	1.55	0.38
Isoleucine	0.95	1.23	0.95	
Phenylalanine	1.18	1.28	0.99	0.19
Valine	1.25	1.28	0.99	0.26
Tryptophan	0.35	0.28	0.22	0.13

[a] Intake from basal diet is not included. Promine D was a different sample from that used in study II. [b] Essential amino acids from 3.09 g of N from Promine D plus added amino acids to equal amino acids from 4.0 g of N from Soyafluff 200W; the amount of N from Promine D based on isoleucine content of flour.

Data Collected. The following data were collected for each dietary treatment: nitrogen intake, based on analyzed values of food composites; urine and fecal nitrogen excretions, based on 24-h urine collections and a 5-day marked fecal collection during the experimental period; creatinine excretion on daily urine composites. Nitrogen and creatinine were determined in urine using a Technicon Autoanalyzer II. Nitrogen was measured using a modification of the Berthelot reaction, and creatinine was measured using a modification of the Jaffe reaction (Technicon Corporation, Tarrytown, N.Y.). Food and fecal nitrogen were determined by a modified Kjeldahl–Gunning–Arnold method (AOAC, 1970). Nitrogen balances were calculated from the above data for the 5-day experimental period. Analysis of variance and Duncan's Multiple Range Test were used to assess responses to dietary treatments (Snedecor and Cochran, 1967; Kramer, 1956).

Rat Studies. *Protein Efficiency Ratios.* PER's were determined according to the AOAC method (1970) for Soyafluff 200W, Promine D, and Promine D plus amino acids to match the pattern of the flour. Diammonium citrate was added to make each treatment isonitrogenous. A fourth group of animals was fed casein as a reference protein. The composition of diets is shown in Table V. Analysis of variance was used to test for differences in response.

RESULTS AND DISCUSSION

Mean daily urine and fecal nitrogen excretions for the 5-day experimental periods of studies I and II are shown in Table VI. There were no significant differences ($p < 0.05$) in urine or fecal nitrogen excretions between the three treatments in study I. Similarly, in study II, urine nitrogen excretions did not differ. However, fecal nitrogens in study II were statistically different as indicated in Table VI. Crude nitrogen retention values (no correction for excretion losses other than in urine and feces) are also shown in Table VI. There were no significant differences ($p < 0.05$) in retention between the three treatment groups in study I or between the four treatment groups in study II.

Apparent nitrogen digestibilities which can be calculated from fecal nitrogen excretions and nitrogen intakes shown in Table VI were not significantly different in either study according to Duncan's Multiple Range Test. However, it is worth noting that the isolates and supplemented isolates in both studies were consistently higher in apparent nitrogen digestibility than the respective flours to which they were compared.

Mean nitrogen retentions were negative for five out of seven treatment groups. This is not surprising in view of the total nitrogen intake level (approximately 5 g) and the sulfur amino acid intake level. The total nitrogen level corresponds to an intake of approximately 0.08–0.09 g of N/kg of body weight for these subjects. Zezulka and Calloway (1976a,b) obtained a corrected mean nitrogen balance of 0.08 g/day when soy protein isolate supplied 4.5 g of nitrogen in a diet with total nitrogen intake of approximately 9 g. The total sulfur amino acid content supplied by the 4 g of soy nitrogen in the diets in the present studies ranged from 550–670 mg/day, while the basal diets for studies I and II supplied 120 and 51 mg/day of methionine. The total sulfur amino acid intake was below the FAO recommended intake of 910 mg/day for a 70-kg man (FAO/WHO, 1973), but above the minimum (540 mg/day) of the requirement range for young adults as determined by Clark et al. (1970). Evidence from Zezulka and Calloway (1976a,b) clearly indicates that a soy

Table IV. Essential Amino Acid Intake Patterns from Soy Flour, Soy Isolate, and Amino Acids Added to Achieve Desired Patterns of Intake for Study II[a]

	Daily intake levels for four treatment groups					
	A	B	C[b]		D[c]	
	Soyafluff 200T, g/4 g of N	Promine D, g/4 g of N	Promine D, g/3.64 g of N	Added amino acids, g	Promine D, g/4 g of N	Added amino acids, g
Lysine	1.60	1.58	1.44	0.16	1.58	
Methionine	0.32	0.32	0.29	0.03	0.32	0.03
Cystine	0.35	0.30	0.27	0.08	0.30	0.08
Threonine	1.05	1.00	0.91	0.14	1.00	
Leucine	1.98	2.08	1.89	0.09	2.08	
Isoleucine	1.25	1.32	1.20	0.05	1.32	
Aromatic amino acid	2.28	2.50	2.28		2.50	
Phenylalanine	0.93	1.02	0.93		1.02	
Tyrosine	1.35	1.48	1.35		1.48	
Valine	1.48	1.35	1.23	0.25	1.35	
Tryptophan[d]	0.35	0.28	0.25	0.10	0.28	

[a] Intake from basal diet is not included. Promine D was a different sample from that used in study I. [b] Amino acids from 3.64 g of N from Promine D plus added amino acids to equal amino acids from 4.0 g of N from Soyafluff 200T; the amount of N from Promine D based on aromatic amino acid content of flour. [c] Amino acids from 4.0 g of N from Promine D plus methionine and cystine. [d] Data from manufacturer.

Table V. Composition of Test Diets and PER's of Different Nitrogen Sources

	Diet, g/100 g of ration			
Component	1 (flour)	2 (isolate)	3 (isolate)	4 (casein)
Vitamin mix[a]	2.2	2.2	2.2	2.2
Mineral mix[a]	5.0	5.0	5.0	5.0
Corn oil	5.0	5.0	5.0	5.0
Alphacel[a]	2.0	2.0	2.0	2.0
Corn starch	66.8	75.2	74.7	74.9
Soy flour[b]	19.2			
Soy isolate[b]		10.8	8.3	
Casein[a]				11.1
Supplemental amino acids				
Lysine[a]			0.11	
Methionine[a]			0.05	
Threonine[a]			0.11	
Leucine[c]			0.15	
Phenylalanine[c]			0.07	
Valine[a]			0.10	
Tryptophan[c]			0.05	
Cystine[c]			0.03	
Diammonium citrate[d]			2.23	
PER[e]	1.6 ± 0.2	1.4 ± 0.2	1.5 ± 0.2	2.7 ± 0.3

[a] ICN Pharmaceuticals, Cleveland. [b] Central Soya, Chicago. [c] Sigma Chemical Co., St. Louis. [d] Fisher Scientific Co., Fair Lawn. [e] Mean ±SD.

isolate (though not the same isolate as in the present studies) can support nitrogen balance in adult subjects at a higher intake level of isolate (6.0 g of nitrogen/day from isolate) or when supplemented with methionine.

The observation that nitrogen retentions are not significantly different for either of the two flours compared to soy isolate is interesting in view of the frequently expressed opinion that, except for the beneficial effect of proper heat processing on raw soy flour, increased processing of soy results in decreased nutritive value (Bressani, 1975). The evidence generally cited in support of this position is PER data, since studies which have tested relative nutritive value of various forms of soy in the same experiment were generally not carried out with human subjects (e.g., Bressani et al., 1967; Longenecker et al., 1964; Cogan et al., 1968). In most human studies, different soy products were not compared to each other but rather to some other protein source. For example, Zezulka and Calloway (1976a,b) compared soy isolate with and without supplemental methionine, acetyl methionine, or inorganic sulfate to egg white as a protein source using the nitrogen balance technique with human subjects. Kies and Fox (1971) and Korslund et al. (1973) compared beef to TVP with and without added methionine in human balance studies with both adults and adolescents. Bressani et al. (1967) fed a soybean protein textured food (SPTF) to children at varying levels of protein intake. Nitrogen balance values obtained with the SPTF were compared to values obtained when milk was the protein source.

In connection with study I, the PER's of Soyafluff 200W and Promine D were determined to be 1.6 ± 0.2 and 1.4 ± 0.2 (casein 2.7 ± 0.3), respectively. Promine D supplemented with amino acids had a PER of 1.5 ± 0.2 (Table V). There were no significant differences in the PER's for the three dietary treatments. This is consistent with the results of human study I. The measured PER for the isolate is similar to the manufacturer's reported value of 1.1–1.2 (casein 2.5; Central Soya, Chicago, Ill.).

Commonly cited literature values for PER's of isolates and toasted flour and the manufacturer's description of Soyafluff 200T as a toasted flour suggested that the protein nutritive value for humans might be different for Soyafluff 200T compared to Promine D. Soy isolates, in general, are reported to have PER's in the range 1.1–1.7 (e.g., Mattil, 1974). Soy flours which are described as untoasted also have PER's of approximately 1.3, but so-called fully toasted flours usually have higher PER's of approximately 2.2 (Kellor, 1974). However, the only statistically significant measured difference between Soyafluff 200T and Promine D in study II was in fecal nitrogen excretion as previously discussed (Table VI).

PER may be particularly unsuitable as the sole means of assessment of the protein nutritive value for humans of processed soy products. Kies and Fox (1971) have reported different relative nutritive values for beef, TVP (Textured Vegetable Protein, Archer Daniels Midland Co., Decatur, Ill.), and TVP + DL-methionine depending on whether the method of assessment was PER (manufacturer's data) or nitrogen balance. Several factors may complicate comparison of the results of the present balance studies with data obtained in rat studies. Trypsin inhibitors may not be as detrimental to humans as to rats (Liener, 1977). If so, the presence or absence of such inhibitors in the soy products could lead to the determination of different relative nutritive values depending on whether a human balance study or a PER study was conducted. Also, processing may produce oxidation of sulfur amino acids and/or isomerization of L to D amino acids (Walker et al., 1975; Cuq et al., 1973; Sen et al., 1977). Little is known about the utilization by humans of oxidized sulfur amino acids; and, in spite of recent work with human subjects (Kies et al., 1975; Zezulka and Calloway, 1976a,b), the relative abilities of rats and man to utilize D-amino acids is still not clear. It has been suggested (Hamdy, 1974)

Table VI. Nitrogen Intake, Excretion, and Retention by Subjects Consuming Soy Flour, Soy Isolate, and Supplemental Diets

	g of nitrogen/day			
		Excretion[a]		
Treatment	Intake	Urinary	Fecal	Retention[a,b]
Study I				
A. Soy flour (Soyafluff 200W)	5.27	4.11 ± 0.22	1.22 ± 0.26	-0.06 ± 0.16
B. Soy isolate[d]	5.28	4.50 ± 0.27	1.08 ± 0.22	-0.30 ± 0.27
C. Soy isolate[d] + amino acids	5.28	4.48 ± 0.71	1.00 ± 0.17	-0.21 ± 0.71
Study II				
A. Soy flour (Soyafluff 200T)	5.16	3.81 ± 0.63	1.44 ± 0.32[ac]	-0.09 ± 0.65
B. Soy isolate[d]	5.20	4.29 ± 0.20	0.74 ± 0.21[b]	0.17 ± 0.38
C. Soy isolate[d] + amino acids	5.30	4.37 ± 0.74	0.87 ± 0.40[b]	0.04 ± 0.98
D. Soy isolate[d] + sulfur amino acids	5.21	4.24 ± 0.40	1.29 ± 0.13[a]	-0.32 ± 0.33

[a] Mean ±SD. [b] Retentions were not significantly different ($p < 0.05$). [c] Fecal excretions (study II) were significantly different ($p < 0.05$) as indicated by different letter superscripts. Statistical analysis included analysis of variance followed by Duncan's multiple range test (Snedecor and Cochran, 1967; Kramer, 1956). [d] Promine D.

that perhaps the difference in assessed values in the Kies and Fox study (1971) was due to a superior ability of the rat compared to humans to utilize D-methionine.

Conventional amino acid analysis of the soy products revealed small changes in amino acid patterns for soy isolate compared to soy flour (Tables III and IV). Either these changes were too small to cause differences in nitrogen retention or they were counteracted by other factors. Feeding soy isolate supplemented with amino acids to match the amino acid pattern of flour or supplemented with small amounts of methionine and cystine led to nitrogen retentions which were not statistically different from retention with flour and isolate alone. Of course, this should not be interpreted to mean that supplementation at higher levels would not improve retention.

In summary, under the conditions of this study there were no detectable differences in protein quality for human subjects between the soy flours, soy isolate, and supplemented soy isolate as measured by the nitrogen balance technique with approximately 5 g total nitrogen intake. Obviously, similar comparisons of other flours and isolates might reveal significant differences in protein value. However, the evidence of these studies clearly indicates that it should not be automatically assumed that either soy flour or soy isolate would be significantly better in protein quality for humans, especially since processing conditions vary widely (Mattil, 1974).

LITERATURE CITED

Association of Official Analytical Chemists, "Official Methods of Analysis", 11th ed, Washington, D.C., 1970.
Badenhop, A., Hackler, L., *Cereal Sci. Today* **15**, 84 (1970).
Badenhop, A., Hackler, L., *J. Food Sci.* **38**, 471 (1973).
Bressani, R., *J. Am. Oil Chem. Soc.* **52**, 254A (1975).
Bressani, R., Viteri, F., Elias, L., de Zaghi, S., Alvarado, J., Odell, A., *J. Nutr.* **93**, 349 (1967).
Central Soya Co., Technical Service Manuals, Chicago, Ill.
Clark, H. E., Howe, J. M., Shannon, B. M., Carlson, K., Kolski, S. M., *Am. J. Clin. Nutr.* **23**, 731 (1970).
Cogan, U., Yaron, A., Berk, Z., Zimmerman, G., *J. Agric. Food Chem.* **16**, 197 (1968).
Cuq, J. L., Provansal, M., Guilleux, F., Cheftel, C., *J. Food Sci.* **38**, 11 (1973).
DeGroot, A., Slump, P., *J. Nutr.* **98**, 45 (1969).
FAO/WHO, "Energy and Protein Requirements", World Health Organization Technical Report No. 522 and FAO Nutrition Meetings Report No. 52, Rome, 1973.
Graham, C., Smith, E., Hier, S., Klein, D., *J. Biol. Chem.* **168**, 711 (1947).
Hamdy, M. M., *J. Am. Oil Chem. Soc.* **51**, 85A (1974).
Harper, A., *Nutr. Rev.* **14**, 225 (1956).
Horan, F., *J. Am. Oil Chem. Soc.* **51**, 67A (1974).
Kellor, R. L., *J. Am. Oil Chem. Soc.* **51**, 77A (1974).
Kies, C., "Symposium: Seed Proteins", Inglett, G. E., Ed., Avi Publishing Co., Westport, Conn., 1972, pp 255–264.
Kies, C., Fox, H. M., *J. Nutr.* **100**, 1275 (1970).
Kies, C., Fox, H. M., *J. Food Sci.* **36**, 841 (1971).
Kies, C., Fox, H. M., *J. Food Sci.* **38**, 1211 (1973).
Kies, C., Fox, H. M., Aprahamian, S., *J. Nutr.* **105**, 309 (1975).
Kofranyi, E., "Protein and Amino Acid Functions", Bigwood, E., Ed., Pergamon Press, Oxford, 1972, pp 1–62.
Korslund, M., Kies, C., Fox, H., *J. Food Sci.* **38**, 637 (1973).
Kramer, C. Y., *Biometrics* **12**, 307 (1956).
Liener, I. E., "Evaluation of Proteins for Humans", Bodwell, C. E., Ed., Avi Publishing Co., Westport, Conn., 1977, pp 288–289.
Longenecker, J., Martin, W., Sarrett, H., *J. Agric. Food Chem.* **12**, 411 (1964).
Mattil, K. F., *J. Am. Oil Chem. Soc.* **51**, 81A (1974).
Moore, S., *J. Biol. Chem.* **238**, 235 (1963).
Parthasarathy, H. N., Doraiswamy, T. R., Panemangalore, M., Rao, M. N., Chandrasekhar, B. S., Swaminathan, M., Sreenivasan, A., Subrahmanyan, V., *Can. J. Biochem.* **42**, 377 (1964).
Robinson, W., Bourne, M., Steinkraus, K., "Development of Soy-Based Foods of High Nutritive Value for Use in the Phillipines", *Natl. Tech. Inf. Ser.* No. PB-213-758, U.S. Department of Commerce, Washington, D.C., 1971.
Rosenfield, D., Hartman, W., *J. Am. Oil Chem. Soc.* **51**, 91A (1974).
Sen, L. C., Gonzalez-Flores, E., Feeney, R. E., Whitaker, J. R., *J. Agric. Food Chem.* **25**, 632 (1977).
Snedecor, G. W., Cochran, W. G., "Statistical Methods", Iowa State University Press, Ames, Iowa, 1967.
Technicon Corporation, Technical Literature, Tarrytown, N.Y.
Walker, H. G., Kohler, G. O., Kuzmicky, D. D., Witt, S. C., "Protein Nutritional Quality of Foods and Feeds", Part I, Friedman, M., Ed., Marcel Dekker, New York, N.Y., 1975, pp 549–567.
Woodard, J., Short, D., *J. Nutr.* **103**, 569 (1973).
Zezulka, A. Y., Calloway, D. H., *J. Nutr.* **106**, 212 (1976a).
Zezulka, A. Y., Calloway, D. H., *J. Nutr.* **106**, 1286 (1976b).

Received for review September 30, 1977. Accepted April 4, 1978.

Precipitation of Proteins from Whey with Bentonite and Lignosulfonate

Janis Cerbulis

A method is presented for the recovery of protein in cheese whey (0.7–0.9%) by precipitation with bentonite and lignosulfonate used separately and in combination. These reagents are compared with the commonly employed precipitant hexametaphosphate. Both bentonite and lignosulfonate precipitated most of the crude protein plus some of the nonprotein nitrogen and lactose from cottage cheese whey, pH 4.6. Precipitation with 3% bentonite yielded 38.5 g/L of dried precipitate containing 5.6 g or 92% of the crude protein, while 1% lignosulfonate yielded 12.5 g/L with 5.2 g or 85% of the crude protein. Hence, both reagents are effective precipitants of whey protein in a one-step procedure carried out at room temperature.

Over 30 billion pounds of whey result from cheese making each year, and only about half of this whey is used as animal or human food; the remainder (15 billion pounds) is wasted and often causes water pollution (Woychik, 1975). Proteins have been recovered from whey by a variety of techniques (Morr, 1976; Woychik, 1975); they have been precipitated with sodium hexametaphosphate (Hartman and Swanson, 1966; Richert, 1973; Hidalgo et al., 1973), carboxymethyl cellulose (Hidalgo and

Eastern Regional Research Center, Science and Education Administration, U.S. Department of Agriculture, Philadelphia, Pennsylvania 19118.